MOLECULAR AND CELLULAR BIOLOGY, July 1996, p. 3429–3436 Vol. 16, No. 7
0270-7306/96/$04.00+0

Yeast Mitochondrial RNase P RNA Synthesis Is Altered in an RNase P Protein Subunit Mutant: Insights into the Biogenesis of a Mitochondrial RNA-Processing Enzyme

VILIUS STRIBINSKIS,[1] GUO-JIAN GAO,[1] PAVOL SULO,[2] YAN LI DANG,[3] AND NANCY C. MARTIN[1]*

Department of Biochemistry, University of Louisville School of Medicine, Louisville, Kentucky 40292[1]; Department of Ophthalmology, Baylor College of Medicine, Houston, Texas 77030[3]; and Institute of Microbiology and Genetics, University of Vienna, A-1030 Vienna, Austria[2]

Received 6 February 1996/Returned for modification 14 March 1996/Accepted 23 April 1996

Rpm2p is a protein subunit of *Saccharomyces cerevisiae* yeast mitochondrial RNase P, an enzyme which removes 5′ leader sequences from mitochondrial tRNA precursors. Precursor tRNAs accumulate in strains carrying a disrupted allele of *RPM2*. The resulting defect in mitochondrial protein synthesis causes petite mutants to form. We report here that alteration in the biogenesis of Rpm1r, the RNase P RNA subunit, is another consequence of disrupting *RPM2*. High-molecular-weight transcripts accumulate, and no mature Rpm1r is produced. Transcript mapping reveals that the smallest RNA accumulated is extended on both the 5′ and 3′ ends relative to mature Rpm1r. This intermediate and other longer transcripts which accumulate are also found as low-abundance RNAs in wild-type cells, allowing identification of processing events necessary for conversion of the primary transcript to final products. Our data demonstrate directly that Rpm1r is transcribed with its substrates, $tRNA_f^{Met}$ and $tRNA^{Pro}$, from a promoter located upstream of the $tRNA_f^{Met}$ gene and suggest that a portion also originates from a second promoter, located between the $tRNA_f^{Met}$ gene and *RPM1*. We tested the possibility that precursors accumulate because the RNase P deficiency prevents the removal of the downstream $tRNA^{Pro}$. Large *RPM1* transcripts still accumulate in strains missing this tRNA. Thus, an inability to process cotranscribed tRNAs does not explain the precursor accumulation phenotype. Furthermore, strains with mutant *RPM1* genes also accumulate precursor Rpm1r, suggesting that mutations in either gene can lead to similar biogenesis defects. Several models to explain precursor accumulation are presented.

Mitochondrial DNA in the yeast *Saccharomyces cerevisiae* codes for components of complexes involved in oxidative phosphorylation and electron transport as well as RNAs and proteins necessary for their expression by the mitochondrial protein synthesizing system (1). Most proteins found in mitochondria, however, are encoded by nuclear genes, translated on cytoplasmic ribosomes, and delivered to mitochondria to function. The mechanisms that coordinate nuclear and mitochondrial gene expression are not known. Even with multicomponent mitochondrial complexes that are composed of subunits from both genomes, it is not clear how their regulated expression and assembly occur. These complexes include ATP synthase, cytochrome oxidase and cytochrome *b*, mitochondrial ribosomes, and mitochondrial RNase P (1). The RNA subunit of mitochondrial RNase P, Rpm1r, is encoded by mitochondrial DNA, whereas proteins necessary for mitochondrial RNase P biogenesis and activity are encoded by nuclear genes and imported into mitochondria (14).

The gene coding for Rpm1r, *RPM1*, was originally identified by correlating the tRNA synthetic ability of petite deletion mutants with the loss or retention of mitochondrial DNA located between the $tRNA_f^{Met}$ and $tRNA^{Pro}$ genes (36). Subsequent studies demonstrated that *RPM1* codes for an AU-rich RNA which varies in size among different yeasts (23, 38). Evidence that *RPM1* codes for an RNase P RNA includes the presence of two short consensus sequences found in all RNase P RNAs (38), the cofractionation of RNase P activity and the RNase P RNA (25), and the dependence of in vivo (25) and in vitro RNase P activity on the presence of the RNA (14).

The expression of *RPM1* has been the subject of several studies, and it is clear that its regulation is complex. Firstly, previous studies have suggested, but not demonstrated, that Rpm1r is transcribed with flanking tRNA substrates (19). Secondly, the relative amounts of transcripts from the $tRNA_f^{Met}$ gene and *RPM1* suggest that transcription attenuation occurs between these two genes (26). Finally, the relative amounts of $tRNA_f^{Met}$ and Rpm1r change significantly during derepression (27, 35). The relative roles that transcription, differential stability, and attenuation play in expression of this region remain to be elucidated.

