Ribosomes are composed of a large, 60S subunit containing three rRNA species—the 25S, 5.8S, and 5S rRNAs—and a small, 40S subunit containing the 18S rRNA species. The 18S, 5.8S, and 25S rRNAs are cotranscribed in the nucleolus as a polycistronic transcript (Fig. 1A) that undergoes a complex series of endonucleolytic cleavages and exonucleolytic processing steps to yield the mature rRNAs (Fig. 1B). Processing of pre-rRNAs occurs within preribosomal particles that contain, in addition to the pre-rRNA and ribosomal proteins, some 200 processing, modification, and assembly factors and 75 small nucleolar RNAs.

The preribosomes that will form the 40S and 60S particles are separated by pre-rRNA cleavage at site A. This cleavage can occur cotranscriptionally, within the small subunit (SSU) processome (31), or posttranscriptionally in the 90S preribosome (Fig. 1B). In either case, the released pre-40S subunit containing the 20S pre-rRNA is rapidly exported from the nucleus to the cytoplasm, where maturation to 18S rRNA is completed (42). 60S subunit assembly requires more extensive rearrangement and processing within the nucleolar and nucleoplastic compartments prior to export to the cytoplasm, and multiple different pre-60S particles have been identified by affinity purification (3, 11, 15, 30, 34, 35). During 60S maturation, the 27S pre-rRNAs are processed to 7S and 6S, the 5′ ends of the 5.8S L and 5.8SS rRNAs, respectively. Sequential cleavage at site C generates the 26S pre-rRNA, which is then processed to 25S rRNA, and 7S L and 7S S, which are processed to 5.8SL and 5.8SS rRNAs, respectively. Processing of 7S pre-rRNA to 5.8S rRNA is a multistep pathway, requiring the activity of several 3′→5′ exonucleases. 7S pre-rRNAs are initially processed to 5.8S+30 (forms of 5.8S L and 5.8S S that are 3′ extended by ~30 nucleotides) by the nuclear exosome complex (1). The 5.8S+30 species are then processed to 6S L and 6S S, heterogeneous populations of pre-5.8S species that are 3′ extended by ~5 to 8 nt. Strains lacking the exosome-associated exonuclease Rrp6 strongly accumulate 5.8S+30 (8). However, a majority of pre-rRNA molecules are correctly processed, making it unclear whether Rrp6 is the only major player in normal 3′ processing of 5.8S+30. Processing of 6S pre-rRNA to mature 5.8S appeared to strictly require the activity of the putative nuclease Ngl2 (10) (Fig. 1B). The situation was, however, complicated by the observation that strains lacking combinations of the Rxx1, Rxx2, and Rxx3 exonucleases (44), or components of the exosome (1), also showed 6S pre-rRNA accumulation. Since strains lacking Ngl2 are viable, 60S ribosomes containing the 6S pre-rRNA are functional in protein synthesis (10). The high-resolution cryo-electron microscopy (cryo-EM) structure of the yeast (Saccharomyces cerevisiae) ribosome (36) indicates that the 3′ end of the 5.8S rRNA is exposed on the surface of the particle, where it is available for processing and would not be predicted to interfere with ribosome function.

Export of 40S and 60S subunits from the nucleus to the cytoplasm requires active transport through nuclear pore complexes. The small GTPase Ran (Gsp1 and Gsp2 in yeast) provides the directionality for this transport system. In the nucleolus, Ran-GTP promotes formation of complexes between export receptors termed “exportins” and export substrates. Following transport, these complexes dissociate in the cytoplasm when GTP is hydrolyzed. The export receptor for both pre-40S and pre-60S subunits in yeast and higher eukaryotes is the exportin Crm1/Xpo1, a member of the karyopherin β family (13, 17, 28, 38, 41). Exportins bind to leucine-rich nuclear export signals (LR-NES) present on either the cargo or on adapter proteins. During 60S export, Crm1/Xpo1 interacts...
scripts can undergo cotranscriptional cleavage (Co-TC) at the A₀, A₁, and generates the 20S and 27SA₂ pre-rRNAs. Alternately, Pol I transcribed A₂ sites, within the small subunit (SSU) processome. Co-TC cleavage at A₂ separates the precursors to the 40S and 60S subunits.

