RESEARCH PAPER

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Immature large ribosomal subunits containing the 7S pre-rRNA can engage in translation in *Saccharomyces cerevisiae*

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Abbreviations: DAPI, 4,6-diamidino-2-phenylindole; FISH, fluorescence *in situ* hybridization; pre-rRNA, precursor rRNA; NRD, Non-functional rRNA decay; r-subunits, ribosomal subunits; r-particles, ribosomal particles; r-proteins, ribosomal proteins; TRAMP complexes, Trf/Air/Mtr4 complexes

Evolution has provided eukaryotes with mechanisms that impede immature and/or aberrant ribosomes to engage in translation. These mechanisms basically either prevent the nucleo-cytoplasmic export of these particles or, once in the cytoplasm, the release of associated assembly factors, which interfere with the binding of translation initiation factors and/or the ribosomal subunit joining. We have previously shown that aberrant yeast 40S ribosomal subunits containing the 20S pre-rRNA can engage in translation. In this study, we describe that cells harbouring the *dob1–1* allele, encoding a mutated version of the exosome-assisting RNA helicase Mtr4, accumulate otherwise nuclear pre-60S ribosomal particles containing the 7S pre-rRNA in the cytoplasm. Polysome fractionation analyses revealed that these particles are competent for translation and do not induce elongation stalls. This phenomenon is rather specific since most mutations in other exosome components or co-factors, impairing the 3′ end processing of the mature 5.8S rRNA, accumulate 7S pre-rRNAs in the nucleus. In addition, we confirm that pre-60S ribosomal particles containing either 5.8S + 30 or 5.8S + 5 pre-rRNAs also engage in translation elongation. We propose that 7S pre-rRNA processing is not strictly required for pre-60S r-particle export and that, upon arrival in the cytoplasm, there is no specific mechanism to prevent translation by premature pre-60S r-particles containing 3′ extended forms of mature 5.8S rRNA.

Introduction

In eukaryotes, ribosome biogenesis is a compartmentalized, multi-component and multi-step process; most of the biogenesis reactions take place in the nucleolus but later events occur in the nucleoplasm and the cytoplasm.[1,2] Although the basic outline of ribosome biogenesis is reasonably well conserved throughout eukaryotes,[3] this process has been best studied in the yeast *Saccharomyces cerevisiae*.[1,2,4-6] In the yeast nucleolus, the mature 18S, 5.8S and 25S rRNAs are transcribed as a single large precursor rRNA (pre-rRNA) by RNA polymerase I, from which the external and internal spacer fragments are removed by endo- and exonucleolytic processing reactions.[7,8] A pre-5S rRNA is transcribed independently by RNA polymerase III, which undergoes exonucleolytic processing at its 3′ end.[8] Concomitant to pre-rRNA processing, pre-rRNAs are extensively modified by base and 2′-O-ribose methylation and pseudouridylation[9,10]; moreover, pre-rRNAs are subjected to diverse structural rearrangements and folding reactions while they associate/dissociate with *trans*-acting factors and assemble with most ribosomal proteins (r-proteins) to form nucleolar pre-ribosomal particles.[2] On their maturation path from the nucleolus to the nucleoplasm, pre-ribosomal particles undergo a series of compositional changes, which finally enable them to recruit export factors and be transported to the cytoplasm,[11-13] where both ribosomal subunits (r-subunits) acquire the competence to engage in translation.[14] Cytoplasmic maturation of pre-40S ribosomal particles (r-particles) involves processing of the 20S pre-rRNA to mature 18S rRNA,[15] the dissociation and recycling of late *trans*-acting factors and the assembly of few r-proteins.[16,17] Cytoplasmic maturation of pre-60S r-particles involves processing of the 6S pre-rRNA to mature 5.8S rRNA[18] (see **Fig. S1**), and, as for pre-40S particles, the release and recycling of late *trans*-acting factors and the assembly of the remaining r-proteins.[19-21]

Translation by aberrantly assembled r-subunits might have severely deleterious consequences to cells, among them, sequestering of translation factors, stalling of translation elongation and reduced fidelity in protein synthesis. Thus, evolution has provided cells with different surveillance mechanisms to monitor and eliminate defective ribosomes (for reviews, see refs. 22,23).

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First, incorrectly assembled pre-ribosomal particles are efficiently retained in the nucle(ol)us and subjected to degradation by a process involving the exosome and the Trf/Air/Mtr4 (TRAMP) complexes.[24-26] Second, the release and/or activity of some of the abovementioned late *trans*-acting factors is clearly impaired when bound to improperly assembled pre-ribosomal particles that escaped the nuclear retention mechanism,[27,28] which is also expected to induce the degradation of these particles. Moreover, r-subunit precursors containing point mutations in essential rRNA sites (i.e. peptidyl-transferase center, decoding site) are recognized and degraded by a distinct pathway, the so-called Nonfunctional rRNA decay (NRD) pathway (see ref. 23). Degradation of aberrant 40S r-subunits by NRD occurs in P-bodies, requires that these particles engage in translation and involves Dom34, Hbs1, Ski7, Xrn1 and the cytoplasmic exosome.[29,30] In contrast, degradation of aberrant 60S r-subunits by NRD does not depend on their engagement in translation and involves the Mms1-Rtt101 E3 ubiquitin ligase, the Cdc48 complex, the proteasome and the cytoplasmic exosome.[31,32]