We report here that *RPM2*, a nuclear gene which codes for a protein subunit of mitochondrial RNase P, also plays a critical role in the expression of *RPM1*. Rpm2p is the most abundant protein in highly purified mitochondrial RNase P and is necessary to remove the 5′ leader sequences from tRNA precursors (24). With a predicted molecular mass of 119 kDa, it is substantially larger than the approximately 14-kDa protein subunits of the prokaryotic enzymes characterized to date (10). A comparison of the protein sequence predicted by the nuclear *RPM2* gene reveals no significant sequence similarity to these smaller proteins (6) nor to another nuclear gene, *POP1*, which codes for a protein component of the distinct nuclear RNase P in *S. cerevisiae* (18). Nonetheless, the evidence that Rpm2p is a subunit of yeast mitochondrial RNase P is compelling. Firstly, Rpm2p was identified by cofractionation with mitochondrial RNase P activity (25). Secondly, antibodies raised to a TrpE-Rpm2p fusion protein immunoprecipitate all RNase P activity and all RNase P RNA from mitochondrial extracts

* Corresponding author. Phone: (502) 852-7797. Fax: (502) 852-6222.

(10). Thirdly, disruption of the *RPM2* gene causes the accumulation of precursor tRNAs in vivo (24).

The large size of Rpm2p compared with other RNase P proteins originally led us to speculate that Rpm2p might have additional functions (10). In work reported elsewhere (15), we have shown that *RPM2* is essential for normal cell growth under conditions that do not require mitochondrial protein synthesis. This essential function, which is currently unknown, does not require RNase P activity (15). We report here the requirement for *RPM2* in the biosynthesis of Rpm1r. This brings the number of functions accommodated in Rpm2p to three. Discovering a role for Rpm2p in the biosynthesis of Rpm1r has enabled us to examine the biosynthetic pathway of Rpm1r more closely.

RNA processing plays a critical role in the expression of mitochondrial DNA. A dodecamer sequence has been implicated in the formation of mRNA 3′ ends directing endonuclease cleavage (29). RNase P is responsible for the maturation of the 5′ ends of tRNAs (21). A 3′ endonuclease prepares mitochondrial tRNAs for the addition of the 3′ CCA (7). Specific cleavage events are necessary for mRNA synthesis, and mRNA and rRNA splicing are critical to mitochondrial biogenesis in yeast cells (11).

Transcription of mitochondrial DNA is carried out by a mitochondrial RNA polymerase consisting of a 150-kDa core polymerase and a 43-kDa specificity factor (33). The promoter used by this polymerase is an octanucleotide consensus sequence, TATAAGTA, and transcription initiates at the last A (30). If the nucleotide located directly downstream is a purine the promoter is strong, and if it is a pyrimidine the promoter is weak (4). Christianson and Rabinowitz mapped many transcription initiation sites on yeast mitochondrial DNA (9). One is located upstream of the $tRNA_f^{Met}$ gene. We show directly here that transcripts arising from this promoter include the $tRNA_f^{Met}$, Rpm1r, and the downstream $tRNA^{Pro}$. There is a second sequence between the $tRNA_f^{Met}$ gene and *RPM1* which deviates from the conserved octanucleotide by one base and which serves as a promoter in vitro (2, 3). We report here a transcript with its 5′ end falling one nucleotide upstream from the predicted initiation site. Thus, two transcriptional start sites may differentially regulate the relative amounts of $tRNA_f^{Met}$ and Rpm1r.

The polycistronic transcripts that arise from the established promoter upstream of the $tRNA_f^{Met}$ gene and the second putative promoter downstream of the $tRNA_f^{Met}$ gene must be processed to release mature Rpm1r and the tRNAs. The processing events that release these three RNAs and that provide mature ends are numerous. Many of the precursors which require processing accumulate in the absence of Rpm2p. Since the final 5′ and 3′ maturation of Rpm1r does not occur, 5′ and 3′ extended precursors are the limit product. Either Rpm2p performs one or both of these processing steps itself, or it presents the proper substrate conformation to distinct processing enzymes. The RNAs that accumulate in the absence of full-length *RPM2* resemble high-molecular-weight RNAs which accumulate in *rpm1* mutants (34). We demonstrate here that disruption of *RPM2* in these *rpm1* strains does not significantly alter the RNA profile. Thus, similar biogenesis defects of Rpm1r can be caused by mutations in either of the two genes known to code for components of yeast mitochondrial RNase P.

MATERIALS AND METHODS

Strains and culture conditions. *S. cerevisiae* W3031A (**a** *ade2-1 his3-11,15 leu2-3,112 ura3-1 trp1-1 can1-100*) contains the D273-10B mitochondrial genome (28); *rpm2*::*LEU2#6* was obtained by inserting the *LEU2* gene into *RPM2* at the unique *Hpa*I site as described by Morales et al. (24). To construct strains with both *RPM1* and *RPM2* altered, the synthetic petite mutants made by biolistic transformation (34) were disrupted with an *RPM2*::*URA3* fragment. This was produced by inserting the *URA3* gene at the unique *Hpa*I site in *RPM2*. Cells were grown in medium containing 1% yeast extract, 1% Bacto Peptone, and 2% glucose or on the appropriate selective medium to promote plasmid retention.

RNA isolation. Total RNA was isolated by hot phenol extraction (16). Mitochondrial RNA was purified by guanidinium extraction (8) of isolated mitochondria (25). RNA was dissolved in 0.5% sodium dodecyl sulfate and stored at −20°C.