Cleavage at A₀,A₁ at the 5′ end of the mature 18S RNA and within ITS1 at A₂. Cleavage at A₂ separates the precursors to the 40S and 60S subunits and generates the 20S and 27SA₁ pre-rRNAs. Alternately, Pol I transcripts can undergo cotranscriptional cleavage (Co-TC) at the A₀, A₁, and A₂ sites, within the small subunit (SSU) processome. Co-TC cleavage is followed by the assembly of the large subunit (LSU) processome. Following either posttranscriptional or cotranscriptional cleavage, the 20S pre-rRNA, contained within a pre-60S particle. Following either posttranscriptional or cotranscriptional cleavage, the 20S pre-rRNA, contained within a pre-60S particle, is exported from the nucleus to the cytoplasm, where maturation to 18S is completed. The pathway of 5.8S and 25S synthesis occurs within a series of pre-60S particles. The 27SA₁ pre-rRNA follows one of two alternate pathways: around 85% is cleaved at the A₁ site within ITS2, followed by 5′→3′ exonucleolytic processing generating the 27SB₁ pre-rRNA. The remaining 15% is processed at site B₁S, which is located 8 nt 5′ to B₁S, yielding the 27SB₁ pre-rRNA. These two alternate forms of 27SB₁ are cleaved within ITS1 at site C₀, yielding 26S pre-rRNA and the long and short forms of 7S. The 7S pre-rRNA is converted to 6S and 6S by the nuclear exosome and Rrp6. Maturation of 26S to 25S rRNA proceeds by a two-step 5′-exonuclease pathway. Subsequently, pre-60S particles are exported to the cytoplasm, where final maturation to 5.8S is completed.

**FIG. 1.** Pre-rRNA processing pathway in *Saccharomyces cerevisiae*. (A) The structure of the 35S rRNA precursor and locations of processing sites. The pre-rRNA encodes the 18S, 5.8S, and 25S rRNAs, which are flanked by the 5′ and 3′ external transcribed spacers (5′-ETS and 3′-ETS) and separated by internal transcribed spacers 1 and 2 (ITS1 and ITS2). The positions of oligonucleotide probes used for Northern hybridization and FISH analysis are shown in blue and red boxes, respectively. (B) The pre-rRNA processing pathway. RNA polymerase I (Pol I) transcripts undergo one of two alternate fates. The 35S precursor, contained within the 90S preribosome, is generated by cleavage at site B₀ within the 3′-ETS. This is followed by posttranscriptional endonucleolytic processing (PTC) within the 5′-ETS, at A₀ and A₁ at the 5′ end of the mature 18S RNA and within ITS1 at A₂. Cleavage at A₂ separates the precursors to the 40S and 60S subunits and generates the 20S and 27SA₁ pre-rRNAs. Alternately, Pol I transcripts can undergo cotranscriptional cleavage (Co-TC) at the A₀, A₁, and A₂ sites, within the small subunit (SSU) processome. Co-TC cleavage is followed by the assembly of the large subunit (LSU) processome. Following either posttranscriptional or cotranscriptional cleavage, the 20S pre-rRNA, contained within a pre-40S particle, is exported from the nucleus to the cytoplasm, where maturation to 18S is completed. The pathway of 5.8S and 25S synthesis occurs within a series of pre-60S particles. The 27SA₁ pre-rRNA follows one of two alternate pathways: around 85% is cleaved at the A₁ site within ITS2, followed by 5′→3′ exonucleolytic processing generating the 27SB₁ pre-rRNA. The remaining 15% is processed at site B₁S, which is located 8 nt 5′ to B₁S, yielding the 27SB₁ pre-rRNA. These two alternate forms of 27SB₁ are cleaved within ITS2 at site C₀, yielding 26S pre-rRNA and the long and short forms of 7S. The 7S pre-rRNA is converted to 6S and 6S by the nuclear exosome and Rrp6. Maturation of 26S to 25S rRNA proceeds by a two-step 5′-exonuclease pathway. Subsequently, pre-60S particles are exported to the cytoplasm, where final maturation to 5.8S is completed.

**MATERIALS AND METHODS**

**Strains and microbiological techniques.** Standard procedures were used for the propagation and maintenance of yeast. A full list of strains used in this study can be found in Table 1. YET151 and YET152 were constructed using a one-step PCR strategy (27). Transformants were selected for resistance to neomycin B (NAT) and screened by PCR and appearance of a cold-sensitive phenotype, conferred by nft4Δ. For pulse-labeling and pulse-chase analyses, strains were transformed with a pUR4 vector to confer uracil prototrophy.