Despite the existence of all these surveillance mechanisms, different examples of relatively stable aberrant r-subunits able to engage in translation have been suggested in yeast. For example, we have previously shown that there is an accumulation of cytoplasmic 40S r-subunits containing the 20S pre-rRNA in both *ubi3*Δ*ub* and *rpl3[W255C]* mutant cells.[28,33] Interestingly, these subunits associate into polysomes and they do apparently not stall on mRNAs, similarly as also reported for *ltv1*Δ *pfa1*Δ and *ltv1*Δ *prp43–414* double mutants,[34] strongly suggesting that, in these cases, the particles are efficient in translation initiation and elongation. Loss of function of *trans*-acting factors such as Fap7,[35] Rio1 and Nob1[36] or r-proteins S0 or S14[37,38] also lead to accumulation of 40S r-particles containing 20S pre-rRNA. However, in these cases, the particles are not or only poorly competent for translation elongation since a much higher 18S rRNA/20S pre-rRNA ratio could be observed in polysomal fractions compared to 80S fractions. The fact that pre-40S can engage in initiation of translation has been clearly shown for particles accumulating upon depletion of Rio1 and Nob1.[36] Therefore, it can be concluded that compositional and/or structural differences may exist between immature cytoplasmic pre-40S r-particles from wild-type and different mutant cells.[34] On the other hand, in the *rrp6*Δ mutant, immature 60S r-subunits containing the otherwise nuclear 5.8S + 30 pre-rRNAs are exported to the cytoplasm and are able to enter into polysomes.[39] In wild-type cells, exported pre-60S r-particles contain the long or the short form of 6S pre-rRNA.[18] In the cytoplasm, the nuclease Ngl2 processes these precursors to 5.8S pre-rRNAs.[40] Since, 6S pre-rRNAs are not fully processed to 5.8S rRNAs in the viable *ngl2*Δ mutant, pre-60S r-particles containing 5.8S+5 pre-rRNAs are predicted to engage in translation.[18,40]

Here, we have studied the export and translation ability of pre-60S r-particles containing 7S pre-rRNAs. Our results show that, in distinct yeast mutants impaired in 3′ end maturation of 5.8S rRNA, a fraction of these otherwise nuclear particles could be exported to the cytoplasm and incorporated into polysomes. Export of these particles is independent of the TRAMP complex. Moreover, these particles are likely able to complete translation since no evidence for an increase in the stall frequency on mRNAs could be found. We propose that 7S pre-rRNA processing is not strictly required for pre-60S r-particle export and that, upon arrival in the cytoplasm, there is neither an impairment of the assembly of the last r-proteins nor a specific mechanism to prevent translation by immature pre-60S r-particles containing 3′ extended forms of mature 5.8S rRNA.

Results

A fraction of 7S pre-rRNAs accumulating in the *dob1–1* mutant is cytoplasmic

Formation of the 3′ end of mature 5.8S rRNAs from the 7S pre-rRNAs is a very complicated multi-step process (**Fig. S1**), which requires the action of different nucleases (for a review, see[8]). The initial step consists in the 3′–5′ trimming of 7S pre-rRNAs to the 5.8S + 30 pre-rRNAs, which is carried out by the exonuclease activity of Rrp44, a subunit of the exosome.[41,42] Next, 5.8S + 30 pre-rRNAs are processed to 6S pre-rRNAs by the exonuclease Rrp6, either in an exosome-dependent or -independent manner.[39,43] In wild-type cells, these 2 reactions occur in the nucleus (**Fig. S1**) and pre-60S r-particles containing 6S pre-rRNA species are then exported to the cytoplasm.[18] There, the 6S pre-rRNAs are successively processed to 5.8S + 5 pre-rRNAs by the 3′–5′ exonucleases Rex1, Rex2 and Rex3[44] and then to mature 5.8S rRNAs by the nuclease Ngl2.[40]

In the nucleus, processing of 7S pre-rRNAs requires additional co-factors, such as the RNA helicase Mtr4/Dob1[45,46] and the non-essential Rrp47/Lrp1 and Mpp6.[47-49] Our group identified in the past *MTR4/DOB1* as the gene complementing the thermo-sensitive *dob1–1* mutation. This mutation leads to a deficit in 60S r-subunits due to the accumulation of 7S pre-rRNAs, which is accompanied by a mild reduction in the levels of mature 5.8S rRNAs.[46] Interestingly and in contrast to other 60S r-subunit biogenesis mutants, *dob1–1* mutant cells are specifically dependent on a high dosage of *TIF3*,[46] which encodes the yeast translation factor eIF4B.[50,51] Furthermore, the *dob1–1* mutant shows a similar hypersensitivity to the protein synthesis inhibitor paromomycin as different translation initiation factor mutants.[46] These results suggested that the *dob1–1* mutation impairs translation, which is likely due to the synthesis of malfunctioning but translation-competent 60S r-subunits rather than the consequence of a general 60S r-subunit deficit.

To test this hypothesis, and taking into account the pre-rRNA processing phenotypes of the *dob1–1* mutant, we first determined whether the *dob1–1* mutant accumulates aberrant pre-60S r-particles containing 7S pre-rRNA in the cytoplasm. To this end, we performed immunoprecipitation experiments in wild-type and *dob1–1* cells expressing TAP-tagged constructs of proteins specific for nuclear (Nop7-TAP), nucleo-cytoplasmic (Arx1-TAP), exclusively cytoplasmic (Lsg1-TAP) pre-60S r-particles, and for mature 60S r-subunits (L24A-TAP). Precipitated RNAs were analyzed by northern hybridization. As shown in **Figure 1A**, and as expected, 7S pre-rRNAs were specifically enriched upon

precipitation of the Nop7-TAP and the Arx1-TAP baits in wild-type cells compared to the untagged control, but were not significantly enriched upon precipitation with the cytoplasmic Lsg1-TAP and L24A-TAP baits. In contrast, in *dob1–1* cells, the Lsg1-TAP and L24A-TAP baits were able to efficiently precipitate 7S pre-rRNAs (**Fig. 1B**). No 27S pre-rRNAs were detected upon affinity-purification from extracts of the Lsg1-TAP bait in either wild-type or *dob1–1* cells (**Fig. S2A** and data not shown). Moreover, no apparent nuclear retention of an Lsg1-GFP construct was observed in the *dob1–1* strain (**Fig. S2B**). Altogether, these results indicate that pre-60S r-particles containing 7S pre-rRNA are being exported in the *dob1–1* mutant.

