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Nuclear RNase MRP Is Required for Correct Processing of Pre-5.8S rRNA in *Saccharomyces cerevisiae*

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RNase MRP is a site-specific ribonucleoprotein endoribonuclease that cleaves RNA from the mitochondrial origin of replication in a manner consistent with a role in priming leading-strand DNA synthesis. Despite the fact that the only known RNA substrate for this enzyme is complementary to mitochondrial DNA, the majority of the RNase MRP activity in a cell is found in the nucleus. The recent characterization of this activity in *Saccharomyces cerevisiae* and subsequent cloning of the gene coding for the RNA subunit of the yeast enzyme have enabled a genetic approach to the identification of a nuclear role for this ribonuclease. Since the gene for the RNA component of RNase MRP, *NME1*, is essential in yeast cells and RNase MRP in mammalian cells appears to be localized to nucleoli within the nucleus, we utilized both regulated expression and temperature-conditional mutations of *NME1* to assay for a possible effect on rRNA processing. Depletion of the RNA component of the enzyme was accomplished by using the glucose-repressed *GAL1* promoter. Shortly after the shift to glucose, the RNA component of the enzyme was found to be depleted severely, and rRNA processing was found to be normal at all sites except the B1 processing site. The B1 site, at the 5′ end of the mature 5.8S rRNA, is actually composed of two cleavage sites 7 nucleotides apart. This cleavage normally generates two species of 5.8S rRNA at a ratio of 10:1 (small to large) in most eukaryotes. After RNase MRP depletion, yeast cells were found to have almost exclusively the larger species of 5.8S rRNA. In addition, an aberrant 309-nucleotide precursor that stretched from the A2 to E processing sites of rRNA accumulated in these cells. Temperature-conditional mutations in the RNase MRP RNA gene gave an identical phenotype. Translation in yeast cells depleted of the smaller 5.8S rRNA was found to remain robust, suggesting a possible function for two 5.8S rRNAs in the regulated translation of select messages. These results are consistent with RNase MRP playing a role in a late step of rRNA processing. The data also indicate a requirement for having the smaller form of 5.8S rRNA, and they argue for processing at the B1 position being composed of two separate cleavage events catalyzed by two different activities.

RNase MRP is a ribonucleoprotein endoribonuclease originally isolated from mammalian cells (3, 4). This enzyme was characterized on the basis of its ability to cleave a mitochondrial transcript in vitro, which is consistent with a role in the formation of a primer for the initiation of mitochondrial DNA synthesis (3). The RNA component of the enzyme is encoded by a nuclear gene and the activity must be imported into the mitochondrion in order to process mitochondrial RNA in vivo (4, 5).

In addition to its mitochondrial localization, most of the RNase MRP RNA was observed in the nucleus (4, 8, 10, 26, 28, 29). In situ localization experiments demonstrated that an antigen associated with RNase MRP RNA, the To autoantigen, was associated with the granular compartment of the nucleolus (8, 13). These facts suggested a role for RNase MRP in the processing of rRNA in the nucleus (8, 10, 19, 29). Recently, we have reported isolation of a similar RNase MRP activity from the yeast *Saccharomyces cerevisiae* (26). The RNA component was subsequently isolated and its gene was cloned (*NME1*); this gene was found to be essential for viability in *S. cerevisiae* (19). All of these results are consistent with a role in nuclear rRNA processing.

In *S. cerevisiae* (30, 32) and other eukaryotic organisms, rRNA is transcribed as a large precursor that is processed into large and small rRNAs (Fig. 1). These reactions occur concomitantly with the assembly of rRNAs with the protein components of the ribosome in the nucleolus. In *S. cerevisiae*, a single 35S rRNA transcript undergoes rapid endonucleolytic cleavages to generate 18S, 25S, and 5.8S rRNAs. Several nucleolar ribonucleoproteins have been implicated in early processing events of the 35S precursor; however, none has been found to be required for a late processing event (27; for reviews, see references 7 and 30).

In an effort to identify the nuclear function(s) of RNase MRP, we have created strains of *S. cerevisiae* that conditionally express the RNA component of the enzyme. After depletion of RNase MRP RNA in *S. cerevisiae*, we examined the effect on rRNA synthesis and identified a specific processing defect associated with the loss of functional enzyme.

MATERIALS AND METHODS

Reagents. Restriction enzymes and T4 DNA ligase were obtained from Boehringer Mannheim. Radiolabeled nucleotides were from New England Nuclear. All other reagents were of the highest grade available.

