



HAL
open science

Roles of eukaryotic ribosomal proteins in maturation and transport of pre-18S rRNA and ribosome function

S. Ferreira-Cerca, Gisela Pöll, Pierre-Emmanuel Gleizes, Herbert Tschochner, Philipp Milkereit

► To cite this version:

S. Ferreira-Cerca, Gisela Pöll, Pierre-Emmanuel Gleizes, Herbert Tschochner, Philipp Milkereit. Roles of eukaryotic ribosomal proteins in maturation and transport of pre-18S rRNA and ribosome function. *Molecular and Cellular Biology*, 2005, 20 (2), pp.263-275. 10.1016/j.molcel.2005.09.005 . hal-00021190

HAL Id: hal-00021190

<https://hal.science/hal-00021190>

Submitted on 1 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Roles of Eukaryotic Ribosomal Proteins in Maturation and Transport of Pre-18S rRNA and Ribosome Function

Sébastien Ferreira-Cerca,^{1,2} Gisela Pöll,¹
Pierre-Emmanuel Gleizes,² Herbert Tschochner,^{1,*}
and Philipp Milkereit^{1,*}

¹Institut für Biochemie, Genetik und Mikrobiologie
Universität Regensburg
Universitätsstr 31
93053 Regensburg
Germany

²Laboratoire de Biologie Moleculaire Eucaryote
LBME-CNRS
118 route de Narbonne
F-32062 Toulouse Cedex
France

Summary

Despite the rising knowledge about ribosome function and structure and how ribosomal subunits assemble *in vitro* in bacteria, the *in vivo* role of many ribosomal proteins remains obscure both in pro- and eukaryotes. Our systematic analysis of yeast ribosomal proteins (r-proteins) of the small subunit revealed that most eukaryotic r-proteins fulfill different roles in ribosome biogenesis, making them indispensable for growth. Different r-proteins control distinct steps of nuclear and cytoplasmic pre-18S rRNA processing and, thus, ensure that only properly assembled ribosomes become engaged in translation. Comparative analysis of dynamic and steady-state maturation assays revealed that several r-proteins are required for efficient nuclear export of pre-18S rRNA, suggesting that they form an interaction platform with the export machinery. In contrast, the presence of other r-proteins is mainly required before nuclear export is initiated. Our studies draw a correlation between the *in vitro* assembly, structural localization, and *in vivo* function of r-proteins.

Introduction

Formation of the two eukaryotic ribosomal subunits is an evolutionarily conserved process that relies on the correct assembly of four rRNA species and more than 70 ribosomal proteins. In addition, about 150 nonribosomal factors are required for synthesis, maturation, and transport of the two subunits. In yeast, the powerful combination of genetic and modern biochemical approaches has shed light on the participation of eukaryotic nonribosomal factors in single steps of ribosome biogenesis. However, much less is known about the roles of eukaryotic r-proteins. A comprehensive analysis of eukaryotic ribosomal protein function in *S. cerevisiae* is difficult because 59 of the 78 r-proteins are encoded by two copy genes and, therefore, those genes could not be analyzed in the genome-wide, sin-

gle knockout approach (Giaever et al., 2002; Winzeler et al., 1999). Because only four of the 19 one-copy genes are not essential for growth, a pivotal role for most of the ribosomal proteins encoded by two gene copies can be anticipated.

In principal, ribosomal protein function can be required for different stages of ribosome biogenesis and/or for distinct steps of the translation process. Accordingly, r-proteins might play a central role in rRNA processing, protein assembly, RNA folding, transport of the ribosomal precursors, stabilization of the subunit structure, and/or interaction with other factors required for either ribosome biogenesis or translation (Blaha, 2004; Nierhaus, 2004). Furthermore, they can also be involved in cotranslational processes like the interaction with protein folding factors at the exit tunnel of the ribosome (Kramer et al., 2002; Pool et al., 2002) or cotranslational translocation (Beckmann et al., 2001; Clemons et al., 2004). R-proteins can also contribute to important enzymatic activities for ribosome function like the mRNA helicase activity of bacterial ribosomes (Takyar et al., 2005).

For eukaryotic r-proteins, only a few functional roles in translation have been so far suggested. rpS0 was shown to interact directly with the subunit Tif32p of the translation initiation factor eIF3, arguing that it is involved in recruiting eIF3 to the small ribosomal subunit (Valasek et al., 2003). Mutants of the small ribosomal subunit protein genes *RPS2* and *RPS9* are able to suppress all three classes of nonsense codons in yeast. Together with rpS28, the corresponding r-proteins are important for translational accuracy, e.g., the rate of missense translation errors, resembling in function their prokaryotic counterparts (Alksne et al., 1993; Stansfield et al., 1998; Syntetos et al., 1996). Furthermore, mutants of *RPL3* and *RPL5* and deletions of the nonessential genes *RPL24*, *RPL39*, and *RPL41* have effects on the peptidyltransferase activity of the resulting ribosomes (Dresios et al., 2003; Meskauskas and Dinman, 2001; Peltz et al., 1999). Whether these ribosomal proteins are also required for ribosome biogenesis is not known.

Although initial biochemical maps of nonribosomal factors in pre-60S and pre-40S ribosomal subunits that were on their path from the nucleolus to the cytoplasm have been established (see Milkereit et al., 2003a; Tschochner and Hurt, 2003 and references therein), ribosomal proteins were pragmatically excluded because they were considered to be nonspecific contaminants — many r-proteins were nonspecifically associated to control beads and were found in more than 3.5% of isolated eukaryotic protein complexes (Gavin et al., 2002; Ho et al., 2002). Most maturation steps of ribosomal precursor particles take place in the nucleolus. First, a common precursor of both ribosomal subunits with the sedimentation constant of 90S is produced, which is then split into precursor particles of the large and small subunit. Components of the small subunit processome (SSU-processome; Dragon et al., 2002), which are also part of the 90S preribosome (Grandi et al., 2002), were suggested to associate cot-

*Correspondence: herbert.tschochner@vkl.uni-regensburg.de (H.T.); philipp.milkereit@vkl.uni-regensburg.de (P.M.)

ranscriptionally with the growing RNA chain (Gallagher et al., 2004). When the U3 snoRNA, together with UTPs (U-three proteins), have assembled to complete the SSU-processome, cleavage at site A2 occurs, and most of the pre-40S factors are then released en bloc and pre-60S factors start to associate with the RNA moiety. After cleavage at site A2, both ribosomal subunits mature in two distinct pathways. The pre-40S ribosomal subunit is rapidly released through the nuclear pore to the cytoplasm (Leger-Silvestre et al., 2004; Trapman and Planta, 1976), where the last processing steps, including the processing of 20S rRNA to mature 18S rRNA, take place. In the 60S pathway, many nucleolar and nuclear pre-ribosomal complexes have been identified that are all involved in proper maturation and transport of the large subunit.

For the 60S-pathway, the roles of a few ribosomal proteins were investigated more closely. Nuclear export of the large subunit is dependent on the interaction between rpL10 and the adaptor protein Nmd3p (Johnson et al., 2002). Apparently rpL10, together with the GTPase Lsg1, is required to release cytoplasmic Nmd3 from the 60S particle to allow Nmd3 recycling into the nucleus (Hedges et al., 2005).

Stabilization of 5S rRNA is mediated by the interaction with rpL5 and rpL16, while rpL12 is involved in the correct assembly of the ribosomal stalk (Briones et al., 1998; Deshmukh et al., 1995; Deshmukh et al., 1993; Tsay et al., 1994).

Interestingly, disruption of some genes coding for ribosomal proteins of the small subunit resulted in related but distinct rRNA processing defects rather than producing just “suboptimal” ribosomes. For instance, rpS15 was shown to be required for late nucleoplasmic maturation steps that make the small subunit precursors competent for nuclear export (Leger-Silvestre et al., 2004). Structural and functional data pointed to a role of rpS14, rpS0, and rpS21 in cytoplasmic rRNA processing steps leading to mature 18S rRNA (Jakovljevic et al., 2004) (Ford et al., 1999; Tabb-Massey et al., 2003).

In conclusion, it can be hypothesized that many eukaryotic small ribosomal subunit proteins that maintain the structural integrity of mature ribosomal subunits also play important roles in ribosome biogenesis and intracellular transport. However many questions remain open such as the following: (1) how many and which small ribosomal subunit proteins are required for growth; (2) which small ribosomal subunit proteins are required for ribosome biogenesis; (3) are they involved in distinct maturation steps along the entire biogenesis pathway; (4) are small ribosomal subunit proteins directly involved in nuclear export; and (5) which of the ribosomal proteins play a crucial role in ribosome function?

We decided to systematically approach these important questions. Yeast strains conditionally expressing r-proteins were generated, and the role of individual ribosomal proteins in growth, pre-18S rRNA processing, localization and export of pre-18S rRNA, and protein translation were analyzed. Our data revealed that most small subunit r-proteins play an essential role in ribosome maturation and transport of rRNA. Shutting down of several essential ribosomal protein genes retard, but

do not completely block, pre-rRNA export, supporting the idea that these ribosomal proteins are directly involved in the export of the small subunit. These r-proteins can be distinguished from those controlling specific maturation steps in the nucleus. From these results, specific ribosomal proteins can now be used as crucial landmarks in the established rRNA processing pathway. Moreover, this in vivo analysis of eukaryotic r-proteins can be compared with structural data and the in vitro assembly map of their bacterial homologs.