Northern (RNA) analysis. Total RNA (30 μg) or mitochondrial RNA (25 μg) was separated on a 4 or 6% polyacrylamide–8 M urea–Tris-borate-EDTA (TBE) gel (as indicated in figure legends), transferred to Zeta-Probe membrane, and processed as recommended by the manufacturer (Bio-Rad). A riboprobe complementary to the $tRNA_f^{Met}$ was transcribed from *Pst*I-digested pMM6 (25). A riboprobe complementary to Rpm1r was transcribed from *Hin*dIII-digested pMH777 (25). A riboprobe complementary to the $tRNA^{Pro}$ was transcribed from *Hin*dIII-digested pT3/T7Pro. All plasmids were transcribed with T3 RNA polymerase by using an Ambion T7/T3 Maxiscript kit as specified by the supplier and [α-^{32}P]UTP (800 Ci/mmol; DuPont NEN).

Primer extension. An oligonucleotide (5′-GATAATATTTATCTTATAAAG-3′) complementary to an internal site in Rpm1r (see Fig. 2A) and an oligonucleotide (5′-CCTGGTAGCAATAATACGATTT-3′) complementary (underlined nucleotides) to the 3′ end of $tRNA_f^{Met}$ were radiolabeled with [γ-^{32}P]ATP (3,000 Ci/mmol; DuPont NEN) and T4 polynucleotide kinase (New England Biolabs) and were separated from unincorporated label by centrifugation through a Sephadex G-25 column (Boehringer Mannheim). Mitochondrial RNA or total RNA from [*rho*^{0}] cells (5 to 10 μg) was precipitated together with 3 to 5 pmol of labeled oligonucleotide. Annealing and primer extension with SuperScript II were performed as recommended by the manufacturer (Gibco BRL), with one exception. Slow cooling to anneal the nucleic acids was done by reducing the temperature from 70°C to less than 37°C over a 15- to 20-min interval. The reaction products were recovered by ethanol precipitation and analyzed on a 6% polyacrylamide–8 M urea–TBE gel alongside labeled molecular weight markers or dideoxy sequencing reaction products. The sequencing reactions were performed with the same primer and a DNA sequencing kit (Sequenase version 2.0; U.S. Biochemicals).

S1 mapping. PCR amplification of *Sau*3AI-digested mitochondrial DNA primed with oligonucleotides complementary to the $tRNA_f^{Met}$ (5′-TGGTTAA CATTTTAGGGTCA-3′) and $tRNA^{Pro}$ (5′-CTGACCTTTTGGCTTCTATC) synthesized a 1,360-bp fragment. The amplified DNA was cloned with the TA cloning kit (Invitrogen), forming pCR/12. A *Hin*fI-*Hpa*II restriction fragment of pCR/12 (see Fig. 3A) was recovered from an agarose gel with a QIAEX gel extraction kit (Qiagen Inc.) and labeled at the 3′ end with [α-^{32}P]dATP and Klenow fragment (Promega). Coding-strand DNA was isolated from the 6% polyacrylamide–8 M urea–TBE gel and used as a probe for 3′-end mapping. Fifteen micrograms of mitochondrial RNA or control *Escherichia coli* tRNA was precipitated together with the gel-purified probe. Hybridization of probe and sample RNA, S1 nuclease digestion, inactivation, and precipitation of reaction products was performed with an SNP-assay kit (Ambion) as specified by the manufacturer. S1 nuclease digestion was carried out at 37°C for 30 min or at 17°C for 2 h. Reaction products were sized on a 6% polyacrylamide–8 M urea–TBE gel by comparison with a Maxam-Gilbert DNA sequencing ladder.

Nucleotide sequence accession number. The accession number of the sequence in Fig. 2A is U46121.

RESULTS

Maturation of Rpm1r is defective in *RPM2* disruption strains. Previous experiments with *RPM2* disruption strains demonstrated that precursor tRNAs accumulated in the mitochondria, and no mature tRNA was detected (24). As predicted for strains defective in mitochondrial protein synthesis (28), strains with the disruption allele of *RPM2* became petite when cultured on glucose medium (24). The expression of *RPM1*, $tRNA_f^{Met}$ and $tRNA^{Pro}$ genes in wild-type cells and in a petite mutant, *rpm2*::*LEU2#6*, which retains these genes, is described here.

To determine whether the biosynthesis of Rpm1r is affected in *rpm2*::*LEU2#6*, Northern analysis was performed. RNAs from wild-type strains and from the petite deletion mutant are different (Fig. 1): no mature Rpm1r is present in the petite mutant, and high-molecular-weight transcripts from *RPM1* accumulate (compare lanes 1 and 3 in Fig. 1). Transformation of the disruption strain with *RPM2* on a plasmid restores mature Rpm1r synthesis and reduced but did not eliminate precursors. The accumulation of higher-molecular-weight transcripts is a