Media and strains. Yeast media and genetic manipulations have been described elsewhere (21, 22). DNA isolation and manipulations have been described elsewhere (15). The *Escherichia coli* strain used for cloning, DH5α, has the genotype ϕ80d*lacZ*ΔM15 *endA1 recA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1* λ^- *gyrA96 relA1* Δ(*lacZYA-argF*)U169 F^-.

Construction of galactose-regulated *NME1*. The pMES150 plasmid was constructed as follows. A 1,042-bp *Eco*RI

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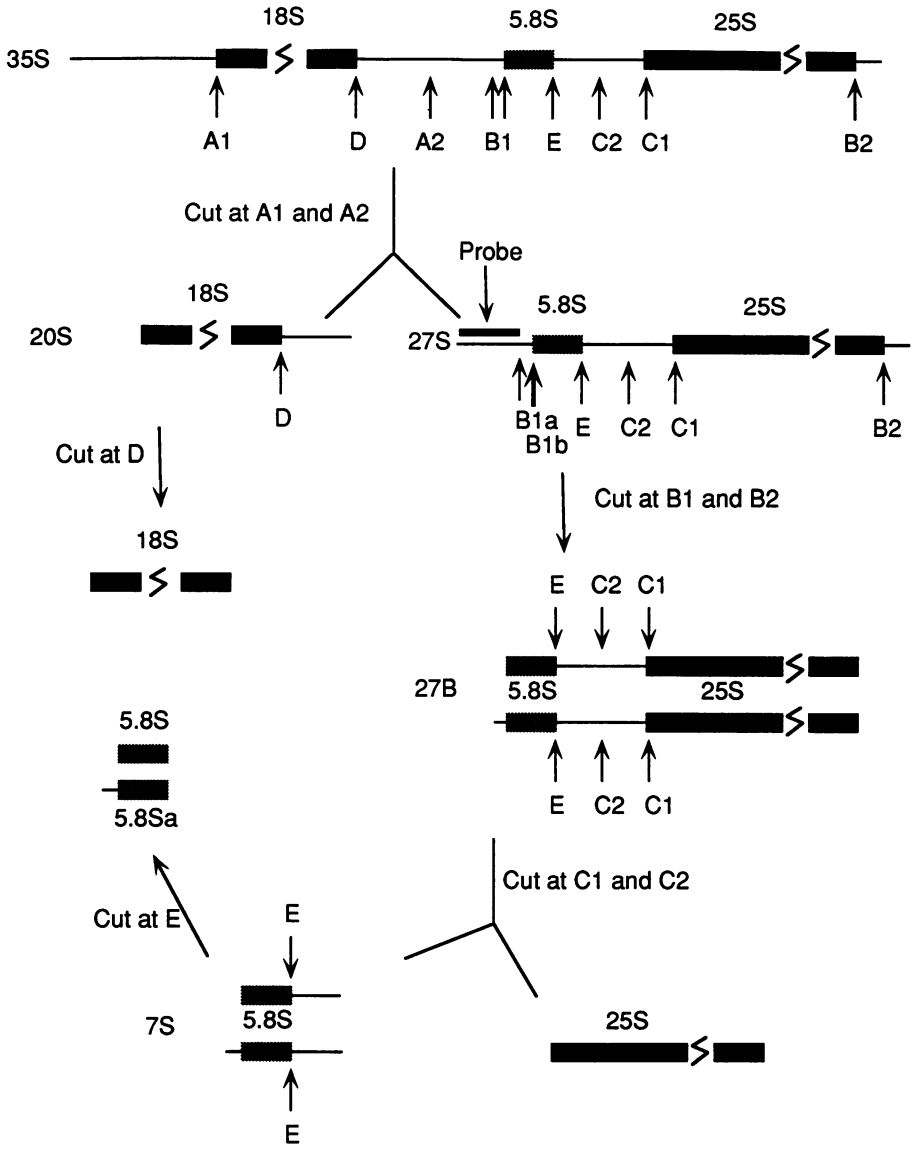

FIG. 1. Schematic of rRNA processing in a yeast cell. An initial 35S RNA transcript is processed through several endonucleolytic cleavage events to produce four mature rRNAs: 18S, 25S, and two species of 5.8S. The two species of 5.8S RNA differ by 7 nt at their 5′ ends. These two species normally exist in a 10-to-1 ratio of smaller to larger RNA in *S. cerevisiae*. The reason for this heterogeneity is unknown. As shown in Fig. 3 to 5, when RNase MRP RNA (*NME1* gene product) is depleted, a shift in the ratio of the 5.8S rRNA species is seen; normal amounts of the 18S and 25S rRNAs are made (data not shown). This result is consistent with a defect at the B1b processing site following RNase MRP depletion.