To further confirm that 7S pre-rRNAs are associated with exported pre-60S r-particles in the *dob1–1* mutant, we studied the localization of different pre-rRNAs by fluorescence *in situ* hybridization (FISH) with probes complementary to the D-A_2 region of ITS1 (probe FISH ITS1), and the E-C_2 (probe FISH ITS2–1) and C_2-C_1 regions of ITS2 (probe FISH ITS2–2). The first probe hybridizes to 35S, 33S, 32S and 20S pre-rRNAs, the second one to 35S, 33S, 32S, all 27S pre-rRNAs and all 3′ extended pre-5.8S precursors, and the third one to 35S, 33S, 32S, all 27S pre-rRNAs and the 5′ extended pre-25S precursors, respectively (**Fig. 2A**). None of these probes hybridize with mature rRNAs. Before performing the FISH experiments, we checked that *dob1–1* cells do not display an enlarged nucleoplasm or nucleus (**Fig. S3**). When probes FISH ITS1 and FISH ITS2–2 were used, similar intensity and distribution of the signals were observed in both wild-type and *dob1–1* cells (**Fig. 2B, C**). However, when the FISH ITS2–1 probe was used, a stronger FISH signal was observed in *dob1–1* mutant cells than in wild-type cells, which is consistent with the 7S pre-rRNA accumulation detected in this strain. More importantly, there is a clear appearance of cytoplasmic ITS2–1 signal in *dob1–1* cells, indicating that 3′ extended forms of 5.8S rRNA, most likely corresponding to 7S pre-rRNAs (**Fig. 1B**), accumulate in the cytoplasm of *dob1–1* mutant cells. However, still a substantial amount of 3′ extended forms of 5.8S rRNA accumulates in the nucleus of *dob1–1* mutant cells.

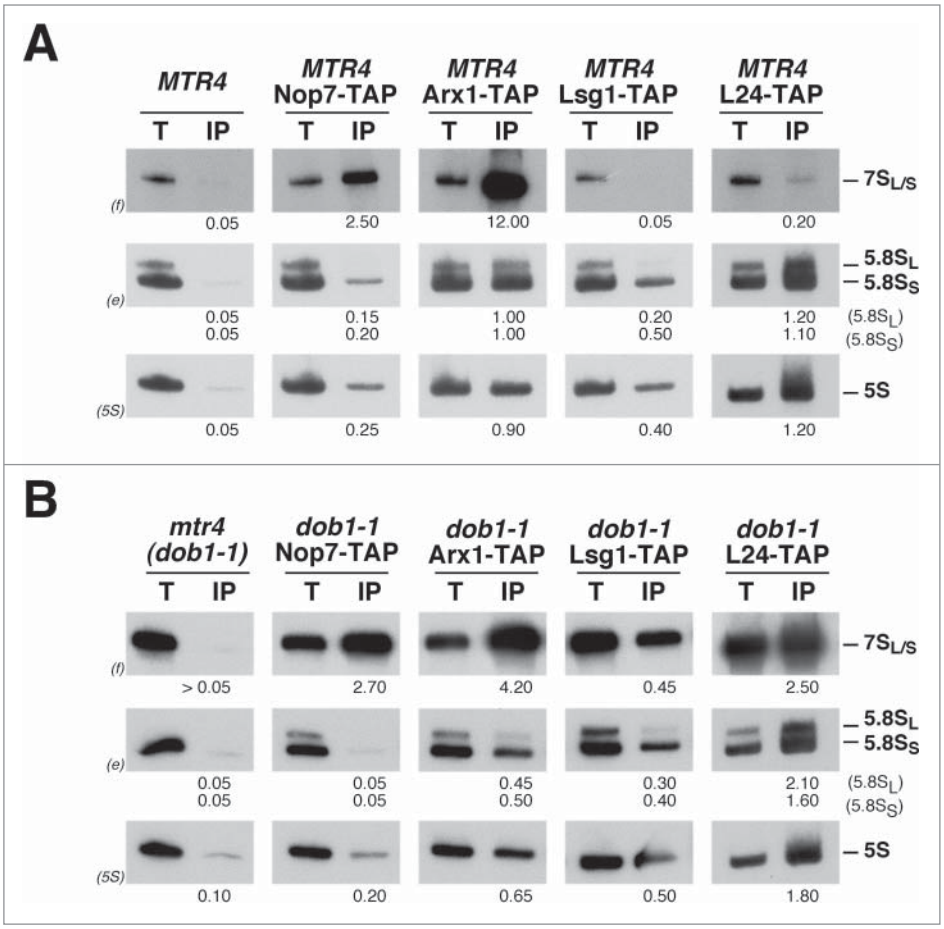

Figure 1. Pre-60S r-particles containing 7S pre-rRNAs are exported to the cytoplasm in the *dob1–1* mutant. Isogenic wild-type (*MTR4*) and *dob1–1* (*mtr4*) strains were grown at 30°C in YPD medium to mid-log phase. Immunoprecipitation experiments were carried out using IgG-Sepharose and whole-cell extracts from wild-type (**A**) and *dob1–1* (**B**) control cells (no TAP bait) or cells expressing TAP-tagged Nop7, Arx1, Lsg1, and L24A. RNA was extracted from the beads (lanes IP) or from an amount of total extract corresponding to 1/100 of that used for the immunoprecipitations (lanes T), separated on a 7% polyacrylamide-8M urea gel, transferred to a nylon membrane and subjected to northern hybridization with the 3 probes indicated in parentheses to detect 7S pre-rRNAs and mature 5.8S and 5S rRNAs. Probes are described in **Figure 2** and **Supplemental Table 2**. Signal intensities were measured by phosphorimager scanning; values (below each IP lane) refer to the percentage of each RNA recovered after purification.