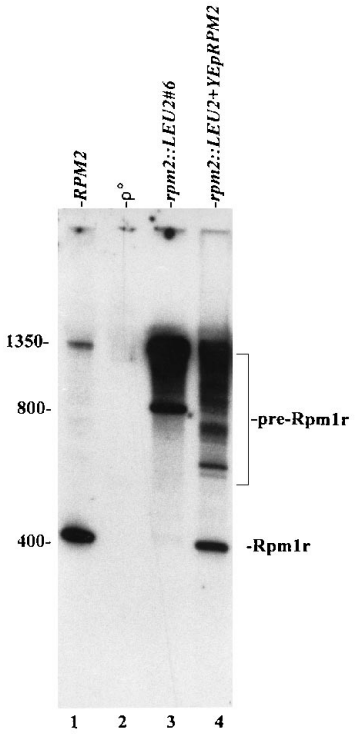

FIG. 1. Disruption of *RPM2* leads to accumulation of high-molecular-weight Rpm1r transcripts. Total cell RNAs were separated on a 6% polyacrylamide denaturing gel, transferred to a nylon membrane, and probed with the Rpm1r-specific riboprobe as described in Materials and Methods. Lane 1, RNA from wild-type cells; lane 2, RNA from [*rho*^{0}] cells; lane 3, RNA from *rpm2*::*LEU2#6* cells; lane 4, RNA from *rpm2*::*LEU2#6* cells transformed with YEp352/*RPM2*. Numbers at the left indicate the length of RNAs (in nucleotides) as determined by migration relative to size standards. Pre-Rpm1r and Rpm1r mark the positions of migration of precursors to Rpm1r and mature Rpm1r, respectively.

characteristic all petite mutants share compared with wild-type cells. Transformation with plasmid alone does not restore synthesis of mature Rpm1r (data not shown). In addition to the accumulation of discrete higher-molecular-weight RNAs, a background smear which becomes more pronounced with longer exposure is observed. Presumably, nuclease attack of the longer RNAs yields fragments with ragged ends that are detected as a smear. No mature Rpm1r can be detected in *rpm2*::*LEU2#6*, even in autoradiograms where the signal from the fragments obscures the discrete RNAs (data not shown; see also Fig. 4, lanes 5 and 6).

A mature Rpm1r 5′ end is not made in *rpm2*::*LEU2#6*. To determine if 5′-end maturation of Rpm1r in the disruption strain was defective, the 5′ ends of transcripts from *RPM1* were mapped by primer extension (Fig. 2). A labeled oligonucleotide complementary to Rpm1r (Fig. 2A) was annealed to wild-type and *rpm2*::*LEU2#6* mitochondrial RNA and extended with Moloney murine leukemia virus reverse transcriptase. The extension products were separated on a 6% denaturing polyacrylamide gel and sized by comparison with denatured DNA molecular weight standards (Fig. 2B) and a DNA sequence generated with the same oligonucleotide (Fig. 2C and D). RNA from [*rho*^{0}] cells did not yield extension products; however, as with other samples some label was retained in the well (Fig. 2B, lane 1; Fig. 2D, lane 5). The most abundant wild-type RNA termination product was the cDNA labeled 5′ end (Fig. 2B, lane 2) resulting from the mature 5′ end of Rpm1r (schematic in Fig. 2A). When this extension product was compared with a DNA sequencing ladder (Fig. 2C, lane 6), a doublet of 122 and 123 nucleotides was evident. This could have occurred if mature Rpm1r has ragged 5′ ends or reverse transcriptase added a nontemplated nucleotide. As we show below, doublets can arise from transcripts with unique ends, showing that the addition of nontemplated nucleotides can occur. No corresponding extension products were made from *rpm2*::*LEU2#6* RNA (Fig. 2B, lane 2; Fig. 2C, lane 7). Thus, 5′ processing is defective in this mutant and 5′ maturation does not occur.

Transcripts other than mature Rpm1r have the same 5′ ends in the wild type and *rpm2*::*LEU2#6*. To determine if there were other processing defects, [*rho*$^+$] and [*rho*$^-$] RNAs were compared. The extension products obtained from both RNAs were SP cDNA, 263 nucleotides; the 3′ Metf, 359 nucleotides; the anticodon loop, 395 nucleotides; and FP, 460 nucleotides (Fig. 2D, lanes 5 and 7). However, wild-type RNA produced fewer anticodon loop and FP cDNAs (Fig. 2B, lane 2). The 460-nucleotide FP product appears to extend to the promoter upstream of the $tRNA_f^{Met}$ gene. To improve the resolution of this product, another primer extension with a labeled oligonucleotide complementary to the 3′ end of the $tRNA_f^{Met}$ was performed. A 111-nucleotide DNA is predicted, because transcription initiates at the last A in the FP promoter sequence, TATAAGTA (9, 20). The extension product resolved into a triplet (Fig. 2E, lane 5) with the least abundant end at the last A and the other two ends at the preceding T and G, again suggesting that the reverse transcriptase adds nontemplated nucleotides. Since transcripts from this promoter are capped in both wild-type (9) and petite (20) cells, it is clear that transcription of *RPM1* initiates from the FP promoter.

The 395-nucleotide product extends to a GA dinucleotide in the anticodon loop of the $tRNA_f^{Met}$. This site could be sensitive to nuclease attack (Fig. 2D, lane 5 and 7). The 5′ end of the 359-nucleotide product is likely formed by cleavage at the 3′ end of $tRNA_f^{Met}$ by an endonuclease preparing it for the addition of CCA (7). Since termination products ranging in size from 310 to 340 nucleotides which fall at the GC-rich sequence (Fig. 2D, lanes 5 and 7) were not found in cDNAs made from a strain missing the GC cluster (data not shown), these represent premature termination. As judged from these extension reactions, the amounts of the longer products are underestimated relative to the 122- and 123-nucleotide doublet and the 263-nucleotide products.