fragment containing the *NME1* gene was cloned into the vector pRS315 (24) to create the plasmid pMES140. A custom oligonucleotide was used in a polymerase chain reaction (PCR) to introduce a *Bam*HI site 3 nucleotides (nt) upstream of the *NME1* TATA box (19) and to delete simultaneously the 5′ end distal to the TATA box to create the plasmid pMES149. The resulting DNA sequence at the TATA box of the *NME1* gene is GGATCCAAATATATA AAAGGAG. A 300-bp *Sau*3AI-to-*Spe*I fragment from pYES2.0 (Invitrogen Corporation) harboring the *GAL1* upstream activation sequence (UAS) was cloned into the larger *Bam*HI-to-*Xba*I fragment of plasmid pMES149 to create plasmid pMES150. This plasmid, pMES150, was transformed into yeast strain MES116 (*MATα lys2-801 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 nme1-Δ2:TRP1* pMES127[pRS316::*NME1 URA3 CEN*]). This strain was crossed to the yeast strain MES110 (*MATa ade2-1 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 nme1-Δ2::TRP1* pMES127[pRS316::*NME1 URA3 CEN*]), and the *URA3*-containing plasmid pMES127 was shuffled out with 5-fluoroorotic acid. This strain, MES124, displayed extremely slow growth on glucose-containing plates and normal growth on galactose-containing plates. As a control in all the experiments, plasmid pMES140 (*NME1* gene under its own promoter) was put into the same genetic background to create strain MES123.

Growth of yeast cells and preparation of total yeast RNA. Yeast strains MES123 and MES124 were grown to mid-log phase in synthetic complete (SC) medium containing 2%

galactose (21). The cells were pelleted by centrifugation, washed once with distilled water, and inoculated into 200 ml of SC medium containing 2% glucose. The culture was grown for 24 h with intermittent dilution to maintain an optical density at 580 nm between 0.3 and 0.8. At 4-h intervals, the optical density at 580 nm of the culture was determined and 25-ml samples were removed for RNA preparation. Total RNA was prepared as previously described (18).

Analysis of yeast RNA. RNA was analyzed either by separation by formaldehyde gel electrophoresis and transfer to a nylon membrane by capillary action (15) or by separation by polyacrylamide gel electrophoresis (PAGE) on a 6% acrylamide–7 M urea gel followed by transfer to a nylon membrane by electroblotting (15). Hybridization probes were made with the Prime-It kit (Stratagene, Inc.) according to the manufacturer's instructions. The *SCR1* probe consisted of a 700-bp *Eco*RI-to-*Spe*I fragment of the gene (9). The *NME1* probe was a 460-bp product generated by PCR, using the *NME1* gene as a template and oligonucleotides OMS-6 (CTAAATAGTGGTCTTCACCTG) and OMS-7 (GTTCGGCATATTTAACAACTATGC) as primers. The probe for ITS1 rRNA was a 157-bp PCR product generated by using oligonucleotides OMS-13 (ATATTTTAAAATTTC CAGTTACGA) and OMS-12 (TCAATACAACACACTGTG GAG) as primers and yeast genomic DNA as the template.

Pulse-chase labeling of RNA. The pulse-chase experiments were carried out essentially as described previously (31).

In vivo translation assay. Cells were grown to log phase in 3 ml of synthetic dextrose medium (21) with the required amino acids and shifted to 37°C or maintained at 24°C for an additional 4 h. The cells were then labeled with 0.1 mCi of [^{35}S]methionine for 1 h and then chased for 5 min with 0.5 ml of 10 mg/ml of nonradioactive methionine. The cells were centrifuged at 3,000 × *g* for 5 min, washed once with water, and transferred to a 1.5-ml microcentrifuge tube. The cells were washed once again with water and resuspended in 50 μl of 2-mercaptoethanol prior to the addition of 600 μl of 2 M NaOH. After 10-min incubation at 4°C, 650 μl of 50% trichloroacetic acid was added, and the sample was incubated for an additional 10 min at 4°C. The sample was then centrifuged at 14,000 × *g* in a microcentrifuge for 2 min. The resulting pellet was washed twice with 1.5 ml of ice-cold acetone and then dried. The pellet was homogenized in 37.5 μl of 4× stacking gel buffer (15) and 75 μl of water. Ten microliters of 2-mercapthoethanol was then added, and the sample was vortexed prior to the addition of 24 μl of 25% sodium dodecyl sulfate (SDS). The samples were then heated at 95°C for 5 min and centrifuged as described above. The supernatant was recovered. Approximately 10^6 cpm was separated onto an SDS-polyacrylamide gel (12% acrylamide) (15). The gel was dried and fluorographed.