Results

Strategy to Generate and Analyze Strains Conditionally Expressing Ribosomal Proteins

A systematic functional study of yeast ribosomal proteins requires in most cases the knockout of two identical copies present in the haploid genome (Figure 1). First, a diploid strain was generated by mating two yeast strains in which either one of the two ribosomal gene copies was replaced by a different selection marker (KanMX4 or HIS3). After transformation with a shuttle vector expressing a wild-type copy of the respective ribosomal protein and the URA3 gene product (pRPSX), tetrad analysis was performed, and haploid strains bearing deletions in both gene copies were selected. Viability of complete knockout strains was then tested on 5-FOA-plates. Twenty six of the 28 small subunit r-proteins studied herein are required for cell growth. Of the remaining four, small subunit r-proteins rpS4 (Synetos et al., 1992) and rpS21 were already reported to be essential (see www.yeastgenome.org) (Tabb-Massey et al., 2003) and r-proteins rpS22 and rpS29 are still under investigation, as no clear phenotype could be observed.

Strains conditionally expressing the respective ribosomal protein were generated by exchanging the plasmid containing the wild-type copy with a vector carrying a conditional allele (for details see Figure 1 and Experimental Procedures). We chose plasmid pGAL-RPSX in which expression of the ribosomal protein is controlled by the tightly regulated GAL1 promoter, yielding high expression levels in the presence of galactose and being strongly repressed after changing to glucose. Our shut-down analysis after transfer of the strains from galactose to glucose was in agreement with the viability test on 5-FOA-plates with only one exception: knockout of *RPS31* was lethal on 5-FOA-plates, whereas shutting down gene expression on glucose resulted in strongly retarded growth. We assume that survival could be due to a highly efficient translation of the rpS31-mRNA, because we could not detect transcription of any of the GAL1-controlled *RPS* genes, including *RPS31*, 2 hr after transfer to glucose by Northern blotting analysis (data not shown).

Viability of all double knockouts could be rescued by overexpression of one of the two r-protein alleles, confirming that the essential function of the respective r-protein is redundant. This observation is in agreement with the systematic single-knockout studies (Giaever et al., 2002; Winzeler et al., 1999).

Plasmids used in the complementation studies contained only the coding sequence of the respective

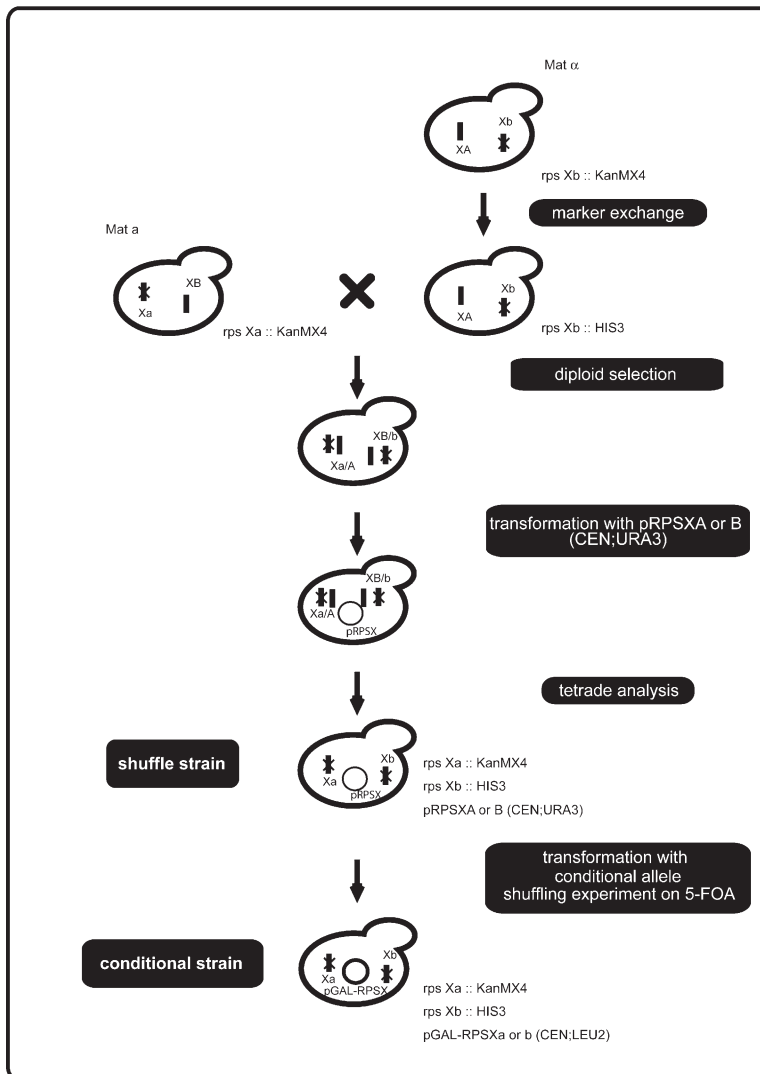


Figure 1. Generation of a Yeast-Strain Collection in which (Duplicated) Genes Coding for Ribosomal Proteins Are Deleted

A strategy to derive knockout strains and strains conditionally expressing r-proteins is depicted. KanMX4 deletion markers of knockout strains were replaced by HIS3 markers. Haploid strains bearing either a KanMX4 or a HIS3 marker in one of the two genes coding for the same r-protein copies were crossed. The resulting diploid cells were transformed with a URA plasmid encoding a wild-type copy of ribosomal protein X. Strains were sporulated, submitted to tetrad analysis, and selected for both markers (HIS3 and Kan) to derive the corresponding shuffle strain for gene RPSX. Any conditional strains can be obtained by exchange of the shuffle plasmid with a plasmid expressing a respective mutant allele, prpsXa.

yeast r-protein, although most of the chromosomal genes contain intron sequences. Interestingly, no significant growth reduction was observed, demonstrating that intron sequences and splicing reaction within ribosomal protein genes play no essential role for cellular processes including maturation and transport of the respective mRNA. Because most ribosomal proteins are required for growth, we used the strains conditionally expressing r-proteins to determine which of the essential ribosomal proteins was required for the production of ribosomes.

Expression of Almost Any Single R-Protein of the Small Subunit Limits Ribosome Production

The dynamics of mature rRNA synthesis were analyzed after shut down of ribosomal protein gene expression in conditional mutants as detailed above (Figure 2). Newly synthesized levels of both 18S rRNA and 25S rRNA were significantly reduced; however, reduction in 18S rRNA synthesis was predominant. Only two exceptions were observed: shut down of *RPS30* and *RPS8* gene

expression affected production of 18S and 25S rRNA to a similar extent, suggesting that both ribosomal subunits are still produced at a comparably low level. Thus, limiting the amounts of any of the r-proteins interferes with efficient production of both mature ribosomal subunits. However, because reduction in 18S synthesis is predominant, the influence of the small ribosomal proteins on proper maturation of the small subunit is more direct. It is a general observation in mutants affecting small subunit production that processing of the large-subunit rRNAs still proceeds, however with reduced kinetics. Even though significantly reduced, levels of newly synthesized 18S rRNA vary in the different conditional strains. Among the strains in which still more than 4% of the wild-type 18S rRNA is generated are GAL-RPS7, GAL-RPS10, and GAL-RPS31, as well as GAL-RPS8 and GAL-RPS30 (see above), indicating that small subunit biosynthesis is not completely blocked in these strains. When the nonessential r-proteins rps12 and rps25 were depleted, similar amounts of 18S and 25S rRNA were produced, however, at a reduced level:

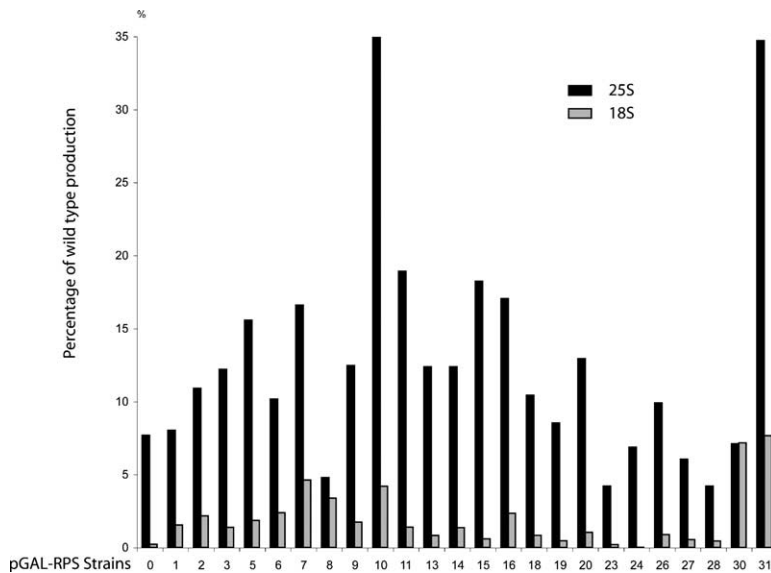


Figure 2. Effect of Small-Subunit R-Protein Depletion on Newly Synthesized Pre-rRNAs

Yeast cells were shifted to glucose containing medium for 2 hr to deplete rpS synthesis and were pulse labeled for 15 min with [³H]uracil. RNAs were isolated and separated by gel electrophoresis and blotted onto a nylon membrane. Membrane slices containing ³H-labeled 25S and 18S rRNA, respectively, were cut, and incorporation of [³H]uracil-activities were counted with a scintillation counter. Background activity was subtracted, and total activity was standardized according to steady-state 25S rRNA (as determined by Northern blotting analysis) in the load. The amount of 25S and 18S rRNA in mutant strains is compared with wild-type conditions.

