Rea1, a Dynein-related Nuclear AAA-ATPase, Is Involved in Late rRNA Processing and Nuclear Export of 60 S Subunits*§

The synthesis of ribosomes is one of the major and most energy-consuming processes in the cell. In Saccharomyces cerevisiae, ribosome biogenesis begins in the nucleolus with the transcription of two rRNA precursors, the 35 S and the pre-5 S RNA, by RNA polymerases I and III, respectively. The 35 S pre-rRNA contains the sequences for the mature 18 S, 5.8 S, and 25 S rRNAs, two external transcribed spacers (ETS) and two internal transcribed spacers (ITS). During the maturation process, the pre-rRNA has to undergo a number of modifications and is subjected to cleavages and trimming events. At least 170 accessory proteins including putative RNA helicases, several putative GTPases and AAA-ATPases as well as small nucleolar ribonucleoprotein particles are involved in the maturation of rRNA and its assembly into ribosomal subunits (1, 2).

Concomitant with rRNA processing, ribosomal and non-ribosomal proteins are assembled on the pre-35 S rRNA, giving rise to a large 90 S pre-ribosomal particle (see Fig. 6B) (3, 4). The initial cleavages at sites A0–A2 separate the two subunits. The pre-40 S subunit is exported relatively rapidly to the cytoplasm, where it undergoes further processing. In contrast, the maturation of the large subunit continues in the nucleoplasm with recruitment of 60 S-specific biogenesis factors and further processing of the 27 S pre-rRNA. This includes the late cleavage and processing in the ITS2 region, which generates mature 5.8 S and 25 S rRNA.

In the last few years, the maturation of 40 S and 60 S pre-ribosomes has been extensively analyzed by purification of pre-ribosomal particles (5–10). Interestingly, a large number of non-ribosomal proteins were identified in pre-60 S particles without an assigned function in RNA metabolism. In contrast to the pre-40 S particles, the nascent 60 S particles contain several putative GTPases and AAA-type ATPases (2, 11).

To understand the events of ribosome biogenesis, we previously purified pre-ribosomal 60 S particles, which represent different maturation states from early nucleolar through cytoplasmic export (5). One of these, the late nucleoplasmic Rix1 particle, was selected for further study. The Rix1 particle is specifically enriched in the products of three uncharacterized open reading frames, YHR085w (Ipi1), YNL182c (Ipi3), and Rea1. Ipi1 has a single ARM repeat motif (12), whereas Ipi3 contains two WD40 domains. In contrast, Rea1, which at 560 kDa is the largest protein identified in the yeast genome, contains several interesting domains and homologies. The N terminus of Rea1 possesses six AAA ATPase protomers and a C-terminal region containing the MIDAS (metal ion-dependent adhesion site) motif. Furthermore, sequence analysis has indicated relatedness to dynein (13).

In this study, we characterize Rea1 and the Rix1 complex containing pre-60 S particle. Our results show that Rea1 and the Rix1 complex exhibit similar subcellular localization and a similar late ITS2 rRNA processing defect. Although the Rix1 complex mutants accumulate the Rpl25-GFP reporter throughout the nucleoplasm, rea1 temperature-sensitive (ts) mutants also show a later defect, with accumulation around the nuclear periphery. Our data demonstrate that Rea1 and the Rix1 complex play an essential role in maturation and nuclear export of nascent 60 S subunits from the nucleoplasm to the cytoplasm.

Experimental Procedures

Yeast Strains and Plasmids—Genomic integration of GFP (HIS3MX6 marker) as a C-terminal tag into yeast strains to create fusion proteins of Ipi1 (strain DS1-2b, MATa, ura3, trp1, his3, leu2) and Ipi3 (strain JBa, MATa trp1 ura3 ade2 ade3 leu2 his3) was performed as described (14). For construction of GAL1::GFP-REA1 and...
Fig. 1. Rix1, Ipi1, and Ipi3 form a salt-stable complex. A, affinity purification of Rix1-TAP under standard conditions (see “Experimental Procedures”). B, Rix1-TAP was affinity-purified as normal until binding to CaM-Sepharose. Then, a stepwise treatment of a single purification with MgCl₂ was performed. Proteins were eluted with 100 mM MgCl₂ (lane 1), 200 mM MgCl₂ (lane 2), and SDS-elution buffer (lane 3). All labeled proteins were confirmed by mass spectrometry. Note that mass spectrometry identified the Ipi1 band both Ipi1 and an Aspergillus contaminant derived from the Calmodulin beads (matrix porin). The enrichment of Ipi1 is greater in the MgCl₂ (B) than in the CaM eluate (A) as determined by mass spectrometry.