**FISH.** For fluorescence in situ hybridization (FISH), cells were fixed in 3.7% paraformaldehyde at room temperature, spheroplasted using Zymolyase, and dehydrated in 70% ethanol overnight at 20°C. Locked-nucleic acid (LNA) modified probe (Exiqon) was fluorescently labeled using a ULS Cy3 kit (GE Lifescience). The sequence of the internal transcribed spacer 2-1 (ITS2-1) probe used is TTTGAGAAGGAAATGACGCT, with a predicted melting temperature (Tₘ) of 74°C. Fluorescence in situ hybridization was performed as previously described (26), with modifications to hybridization and wash conditions. Hybridization was performed in 80% formamide–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 45°C. Following hybridization, coverslips were washed extensively under the following conditions. Two washes in 80% formamide–2× SSC at 45°C, 1 wash in 2× SSC–0.1% Triton at 25°C, 1 wash in 2× SSC at 25°C, and a final wash in 1× phosphate-buffered saline (PBS). Cells were stained with DAPI (4′,6-diamidino-2-phenylindole) to visualize DNA and coverslips were mounted in Pro-Long mounting medium (Invitrogen). All Images were captured using a CoolSnap charge-coupled device (CCD) camera fitted to the DeltaVision RT Restoration imaging system based on the Olympus IX71 microscope using ×100 objective with an NA of 1.4. Images captured using the DeltaVision system were subjected to real-time two-dimensional deconvolution algorithms. Single optical sections were selected following deconvolution and assembled using Image J software.
TABLE 1. Yeast strains used and constructed in this study

<table>
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<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMA38</td>
<td>MATa his3Δ200 leu2-3,112 ura3-1 trp1Δ ade2-1</td>
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</tr>
<tr>
<td>BY4741</td>
<td>MATa his3Δ1 leu2α met15α ura3Δ0</td>
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<td>CEN</td>
<td>MATa ura3-52 trp1-289 leu2-3,112 his3 D1</td>
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<td>W303</td>
<td>MATa ura3-32 trp1Δ2 leu2-3,112 his3-11 ade2-1 can1-100</td>
<td>29</td>
</tr>
<tr>
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<td>29</td>
</tr>
<tr>
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<td>29</td>
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<td>Same as MNY7, but pURA</td>
<td>This study</td>
</tr>
<tr>
<td>YET21</td>
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<td>This study</td>
</tr>
<tr>
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<td>14</td>
</tr>
<tr>
<td>YET23</td>
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<td>14</td>
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</tr>
<tr>
<td>YET32</td>
<td>As MNY8, but trf4Δ::NAT MX6</td>
<td>This study</td>
</tr>
</tbody>
</table>

Affinity purification of TAP-tagged strains. One-step affinity purification of tandem affinity purification (TAP)-tagged strains was performed as previously described (33) with the following modifications. Exponentially growing yeast cells (OD₆₀₀ of 0.6) were harvested by centrifugation and frozen in liquid nitrogen. Total extract was prepared by adding 1 packed cell volume (PCV) of zirconia beads and 1 PCV of immunoprecipitation (IP) buffer (100 mM KCl, 50 mM HEPES [pH 7.5], 5 mM MgCl₂, 0.1% NP-40, 1 mM dithiothreitol [DTT]). Complete protease inhibitor tablet and vortexed for 5 min. Lysed cells were incubated with IgG-Sepharose for 1 h. Beads were extensively washed in IP buffer prior to RNA extraction. Associated pre-RNAs were recovered from the IgG beads or the total extract by guanidinium and phenol-chloroform extraction and ethanol precipitation. RNA was analyzed by Northern hybridization or primer extension.

RESULTS

5.8S rRNA precursors can be detected by FISH in the cytoplasm. To localize 3′-extended 5.8S precursors, fluorescent in situ hybridization (FISH) was performed using a 20-nt locked nucleic acid (LNA) probe (39). The ITS2-1 probe is complementary to the region traversing the 5.8S-ITS2 junction and hybridizes to all precursors of 5.8S, including 6S and 7S, but not to the mature rRNA. This probe gave a strong nucleolar signal, corresponding to nascent transcripts of 35S, 27S, and 7S pre-rRNAs. However, a weak signal throughout the cytoplasm was also observed in three different wild-type (WT) strains tested (Fig. 2A), indicating that the presence of cytoplasmic pre-5.8S species may be a general feature of yeast pre-rRNA processing.