Cytoplasmic pre-60S r-particles containing the 7S pre-rRNA get incorporated into translating ribosomes

To assess whether the cytoplasmic, 7S pre-rRNA containing pre-60S r-particles present in *dob1–1* cells are incorporated into translating ribosomes, we analyzed the distribution of the 7S pre-rRNAs in polysome profiles of *dob1–1* mutant cell extracts by sucrose gradient fractionation and northern blotting and compared it to that of wild-type cells (**Fig. 3**). In wild-type cells, 7S pre-rRNAs co-migrated exclusively with the 60S r-subunit peak, which is indicative of its presence in nuclear pre-60S r-particles. In contrast, 7S pre-rRNAs were not only associated with the 60S r-subunit peak, but also co-sedimented significantly with polysomes in the *dob1–1* mutant.

To confirm that the 7S pre-rRNA containing particles sedimenting with polysomes in the *dob1–1* mutant were not simply

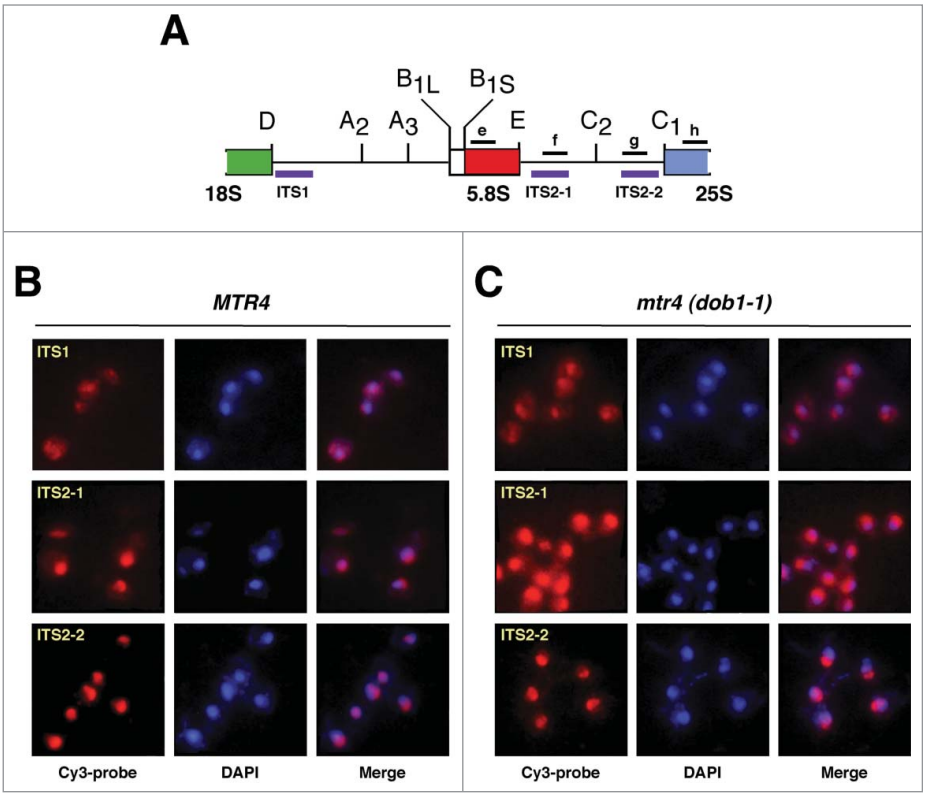

Figure 2. Detection of pre-ribosomal particles by fluorescence *in situ* hybridization (FISH). (**A**) Primary structure of the 2 internal transcribed spacers, ITS1 and ITS2, of the 35S pre-rRNA. Processing sites are shown. The location of the 3 Cy-3 labeled probes used for FISH (purple) and of the oligonucleotides probes used for northern hybridization (black) is indicated. (**B and C**) Wild-type (*MTR4*) and *dob1–1* (*mtr4*) cells were grown at 30°C in YPD medium to mid-log phase. Distinct pre-rRNAs were detected by FISH using Cy-3 labeled ITS1, ITS2–1 or ITS2–2 probes (red; purple in merge panels). Cells were further stained with DAPI to visualize the nucleoplasm (blue). All images shown were captured using identical exposure times and processed in the same manner.

aggregates, cell extracts of both wild-type and *dob1–1* cells were prepared under polysome run-off conditions, by omission of cycloheximide, either in standard buffer or in a buffer lacking Mg^{2+}, which causes dissociation of 80S ribosomes into 40S and 60S r-subunits.[52] Under these conditions, the bulk of 7S pre-rRNAs and 5.8S rRNAs was shifted to positions of the 80S and 60S fractions in the *dob1–1* mutant, similarly as observed for the 5.8S rRNAs in wild-type cells (**Fig. S4**). This result clearly reveals that the fractions containing 7S pre-rRNA were indeed associated with polysomes. Moreover, as shown in **Figure 3**, when the 7S pre-rRNAs levels were quantified within the different fractions from standard sucrose gradients and compared to those of $5.8S_S$ rRNA in the same fractions in both wild-type and *dob1–1* cells, we found that 7S pre-rRNA levels were particularly high in the 60S r-subunit peak compared to those of $5.8S_S$ rRNA in the *dob1–1* mutant, which indicates that this peak consists of both nuclear and cytoplasmic pre-60S r-particles containing the 7S precursor. However and remarkably, the $7S/5.8S_S$ RNA ratio remains constant along the 80S and polysome fractions in the *dob1–1* mutant. Altogether, these findings clearly reveal that the cytoplasmic pre-60S r-particles containing 7S pre-rRNA observed in *dob1–1* mutant cells are competent for both translation initiation and elongation.