GAL1::HA-REA1 strains, GAL1::GFP and GAL1::HA cassette containing the TRP1 marker were integrated 5′ upstream of the ATG start codon of REA1 (strain RS453). Integration of the GFP and HA tags was confirmed by Western blot. The REA1 shuffle strain was obtained by transforming plasmid pYCG-YLR106c (REA1) into the heterozygous strain BY4743 (rea1Δ:kanMX4/REA1; derived from EUROSCARF) and selection of kan^+ haploid progeny after tetrad dissection. The degron yeasts strains ipi3-td, ipi1-td, and rix1-td as well as the isogenic wild type (wt-td) (15), strains expressing Rix1-TAP and Rix1-GFP (5), and Ipi3-TAP (16) were described previously. The degron strains were grown at 23°C in selective raffinose-glucose medium containing CuSO₄ (permissive condition) before shift to galactose medium lacking CuSO₄, first for 40 min at 23°C for induction of Ubr1 expression and then to 37°C to induce protein degradation of the mutant proteins (15, 17). For RNA analysis, cells were grown similarly except that the raffinose medium contained no glucose and that cells were washed twice with prewarmed galactose medium without CuSO₄. The following described plasmids were used in this study: pFA6a-TRP1-PGAL1-GFP, pFA6a-TRP1-PGAL1-3HA (14), pRS316-RPL25-GFP (18), pRS314-RPS2-GFP (10) or the wild-type pRS316-REA1 plasmid. Oligonucleotides used were: 003, 5′-TGT TAC CTC TGG GCC C-3′; 004, 5′-GGG TTT TAA TTG TCC TA-3′; 005, 5′-AGA TTA TCA GCC GCA GGT GG-3′; 006, 5′-CTC CGC TTA TTA ATG ATC TGG GAG AC-3′; 017, 5′-GCG TTT TAA AGA TTA GCC GCA GGT GG-3′; 020, 5′-TGA GAA GGA AAT GAC GCT-3′; 041, 5′-CTA CTC GGT CAG GCT C-3′; 250, 5′-ATC CCGGGGCGCTCCTACGAC-3′; 306, 5′-GCA TCT TAC GAT ACC TG-3′.

Miscellaneous—Western blot analyses were performed according to Ref. 24. Fluorescence microscopy was done as described (11). The fluorescence-based visual assay to analyze the nuclear export of large and small ribosomal subunits using the Ipi25-GFP and Rps2-GFP reporters, respectively, in living cells was performed according to Refs. 18, 19, and 25. Sedimentation analysis of ribosomes under low salt conditions by sucrose gradient centrifugation was performed as described (10). Mass spectrometry using tryptic digests from Coomassie Blue-stained bands derived from SDS-PAGE was performed as described (10). Synthetic lethality was determined by tetrad dissection of either the rix1-1 (10) or the rix1-7 (11) strain mated to the rea1 deletion strain complemented with the pRS316-REA1 plasmid. Haploid progeny containing either the rix1-1 or the rix1-7 mutation and the real1 deletion were transformed with pRS315 plasmids containing no insert or real1-7 or real1-21 mutant alleles. Strains were considered synthetic lethal if they could not grow on 5-fluoroorotic acid, i.e. if they could not lose the wild-type pRS316-REA1 plasmid.