To determine whether the cytoplasmic pre-rRNA species were being actively exported, FISH analyses were performed in a strain defective for ribosome subunit export. A single amino acid alteration (T₅₃₉C) in the gene encoding the exportin Crm1 renders it sensitive to inhibition by leptomycin B (LMB) (29). Following LMB treatment, the cytoplasmic signal in the crm1(T₅₃₉C) strain was clearly decreased relative to the CRM1 strain (Fig. 2B). This indicates that pre-60S particles contain-
5.8S rRNA synthesis is rapidly inhibited in an export mutant. If the exported pre-60S particles predominately contain 5.8S rRNA precursors, then blocking export should lead to a rapid inhibition of 5.8S synthesis. To assess the effect of inhibiting export on 5.8S rRNA synthesis, \emph{in vivo} pulse-labeling with \textsuperscript{3}H\textsubscript{3}H]uracil was performed in \textit{CRM1} and \textit{crm1(T539C)} strains. In initial experiments, cells were treated with LMB for 15 min prior to labeling with \textsuperscript{3}H\textsubscript{3}H]uracil (Fig. 3A). Under these conditions, no mature 5.8S rRNA was synthesized in the \textit{crm1(T539C)} mutant strain. In contrast, the independently transcribed SS rRNA accumulated with identical kinetics in \textit{CRM1} and \textit{crm1(T539C)} strains. This indicates that treatment with LMB caused a rapid block in the synthesis of 5.8S rRNA.

To better define the kinetics of the inhibition of 5.8S synthesis, \emph{in vivo} labeling was repeated with preincubation for 5 min with \textsuperscript{3}H\textsubscript{3}H]uracil prior to treatment with LMB. Incorporation into 5.8S rRNA is seen during preincubation prior to drug treatment, but the rates of incorporation in the wild-type and mutant strains rapidly deviate following LMB addition (Fig. 3B and C). As early as 5 min after LMB addition, a reduction in 5.8S synthesis was evident, and by 7 min the difference in accumulation was significant.

The \textsuperscript{3}H\textsubscript{3}H]-extended precursors to 5.8S rRNA, the 7S, 5.8S+30, and 6S pre-rRNAs, are not readily quantified by \emph{in vivo} labeling. These were therefore analyzed by Northern hybridization (Fig. 4A) using probe 020, which hybridizes across the 5' end of ITS2 (see Fig. 1A). In the \textit{CRM1} wild-type (WT) strain, the ratio of 6S to 7S remains relatively constant, as would be expected for unperturbed steady-state RNAs. In contrast, the ratio was drastically affected in the LMB-sensitive strain, where the level of 6S fell with respect to 7S. A striking reduction of 6S levels could be seen following 6 min of LMB treatment, just prior to the marked decrease seen for 5.8S synthesis detected by pulse-labeling (Fig. 4B).

No accumulation of the 7S or 5.8S+30 pre-rRNAs was seen, suggesting that the decrease in levels of 6S pre-rRNA is not caused by inhibition of 7S-to-6S processing, but rather is due to 6S pre-rRNA being rapidly degraded when export is blocked. To test this prediction, we deleted the gene encoding Trf4, the poly(A) polymerase from the major TRAMP nuclear RNA surveillance complex (24, 43), in the \textit{CRM1} and \textit{crm1(T539C)} strains. In the \textit{CRM1 trf4} strain, there was little variation in the 6S/7S ratio (Fig. 4B), consistent with previous analyses that showed no major alteration in pre-rRNA levels in \textit{trf4} strains (19). In the LMB-sensitive \textit{crm1(T539C) trf4} strain, the 6S/7S ratio initially increased, following 5 min of LMB treatment, consistent with an inhibition of 6S processing, followed by a modest decrease. The 6S/7S ratio was clearly stabilized in the \textit{crm1(T539C), trf4} double mutant relative to the \textit{crm1(T539C)} single mutant. This indicates that when export is blocked, 6S pre-rRNA is synthesized but is subject to degradation, for which Trf4 is partially responsible.