65% and 30%, respectively (data not shown). These data clearly suggest that by far most r-proteins of the small subunit that are essential for growth are also required for the proper production of the small subunit. Thus, ribosomal proteins can either play a direct role in the maturation pathway or their presence might be mandatory to ensure that only properly assembled ribosomal subunits reach their place of function.

Most Ribosomal Proteins of the Small Subunit Have a Distinct Role in 18S rRNA Processing

Next, we investigated whether ribosomal proteins are required for distinct steps in the rRNA processing pathway. Therefore, we analyzed steady-state levels of rRNA intermediates leading to mature 18S rRNA after 2 and 4 hr shift from galactose to glucose (Figure 3). Transblotted rRNA species were detected by using specific probes (see legend to Figure 3 and Experimental Procedures). According to their different RNA processing patterns, strains could be divided into six different groups and subgroups (Figures 3C and 3D), demonstrating that almost all essential small ribosomal proteins are involved in the control of distinct maturation steps. Thus, strains could be separated into two main groups, one in which 20S rRNA is not detectable (group I, Figure 3C) and one in which 20S rRNA is produced with either wild-type or slightly reduced efficiency (group II, Figure 3C). 20S rRNA occurs after processing of pre18S rRNA at sites A0, A1, and A2 (Figure 3A). However, both the slightly elevated levels of 35S and 32S rRNA and the detectable amounts of 23S rRNA in the same mutants indicate that cleavage at sites A0, A1, and A2 is either delayed or not as efficient as in wild-type yeast. Three subgroups of mutant strains producing 20S rRNA could further be distinguished: strains that accumulate either 21S rRNA (like pGAL-RPS20), 22S rRNA (like pGAL-RPS10), or none of these two species (like pGAL-RPS30 or the recently published pGAL-RPS15 [Leger-Silvestre et al., 2004]). Appearance of 22S rRNA indicates cleavage at site A0 but defective

processing at sites A1 and A2, whereas 21S rRNA results from inefficient cleavage at site A2 and cleavage occurring at sites A0 and A1. These results underline that a distinct subset of r-proteins supports each cleavage step leading to 20S rRNA, the export-competent form of pre-18S rRNA. Although 20S rRNA is made in these strains, its steady-state amount is more or less reduced.

Mutant strains in which 20S rRNA is not detectable accumulate 23S rRNA (Figures 3B and 3C), representing either an aberrant RNA intermediate that is normally degraded (Venema and Tollervey, 1999) or an intermediate of an alternative pathway to mature 18S rRNA (Granneman and Baserga, 2004). 23S rRNA is generated when cleavage at site A3 precedes processing at sites A0, A1, and A2. Accordingly, accumulation of the 23S rRNA indicates that the respective depleted r-protein is involved in early cleavage steps at sites A0, A1, and A2. Two further subgroups in which 23S rRNA is accumulated can be distinguished, one in which cleavage at A0 is affected but occurs at a low rate to yield 22S rRNA and one in which A0, A1, and A2 are equally inhibited. Depletion of rpS27 resulted particularly in 23S rRNA accumulation. However, in this strain, an rRNA intermediate was also detected with probes specific for the region upstream of A0 and upstream of the 3' boundary of 18S rRNA, whereas probes complementary to ITS1 did not recognize this species (data not shown). We conclude that in this mutant strain, cleavage at site A0 and A1 is inhibited, whereas processing at site A2 is delayed, leading to a pronounced level of 23S rRNA. Apparently in this mutant, a minor fraction of rRNA is cleaved at the 3' boundary of the 18S rRNA (site D) before cleavage at A2 occurs, resulting in an intermediate slightly larger than the 22S rRNA with extension to the +1 site of transcription. A similar fragment spanning the region from +1 to site D was recently described (Kufel et al., 2003; Vos et al., 2004). It will be interesting to see whether in this case D cleavage occurs in the nucleus or whether the unprocessed rRNA

can be transported out of the nucleus prior to the cleavage reaction at site D. To bypass the conventional maturation pathway, depletion of rpS27 thus tethers the cytoplasmic processing machinery to the nucleus or allows the passage of unprocessed rRNA through the nuclear membrane.

Ribosomal Proteins Control Export of Pre-18S and Late rRNA Processing Steps

Mutants defective in 18S rRNA synthesis but producing 20S rRNA are inhibited in making 20S rRNA export competent, are directly impaired in export of 20S rRNA from the nucleus to the cytoplasm, or are deficient in the cytoplasmic processing steps. To differentiate between these possibilities, a dynamic maturation assay was applied that helps to distinguish spatially and temporally between rRNA species at high resolution (Figure 4A). We performed short (15 min) ³H-uracil pulse-labeling experiments with the respective conditional yeast strains and localized de novo-synthesized rRNA species by subsequent cell fractionation. Northern blot analysis revealed that the amounts of steady-state rRNAs did not change during the first 2 hr of Gal depletion. Thus, samples were normalized according to their content of steady-state 25S rRNA. Previous studies demonstrated that this assay allows the comparison of the maturation and export of 20S rRNA in wild-type cells to that in mutant cells in which the expression of distinct (pre-) ribosomal factors was blocked (Leger-Silvestre et al., 2004). Strains having a low amount of steady-state 20S rRNA after depletion of r-proteins (rpS5, rpS18, and rpS19 (see Figure 3B)) show only a minor amount of neosynthesized 20S rRNA in nuclei and no 20S rRNA in the cytoplasmic fraction (Figure 4A). We conclude that 20S rRNA cannot reach the cytoplasm because either it is unstable, and/or it is exported at such a reduced level that it cannot be detected. Anyway, the primary defect of these mutants is the low occurrence of the precursor rRNA that should be exported to the cytoplasm.

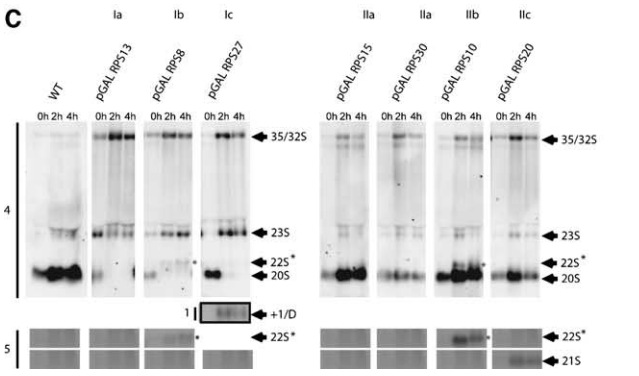
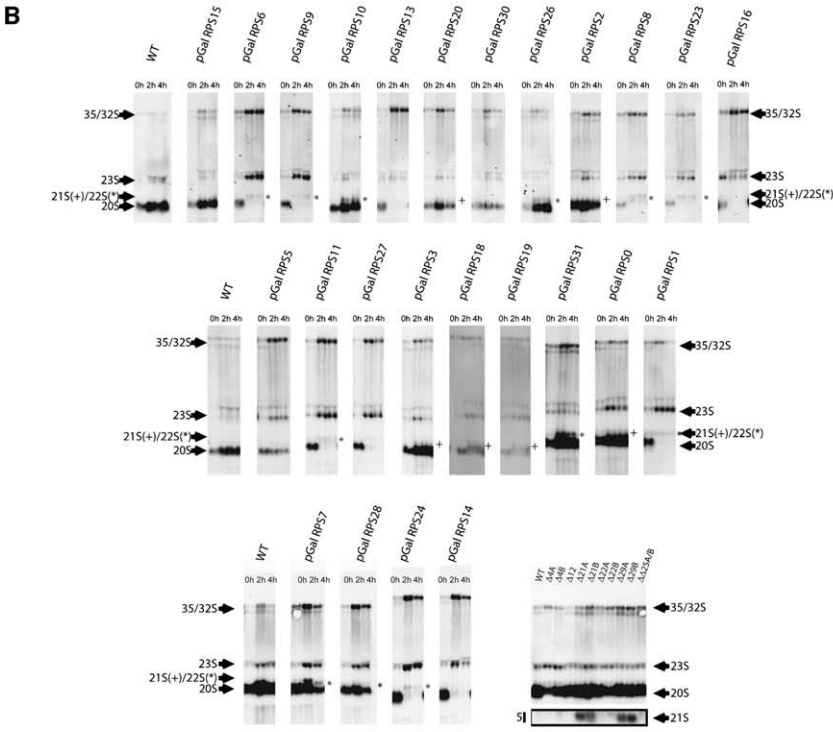
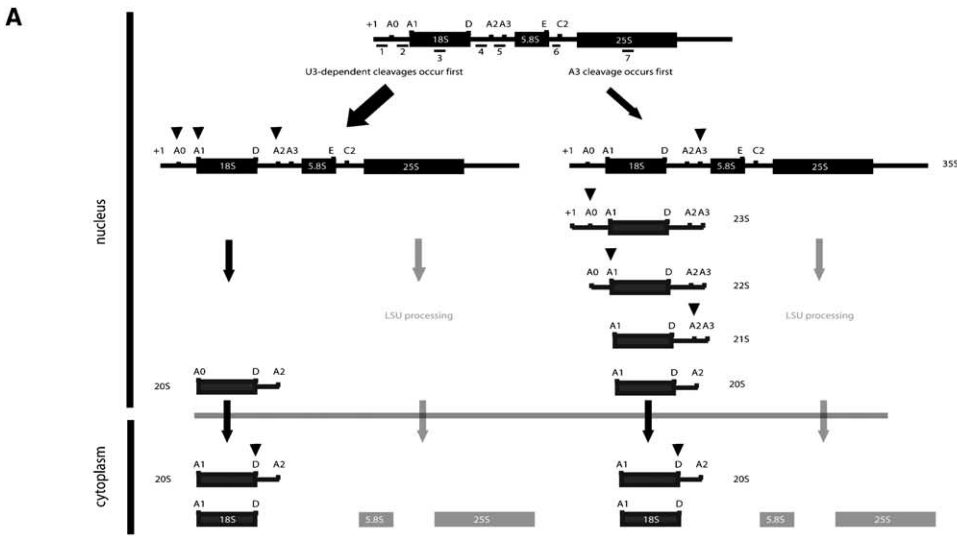
In contrast, in conditional mutant strains yielding high levels of steady-state 20S rRNA (strains depleted in rpS15, rpS10, rpS26, rpS3, rpS2, rpS30, rpS20, rpS0, or rpS31, see Figure 3B), similar amounts (between 50% and 110% of wild-type levels) of neosynthesized 20S rRNA are found in nuclear fractions as in wild-type nuclei (Figure 4A, upper). If rpS20, rpS31, rpS7, or rpS30 is lacking, a significant amount of newly made 20S rRNA reaches the cytoplasm. In strains depleted in rpS7, rpS30, and rpS31, some of the cytoplasmic 20S rRNA can be further processed to 18S rRNA (Figure 4A, see also Figure 2). Accordingly, r-proteins rpS7, rpS30, and rpS31 seem to be not strictly required for rRNA maturation and nuclear export, indicating that their primary role might be in protein translation. No neosynthesized 18S rRNA is visible after depletion of rpS20, suggesting that 20S rRNA can be exported, but not converted into 18S rRNA.