Analysis of mutants affected in 3′ end maturation of 5.8S rRNA

As abovementioned, 3′ end maturation of 5.8S rRNA in yeast is a multi-step and multi-component pathway (see **Fig. S1**). Thus, we next determined whether immature, translation-competent pre-60S r-particles containing 3′ extended forms of 5.8S rRNA are present in other mutants affecting this maturation process (see **Table S1**). Total RNA was extracted from these mutant strains, either grown at 30°C or shifted to 37°C, and subjected to northern blot analysis with probes hybridizing to the different precursors of mature 5.8S rRNAs. As shown in **Figure 4**, the *csl4–1, mtr3–1*, and *rrp4–1* mutants, as previously reported,[53,54] accumulated 7S pre-rRNAs. The strains lacking Rrp6 and Rrp47[39,47] were selected since they accumulated slightly 7S pre-rRNAs and significantly 5.8S + 30 pre-rRNAs. Finally, the *ngl2*Δ mutant[40] accumulated the 5.8S + 5 pre-rRNA species and did not produce completely matured 5.8S rRNAs.

We next assessed the ability of the corresponding pre-60S r-particles containing the above pre-5.8S rRNA species from the different mutants to engage in translation. Similarly as done for the *dob1–1* mutant, cell extracts were prepared under polysome-preserving conditions from the wild-type strain and the different mutants and subjected to sucrose gradient fractionation; then, RNA was prepared from individual fractions, separated by electrophoresis and analyzed by northern blotting. As shown in **Figure 5**, pre-60S r-particles containing 7S pre-rRNAs were predominantly found in 60S fractions but not in polysomes in extracts from the *csl4–1, mtr3–1*, and *rrp4–1* mutants grown at 30°C. The 7S pre-rRNAs were neither found in polysomes from the *rrp47*Δ and *ngl2*Δ strains and very faintly in polysome-containing fractions in extracts from the *rrp6*Δ mutant (**Fig. 5**). Interestingly, when the *rrp4–1* and *rrp6*Δ mutants were subjected to a shift at 37°C for 4 h, 7S pre-rRNAs did associate into polysomes (**Fig. 5** and data not shown). In contrast, 7S pre-rRNA containing pre-60S r-particles from the *csl4–1, mtr3–1*, and *rrp47*Δ mutants subjected to a similar shift did not enter polysomes (**Fig. 5** and data not shown). We further studied the *rrp4–1* mutant to determine whether 7S pre-rRNA species were exported at 30°C and 37°C. To this end, we performed immunoprecipitation experiments in *rrp4–1* cells expressing TAP-tagged constructs of either Nop7 or Lsg1 and

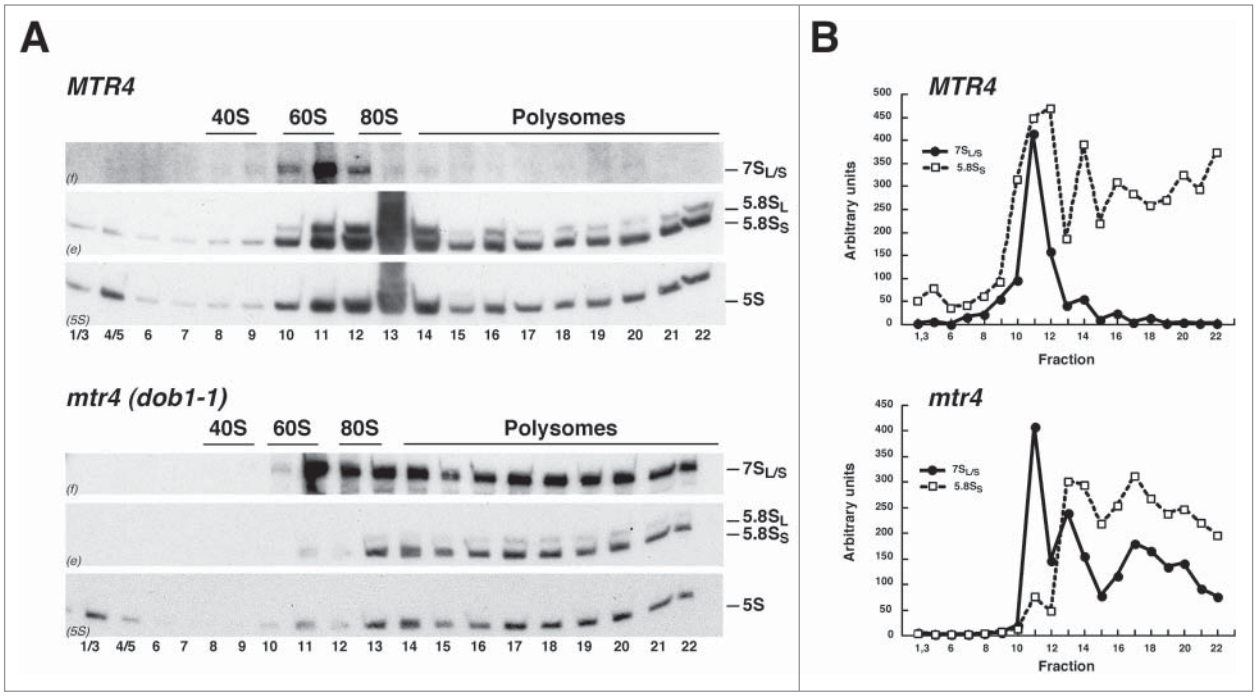

Figure 3. Pre-60S r-particles containing 7S pre-rRNAs engage in translation in the *dob1–1* mutant. Wild-type (*MTR4*) and *dob1–1* (*mtr4*) cells were grown at 30°C in YPD medium to mid-log phase. (**A**) Cells extracts were prepared and 10 A_{260} units of each extract were resolved in 7–50% sucrose gradients and fractionated. RNA was extracted from each fraction and analyzed by northern blotting using probes f, e and 5S, which reveal 7S pre-rRNAs and mature 5.8S and 5S rRNAs, respectively. The position of free 40S and 60S r-subunits, 80S ribosomes and polysomes, obtained from the recorded A_{254} profiles, are shown. (**B**) Signal intensities of the 7S pre-rRNAs (black dot, continuous line) and 5.8S_S rRNA (white squares, dashed line) were determined for each fraction by phosphorimager scanning and represented as arbitrary units.