Construction of temperature-conditional mutants. Details of the *NME1* mutagenesis approach and genetic screen will be published elsewhere. The P6 mutant was generated by a random mutagenesis approach and contains a G-to-A transition at position 122 of *NME1* (19). The G-Δ2 mutation was generated by site-directed mutagenesis and is a deletion of positions 237 to 252 of *NME1* (19). Both mutations are in the *NME1* gene on plasmid pMES140. These mutations were maintained in yeast strain MES116 after removal of the *URA3*-containing plasmid, and their phenotypes were always compared with that of the wild-type pMES140 plasmid in the same strain.

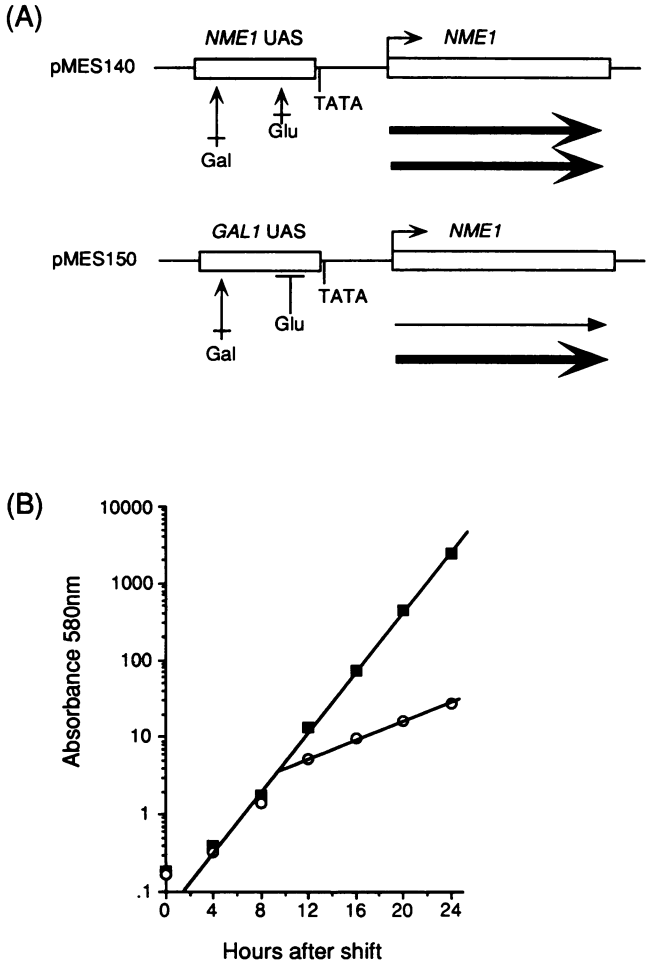

FIG. 2. Schematic of *NME1* Gal control constructs and the growth curves of strains harboring these constructs. (A) A 300-bp *Spe*I-to-*Sau*3AI fragment from vector pYES1.0 containing the *GAL1* promoter was placed directly upstream of the *NME1* TATA box, essentially replacing the original *NME1* promoter. Both the wild-type gene and the *GAL1* promoter fusion gene were inserted into a yeast *CEN LEU2* vector (pRS315) to create pMES140 and pMES150, respectively. Relative levels of transcription from the two promoters with either galactose or glucose as a carbon source are indicated by the thickness of the line. (B) Yeast strains carrying either the plasmid pMES140 (wild type) or pMES150 (Gal fusion) were grown on SC medium containing 2% galactose to mid-log phase. The cells were washed twice with water and then used to inoculate SC medium containing 2% glucose. The cells were maintained in logarithmic growth phase by dilution into fresh medium, and the cell density was monitored by A_{580} and plotted against time.

RESULTS

Conditional expression of RNase MRP RNA. A diploid yeast strain was constructed with both chromosomal copies of the *NME1* gene encoding the yeast RNase MRP RNA (19) deleted. This yeast strain maintains viability because of the presence of the *NME1* gene on an episomal plasmid. Transcriptional control was accomplished by placing a *GAL1* UAS construct upstream of the *NME1* gene (Fig. 2A). When yeast cells harboring this construct are grown on galactose, the *NME1* gene is transcribed normally; however, when they are shifted to glucose, *NME1* transcription is repressed.