Surprisingly, in strains depleted in rpS15, rpS10, rpS26, rpS3, rpS0, or rpS2, the amount of neosynthesized 20S rRNA in the nuclear fractions corresponds to wild-type levels, but the amount of newly made 20S rRNA reaching the cytoplasm during the 15 min of pulse

is strongly reduced. This result shows that exit of pre-18S rRNA from the nucleus is clearly retarded, but not completely inhibited, which can be explained by a direct export defect in these mutants. From these data, we suggest that r-proteins rpS15, rpS10, rpS26, rpS3, rpS0, and rpS2 are an integral part of the export machinery for 20S rRNA. By comparison, r-proteins rpS5, rpS18, and rpS19 can be required for a late nuclear pre-40S maturation step before the passage of 20S rRNA through the nuclear pore. Such a late processing step could be the proper assembly of export-competent pre-40S particles that contain 20S rRNA and that might represent the immediate prerequisite for the contact between the pre-40S particles and the nuclear export factors. In fact, proper assembly of pre-40S-specific factors with 20S rRNA is disturbed in rpS19-depleted cells (P.-E.G., unpublished data).

Analysis of the steady-state levels of pre-18S rRNA of rpS mutants confirmed the suggested roles of the r-proteins in either export or late processing steps. We used the same blot as in Figure 4A to determine steady-state levels of pre-18S rRNAs in nuclei and cytoplasm by using Dig-labeled ITS1 as a probe for the Northern blot (Figure 4B). As anticipated, mutants having a retarded nuclear export rate (rpS15, rpS10, rpS26, rpS3, rpS0, and rpS2) accumulate steady-state 20S rRNA in the nucleus. Surprisingly, cytoplasmic 20S rRNA of these mutants is rather stable, as it apparently accumulates over time and —after two hours depletion —it reaches levels between 40% and 120% of the cytoplasmic 20S rRNA intermediate in wild-type cells (In wild-type cells about 80% of 20S rRNA is found in the cytoplasm). These findings are in agreement with previous studies on rpS0, which showed that cytoplasmic 20S rRNA is not efficiently processed in haplo-insufficient RPS0-cells (Ford et al., 1999; Tabb-Massey et al., 2003). In this group of mutants with a retarded rRNA export, yeast cells depleted in rpS15 clearly accumulate the lowest amount of cytoplasmic 20S rRNA over time, explaining the low cytoplasmic signal in fluorescence in situ hybridization analysis (FISH) of pre 18S rRNA (Leger-Silvestre et al., 2004; see below). No cytoplasmic 20S rRNA was detected in strains lacking rpS5, rpS18, or rpS19, in agreement with a primary role for these proteins in late nuclear ribosome biogenesis.

FISH analysis using ITS1 as probe verified the steady-state distribution of 20S rRNA and its precursors in the nuclei and cytoplasm of the mutants (Figure 4C). Exclusively nucleolar and nuclear localization of rRNA intermediates hybridizing with ITS1 was visible in mutants depleted of rpS5, rpS18, and rpS19. In mutants that are able to export 20S rRNA at a similar rate as wild-type cells (strains depleted in rpS20, rpS30, and rpS31) or that can accumulate 20S rRNA over time (strains depleted in rpS10, rpS26, rpS3, rpS0, and rpS2), nuclear and cytoplasmic FISH signals similar or only slightly reduced to those obtained with wild-type cells were observed. This result emphasizes that FISH analysis cannot identify mutants in which the dynamics of nuclear export are impaired if these mutants can slowly accumulate unprocessed 20S rRNA in the cytoplasm (see Figures 4A and 4B). In agreement with previous observations (Leger-Silvestre et al., 2004), depletion of rpS15 has a pronounced impact on pre-18S



D

Group	I			II		
	20S is not detectable					
	a	b	c	a	b	c
Phenotyp	20S is not detectable					
Sub-Group	a	b	c	a	b	c
rRNA species accumulating after depletion	35S/23S A0,A1,A2,D inhibited A3 delay	35S/23S/22S A1,A2,D inhibited AB delay	35S/23S/+1-D A0,A1,A2, inhibited A3 delay, D ? can occur with delay	35S/23S/20S A0,A1,A2,A3 delay	35S/23S/22S A0,A1,A2,A3 delay	35S/23S/21S A0,A1,A2,A3 delay
RPS	RPS13 RPS14 RPS16	RPS1 RPS6 RPS8 RPS9 RPS11 RPS23 RPS24	RPS27	RPS5 RPS15 RPS30	RPS7 RPS10 RPS26 RPS28 RPS31	RPS0 RPS2 RPS3 RPS18 RPS19 RPS20

export. Steady-state amounts of cytoplasmic 20S rRNA is clearly reduced in rpS15-depleted cells according to our FISH and Northern blot analyses (Figures 4B and 4C), though higher amounts (about 110%) of nuclear 20S rRNA is produced compared to wild-type cells (see Figure 4A).

In summary, these results demonstrate that ribosomal proteins have different functional roles and can be involved in several important 18S rRNA maturation steps. R-proteins rpS10, rpS26, rpS3, rpS0, rpS15, and rpS2 appear to be directly involved in nuclear export. Defects in rpS5, rpS18, and rpS19 impair late nuclear processing and/or quality control steps leading to nuclear 20S rRNA that is not exported. Finally, lack of rpS20 blocks cytoplasmic 20S rRNA processing whereas rpS7, rpS30, and rpS31 play no primary role for 18S rRNA maturation.

Ribosomal Proteins Are Involved in the Process of Translation

rpS7, rpS30, and rpS31 were essential ribosomal proteins whose depletion did not completely block production of mature 18S rRNA (see Figure 2 and Figure 4). It is possible that these ribosomal proteins, together with the two nonessential proteins rpS12 and rpS25, function predominantly in protein translation. We investigated this possibility by analyzing polysome assembly in strains in which the respective ribosomal protein is missing. Polysome profiles obtained from strains lacking either rpS12 or rpS25 were indistinguishable from those of wild-type cells (Figure 5A), indicating that both proteins are not required for formation of mRNA-associated ribosomes. However, depletion of the essential ribosomal proteins rpS30, rpS31, and rpS7 showed a significant reduction in free 40S subunits and polysomes, whereas the levels of 80S and/or free 60S ribosomes were increased. A simple explanation for this result is that 40S subunits are incorporated in 80S ribosomes that are either not associated with mRNA (Cigan et al., 1991; Hartwell and McLaughlin, 1969) or are incompetent for translation initiation or elongation. Accordingly, the polysome:80S ratio drops (Deloche et al., 2004). In contrast, mutant strains with a pronounced defect in 40S biosynthesis show a clear reduction of the 40S peak and, in addition, a decrease in both 80S and polysomes peaks (but not in free 60S subunits) (Figure 5A, Gal RPS0, 2 hr YPD incubation). This finding is observed in small subunit biogenesis mutants, un-

derlining that association of 40S with the 60S subunit cannot occur because production of the small subunit is impaired (Tabb-Massey et al., 2003; Dragon et al., 2002; Grandi et al., 2002; Milkereit et al., 2003b). From these data, we conclude that the ribosomal proteins rpS30, rpS7, and rpS31, but not rpS12 and rpS25, are required to form properly assembled polysomes, but it remains to be shown how these r-proteins affect ribosome function.

Although rpS12 and rpS25 do not directly participate in the proper formation of a cytoplasmic ribosome, they can still be important for the translation reaction. Therefore, we analyzed strains lacking the respective proteins in their sensitivity against cycloheximide, an inhibitor of protein biosynthesis (Dresios et al., 2001). Cycloheximide blocks the peptidyltransferase activity of mRNA-associated ribosomes, thereby inhibiting both translational initiation and elongation. In the presence of 0.5 μ g/ml cycloheximide, wild-type cells showed only a slight growth reduction whereas strains bearing a knockout of either RPS12 or RPS25 alleles are significantly growth inhibited (Figure 5B). Consistent with a distinguished role of rpS30 in ribosome function, a single knockout of one of the two RPS30 alleles also results in growth inhibition in the presence of cycloheximide (Figure 5B). In which specific step of protein translation these ribosomal proteins are involved remains to be determined.