analyzed the precipitated RNAs by northern hybridization. As shown in **Figure 6**, at either 30°C or 37°C, 7S pre-rRNAs were specifically enriched upon precipitation of the Nop7-TAP bait; however, these precursors were only significantly enriched upon precipitation with the cytoplasmic Lsg1-TAP bait upon the shift to 37°C.

These results suggest that the pre-60S r-particles containing 7S pre-rRNAs might not be similar in the different mutants. Thus, particles unable to exit to the cytoplasm are expected to either retain factors that prevent their export or lack factors that prepare them for export competence. In addition, for those able to arrive in the cytoplasm, there might be no apparently specific mechanism to prevent their engagement in translation.

Discussion

With this study, we provide, to the best of our knowledge, the first report on cytoplasmic pre-60S r-particles containing 7S pre-rRNAs. These particles, which are normally restricted to the nucleus in wild-type cells, are exported to the cytoplasm in certain mutant conditions where 7S pre-rRNA processing is affected, such as in *dob1–1, rrp4–1*, and *rrp6*Δ mutant cells.

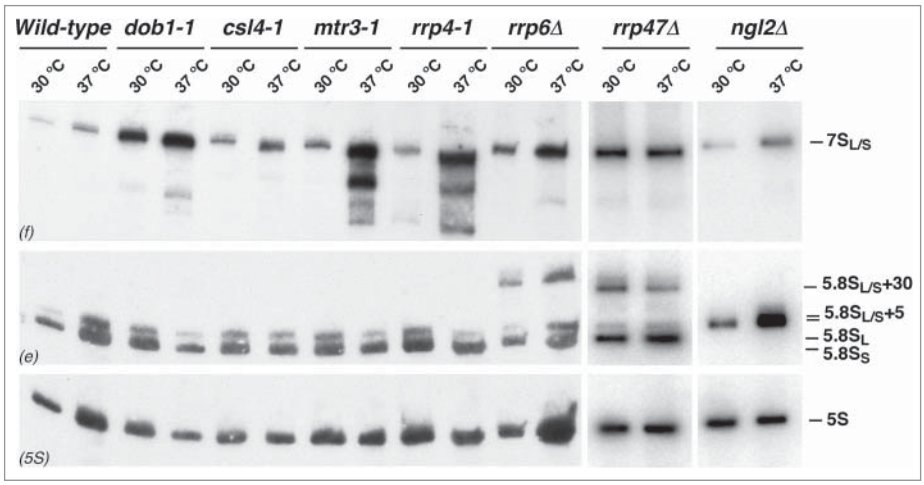

Figure 4. Pre-rRNA processing of the 7S pre-rRNAs in different mutants affected in 5.8S rRNA maturation. A wild-type strain and the indicated mutants were grown at 30°C in YPD medium to mid-log phase or shifted for 4 h to 37°C. Total RNA was prepared and 5 μg was separated on a 7% polyacrylamide-8M urea gel, transferred to a nylon membrane and subjected to northern hybridization with the 3 probes indicated in parentheses to detect 7S pre-rRNAs and mature 5.8S and 5S rRNAs. Note that probe e also reveals 5.8S + 30 and 5.8S + 5 pre-rRNAs in the *rrp6*Δ and *rrp47*Δ, and *ngl2*Δ mutants, respectively.