The effect of glucose repression was apparent on SC plates

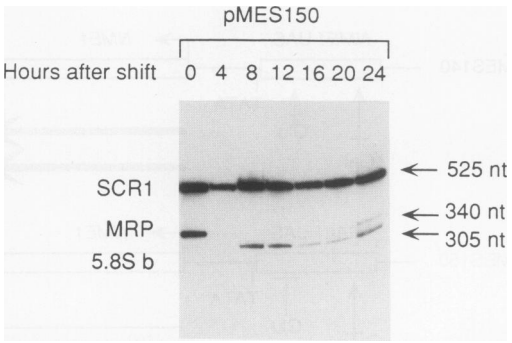

FIG. 3. Depletion of RNase MRP RNA (*NME1*) after shift to glucose and accumulation of an aberrant rRNA precursor. After the shift to glucose-containing medium, samples were removed every 4 h and used to prepare total RNA. After gel electrophoresis and transfer to nylon, the RNA was probed for *SCR1* (the yeast signal recognition particle RNA, a loading control) and RNase MRP RNA (*NME1*) or with a probe to the ITS1 of the rRNA precursor (cleavage sites A2 to B1). The aberrant precursor stretches from the A2 to E processing sites (Fig. 1).

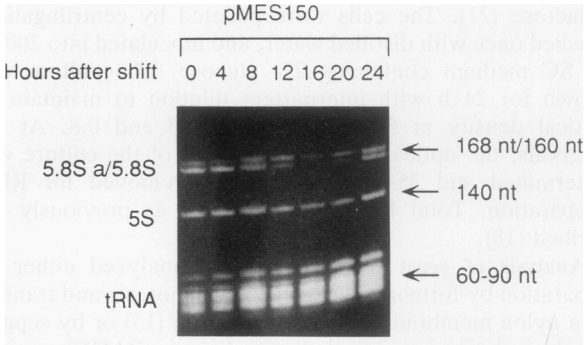

FIG. 4. Shift in the ratio of the 5.8S rRNAs after depletion of the RNase MRP RNA (*NME1*). RNA was isolated from yeast cells after growth in glucose and examined by staining with ethidium bromide after PAGE (15). No change in either the tRNA or 5S rRNA profile was seen; however, a dramatic shift in the ratio of the 5.8S rRNAs is apparent.

containing 2% glucose on which the galactose-regulated strain, MES124, grew very slowly. To measure the exact growth rate of this strain, a galactose-grown culture was used to inoculate SC medium containing 2% glucose and the optical density at 580 nm of the culture was monitored for 24 h (Fig. 2B). Compared with the control strain, the galactose control strain grew at a normal rate for approximately 8 h, when its growth rate rapidly declined. At later time points, the wild-type strain maintained a doubling time of 110 min, while the doubling time of the galactose control strain was 6 h. The small amount of residual growth of the galactose control strain was probably due to a basal level of transcription of the *NME1* gene, even under glucose-repressing conditions. This phenomenon has been seen by other investigators (12, 20).

At 4-h intervals during growth on glucose, samples of the culture were taken and total RNA was isolated. The RNA was separated by electrophoresis on a 6% acrylamide–7 M urea gel, transferred to a nylon membrane, and probed for both the RNase MRP RNA (*NME1*) and yeast signal recognition particle RNA (*SCR1*) (Fig. 3). The *SCR1* signal is a control for loading differences in the gel, since its levels should remain unaltered by glucose. At the earliest time point (4 h), RNase MRP RNA is severely depleted. At the 8-h time point, RNase MRP RNA is nearly absent, though still detectable on a longer exposure (Fig. 3). Interestingly, at the 24-h time point the levels of RNase MRP RNA are increasing. The most probable causes are amplification of the plasmid or a loss of tight glucose repression, since the cells are ill.

Depletion of RNase MRP RNA causes a change in the distribution of 5.8S rRNA. The same total RNA samples were examined by agarose gel electrophoresis and Northern (RNA) analysis to examine maturation of the larger 18S and 25S rRNAs. In the *NME1*-depleted strain, normal amounts of both the 18S and 25S rRNAs were produced, and there was no evidence for an increase in any of the normal rRNA precursors. Neither was there any large aberrant precursor detected (data not shown). This level of analysis does not eliminate the possibility of subtle changes in such rRNA species.

Accumulations of 5S and 5.8S rRNAs were also examined in this strain. Total RNA was separated by electrophoresis on a 6% acrylamide–7 M urea gel and visualized by staining with ethidium bromide (Fig. 4). At the 8-h time point, when culture growth is slowing and RNase MRP RNA is essentially depleted (less than 5% normal levels) (Fig. 2 and 3), the 5.8S rRNA population begins to shift from predominantly the smaller species (5.8S) to the larger species (5.8Sa) (7 nt longer) (23). This change in the 5.8S rRNA population was most evident at the 12- to 20-h time points. At the 24-h time point, concomitant with the appearance of small amounts of RNase MRP RNA (Fig. 3), the 5.8S rRNA population begins to return to normal. This shift in the 5.8S ratio appears to be composed of both a decrease in the 5.8S molecules and an increase in the number of 5.8Sa molecules (compare the 4-, 8-, and 12-h time points in Fig. 4), though clearly the bulk of the change is in the decrease of the 5.8S rRNA. The 5S rRNA and tRNAs appeared completely normal throughout the time course of the experiment.