Discussion

Special Demands on the Function of Eukaryotic Ribosomal Proteins

We started a systematic approach to study the roles of yeast small subunit r-proteins in production, maturation, and export of 18S rRNA, the rRNA component of the small ribosomal subunit. The r-protein families of both ribosomal subunits can be divided into two large groups: those conserved between Bacteria, Archaea, and Eucarya and those only common in Archaea and Eucarya. Fifteen r-proteins of the 32 yeast small subunit proteins have homologs in all three evolutionary domains; 13 are conserved between Archaea and Eucarya and only four do not have counterparts in Archaea or in Bacteria (Lecompte et al., 2002). At least one-third of r-proteins are not required for growth in *E. coli* at 37°C, suggesting that many r-proteins are dispensable for ribosomal structure or function (Dabbs, 1991; Nierhaus,

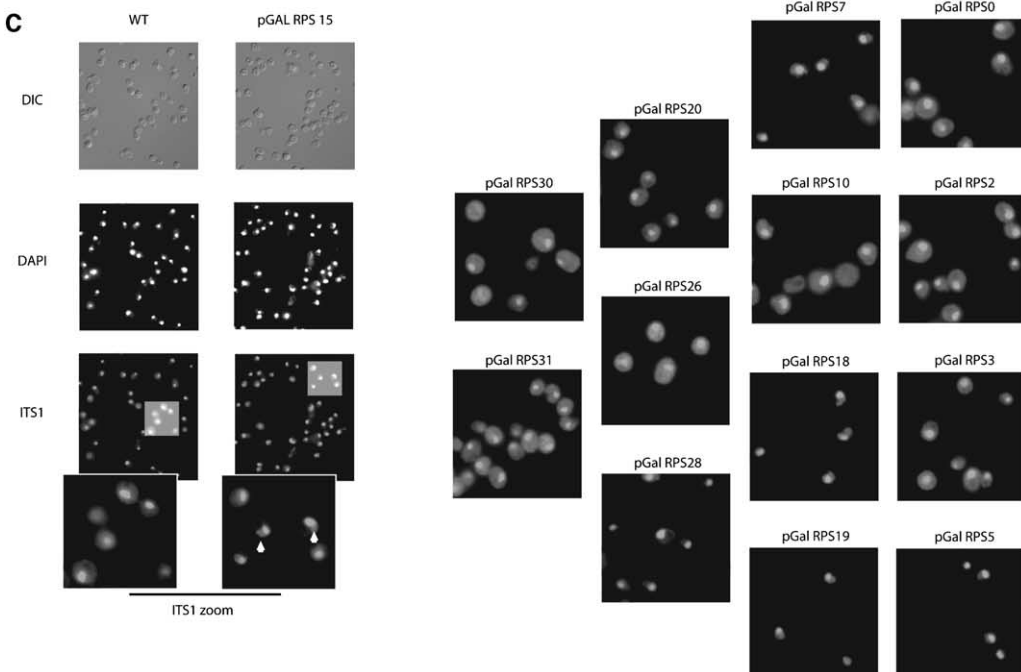
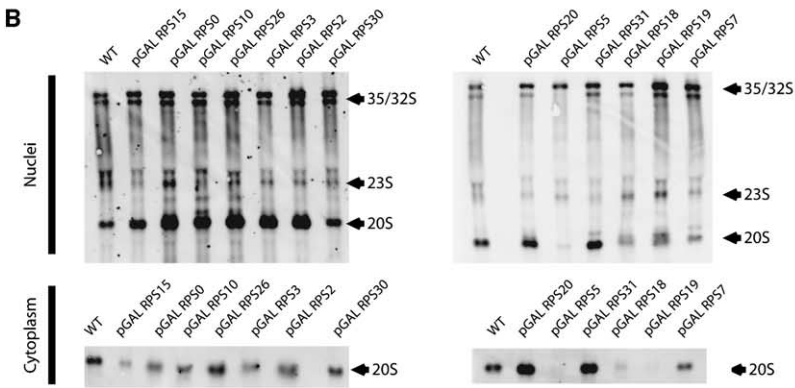
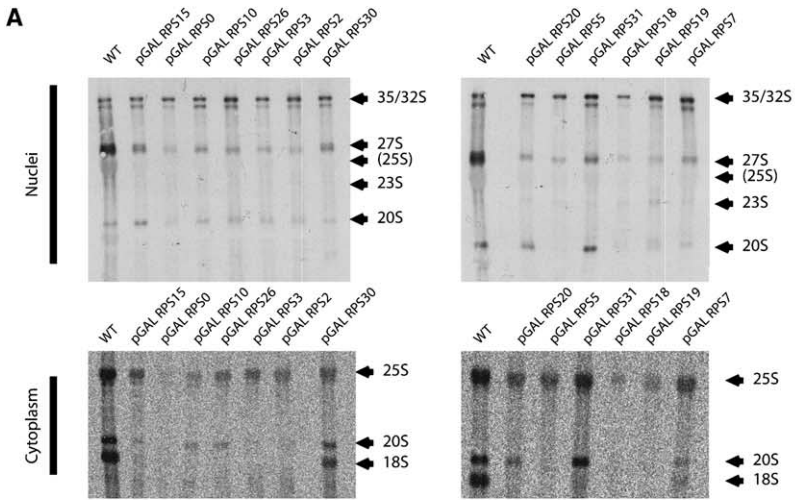
Figure 3. R-Proteins Control Distinct Pre-rRNA Processing Steps

(A) Scheme of pre-rRNA processing in *S. cerevisiae* according to Gallagher et al. (2004). Probes 1–7 were used to map the different rRNA species.

(B) Analysis of pre-rRNA processing in strains depleted in r-proteins by Northern blot. RNA from the same amount of cells was extracted from either wild-type or Gal-RPS strains grown in YP-galactose (0 hr) or after a 2 hr or 4 hr shift to YP-glucose. RNA probes (4) ITS1 and (5) (bottom lane) were used to detect specific RNA intermediates. The plus sign and asterisk indicate the detection of 21S and 22S rRNA, respectively. The presence of 21S and 22S rRNA intermediates was further confirmed with probes (2) and (5) (see Supplemental Data for sequences).

(C) An overview of representative strains resulting in a similar processing pattern is depicted. Northern blot hybridized with probe ITS1 (4, upper) is shown. Detection of 21S/22S using probes A2/A3 (5) and fragment +1-D rRNA using probe +1-A0 (1) is shown (lower).

(D) Classification of r-proteins according to their pre-rRNA processing defects. Ribosomal proteins are sorted into group I if their depletion results in loss of 20S rRNA. If 20S rRNA is still produced, they belong to group II. Subgroups were classified according to the presence or absence of 21S, 22S, or +1-D rRNA species (Ia, Ib, or Ic and IIa, IIb, or IIc, respectively).



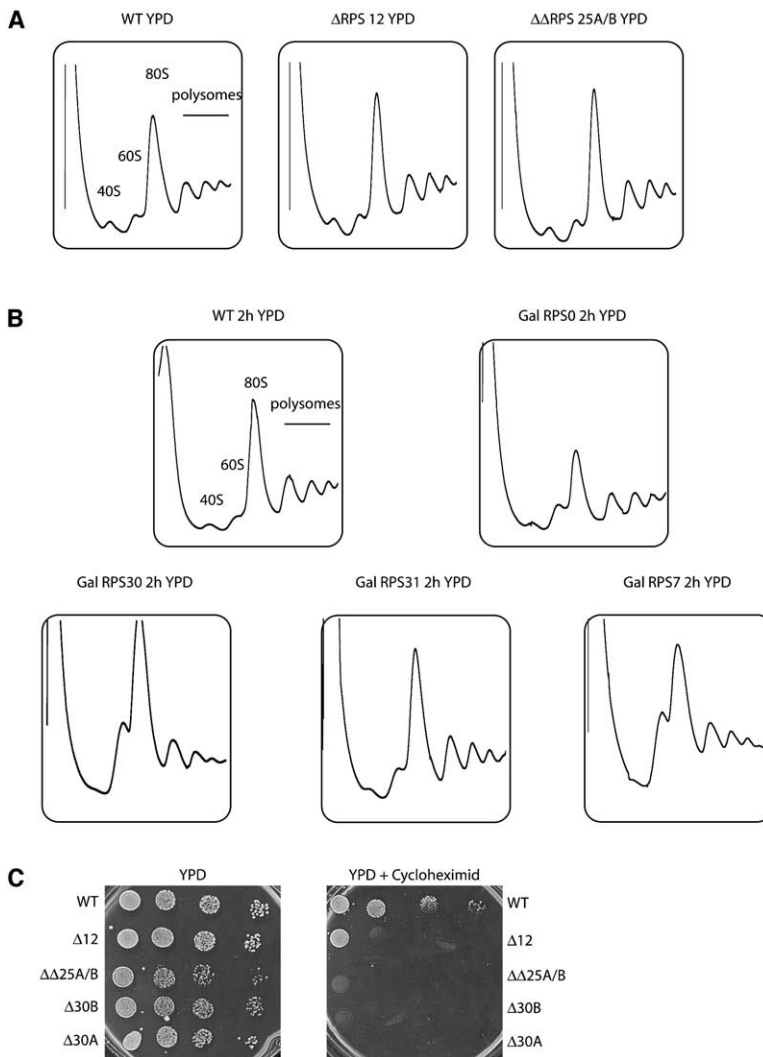


Figure 5. R-Protein Mutants that Allow Production of Mature 18S rRNA Are Impaired in Ribosome Function

(A) Polysome gradients of strains depleted for the two nonessential ribosomal proteins rpS12 and rpS25 (growth on YPD). Cycloheximide-treated cell extracts were fractionated on a gradient containing 8%–36% sucrose. Absorbance at 260 nm was monitored. (B) Comparison between polysome gradients from strains deficient in 18S rRNA production (RPS0) and conditional lethal strains that still produce 18S rRNA (RPS30, RPS31, and RPS7). The strains were depleted in r-protein expression for 2 hr in glucose-containing medium, treated with cycloheximide, and fractionated on an 8%–36% sucrose gradient. (C) Cycloheximide sensitivity of cells with the two nonessential genes rpS12 and rpS25 deleted as well as for strains with deletions for genes rps30A or rps30B. The dilution series was plated on YPD with and without 0.5 μ g/ml cycloheximide.