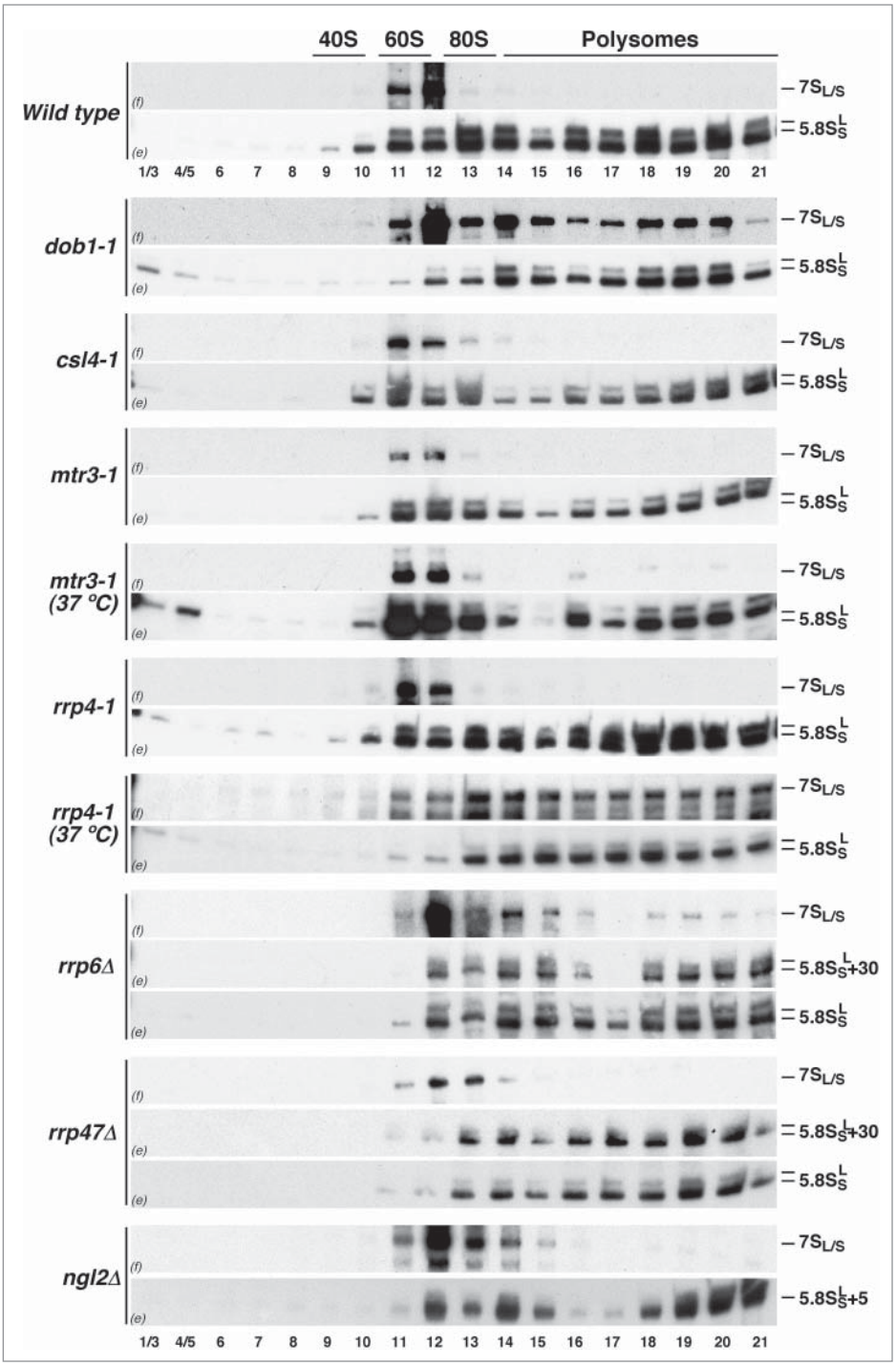

Figure 5. Ability of pre-60S r-particles containing 3′ end extended precursors of 5.8S rRNAs to engage in translation in different mutant strains. A wild-type strain and the indicated mutants were grown at 30°C in YPD medium to mid-log phase. The *mtr3–1* and *rrp4–1* mutants were also shifted for 4 h to 37°C. Cell extracts were prepared and 10 A_{260} units of each extract were resolved in 7–50% sucrose gradients and fractionated. RNA was extracted from each fraction and analyzed by northern blotting using probes f and e, which reveal 7S pre-rRNAs and mature 5.8S rRNAs, respectively. Note that probe e also reveals 5.8S + 30 and 5.8S + 5 pre-rRNAs in the *rrp6*Δ and *rrp47*Δ, and *ngl2*Δ mutants, respectively. The position of free 40S and 60S r-subunits, 80S ribosomes and polysomes, obtained from the recorded A_{254} profiles, are indicated.

Whether these particles are being actively exported to the cytoplasm and/or arrive there as a result of leaking passively through nuclear pores remains to be elucidated. Moreover, these aberrant cytoplasmic pre-60S r-particles are competent for translation, as indicated by their occurrence in polysome fractions. Importantly, the ratio between 7S pre-rRNA and mature 5.8S and 5S rRNAs was comparable in all the polysome fractions in these mutants; thus, indicating that these particles efficiently perform translation elongation without being stalled on the mRNAs. The TRAMP complexes have been described as major nuclear co-factors for RNA degradation of pre-rRNA precursors present in defective nuclear pre-60S r-particles.[26,55] Thus, if a leakage process for export would operate, it would be expected that inactivation of the TRAMP complex would increase the bulk of cytoplasmic pre-60S r-particles in mutants affected in 7S pre-rRNA processing. However, when we assessed the effects of the absence of Trf4 alone or in combination with Air1 in the *csl4–1, mtr3–1*, and *rrp4–1* mutants, we only detected a slight increase in the amount of pre-60S particles engaged in translation (**Fig. S5**). Therefore, these results argue in favor of an active export for pre-60S r-particles containing 7S pre-rRNA in these mutants. Indeed, a predominant leakage process for export is unlikely since pre-60S containing 7S pre-rRNAs do accumulate in other mutants impaired in 5.8S rRNA maturation, but are efficiently retained in the nucleus (for an example, see **Figs. 4** and **6**). It remains a challenging task to understand at a molecular level what promotes the retention or induces the export of pre-60S r-particles containing 7S pre-rRNAs in the different mutants. In this respect, the *dob1–1* mutation changes the glutamate codon (GAG) at amino acid position 796 to a lysine codon (AAG). Strikingly, E796 is located within the major helix of the so-called KOW domain in the "arch" of Mtr4.[56,57] This domain has been shown to contribute to RNA