The 263-nucleotide extension product from wild-type and *rpm2*::*LEU2#6* RNA could have resulted from processing or from an independent primary transcript initiating between the $tRNA_f^{Met}$ and *RPM1* gene. The latter possibility is supported by the sequence TATAAGAA between these two genes serving as an in vitro promoter (2, 3). The majority of the extension product fell at the G of the proposed promoter (Fig. 2D, lanes 5 and 7). Minor products stopped at the A flanking the G.

Extension products longer than those falling at the FP promoter were observed only with mutant *rpm2*::*LEU2#6*. These transcription events must arise upstream of the FP promoter with RNA synthesis continuing through it. Since these transcripts were not detected in wild-type cells, they have not been characterized.

A mature Rpm1r 3′ end is not made in *rpm2*::*LEU2#6*. To determine if 3′-end maturation occurred, S1 mapping was used. A 198-bp fragment, extending from the *Hin*fI site at nucleotide 320 (numbering from the 5′ end of mature Rpm1r) to the *Hpa*II site downstream of *RPM1*, was end labeled and strand separated, and the complementary strand was hybridized to mitochondrial RNA from wild-type and *rpm2*::*LEU2#6* cells. The position of the probe relative to the 3′ end and a

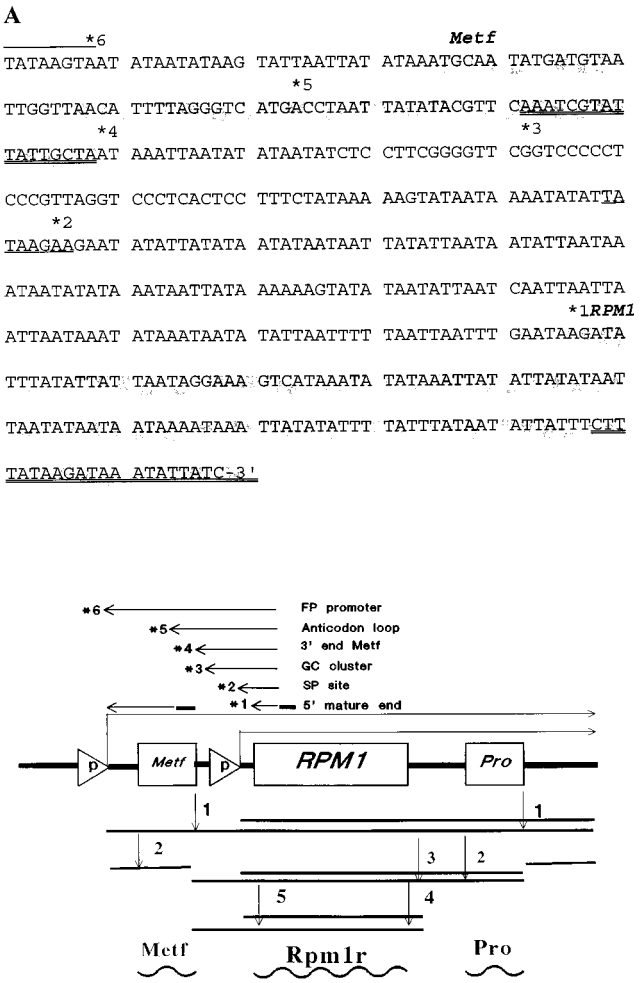

FIG. 2. Mapping of *RPM1* transcript 5′ termini. RNA 5′ ends were determined by extending a primer complementary to a sequence in Rpm1r (B to D) or in $tRNA_f^{Met}$ (E). Primers are shown as dark blocks above the $tRNA_f^{Met}$ and *RPM1* genes in the schematic in panel A. (A) Sequence of the region with ends of extension products indicated. Shown are the DNA sequence (noncoding strand) of the $tRNA_f^{Met}$ gene and a portion of *RPM1* (represented by shaded nucleotides) from W3031A. The FP promoter sequence is overlined, and the SP site is underlined. Double-underlined nucleotides correspond to the complementary sites of oligonucleotides used in reverse transcription reactions. The sequence is from nucleotide 31 to 468. Numbered asterisks above the sequence indicate the last nucleotide of extension products numbered *1 through *6 and named in the schematic diagram. *3 is placed in the center of the sequence in which the numerous 5′ ends, produced by termination in the GC-rich cluster, occur. It is represented by a discrete line labeled *3 in the schematic diagram. Wavy lines correspond to mature $tRNA_f^{Met}$, Rpm1r, and $tRNA^{Pro}$. Transcripts which originate at consensus promoter sequences (P) are represented by narrow lines above the schematic gene map, and putative processing intermediates are represented by solid lines below the gene map. Arrows labeled with numbers correspond to processing events discussed in the text. (B) Extension products from [*rho*^{0}] (lane 1), wild-type (lane 2), and *rpm2*::*LEU2#6* (lane 3) RNA separated on a 6% denaturing gel. Numbers indicate the length (in nucleotides) of single-stranded DNA size standards. (C) A dideoxy sequencing ladder (lanes 1 to 4) and extension products from [*rho*^{0}] (lane 5), wild-type (lane 6), and *rpm2*::*LEU2#6* (lane 7) RNAs were allowed to migrate for 3 h to resolve transcripts arising from the mature 5′ end. (D) Dideoxy sequencing ladders (lanes 1 to 4 and 8 to 11) and extension products from wild-type (lane 5), [*rho*^{0}] (lane 6), and *rpm2*::*LEU2#6* (lane 7) RNAs were allowed to migrate 6 h to resolve transcripts arising from high-molecular-weight *RPM1* transcripts. (E) Shown are $tRNA_f^{Met}$ extension products from *rpm2*::*LEU2#6* cells (lane 5) produced with an oligonucleotide complementary to the 3′ end of $tRNA_f^{Met}$ (A) separated on a sequencing gel and compared to a dideoxy sequencing ladder (lanes 1 to 4) generated with the same primer. See Results for details.