2004). However, in yeast, by far most ribosomal proteins are essential for growth, indicating that they have acquired additional fundamental roles in addition to participating in a stable ribosomal structure.

With the exception of three r-proteins (rpS7, rpS30, and rpS31), all essential r-proteins of the small subunit investigated were required for the proper generation of mature 18S RNA, which emphasizes that one major

task of eukaryotic ribosomal proteins is to control assembly and maturation of the ribosomal subunits. Interestingly, depletion of r-proteins blocks ribosome maturation at many specific stages. These findings demonstrate a direct involvement of r-proteins in distinct processing steps, showing that reduction of small subunit synthesis is not a general effect when the level of one ribosomal protein is decreased.

Figure 4. Ribosomal Proteins Control Competence and Efficiency of Pre-18S Export

Nuclear export of 20S rRNA was analyzed in Gal-RPS strains 2 hr after transfer into YP-glucose by using the following three approaches. (A) Analysis of newly synthesized pre-18S rRNA nuclear export by metabolic labeling and cell fractionation. After a 2 hr shift to glucose-containing medium, spheroplasts were labeled with [3 H]uracil for 15 min and fractionated in nuclei and cytoplasm (see [Experimental Procedures](#) for details). RNAs were isolated and steady-state amounts of 25S rRNA were determined by dot blot analysis. Equal amounts of steady-state 25S rRNA of cytoplasmic and nuclear fractions, respectively, were separated by gel electrophoresis and blotted. Newly synthesized 3 H-labeled RNAs were detected by phosphorimaging and quantified using Image Gauge software (Fuji). (B) Steady-state analysis of rRNA in nuclei and cytoplasm. The same blots as in (A) were developed using digoxigenin-labeled ITS1-probe 4 (see [Figure 3A](#)). (Note that the cytoplasmic fraction of wild-type strains contained about 80%–90% of total 20S rRNA). 20S rRNA was quantified with the Fuji imaging system LAS3000, applying the AIDA software (Raytest). (C) FISH of pre-18S rRNA. A probe complementary to the D-A2 segment of ITS1 was used to localize 20S rRNA after depletion of rpS expression. Arrowheads indicate the nucleoplasm that was visualized by DAPI staining.

Table 1. Summary of the Effects of R-Protein Depletion on the 18S rRNA Processing Pathway

Group	I			II		III			IV	
Phenotype	20S Is Not Detectable			20S Is Made and Not Exported		20S Is Made and Exported or Retarded but Not Converted into 18S			20S Is Made, Exported, and Converted into 18S	
Subgroup	a	b	c	a	c	a	b	c	a	b
rRNA species	35S/23S	35S/23S/22S	35S/23S/+1-D	35S/23S/20S	35S/23S/21S	35S/23S	35S/23S/22S	35S/23S/21S	35S/23S	35S/23S/22S
accumulating after depletion	A0, A1, A2, D inhibited; A3 delay	A1, A2, D inhibited; A0 Delay	A0, A1, A2, A3 delay; D (?) can occur with delay	A0, A1, A2, A3 delay	A0, A1, A2, A3 delay	A0, A1, A2, A3 delay; D is inhibited	A0, A1, A2, A3 delay; D is inhibited	A0, A1, A2, A3 delay; D is inhibited	A0, A1, A2, A3 D delay	A0, A1, A2, A3 D delay
RPS	RPS13 RPS14 RPS16	RPS1 RPS6 RPS8 RPS9 RPS11 RPS23 RPS24	RPS27	RPS5	RPS18 RPS19	RPS15	RPS10 RPS26 RPS28	RPS0 RPS2 RPS3 RPS20 ^a	RPS30	RPS7 RPS31

^aNote that 20S rRNA is exported almost with wild-type efficiency in rpS20-depleted cells, but cytoplasmic processing does not take place.

The Roles of R-Proteins in Processing and Transport of Pre-18S rRNA

A summary about the different rRNA processing steps in which r-proteins are involved is depicted in Table 1. Eleven r-proteins are required for efficient early cleavages: after their depletion, 35S and 23S rRNA accumulate, whereas 20S rRNA becomes not detectable. According to their involvement in specific cleavage steps, they can be sorted into three further groups, each representing a different phenotype. In the absence of any of the remaining 15 essential r-proteins, 20S rRNA is generated, at least to some extent. Four different phenotypes could be distinguished after depletion of these respective r-proteins: (1) strains in which 20S rRNA is produced, but not exported to the cytoplasm, (2) strains with a significantly reduced export efficiency, (3) one strain in which 20S rRNA is exported, but not converted into 18S rRNA, and (4) strains that can export 20S rRNA and convert it into 18S rRNA.

Interestingly, lack of rpS5, rpS18, and rpS19 completely inhibits 20S rRNA export, yielding unstable RNA intermediates. We suggest, therefore, that these r-proteins are either actively involved to render pre-40S particles export competent or they participate in a quality-control step that is required to overcome retention of the small subunit in the nucleus. Apparently, one common crucial point of preribosomal quality control is on the level of 20S rRNA-containing particles, because export-inhibited 20S rRNA is still slightly produced in these conditional mutants, though cleavage at other sites is also retarded. We suggest that in mutants with a complete block of 20S export, the pre-40S particle is not able to contact the export machinery, resulting in its subsequent degradation.

Depletion of any of the r-proteins rpS15, rpS10, rpS26, rpS2, rpS0, or rpS3 resulted in a strongly re-

duced export rate of 20S rRNA to the cytoplasm. These findings are in accordance with previous results obtained for rpS15, which was the first ribosomal protein shown to play a crucial role in a late nuclear step of the pre-40S pathway (Leger-Silvestre et al., 2004). Three simple explanations for the reduced transport efficiencies are possible. First, these r-proteins communicate directly or indirectly with different parts of the export machinery. Second, incomplete folding or incomplete assembly of the particles could interfere with the export efficiency. Third, each of the respective r-proteins is necessary to correctly position and shield the pre-40S particle during its passage through the nuclear pore. It could be that the spatial organization of these r-proteins on the surface of the ribosomal subunit might be required to insulate the hydrophilic rRNA moiety from the hydrophobic regions of the nucleoporins.

Comparison of In Vivo Function, In Vitro Assembly, and Structural Data of R-Proteins

The structure of the bacterial ribosomal subunits was determined to atomic resolution (Ban et al., 2000; Schluenzen et al., 2000; Wimberly et al., 2000; Yusupov et al., 2001), elucidating the interaction of r-proteins with 16S rRNA in 3D (Brodersen et al., 2002). In addition, in vitro reconstitution experiments (Held et al., 1974; Traub and Nomura, 1968) revealed an assembly gradient of primary, secondary, and tertiary binding proteins to the rRNA (Brodersen et al., 2002; reviewed in Nierhaus, 2004). Strikingly, three out of five bacterial primary binders (S17, S4, and S15) have homologs in yeast (rpS11, rpS9, and rpS13) that are involved in early rRNA processing, suggesting that early assembly correlates with early maturation steps. Bacterial r-protein S15 organizes the central domain (platform) of the rRNA; S4 assembles the lower part of the 3' major do-

main (head); and S7 and S17 organize different parts of the 5' and central domain (body and platform). It is possible that the corresponding yeast proteins also fulfill similar early roles in the yeast ribosomal assembly.

The bacterial homologs of yeast rpS0, rpS2, rpS3, rpS15, and rpS20 (S2, S5, S3, S19, and S10, respectively) are all tertiary binding proteins, which together with S14 are important for forming a cluster at the 3' major 16S rRNA, the head structure of the small subunit (Brodersen et al., 2002). Strikingly, the corresponding yeast proteins were all grouped into a common class of r-proteins involved in the same two late biogenesis steps (Table 1, lane IIIa and IIIc). Haplo-insufficient strains of rpS29, the homolog of bacterial S14, also show a late RNA processing defect (data not shown). Apparently, the sequence by which r-proteins participate in the formation of ribosomal (sub-) structures was retained in evolution. However, due to the extra demands of a eukaryotic cell, yeast r-proteins have acquired additional functions. Alternatively, formation of a distinct structure, such as the head-like cluster, might represent a necessary prerequisite to also fulfill eukaryotic-specific functions, like nuclear export or cytoplasmic processing. Two further eukaryotic-specific r-proteins, rpS10 and rpS26, might also participate in the formation of such a structural entity, as they are involved in the same late biogenesis steps.