RESULTS

High Salt Dissociates Real and 60 S Subunits from the Rix1 Complex—We have previously reported the isolation and identification of 60 S pre-ribosomal particles, which are characterized by the presence of different ribosomal precursors depending on the maturation state of each of the particles (5, 10). The Rix1 particle represents a late intermediate in 60 S biogenesis and is highly enriched in the AAA-ATPase Real1/Mdn1, as well as in two other non-ribosomal proteins, Yhr085/Ip1 and Ynl182/Ip3 (Fig. 1A; see also Ref. 5). All three proteins are specifically associated with the Rix1 particle since they are largely absent both from earlier and from later pre-60 S subunits. The observation that Rix1, Ip1, Ip3, and Real1 co-enrich during biochemical purification indicates that these four proteins could be organized in a complex attached to nascent 60 S subunits. To test this possibility, we affinity-purified Rix1-TAP by the tandem affinity purification method. However,
instead of eluting the purified complexes from the final CaM-Sepharose with EGTA (as in Fig. 1A), we treated the beads with increasing concentrations of MgCl₂ (Fig. 1B). The additional salt should release the interacting proteins that are not tightly bound with the affinity-purified bait proteins (26). When Rix1-TAP, which is immobilized on CaM beads, was incubated with a MgCl₂ step gradient, Rea1 and 60 S subunit proteins were released with 100 mM salt (Fig. 1B, lane 1). However, Rix1, Ipi1, and Ipi3 remained bound under these conditions and were also resistant to 200 mM MgCl₂, but they eluted by SDS-sample buffer (Fig. 1B, lane 3). Ipi1 reproducibly appeared substoichiometric after salt washing. It remains to be shown whether Ipi1 is present in lower amounts in the Rix1 complex or is not stained effectively by Coomassie Blue. Similar results were obtained for salt-treated Ipi3-TAP purification (data not shown). We conclude that Rix1, Ipi1, and Ipi3 form a salt-stable complex, to which Rea1 and the nascent 60 S subunit are less tightly attached. Recently, Krogan et al. (27), in a large scale effort to isolate RNA processing complexes by TAP purification following a ultracentrifugation step to remove ribosomes, have reported a similar complex. Finally, since Rea1 was observed to be specifically enriched in the Rix1-TAP purification, we examined whether a synthetic lethal relationship exists between the rea1 ts mutants and the rix1-1 mutant (10). However, we did not observe any sl phenotype (data not shown).

Rea1 and the Rix1 Complex Members Exhibit a Nucleoplasmic Location—Since Rea1 and the Rix1 complex are co-enriched, we wanted to know whether their subcellular distribution is also similar. Previously, we showed that Rix1 is localized in the nucleus (5). Ipi1 and Ipi3 were genomically tagged with the GFP epitope at their C terminus, which ensures that protein expression remains under the control of the native promoter. Expression of the genomically N-terminally tagged GFP-Rea1 is under the GAL promoter due to difficulties in obtaining a fully functional C-terminal GFP fusion protein.² After confirming that the GFP tagging had no effect on the growth rate of the strains (Supplemental Fig. 1), we examined the yeast cells under the fluorescence microscope. Similar to Rix1, Rea1, Ipi1, and Ipi3 were localized throughout the nucleoplasm (Fig. 2). We also expressed the DsRed-tagged nuclear protein Nop1 in the GFP-tagged strains, and we did not observe nucleolar concentration for any of the proteins as this can be judged by the lack of colocalization between the GFP and the DsRed signals (Supplemental Fig. 2). These results are in agreement with the previously reported localization of Ipi1 and Rea1 proteins (28) and Rix1 (5). Thus, Rea1 and the Rix1 complex could function in late nucleoplasmic maturation of pre-60 S subunits and/or their export to the cytoplasm. Taken together, the identified Rix1 complex is present together with the AAA-type ATPase Rea1 in a late pre-60 S particle that is located in the nucleoplasm.

Rea1 and the Rix1 Complex Are Required for Nuclear Export of the 60 S Subunit—We wanted to determine whether Rea1 and the components of the Rix1 complex are involved in late nucleoplasmic steps during 60 S subunit biogenesis. Previously, we showed that the rix1-1 ts mutant is strongly impaired in 60 S subunit export, but rRNA processing was not significantly affected (10). We followed different strategies to obtain conditional-lethal mutants of the Rea1 protein and Rix1 complex members. For REA1, both a repressible GAL1::REA1 construct and ts rea1 alleles were generated (see “Experimental Procedures”). For the essential Rix1, Ipi1, and Ipi3, conditional-lethal degron (td) mutants were used. These degron mutants target the proteins for rapid degradation in vivo upon shift to 37°C (15). Furthermore, we have confirmed that rea1-7 and rix1-21 ts mutants could be rescued by the presence of a plasmid carrying the REA1 wild-type gene (Supplemental Fig. 3A). In the case of the td mutants, we used the available RIX1 wild-type plasmid (10) to verify that the expression of the wild-type gene is sufficient to rescue the lethal phenotype of rix1-td mutant (Supplemental Fig. 3B).