The rapid kinetics and specific inhibition of the 6S-to-5.8S synthesis suggest that blocking export is the direct cause of the defect, rather than a secondary effect. We conclude that pre-60S export is required for 3' processing of 6S pre-rRNA to 5.8S rRNA.

**Shuttling and cytoplasmic 60S synthesis factors precipitate 6S pre-rRNA.** To determine which 5.8S precursors are associated with the exported pre-60S particles, immunoprecipitation was performed using three TAP-tagged bait proteins, Arxl, Nmd3, and Lsg1, which associate with different sets of late pre-60S particles (Fig. 5A). Copurifying RNAs were analyzed by Northern hybridization and primer extension. Arxl associates with pre-60S particles in the nucleus and accompanies the pre-60S particles to the cytoplasm. As previously reported (30), Arxl was strongly associated with 7S pre-rRNA. However, additional processing intermediates, including the 6S pre-rRNA, and the mature 5.8S were also enriched (Fig. 5B and C).
Nmd3 is the major export adapter for pre-60S particles and is proposed to be among the last factors that bind prior to export. The Nmd3 precipitation therefore characterizes an even later nucleoplasmic stage than Arx1. Consistent with this, Nmd3 did not enrich 7S pre-rRNA, compared to either the tagged or untagged control precipitations, but did enrich 6S pre-rRNA and 5.8S (Fig. 5B and C). This indicates that very late nucleoplasmic preribosomes that shuttle to the cytoplasm contain the 6S pre-rRNA. Finally, the cytoplasmic GTPase Lsg1 also showed enrichment of 6S and 5.8S. Lsg1 is essential for release of Nmd3 and for 60S subunit synthesis (16), and its association of with 6S pre-rRNA indicates that Lsg1 is present in the pre-60S particles that are exported to the cytoplasm. In contrast, purification of the exportin Crm1 yielded no coprecipitated pre-RNAs. During 60S export, Crm1 is associated with Gsp1 (yeast RAN) bound to GTP (reviewed in reference 22). On arrival in the cytoplasm, the RAN-GAP Rna1 stimulates hydrolysis of Gsp1-GTP to Gsp1-GDP, triggering pre-60S release. Following cell lysis, we expect that GTP hydrolysis by Gsp1 will be stimulated by Rna1, resulting in the dissociation of Crm1 from its preribosome cargo and preventing recovery of associated RNAs.
The 25S rRNA is 5' matured within late pre-60S particles, and this was analyzed by primer extension using RNA precipitated with the same bait proteins. Only Arx1-TAP precipitated the 5'-extended 25S species, which is extended by some 8 nucleotides at the 5' end (Fig. 5D). This indicates that 5' maturation of 25S rRNA takes place in late, nucleoplasmic pre-60S particles prior to export.

We conclude that the exported pre-60S particles contain mature 25S rRNA together with the 6S pre-rRNA.

Localization of 5.8S precursors in processing mutants. Previous analyses have revealed a multistep 3' processing pathway for the 5.8S rRNA (see Fig. 7 below) (1, 8, 10, 44). The 7S pre-rRNA (5.8S 3' extended by 150 nt) is generated by 3' cleavage at site C2 by an unidentified endonuclease. The nuclear exosome then processes from 7S to the 5.8S 30 pre-rRNA (5.8S 3' extended by 30 nt) (1), followed by processing from 5.8S+30 to 6S (5.8S 3' extended by ~6 nt) by the nuclear exonuclease Rrp6 (8). Finally, Ngl2 processes 6S to mature 5.8S (10). Ngl2 was reported to be cytoplasmic in genome-wide green fluorescent protein (GFP) localization studies (20, 23); however, we were unable to clearly localize Ngl2-GFP expressed at endogenous levels (data not shown). These data suggest that strains lacking Rrp6 should accumulate nuclear 5.8S+30 pre-rRNA, whereas strains lacking Ngl2 would accumulate 6S pre-rRNA in the cytoplasm.