schematic diagram of the products are shown in Fig. 3A. The reaction products were analyzed on sequencing gels and sized against a chemical sequencing ladder of the same fragment (Fig. 3B, lanes 1 to 4). *E. coli* tRNA was used in control reactions, and no protection was observed (data not shown). The temperature of digestion was critical for protection in these experiments. No specific protection was observed when samples were digested at 37°C (data not shown), while digestion at 17°C yielded clear results. The fragment protected by wild-type RNA is labeled "3′ end" (Fig. 3B, lane 6). This 100-nucleotide fragment marked the mature 3′ end of Rpm1r and together with the established 5′ end makes the wild-type RNA 418 or 419 nucleotides long. No corresponding reaction product was obtained from *rpm2*::*LEU2#6* (Fig. 3B, lane 7); the vast majority of the protected fragment is the full-length probe. Even in this exposure, in which the signals from the full-length probe in lanes 5 and 7 extend into lane 6, little full-length probe remained in the wild-type RNA sample (Fig. 3B, lane 6). These results demonstrate that the 3′ end of

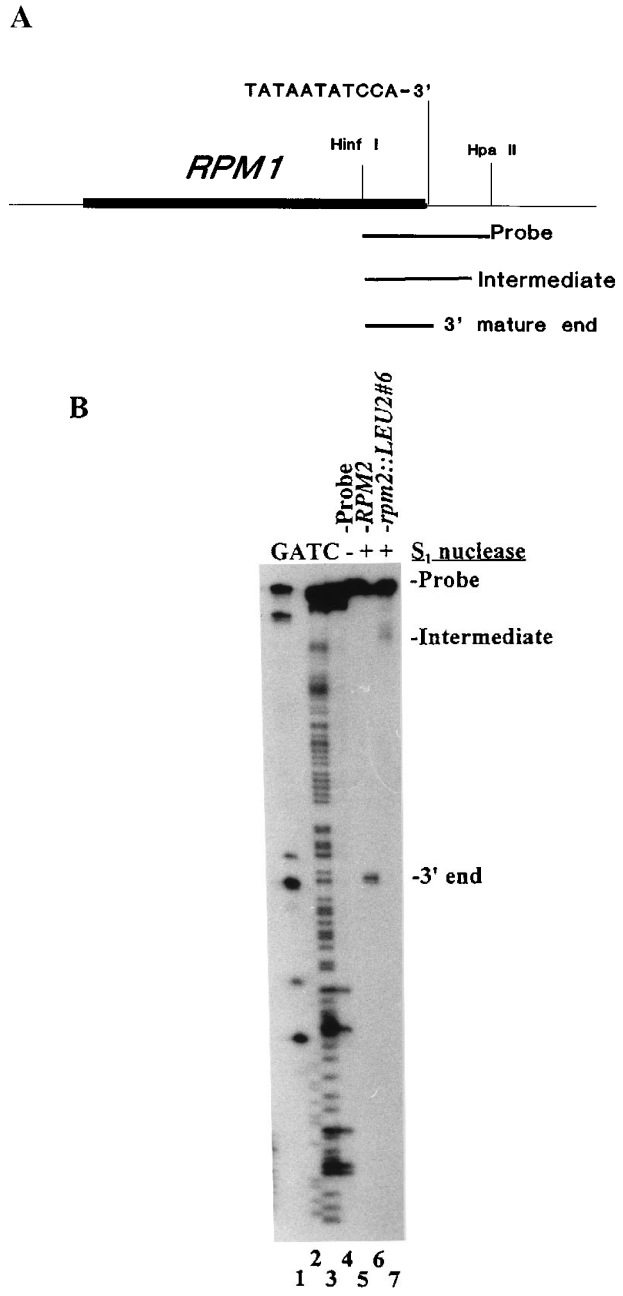

FIG. 3. Mapping of Rpm1r 3′ termini. A 198-nucleotide single-stranded DNA probe was hybridized with mitochondrial RNA. The samples were digested with S1 nuclease, and protected fragments were sized by comparison to a sequencing ladder. (A) Schematic illustration of the probe used in this experiment and products observed. *Hpa*II and *Hin*fI represent the 5′ and 3′ end of the DNA probe, respectively. (B) Maxam-Gilbert sequencing ladders (lanes 1 to 4), probe alone (lane 5), probe plus S1 nuclease in the presence of wild-type (lane 6), and *rpm2*::*LEU2#6* RNA (lane 7). 3′ end represents the mature 3′ end of Rpm1r; Intermediate marks a putative processing intermediate.