The Rpl25-GFP reporter assay was developed to monitor 60 S export in vivo (18, 29). In wild-type cells, Rpl25-GFP is localized in the nucleolus or throughout their nucleoplasm (Fig. 2, 1-h shift). At a later time point, all td mutants exhibited strong nuclear accumulation, whereas the nucleolus was either similar to nucleoplasmic staining or else devoid of any signal (Fig. 3A, 2-h shift; Supplemental Fig. 4). A similar assay for nuclear export of the small subunit showed cytoplasmic localization of Rps2-GFP comparable with wild-type in all of the mutants (Fig. 3). Both the rea1-21 ts and the repressible

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² K. Galani, unpublished data.
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GAL::REA1 mutants accumulated the Rpl25-GFP in their nucleoplasm upon shift to restrictive conditions (Fig. 3, B and C). Interestingly, in the case of the rea1-21 ts mutant, ~25% of the cells accumulated the Rpl25-GFP protein at their nuclear periphery after 9 h of shift. The same phenotype was also observed with the rea1-7 ts mutant, albeit to a lesser extent (15%; data not shown). The fact that pre-60 S ribosomes accumulated in the entire nucleoplasm or at the nuclear periphery (Fig. 3, rea1-21 insert; Supplemental Fig. 5) suggests that a late step in nuclear export from the nucleoplasm to the cytoplasm is blocked in rea1 and rix1 complex mutants.

Rea1 and the Rix1 Complex Are Required for Normal Levels of the 60 S Subunit—As the export is impaired for Rea1 and the Rix1 complex mutants, we wished to see whether the overall production of 60 S subunits is likewise reduced. For this reason, we analyzed the ribosomal and polysomal profiles in rea1 and rix1 complex mutants by sucrose density gradient centrifugation. This analysis revealed a significant reduction of 60 S subunits as compared with 40 S in the mutants. Moreover, the appearance of half-mer polysomes was observed in mutant strains (Fig. 4), indicating a lack of mature 60 S to bind to the 43 S preinitiation complex. These data are consistent with the export defect, demonstrating that it is specific to the 60 S pathway.

Depletion of Rea1 or the Rix1 Complex Inhibits Synthesis of the 5.8 S rRNA—To determine whether the rea1 and rix1 complex member mutants are impaired in pre-rRNA processing, we performed Northern hybridization and primer extension analyses. The locations of oligonucleotide probes are indicated in Fig. 6A. Analyses of low molecular weight RNAs (Fig. 5A, upper panel) revealed that genetic depletion of Rea1 resulted in strong inhibition of processing from 7 S pre-rRNA to 5.8 S and 6 S pre-rRNA, as shown by their substantial loss 16 h after transfer of the GAL::REA1 strain to glucose medium. Since the 7 S pre-rRNA was mildly accumulated, whereas the normal products of its processing were drastically reduced, it is likely that much of the 7 S pre-rRNA is degraded, presumably reflecting degradation of the entire pre-60 S particle. It is, however, difficult to directly assess this experimentally since 7 S is only faintly visible in pulse-chase labeling of wild-type strains, and the 5.8 S and 6 S pre-rRNAs are not observed (data not shown). Mature 5.8 S was reduced relative to the tRNA<sub>Leu</sub> loading control, as was the 5 S rRNA component of the 60 S subunit. Analyses of high molecular weight RNA by Northern hybridization (Fig. 5A, middle panel) and primer extension (Fig. 5A, lower panel) showed the accumulation of the 35 S pre-rRNA and 23 S RNA, accompanied by a mild reduction in the 20 S and 27 S pre-rRNA. Very similar processing defects were observed in the rea1-21 ts strain 4 h after transfer to the non-permissive temperature (data not shown). These phenotypes indicate a delay in early pre-rRNA processing at sites A<sub>0</sub>, A<sub>1</sub>, and A<sub>2</sub> (Fig. 6B), which are very frequently seen in strains defective in 60 S subunit synthesis (30). The 27 SB pre-rRNAs were mildly accumulated, with little alteration in...
the levels of processing at the alternative B_{18} and B_{12} sites, indicating that the early steps in 60 S synthesis continue in the Real-depleted strain.