An aberrant rRNA precursor appears after depletion of RNase MRP RNA. After RNase MRP RNA depletion, RNA was hybridized with a probe from ITS1, which was complementary to RNA sequence from the A2 to B1 processing sites of rRNA. This probe detected an aberrant 305-nt 5.8S rRNA (5.8Sb) (11, 23) (Fig. 3). This RNA species stretches from the A2 processing site to the E processing site and apparently results from a failure to cleave at the B1 processing site, while still processing normally at all the other sites (Fig. 1). Again, this RNA species appears concomitantly with the disappearance of the RNase MRP RNA. This longer rRNA species was not seen at any appreciable level in wild-type strains, so it is not believed to be a normal rRNA precursor. A wild-type strain was examined in parallel with the galactose-regulated strain, and no appreciable change of any of the RNA species examined was seen throughout the time course of the experiment (data not shown).

Strains with temperature-sensitive conditional mutations in the *NME1* gene show an identical rRNA processing defect. As a second experimental approach, we utilized two temperature-conditional mutations in the *NME1* gene. Both mutants, P6 and G-Δ2, fail to grow at 37°C when grown on SC medium containing 2% glucose. In addition, the P6 mutant grows much slower than the wild type at 30°C and is therefore believed to represent a stronger mutation than G-Δ2. The two mutant strains were grown in parallel with the isogenic, wild-type strain at the permissive temperature, 24°C, until

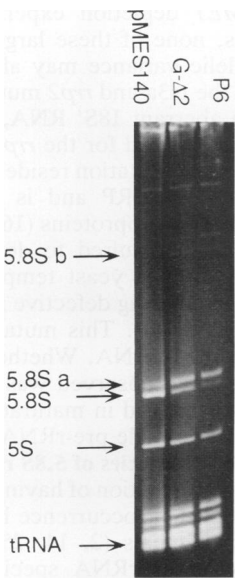

FIG. 5. *NME1* conditional mutants show the same rRNA processing defect. Yeast strains were grown at the permissive temperature (24°C) and then shifted to the nonpermissive temperature (37°C) for 6 h. Total RNA was prepared, fractionated by PAGE, and then visualized with ethidium bromide. A description of the strains is given in Materials and Methods. The identity of the 305-nt 5.8Sb rRNA was confirmed by Northern hybridization (data not shown).

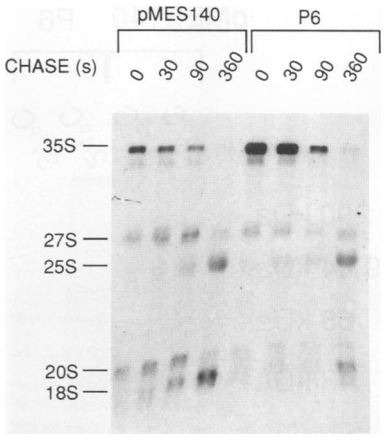

FIG. 6. Pulse-chase labeling of rRNA in wild-type cells and a *NME1* conditional mutant. Log-phase cultures of isogenic strains carrying either a wild-type *NME1* gene (pMES140) or a temperature-conditional mutation (P6) grown at 24°C were shifted to 37°C and grown for 4 h. RNA was then labeled in vivo with [*methyl*-^{3}H] methionine for 2.5 min and then chased with unlabeled methionine for the number of seconds indicated, when 1-ml samples were then withdrawn. RNA was prepared, and 2.5×10^4 cpm was analyzed on a 1.0% agarose gel. The gel was treated with DuPont Enlightning, dried, and visualized by fluorography.

the culture was in mid-log phase. The cultures were then shifted to 37°C and grown for an additional 6 h before total RNA was prepared. The RNA from these strains can be seen in Fig. 5 after PAGE and visualization with ethidium bromide. As in the case of depletion of the *NME1* RNA, a clear shift in the ratio of the 5.8S to 5.8Sa rRNA is seen in both conditional mutants. Also, enough of the 305-nt 5.8Sb rRNA has accumulated such that it is clearly visible by ethidium bromide staining. The more-stringent P6 mutation shows a more drastic shift in the ratio of the two 5.8S rRNAs. The identity of the 305-nt 5.8Sb rRNA was confirmed by Northern hybridization (data not shown).

The overall processing of the large rRNAs was also examined in these conditional strains, and the large rRNA species were found to be normal, as was the case in the RNase MRP depletion experiments (data not shown). Also, the amount of 5S rRNA and the processing of tRNAs appeared unchanged in these conditional strains under restrictive growth conditions.