Rpm1r transcripts in *rpm2*::*LEU2#6* was extended relative to the wild-type mature 3′ end and that most of the transcripts in the mutant extend past the end of the probe. There is a signal present in *rpm2*::*LEU2#6* (Fig. 3B, lane 7 [Intermediate]) which could have arisen from RNA extended 80 nucleotides downstream of the mature 3′ end of Rpm1r. When these results are considered with the 5′ ends determined for *rpm2*::*LEU2#6* (Fig. 2), RNAs of about 640 and 740 nucleotides are predicted. These discrete products were detected by Northern analysis (Fig. 1 and 4). Interestingly, there appears to be more of the latter in *rpm2*::*LEU2#6* cells and more of the former in *rpm2*::*LEU2#6* transformed with *RPM2* (Fig. 1, lanes 3 and 4).

Transcripts which accumulate in *rpm2*::*LEU2#6* are also present as low-abundance RNAs in wild-type cells. It is clear from the above-described experiments that the maturation of Rpm1r is compromised in *rpm2*::*LEU2#6*. However, primer extensions and S1 analyses cannot establish the identity of the RNAs that do accumulate. To characterize transcripts from the three genes located in this region of mitochondrial DNA, Northern analysis using specific RNA probes was carried out (Fig. 4). With the $tRNA_f^{Met}$ probe, the mature tRNA was detected in wild-type RNA (Fig. 4, lane 1). In *rpm2*::*LEU2#6* RNA, the major RNA detected corresponded in size to the previously characterized pre-$tRNA_f^{Met}$ (Fig. 4, lane 2), which contains a 28-nucleotide 5′ leader and a fully processed 3′ end (20). No longer transcripts were detected with this tRNA probe. Since most of the $tRNA_f^{Met}$ was the pre-tRNA and was separated from the higher-molecular-weight RNA, the 5′ extension on the $tRNA_f^{Met}$ did not inhibit separation of the tRNA from downstream sequence. However, by primer extension it is clear that there are cotranscripts containing $tRNA_f^{Met}$ and Rpm1r (Fig. 2).

Hybridization of a duplicate filter with a probe complementary to mature Rpm1r again identified the major wild-type RNA to be the mature Rpm1r (Fig. 4, lane 5). In contrast, the major RNAs in *rpm2*::*LEU2#6* samples (Fig. 4, lane 6) were much longer than mature Rpm1r, although their identity was obscured by the intensity of the signal (also Fig. 1). With a shorter exposure, the two major RNAs in *rpm2*::*LEU2#6* are

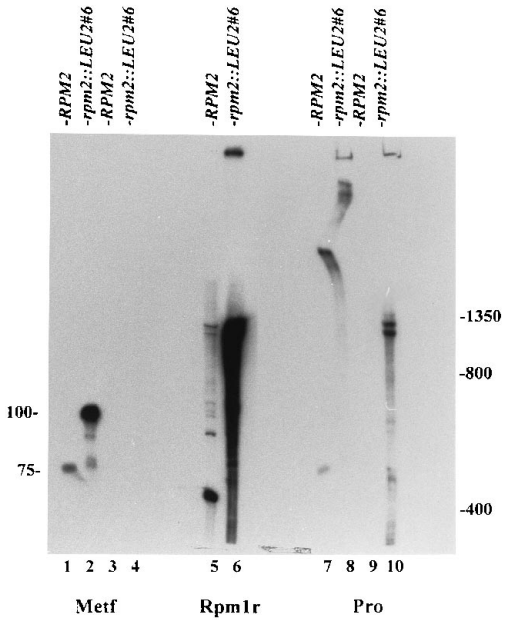

FIG. 4. Rpm1r is transcribed with both $tRNA_f^{Met}$ and $tRNA^{Pro}$. Northern analysis of wild-type (odd-numbered lanes) and *rpm2*::*LEU2#6* (even-numbered lanes) mitochondrial RNAs. RNAs in lanes 1, 2, 7, and 8 were run for a shorter time than those in lanes 3 to 6, 9, and 10 to resolve small RNAs on a 4% polyacrylamide denaturing gel. Numbers at the left indicate the length of RNAs (in nucleotides) as determined by their migration relative to size standards. Lanes 3 to 6, 9, and 10 resolve large RNAs, and numbers at the right indicate the length of these longer RNAs (in nucleotides) as determined by their migration relative to size standards. Shown are $tRNA_f^{Met}$ probe (lanes 1 to 4), an Rpm1r probe (lanes 5 and 6), and a $tRNA^{Pro}$ probe (lanes 7 to 10).