Related defects in 5.8 S synthesis were seen in strains depleted of Rix1, Ipi1, or Ipi3 (Fig. 5B, upper panel). In each case, the 7 S pre-rRNA was accumulated relative to the wild-type control following transfer to non-permissive conditions, accompanied by reduced 6 S pre-rRNA. The level of the 5.8 S was reduced in the wild-type following transfer to 37 °C but was further reduced in the mutant strains. Some reduction in the levels of the mature 5.8 S was seen after 4 h in non-permissive conditions. Some reduction in the levels of the mature 5.8 S was seen after 4 h in non-permissive conditions. The mature rRNAs are not generally turned over and are therefore depleted only by growth under non-permissive conditions. Greater depletion would not therefore have been expected over this period. Few clear alterations were seen in the processing of the earlier, high molecular pre-rRNAs. However, some increase in the level of 35 S was seen in the rix1-td strain at later time points, whereas 23 S accumulated in the ipi1-td strain, both indicative of a mild delay in the early pre-rRNA processing steps (Fig. 5B, middle panel).

We conclude that depletion of Real, Ipi1, Ipi3, or Rix1 each inhibits the processing of the 7 S pre-rRNA to 5.8 S + 30 and 6 S rRNA accompanied by degradation of the pre-60 S particle, with consequent reduced synthesis of 5.8 S rRNA. Mild defects seen in early pre-rRNA processing steps are probably indirect, as many other mutations leading to impaired 60 S subunit synthesis have been reported to have similar effects (30).

**DISCUSSION**

Biogenesis of ribosomal subunits is a complicated process, which requires the spatial and temporal coordinated function of greater than 170 trans-acting factors. A major challenge now is to assign functions to these many components, determine their nearest neighbor relationships and organization in subcomplexes, group them in classes with similar functions, and map their positions within the structure of the nascent pre-60 S subunit.

In this study, we focused on a late nucleoplasmic pre-60 S intermediate that is close to export to the cytoplasm. This particle contains the 560-kDa AAA-type ATPase Real1, which is distantly related to the motor protein dynein, and the Rix1 complex, which is composed of Rix1, Ipi1, and Ipi3 subunits. Mutants of these non-ribosomal factors result in the defective 3′ processing of the 5.8 S rRNA, a late pre-rRNA processing step, and export of the 60 S subunit from the nucleoplasm to the cytoplasm.

Real1 is predicted to be the largest yeast protein at 560 kDa and belongs to the family of AAA^+^ type ATPases (31). Members of this family are involved in diverse processes such as membrane fusion, proteolysis, DNA replication and recombination, microtubule organization, and intracellular motility (32, 33). All the functions of the AAA family can be linked to its central property of affecting protein-protein interactions. Their common feature is the presence of an AAA-ATPase module, which is required for ATP binding and hydrolysis. Structural analysis
revealed that the AAA domain forms hexameric or heptameric rings after oligomerization, which change their conformation depending on the bound nucleotide (34, 35). Instead of the more common one or two AAA protomers per molecule, Rea1 possesses six domains, similar to the AAA motor protein dynein. Detailed sequence analysis of the AAA protomers, in both dynein and Rea1, demonstrate a closer relatedness to each other than to any other AAA protein, suggesting that they evolved from a common ancestor (13). If Rea1 performs a function related to dynein, it could be involved in intranuclear movement or export of pre-60 S particles from the nucleoplasm to the cytoplasm.

In addition to the N-terminal AAA motifs, Rea1 has a MIDAS domain located at the carboxyl terminus. The MIDAS...
domain, most notably present in integrins, is involved in protein-protein interactions. Interestingly, deletion of the C terminus of Rea1 containing the MIDAS motif is lethal.

Rea1 represents the second AAA ATPase shown to have a role in ribosome biogenesis. Rix7 was the first such protein found to be required for large subunit formation (11). RNA processing analyses indicate that the \textit{rix7} mutation (11) inhibits an earlier step in the biogenesis process than do the \textit{rea1} mutant or Rea1 depletion. We examined whether the \textit{rix7} mutation was synthetic lethal with either of the \textit{rea1} mutant alleles (data not shown), but no sl phenotype was observed.