Strains lacking Rrp6 or Ngl2 are viable (8, 10), and we therefore performed pulse-chase labeling in deletion mutants to confirm the defects in processing of newly synthesized pre-rRNA (Fig. 6A and 7A). In the rrp6Δ strain, an initial, complete block to mature 5.8S rRNA synthesis was seen with strong accumulation of 5.8S+30 (Fig. 6A). A low level of mature 5.8S rRNA was synthesized only at later time points, but there was a substantial delay between the appearance of labeled 5.8S+30 (10 min) and mature 5.8S (40 min). This long delay suggests that in the absence of Rrp6, no 5.8S+30 undergoes processing through the normal pathway and confirms that Rrp6 is indeed solely responsible for normal processing of 5.8S+30. However, alternative pathways can eventually mature some of the accumulated pre-rRNA. The localization of the accumulated pre-5.8S+30 RNA was assessed using the ITS2-1...
FISH probe (Fig. 6B), which revealed very strong decoration of the nucleolus. Additionally, many *rrp6Δ* cells exhibited pre-5.8S signal at the nuclear periphery, presumably corresponding to pre-60S particles that contain 5.8S+30 and fail to be efficiently exported. The cytoplasmic signal was not clearly above the background in *rrp6Δ*, although the strength of the nucleolar signal made this difficult to image. In previous analyses, 5.8S+30 was detected in polysomes (8), indicating that some leakage does take place.

In the *ngl2Δ* strains, the 6S pre-rRNA species accumulated and no mature 5.8S rRNA was synthesized (Fig. 7A), confirming that Ngl2 is solely responsible for normal processing of 6S pre-rRNA. To confirm the identities of the RNA species observed by pulse-labeling, the same filter was hybridized with 32P-labeled probes directed against 3' extended 5.8S (probe 020) or the mature 5.8S rRNA (probe 017) (Fig. 7B; the lanes corresponding to the 10-min pulse-chase samples are shown). FISH analyses were performed in the *ngl2Δ* strain to localize the 6S pre-rRNA (Fig. 7C), which showed a strong signal throughout the cytoplasm.

We conclude that the 5.8S+30 pre-rRNA is predominately nuclear restricted, whereas 6S pre-rRNA accumulates in the cytoplasm, consistent with the immunoprecipitation data (Fig. 5).

**DISCUSSION**

The data we present here provides the first direct evidence in yeast of a cytoplasmic phase of 5.8S rRNA maturation. Based on the data presented, we propose a revised model for the synthesis of 5.8S rRNA (Fig. 8). This proposes that nuclear pre-60S particles that contain 6S pre-rRNA are competent for binding the export adapter Nmd3 and exportin Crm1 and are exported to the cytoplasm. Once in the cytoplasm, the GTPase Lsg1 binds the pre-60S and the putative nuclease Ngl2 completes the 3' maturation of 5.8S rRNA. Finally, Nmd3 is displaced by Lsg1 and recycled to the nucleus.

These findings may help answer the question of how and when ribosome subunits attain export competence and how immature subunits are excluded from the, essentially irreversible, step of nuclear export. The finding that 6S pre-rRNA was the only precursor species coprecipitated with Nmd3 (Fig. 5A and B) indicates that removal of ~20 nt of RNA from 5.8S+30
to 6S is coupled to the acquisition of export competence. The simplest hypothesis might be that this region binds one or more protein factors that are lost on processing. These factors might mediate nuclear retention by preventing binding of Nmd3, either directly or by imposing a particular preribosomal structure. Experiments to characterize the proteins bound to this region of the pre-rRNA are under way.

The TRAMP4 complex, containing Trf4, is involved in the turnover of the nuclear retained 6S pre-rRNA when export is blocked. The TRAMP complexes act through addition of short poly(A) tails to the RNAs to be degraded and target RNAs for exosome-mediated degradation. We predict that when export is blocked the nuclear retained pre-rRNAs are subject to oligoadenylation and subsequent degradation. The incomplete stabilization observed in the ccm1(Ts)pC trf4A strain is likely due to the activity of additional surveillance factors. Functional redundancy appears to be a general property of the nuclear RNA surveillance system (reviewed in reference 18). In addition to Ngl2, mutations in a surprising number of other 3’ exonucleases have been shown to accumulate 6S pre-rRNA, including members of the RNase D family of exonucleases Rex1, Rex2, and Rex3 (44) and core components of the exosome (1). We speculate that this 6S accumulation reflects reduced surveillance of late, nuclear preribosomal particles. Export of preribosomes to the cytoplasm is a key, irreversible step in ribosome synthesis, and other analyses suggest that surveillance of late, nuclear preribosomal particles is coupled to the acquisition of export competence. The 6S pre-rRNA is coupled to the nuclear export of the large ribosomal subunit. This work was supported by the Wellcome Trust.

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