Pulse-chase analysis of a temperature-conditional mutant. The processing of newly synthesized pre-rRNAs can be monitored by a pulse-chase incorporation analysis, thereby providing a more sensitive analysis of pre-rRNA processing than standard Northern analysis (27). We examined the P6 temperature-conditional mutation in this way to analyze the relative kinetics of synthesis of the 18S and 25S rRNAs with the loss of RNase MRP RNA (Fig. 6). The P6 mutant was found to continue to process correctly the 35S pre-rRNA to the 18S and 25S rRNAs at the nonpermissive temperature; however, the rate of their maturation was slowed, as evidenced at the 0-, 30-, and 90-s time points. There were no aberrant products seen with this analysis.

In vivo translation from ribosomes depleted of 5.8S rRNA. Since the defect associated with RNase MRP was so subtle, we examined whether the shift from 5.8S rRNA to 5.8Sb rRNA would lead to a defect in translation and hence point to the lethality associated with the loss of RNase MRP (19). Log-phase cells carrying the P6 temperature-conditional mutation and the wild-type strain were each shifted, as described above, to 37°C for 4 h (the ratio of 5.8S to 5.8Sa is approximately 1:4 at this point in the mutant strain). The cells were then labeled with methionine for either 5 min (data not shown) or 1 h (Fig. 7). Surprisingly, incorporation of methionine into protein in the P6 mutant at the nonpermissive temperature was extremely vigorous and actually outpaced that of the wild-type strain (pMES140). When the total cellular protein was examined after SDS-PAGE, there appeared to be selective translation or loss of translation of several proteins (compare proteins marked with arrows in the pMES140 37°C lane with the P6 37°C lane in Fig. 7). The identities of these proteins have not yet been determined.

DISCUSSION

The B1 processing site in rRNA is actually two distinct sites, B1a and B1b. The B1 processing cleavage has been considered a single occurrence; however, it generates two separate products with ends differing by 7 nt at the 5′-end region of 5.8S rRNA (14, 23). The results presented in this article demonstrate that these two 5.8S rRNAs can be distinctly separated and that they probably result from two different events in *S. cerevisiae*. Henceforth we will refer to the two sites as B1a and B1b (Fig. 1). In order to maintain a proper ratio of the two 5.8S rRNAs in a cell, the activities that produce these two cleavages must be tightly regulated. Cleavages at the B1a and B1b sites may occur independently or be processive in nature. Future isolation of mutants with defective processing at the B1a site and not the B1b site should help clarify the issue.

RNase MRP RNA is required for processing at the B1b site in rRNA. We have shown in this work that the presence of RNase MRP RNA is required for proper processing and maturation of 5.8S rRNA, but we do not know whether the

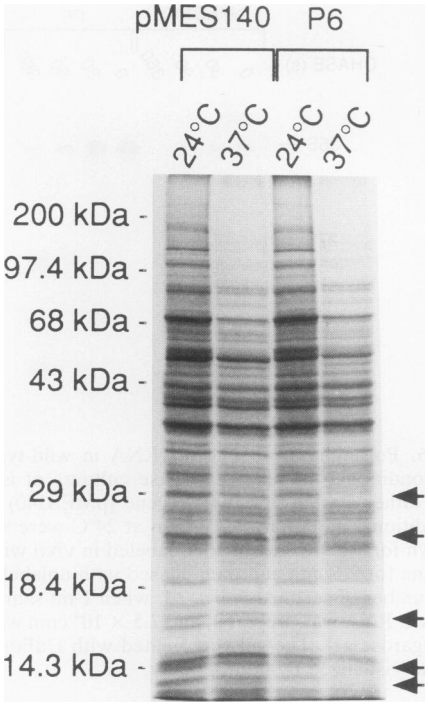

FIG. 7. In vivo labeling of ribosomal translation products. The same isogenic strains as in Fig. 6 were labeled with [^{35}S]methionine as described in the Materials and Methods. Equal amounts of total yeast protein were separated on a 12% polyacrylamide gel and then exposed to film. Protein bands that showed selective translation in either the wild type or the P6 mutant are indicated with arrows. The sizes of molecular mass standards are indicated.

enzyme catalyzes this cleavage directly or indirectly (see below). The data would fit our hypothesis that two separate enzymes are required for independent B1a and B1b cleavages. When cells were depleted of RNase MRP RNA, there was failure to cleave at the B1b processing site; however, if the B1a endonuclease could still produce its cleavage product, the observed change in the ratio of the two species would occur. The B1a endonuclease may be normally present in limiting amounts so that most of the RNA is cleaved by the B1b (RNase MRP?) enzyme to produce the final 1:10 ratio normally seen (14). With the depletion of the B1b endonuclease, 5.8Sb rRNA would become predominantly a substrate for the B1a endonuclease such that more 5.8Sa rRNA would be produced. However, with limiting amounts of the B1a endonuclease, all of the pre-5.8S rRNA may not be efficiently cleaved and the aberrant 305-nt 5.8Sb rRNA is thereby produced.