Rea1 and the Rix1 complex are required for maturation of the 5.8 S rRNA, which is inhibited in \textit{rea1} and \textit{rix1} complex mutants. No accumulation of intermediates between 7 S and 6 S pre-rRNA were observed in strains depleted of Rea1, Ipi3, Ipi1, or Rix1. This is in contrast to the effects of depletion of components of the exosome complex, which is believed to be directly responsible for this processing reaction. This suggests that in the absence of Rea1 and the Rix1 complex members, 3' processing cannot be initiated from site C\textsubscript{2}, perhaps because the 3' end of the pre-rRNA is sequestered in the pre-rRNA structure (Fig. 6B). It is notable that in the predicted structure of ITS2, the C\textsubscript{2} cleavage site is indeed predicted to lie within a base-paired region (Ref. 36 and references therein).

Recently, Krogan \textit{et al.} (27) examined rRNA processing in strains carrying tet-regulated alleles of \textit{RIX1}, \textit{IPI1}, and \textit{IPI3}. They reported overall defects in RNA processing with specific reduction in 25 S rRNA, 20 S pre-rRNA, and 18 S rRNA and accumulation of 7 S levels. It is possible that these more general effects are due to the extended 24-h depletion time course used. Here we used degron constructs that result in rapid protein depletion and observed defects in the ITS2 processing within 1 h of shifting to restrictive conditions. Our data suggest that depletion of the Rix1 complex proteins alters the structure of a late nuclear pre-60 S particle, leading to inhibition of the 3' processing of 7 S pre-rRNA and degradation of the pre-ribosome.

We also report that Rea1 and the Rix1 complex members are required for the export of the large ribosomal subunit. This defect is apparently specific as small subunit export was impaired. Strikingly, the ts mutants of \textit{rea1} accumulated Rpl25-GFP at the nuclear periphery, suggesting an involvement just prior to or during export. This would be consistent with Rea1 being responsible for removal of proteins that retain the nascent ribosome in the nucleus.

A possible explanation for our results is that the ATPase activity of the Rea1 complex is used for the structural remodeling of the pre-ribosome, including the separation of the 5' and 3' regions of ITS2. The processing of ITS2, triggered by the Rea1/Rix1 complex-induced pre-60 S remodeling, could act as a quality control step required to allow maturation of 60 S subunits into export-competent particles. Delays in ITS2 processing have also been reported in strains defective for other late-

![Fig. 6. Ribosomal RNA processing pathway and locations for oligonucleotide annealing. A, the locations of oligonucleotides used for primer extension and Northern hybridization. Oligonucleotide 020 lies across the 5.8 S/ITS2 boundary (positions +10 to 8 with respect to the 3' end of the mature 5.8 S rRNA). B, a schematic diagram of the 35 S pre-rRNA processing showing processing sites. The transcribed spacer regions are indicated with narrow lines, and mature rRNA is indicated with rectangles.](image-url)
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acting factors required for pre-60 S export, including Nmd3, Gsp1, and Rpr12 (37–39).

Reduction of the levels of two of the putative GTPases, Nog1 and Nog2, which were found associated with the Real/Rix1 complex containing pre-60 S particles also exhibits defects in ITS2 processing. Disruption of Nog1 function through RNA interference led to a dramatic decrease in the levels of free 60 S particles and the appearance of an atypical rRNA intermediate in which ITS2 was not cleaved (40). Likewise, depletion of Nog2 resulted in a dramatic decrease in 5.8 S rRNA levels and accumulation of the 7 S precursor as well as an intermediate in which ITS2 was not cleaved (40). Likewise, RNA interference led to a dramatic decrease in the levels of ITS2 processing. Disruption of Nog1 function through the Rix1 complex containing pre-60 S particles also exhibits defects in ITS2 processing. Reduction of the levels of two of the putative GTPases, Rix1, Rix2, and Rix3, prior to the irreversible step of nuclear export.

These GTPases may act in concert with the Rea1 ATPase to promote local accumulation of ITS2-containing precursors (6). Disruption of Nog1 and Nug2, which were found associated with the Rea1/Rea2 ATPase, also exhibits defects in ITS2 processing. Disruption of Nog1 function through RNA interference led to a dramatic decrease in the levels of free 60 S particles and the appearance of an atypical rRNA intermediate in which ITS2 was not cleaved (40). Likewise, RNA interference led to a dramatic decrease in the levels of ITS2 processing. Disruption of Nog1 function through the Rix1 complex containing pre-60 S particles also exhibits defects in ITS2 processing.

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