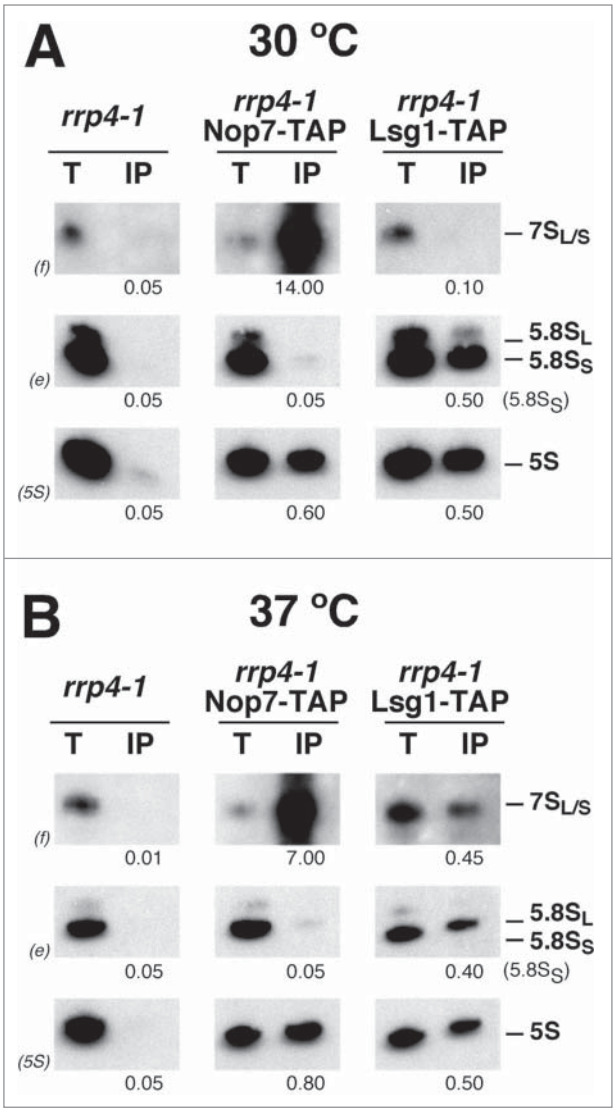

Figure 6. Pre-60S r-particles containing 7S pre-rRNAs are exported to the cytoplasm in the *rrp4–1* mutant at the non-permissive temperature. The *rrp4–1* strain was grown at 30°C in YPD medium to mid-log phase (**A**) or shifted for 4 h to 37°C (**B**). Immunoprecipitation experiments were carried out using IgG-Sepharose and whole-cell extracts from untagged or TAP-tagged Nop7 and Lsg1 cells. RNA was extracted from the beads (lanes IP) or from an amount of total extract corresponding to 1/100 of that used for the immunoprecipitations (lanes T), separated on a 7% polyacrylamide-8M urea gel, transferred to a nylon membrane and subjected to northern hybridization with the probes indicated in parentheses to detect 7S pre-rRNAs and mature 5.8S and 5S rRNAs. Signal intensities were measured by phoshorimager scanning; values (below each IP lane) refer to the percentage of each RNA recovered after purification.

binding,[57] suggesting that it might be required to present RNA substrates, including 7S pre-rRNAs, to the Mtr4 helicase core and, thus, to the exosome. Therefore, we envisage that the acquisition of export competence by pre-60S r-particles may rely, among other mechanisms, on the non-recruitment or efficient release of the exosome from the particles.

Pre-60S r-particles containing 7S pre-rRNA are not the sole aberrant pre-60S r-particles able to engage in translation. In a previous work, it has been reported that a fraction of pre-60S r-particles containing 5.8S + 30 pre-rRNAs can enter into polysomes in the *rrp6*Δ mutant[39]; our study confirms this finding and furthermore reveals that this also occurs in the *rrp47*Δ mutant. Moreover, since the *ngl2*Δ strain is viable and has very little effect on cellular growth ([40] and our own results), pre-60S r-particles containing 5.8S + 5 pre-rRNA were predicted to be able to engage in translation, an assumption that we have herein corroborated. Altogether, these results indicate that the 3′-end extension of mature 5.8S rRNA, as previously presumed,[18] does not significantly hamper the structure or the role of the relevant functional sites of the ribosome during translation (see **Fig. S6**). We propose that in wild-type conditions nuclear export acts as an efficient barrier preventing immature pre-60S r-particles containing 3′ extended forms of mature 5.8S rRNA to enter the pool of translating ribosomes in the cytoplasm. Therefore, if these particles escape to the cytoplasm, they obligatorily engage in translation without affecting elongation efficiency. Further experiments are required to elucidate whether differences do nevertheless exist for the translation of specific mRNAs when carried out either by normal or "aberrant" 7S pre-rRNA containing ribosomes.

Materials and Methods

Yeast strains, media and microbiological methods

All yeast strains used in this study are listed in **Supplemental Table 1**. Growth and handling of yeast and standard media were done following established procedures.[58]

Sucrose gradient centrifugation

Cell extract for polysome and r-subunit analyses were performed according to Foiani et al.,[59] as previously described[52] using an ISCO UA-6 system equipped to continuously monitor A_{254}. Fractions of 0.5 ml were collected from the gradients and RNA was extracted from the different fractions and analyzed as described below.

RNA analyses

Total RNA was extracted by the acid-phenol method.[60] RNA was also extracted from sucrose gradient fractions as exactly described.[61] Equal amounts of total RNA (5 μg) or equal volumes of each fraction were loaded on 1.2% agarose-6% formaldehyde or on 7% polyacrylamide-8M urea gels as described.[62] Northern hybridization was performed as previously described.[46] Signal intensities were quantified using a FLA-5100 imaging system and Image Gauge (Fujifilm). The sequences of the oligonucleotides used for northern hybridization are listed in **Supplemental Table 2**.

Fluorescence microscopy

Fluorescence *in situ* hybridization (FISH) was carried out in fixed cells as previously described,[33,63] using Cy3-labeled ITS1- or ITS2-specific probes (see **Table S2**). Cells were also stained with DAPI (4,6-diamidino-2-phenylindole) to visualize DNA.

Images were acquired using an Olympus BX61 fluorescence microscope equipped with a digital camera. Images were analyzed using the Cell Sens software.

The localization of GFP-tagged Lsg1 was inspected by fluorescence microscopy as previously described.[64]

RNA precipitations

Extracts from distinct TAP-tagged assembly factors were used to affinity-purified pre-ribosomal particles on the road to mature ribosomes by IgG-Sepharose beads as previously described.[65] Pre-rRNAs copurifying with these particles were recovered from the beads by phenol-chloroform extraction, as described in,[65] and assayed by northern hybridization as indicated above.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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