Bipartite Functions of Small Subunit R-Proteins

Of course, r-proteins required for early rRNA processing can also participate in nuclear exit, in cytoplasmic rRNA processing, or in ribosome function. However, because their essential role is tightly linked to early maturation steps, they could not be assayed for other functions in this screen. Future mutational analysis of such "early" r-proteins will reveal whether they cover bipartite functions. Such a dual role was shown for rpS14, as it was found to be involved in early rRNA processing (Moritz et al., 1990; see also Figure 3) and its carboxy-terminal extension was also recently described to play an important role in late cytoplasmic rRNA processing (Jakovljevic et al., 2004). Furthermore, it is possible that r-proteins other than the three essential ones—rpS7, rpS30, and rpS31—participate in activities that are more related to ribosome function. For instance, rpS9 and rpS2 that are required for early and late biogenesis steps (Table 1), respectively, are also important for translational accuracy (Alksne et al., 1993; Stansfield et al., 1998; Synetos et al., 1996). Further studies are required to dissect other possible dual or multiple functions and to find out whether and how r-proteins support the activities of eukaryotic ribosomal subunits.

Experimental Procedures

Standard techniques in molecular biology, plasmids, oligonucleotides, and yeast strains are described in the Supplemental Data available with this article online.

In Vivo Labeling Experiments

Logarithmically growing cells in YPG were resuspended in prewarmed YPD media (OD 0.5) and incubated for 2 hr at 30°C. 3 ml of cells was pelleted and resuspended in 1 ml of YPD including 20 μ Ci of 5',6' [³H] uracil (Amersham) for 15 min at 30°C. RNA was extracted, and the same amounts of radioactivity (cpm) were

loaded onto a denaturing agarose gel and then transferred onto a Positive membrane (Qbiogene). The membrane was exposed to a BAS-TR 2040 screen for 1 week.

Radioactivity on membrane slices containing 25S and 18S rRNA was counted using a scintillation counter (Packard Tri-Carb 1600TR). Background activity was subtracted from the values obtained.

The exact procedure was performed in duplicate for standardization with the only exception being that the volumes of RNA loaded were the same. The resulting membranes were hybridized with a ³²P-labeled 25S probe. The amounts of labeled 25S rRNA in each strain were quantified using a PhosphorImager (Fuji).

[³H] activity was adjusted according to equal amounts of 25S rRNA on the gel and was expressed as a percentage of labeled wild-type RNA.

Cell Fractionation and Metabolic Labeling of Neosynthesized RNA

Cells were grown overnight in YP-Galactose medium and were resuspended in prewarmed YP-Glucose medium for 1 hr. Twelve ml of cells at OD 1.2 was centrifuged and washed in 2 mM DTT and resuspended in recovery buffer (2% glucose, 1% peptone, 0.6% malt extract, 0.01% yeast extract, 12% mannitol, and 17.8 mM magnesium acetate) plus 1 mM DTT and 1 mg/ml zymolyase 100 T (Seikagaku Corporation) for 30 min at 30°C with mild agitation. Cells were resuspended for 30 min at 30°C in recovery buffer and pulse labeled with 20 μ Ci of 5',6' [³H] uracil (Amersham) for 15 min at 30°C. Nuclear and cytoplasmic fractions were then prepared as described in (Leger-Silvestre et al., 2004).

For normalization, steady-state rRNA amounts in each fraction were determined by using a dot blot experiment with a ³²P labeled 25S probe. Same amounts of cold RNA were then loaded on a denaturing agarose gel and transferred onto a Positive membrane from Qbiogene.

Steady-state amounts of pre-rRNA were analyzed after hybridization by using a Dig-labeled ITS1 probe (see Northern Blotting Analysis in the Supplemental Data available with this article online).

Neosynthesized rRNA was visualized either by phosphorimaging (BAS-TR2040 screen, Fuji; one week exposure) or by exposure of the blot to a BioMax MS film including a BioMax TransScreen (Kodak) at -80°C.

Fluorescence In Situ Hybridization Microscopy

Cells growing in YPG were resuspended in prewarmed YPD media (OD 0.5) for 2 hr. Cells were fixed with 4% paraformaldehyde in YPD for 30 min at 30°C and handled as described by (Gleizes et al., 2001) and using the same oligonucleotidic probe as in (Leger-Silvestre et al., 2004).

Images were captured with an AxioCam MR CCD camera mounted on an Axiovert 200M Zeiss microscope and processed with Adobe Photoshop.

Polysome Profile Analysis

Polysome profile analyses were carried out as described in Milker-Eit et al. (2001) except that extracts containing 500 μ g of protein were loaded onto a 10.5-ml, 8%–36% sucrose gradient and centrifuged in a SW40 rotor for 3 hr at 39,000 rpm at 4°C. Gradients were analyzed using a BioLogic UV detection system coupled to LP analysis software (Bio-Rad).

Supplemental Data

Supplemental Data including three tables and Supplemental Experimental Procedures are available online with this article at <http://www.moleculer.org/cgi/content/full/20/2/263/DC1/>.

Acknowledgments

We thank Stephan Jellbauer for constructing deletion vectors Top 234 and Top238; Anja Ackermann and Ulrike Stöckl for preparing media and tools for molecular biology; Drs. Ludwig Lehle, Jürgen Stolz, and Widmar Tanner (all University of Regensburg) for sharing facilities; and Dr. Joachim Griesenbeck for critically reading the

manuscript. This work was supported by Deutsche Forschungsgemeinschaft grants to H.T. and P.M.