There have been three other reports in the literature of a similar defect in rRNA processing. A temperature-sensitive lethal mutation, *rrp2*, that is allelic to a separately isolated mutation, ts3a (11), has been described (23). The ts3a mutant also has a shifted ratio of 5.8S to 5.8Sa rRNA, and both the *rrp2* and ts3a mutants accumulate the same 305-nt aberrant precursor that stretches from the A2 to E processing sites. However, these mutants were reported to accumulate larger rRNA intermediates at the nonpermissive temperature; these intermediates include an aberrant 24S RNA (11, 23) composed of the entire 5′ external transcribed spacer (ETS), 18S, ITS1, and the 5.8S rRNA sequences and an aberrant 18S′ RNA (23) that included 5.8S, ITS2, and part of the 18S rRNA. In the *NME1* depletion experiments and in the conditional mutants, none of these large pre-RNA defects were observed. Allelic variance may also account for the differences, indeed the ts3a and *rrp2* mutants differed in the accumulation of an aberrant 18S′ RNA, and the change in 5.8S rRNA was not reported for the *rrp2* mutant (23). It is conceivable that the *rrp2* mutation resides in a protein that is a component of RNase MRP and is shared with other relevant nucleolar ribonucleoproteins (16); mapping or cloning of the *rrp2* gene is required to determine any direct relationship with *NME1*. A yeast temperature-conditional lethal mutant isolated as being defective in mRNA transport (*rat1*) has been reported (1). This mutant shows a similar defect in processing 5.8S rRNA. Whether or not any other aberrant precursors were observed was not reported. The *rat1* protein may be involved in maturation or transport of RNase MRP RNA or possible pre-rRNA substrates.

The two heterogeneous species of 5.8S rRNA are not equivalent. The physiological function of having two different 5.8S rRNAs is unclear, but their occurrence has been evolutionarily conserved in mammals (2, 14, 25). Ribosomes that contain one or the other rRNA species might translate different sets of messages selectively (e.g., heat shock mRNAs or mRNAs destined for a particular compartment such as the mitochondria). This would provide yet another level of control on cellular metabolism. Clearly, from the in vivo translation experiments, individual ribosomes containing one of the two different rRNA species are not equivalent, because there is an apparent requirement for the smaller 5.8S rRNA species to translate certain proteins for which the larger 5.8Sb species cannot compensate, even when the larger rRNA is relatively abundant. This should provide an interesting area for further research.

In vitro cleavage of the pre-5.8S rRNA by RNase MRP. The most direct involvement of RNase MRP would be the demonstration of its processing at the B1b site in an in vitro cleavage assay. We have performed experiments with eight different potential rRNA substrates and have been unable to find any evidence for endonucleolytic cleavage with purified RNase MRP in in vitro reactions (unpublished observations). We cannot rule out the possibility that we have yet to provide the proper substrate, since because of its size, it was not feasible to present the enzyme with the predicted 29S pre-rRNA substrate which would extend from the A2 to B2 sites (Fig. 1). Partial assembly of the pre-rRNA with ribosomal proteins may be essential for RNase MRP cleavage, making it impossible to demonstrate cleavage in a substrate RNA-only reaction. Also, we note that there is no obvious sequence homology between the RNase MRP mitochondrial RNA substrate and the predicted pre-rRNA substrate. It may be that the enzyme requires recognition of an RNA structure that is not easily predicted by sequence alone. This is certainly the case for RNase P, a close relative of RNase MRP (6, 8, 17).

Another possibility is that RNase MRP is needed to cleave some other RNA that, in turn, is a required component of the processing complex active at the B1b site in pre-5.8S rRNA. Indeed, one could propose an RNA processing role for the enzyme that was critical for any RNA species needed directly or indirectly (even as a mRNA) to support correct production of mature 5.8S rRNA.

Other nuclear functions of RNase MRP. Although the data reported here represent the first information on a nuclear function of RNase MRP, they do not exclude additional roles for RNase MRP activity in the nucleus, nor do they prove that the defect observed reflects an essential function of the

enzyme. Examination of other nuclear metabolic processes as well as an analysis of suppressor mutations of RNase MRP-specific defects should yield further insights.

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