Received: July 4, 2005

Revised: August 8, 2005

Accepted: September 6, 2005

Published: October 27, 2005

References

- Alksne, L.E., Anthony, R.A., Liebman, S.W., and Warner, J.R. (1993). An accuracy center in the ribosome conserved over 2 billion years. *Proc. Natl. Acad. Sci. USA* **90**, 9538–9541.
- Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. (2000). The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**, 905–920.
- Beckmann, R., Spahn, C.M., Eswar, N., Helmers, J., Penczek, P.A., Sali, A., Frank, J., and Blobel, G. (2001). Architecture of the protein-conducting channel associated with the translating 80S ribosome. *Cell* **107**, 361–372.
- Blaha, G. (2004). Structure of the ribosome. In *Protein Synthesis and Ribosome Structure*, K.H. Nierhaus and D.N. Wilson, eds. (Weinheim, Germany: Wiley-VCH), pp. 85–106.
- Briones, E., Briones, C., Remacha, M., and Ballesta, J.P. (1998). The GTPase center protein L12 is required for correct ribosomal stalk assembly but not for *Saccharomyces cerevisiae* viability. *J. Biol. Chem.* **273**, 31956–31961.
- Brodersen, D.E., Clemons, W.M., Jr., Carter, A.P., Wimberly, B.T., and Ramakrishnan, V. (2002). Crystal structure of the 30 S ribosomal subunit from *Thermus thermophilus*: structure of the proteins and their interactions with 16 S RNA. *J. Mol. Biol.* **316**, 725–768.
- Cigan, A.M., Foiani, M., Hannig, E.M., and Hinnebusch, A.G. (1991). Complex formation by positive and negative translational regulators of GCN4. *Mol. Cell. Biol.* **11**, 3217–3228.
- Clemons, W.M., Jr., Menetret, J.F., Akey, C.W., and Rapoport, T.A. (2004). Structural insight into the protein translocation channel. *Curr. Opin. Struct. Biol.* **14**, 390–396.
- Dabbs, E.R. (1991). Mutants lacking individual ribosomal proteins as a tool to investigate ribosomal properties. *Biochimie* **73**, 639–645.
- Deloche, O., de la Cruz, J., Kressler, D., Doere, M., and Linder, P. (2004). A membrane transport defect leads to a rapid attenuation of translation initiation in *Saccharomyces cerevisiae*. *Mol. Cell* **13**, 357–366.
- Deshmukh, M., Stark, J., Yeh, L.C., Lee, J.C., and Woolford, J.L., Jr. (1995). Multiple regions of yeast ribosomal protein L1 are important for its interaction with 5 S rRNA and assembly into ribosomes. *J. Biol. Chem.* **270**, 30148–30156.
- Deshmukh, M., Tsay, Y.F., Paulovich, A.G., and Woolford, J.L., Jr. (1993). Yeast ribosomal protein L1 is required for the stability of newly synthesized 5S rRNA and the assembly of 60S ribosomal subunits. *Mol. Cell. Biol.* **13**, 2835–2845.
- Dragon, F., Gallagher, J.E., Compagnone-Post, P.A., Mitchell, B.M., Porwancher, K.A., Wehner, K.A., Wormsley, S., Settlage, R.E., Shabanowitz, J., Osheim, Y., et al. (2002). A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. *Nature* **417**, 967–970.
- Dresios, J., Panopoulos, P., Frantziou, C.P., and Synetos, D. (2001). Yeast ribosomal protein deletion mutants possess altered peptidyltransferase activity and different sensitivity to cycloheximide. *Biochemistry* **40**, 8101–8108.
- Dresios, J., Panopoulos, P., Suzuki, K., and Synetos, D. (2003). A dispensable yeast ribosomal protein optimizes peptidyltransferase activity and affects translocation. *J. Biol. Chem.* **278**, 3314–3322.
- Ford, C.L., Randal-Whitis, L., and Ellis, S.R. (1999). Yeast proteins related to the p40/laminin receptor precursor are required for 20S ribosomal RNA processing and the maturation of 40S ribosomal subunits. *Cancer Res.* **59**, 704–710.
- Gallagher, J.E., Dunbar, D.A., Granneman, S., Mitchell, B.M., Osheim, Y., Beyer, A.L., and Baserga, S.J. (2004). RNA polymerase I transcription and pre-rRNA processing are linked by specific SSU processome components. *Genes Dev.* **18**, 2506–2517.
- Gavin, A.C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J.M., Michon, A.M., Cruciat, C.M., et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**, 141–147.
- Giaever, G., Chu, A.M., Ni, L., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., Andre, B., et al. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418**, 387–391.
- Gleizes, P.E., Noaillic-Depeyre, J., Leger-Silvestre, I., Teulier, F., Dauxois, J.Y., Pommet, D., Azum-Gelade, M.C., and Gas, N. (2001). Ultrastructural localization of rRNA shows defective nuclear export of preribosomes in mutants of the Nup82p complex. *J. Cell Biol.* **155**, 923–936.
- Grandi, P., Rybin, V., Bassler, J., Petfalski, E., Strauss, D., Marzioch, M., Schafer, T., Kuster, B., Tschochner, H., Tollervey, D., et al. (2002). 90S pre-ribosomes include the 35S pre-rRNA, the U3 snoRNP, and 40S subunit processing factors but predominantly lack 60S synthesis factors. *Mol. Cell* **10**, 105–115.
- Granneman, S., and Baserga, S.J. (2004). Ribosome biogenesis: of knobs and RNA processing. *Exp. Cell Res.* **296**, 43–50.
- Hartwell, L.H., and McLaughlin, C.S. (1969). A mutant of yeast apparently defective in the initiation of protein synthesis. *Proc. Natl. Acad. Sci. USA* **62**, 468–474.
- Hedges, J., West, M., and Johnson, A.W. (2005). Release of the export adapter, Nmd3p, from the 60S ribosomal subunit requires Rpl10p and the cytoplasmic GTPase Lsg1p. *EMBO J.* **24**, 567–579.
- Held, W.A., Ballou, B., Mizushima, S., and Nomura, M. (1974). Assembly mapping of 30 S ribosomal proteins from *Escherichia coli*. Further studies. *J. Biol. Chem.* **249**, 3103–3111.
- Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S.L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., et al. (2002). Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* **415**, 180–183.
- Jakovljevic, J., de Mayolo, P.A., Miles, T.D., Nguyen, T.M., Leger-Silvestre, I., Gas, N., and Woolford, J.L., Jr. (2004). The carboxy-terminal extension of yeast ribosomal protein S14 is necessary for maturation of 43S preribosomes. *Mol. Cell* **14**, 331–342.
- Johnson, A.W., Lund, E., and Dahlberg, J. (2002). Nuclear export of ribosomal subunits. *Trends Biochem. Sci.* **27**, 580–585.
- Kramer, G., Rauch, T., Rist, W., Vorderwulbecke, S., Patzelt, H., Schulze-Specking, A., Ban, N., Deuerling, E., and Bukau, B. (2002). L23 protein functions as a chaperone docking site on the ribosome. *Nature* **419**, 171–174.
- Kufel, J., Allmang, C., Petfalski, E., Beggs, J., and Tollervey, D. (2003). Lsm proteins are required for normal processing and stability of ribosomal RNAs. *J. Biol. Chem.* **278**, 2147–2156.
- Lecompte, O., Ripp, R., Thierry, J.C., Moras, D., and Poch, O. (2002). Comparative analysis of ribosomal proteins in complete genomes: an example of reductive evolution at the domain scale. *Nucleic Acids Res.* **30**, 5382–5390.
- Leger-Silvestre, I., Milkereit, P., Ferreira-Cerca, S., Saveanu, C., Rousselle, J.C., Choesmel, V., Guinefoleau, C., Gas, N., and Gleizes, P.E. (2004). The ribosomal protein Rps15p is required for nuclear exit of the 40S subunit precursors in yeast. *EMBO J.* **23**, 2336–2347.
- Meskauskas, A., and Dinman, J.D. (2001). Ribosomal protein L5 helps anchor peptidyl-tRNA to the P-site in *Saccharomyces cerevisiae*. *RNA* **7**, 1084–1096.
- Milkereit, P., Gadal, O., Podtelejnikov, A., Trumtel, S., Gas, N., Petfalski, E., Tollervey, D., Mann, M., Hurt, E., and Tschochner, H. (2001). Maturation and intranuclear transport of pre-ribosomes requires Noc-proteins. *Cell* **105**, 499–509.
- Milkereit, P., Kühn, H., Gas, N., and Tschochner, H. (2003a). The pre-ribosomal network. *Nucleic Acids Res.* **31**, 799–804.
- Milkereit, P., Strauss, D., Bassler, J., Gadal, O., Kühn, H., Schütz, S., Gas, N., Lechner, J., Hurt, E., and Tschochner, H. (2003b). A

Noc-complex specifically involved in maturation and nuclear export of ribosomal 40S subunits. *J. Biol. Chem.* 278, 4072–4081.

Moritz, M., Paulovich, A.G., Tsay, Y.F., and Woolford, J.L., Jr. (1990). Depletion of yeast ribosomal proteins L16 or rp59 disrupts ribosome assembly. *J. Cell Biol.* 111, 2261–2274.

Nierhaus, K.H. (2004). Assembly of the prokaryotic ribosome. In *Protein Synthesis and Ribosome Structure*, K.H. Nierhaus and D.N. Wilson, eds. (Weinheim, Germany: Wiley-VCH), pp. 85–106.

Peltz, S.W., Hammell, A.B., Cui, Y., Yasnachak, J., Puljanowski, L., and Dinman, J.D. (1999). Ribosomal protein L3 mutants alter translational fidelity and promote rapid loss of the yeast killer virus. *Mol. Cell. Biol.* 19, 384–391.

Pool, M.R., Stumm, J., Fulga, T.A., Sinning, I., and Dobberstein, B. (2002). Distinct modes of signal recognition particle interaction with the ribosome. *Science* 297, 1345–1348.

Schlutzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., and Yonath, A. (2000). Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. *Cell* 102, 615–623.

Stansfield, I., Jones, K.M., Herbert, P., Lewendon, A., Shaw, W.V., and Tuite, M.F. (1998). Missense translation errors in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 282, 13–24.

Synetos, D., Dabeva, M.D., and Warner, J.R. (1992). The yeast ribosomal protein S7 and its genes. *J. Biol. Chem.* 267, 3008–3013.

Synetos, D., Frantziou, C.P., and Alksne, L.E. (1996). Mutations in yeast ribosomal proteins S28 and S4 affect the accuracy of translation and alter the sensitivity of the ribosomes to paromomycin. *Biochim. Biophys. Acta.* 1309, 156–166.

Tabb-Massey, A., Caffrey, J.M., Logsdon, P., Taylor, S., Trent, J.O., and Ellis, S.R. (2003). Ribosomal proteins Rps0 and Rps21 of *Saccharomyces cerevisiae* have overlapping functions in the maturation of the 3' end of 18S rRNA. *Nucleic Acids Res.* 31, 6798–6805.

Takyar, S., Hickerson, R.P., and Noller, H.F. (2005). mRNA helicase activity of the ribosome. *Cell* 120, 49–58.

Trapman, J., and Planta, R.J. (1976). Maturation of ribosomes in yeast. I. Kinetic analysis by labelling of high molecular weight rRNA species. *Biochim. Biophys. Acta.* 442, 265–274.

Traub, P., and Nomura, M. (1968). Structure and function of *E. coli* ribosomes. V. Reconstitution of functionally active 30S ribosomal particles from RNA and proteins. *Proc. Natl. Acad. Sci. USA* 59, 777–784.

Tsay, Y.F., Shankweiler, G., Lake, J., and Woolford, J.L., Jr. (1994). Localization of *Saccharomyces cerevisiae* ribosomal protein L16 on the surface of 60 S ribosomal subunits by immunoelectron microscopy. *J. Biol. Chem.* 269, 7579–7586.

Tschochner, H., and Hurt, E. (2003). Pre-ribosomes on the road from the nucleolus to the cytoplasm. *Trends Cell Biol.* 13, 255–263.

Valasek, L., Mathew, A.A., Shin, B.S., Nielsen, K.H., Szamecz, B., and Hinnebusch, A.G. (2003). The yeast eIF3 subunits TIF32/a, NIP1/c, and eIF5 make critical connections with the 40S ribosome in vivo. *Genes Dev.* 17, 786–799.

Venema, J., and Tollervey, D. (1999). Ribosome synthesis in *Saccharomyces cerevisiae*. *Annu. Rev. Genet.* 33, 261–311.

Vos, H.R., Faber, A.W., de Gier, M.D., Vos, J.C., and Raue, H.A. (2004). Deletion of the three distal S1 motifs of *Saccharomyces cerevisiae* Rrp5p abolishes pre-rRNA processing at site A(2) without reducing the production of functional 40S subunits. *Eukaryot. Cell* 3, 1504–1512.

Wimberly, B.T., Brodersen, D.E., Clemons, W.M., Jr., Morgan-Warren, R.J., Carter, A.P., Vornrhein, C., Hartsch, T., and Ramakrishnan, V. (2000). Structure of the 30S ribosomal subunit. *Nature* 407, 327–339.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., et al. (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285, 901–906.

Yusupov, M.M., Yusupova, G.Z., Baucom, A., Lieberman, K., Earnest, T.N., Cate, J.H., and Noller, H.F. (2001). Crystal structure of the ribosome at 5.5 Å resolution. *Science* 292, 883–896.