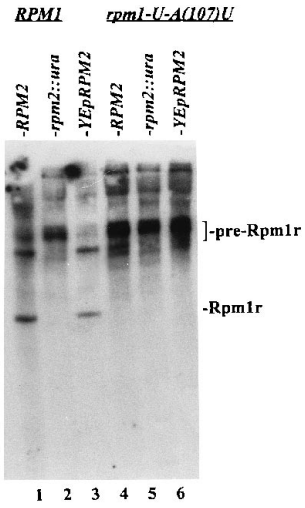

FIG. 5. Northern analysis of Rpm1r transcripts in synthetic petite mutants lacking a 3′-flanking tRNA. Synthetic petite mutants with a wild-type *RPM1* gene (lanes 1 to 3) containing wild-type *RPM2* (lane 1), an *rpm2*::*URA3* disruption (lane 2), and wild-type *RPM2* on the multicopy plasmid YEp352 are shown. Synthetic petite mutants with the *rpm1-U-A(107)U* gene (lanes 4 to 6) containing wild-type *RPM2* (lane 1), an *rpm2*::*URA3* disruption (lane 2), and wild-type *RPM2* on the multicopy plasmid YEp352 are also shown. The Rpm1r riboprobe was used to identify the positions of migration of precursors to Rpm1r (pre-Rpm1r) and mature Rpm1r (Rpm1r), respectively.

the same as two RNAs of approximately 1,300 nucleotides found in wild-type cells and the same as the RNAs detected with the $tRNA^{Pro}$ probe (Fig. 4, lane 10).

A triplicate filter was probed with an oligonucleotide complementary to $tRNA^{Pro}$ (Fig. 4, lanes 7 to 10). In wild-type RNA at this exposure, only a faint signal was seen for mature $tRNA^{Pro}$ (Fig. 4, lane 7) and precursors were not evident (Fig. 4, lane 9). However, in longer exposures of wild-type RNA and in shorter exposures with *rpm2*::*LEU2#6* RNA, longer RNAs are seen (Fig. 4, lane 10). These longer RNAs are the same size as those detected with the Rpm1r probe in lanes 5 and 6. They must still have $tRNA^{Pro}$ at their 3′ ends, and their 5′ ends include *RPM1* transcripts but not $tRNA_f^{Met}$ transcripts. They differ in size by 100 nucleotides, as predicted from the primer extension experiments for the case in which one end falls at the 3′ end of the $tRNA_f^{Met}$ and the other falls at the SP promoter. The sizes of these two transcripts suggest that the $tRNA^{Pro}$ contained within them is processed at its 3′ end. As in the processing at the 3′ end of $tRNA_f^{Met}$, 3′ end processing at or near the 3′ end of $tRNA^{Pro}$ occurs quickly relative to other processing events and is not inhibited by 5′ extension of $tRNA^{Pro}$. Transcription continues 500 to 600 nucleotides past the $tRNA^{Pro}$ gene, since a downstream probe detected RNA (data not shown). No discrete pre-$tRNA^{Pro}$s other than the 1,200- and 1,300-nucleotide RNAs were detected in *rpm2*::*LEU2#6* RNA, although $tRNA^{Pro}$-containing transcripts would be predicted to arise from the cleavage 80 nucleotides downstream of the 3′ end of mature Rpm1r. If transcripts with $tRNA^{Pro}$ were not substrates for this cleavage, only those transcripts without $tRNA^{Pro}$ would be cleaved in *rpm2*::*LEU2#6*. Since some random degradation of long RNAs occurred, some $tRNA^{Pro}$ might be separated from the long transcripts nonspecifically. Those RNAs could then become substrates for the nuclease that cleaves at the 80-nucleotide-downstream site. An inability to process Rpm1r because $tRNA^{Pro}$ could not be removed from the 1,200- and 1,300-nucleotide transcripts in *rpm2*::*LEU2#6* could lead indirectly to the lack of mature 3′ ends in RNA from the disruption strain.

The defect in Rpm1r maturation is independent of flanking tRNAs. To determine whether accumulation of longer Rpm1r RNAs could be due to retention of the $tRNA^{Pro}$ sequences, we took advantage of a synthetic petite mutant created previously (34). In the previous study, wild-type or mutant *RPM1* genes, cloned into a plasmid with a reporter $tRNA^{Asp}$ and a selectable marker, were introduced into yeast mitochondria by biolistic transformation. The $tRNA^{Asp}$ gene is upstream of *RPM1*, and no tRNA is present downstream (34). Figure 5 shows a Northern blot containing RNA samples from this synthetic petite mutant with a wild-type *RPM1* gene that supports the synthesis of mature Rpm1r (lane 1). Like other petite mutants, this strain accumulates higher-molecular-weight transcripts as well as mature RNAs. Since the sequences flanking *RPM1* are different from those flanking *rpm2*::*LEU2#6*, the pattern of transcripts is expected to be different. When this petite mutant is engineered to contain an *RPM2* disruption at the *Hpa*I site, the mature Rpm1r disappears and higher-molecular-weight precursor RNAs accumulate (lane 2). Regardless of whether Rpm1r is flanked by tRNAs, the Rpm1r synthesis defect remains. Lane 3 contains RNA from the same petite mutant transformed with *RPM2* on a multicopy plasmid. The additional copies of *RPM2* had no significant effect on the transcripts (compare lanes 1 and 3).

Mutations in either *RPM1* or *RPM2* lead to the accumulation of higher-molecular-weight RNAs. The Rpm1r RNA processing defect observed in the *RPM2* disruption mutants resembled an RNA processing defect we had observed in *rpm1* mutants (34). To determine whether the accumulated RNAs in both types of mutants were similar, an *RPM2* disruption was created in an *rpm1* mutant. RNA obtained from a strain with a wild-type *RPM2* gene and that from the disruption gene were compared. RNA from the *RPM1* mutant *rpm1-U→A(107)ΔU (113)* (34) contained a mutation predicted to interrupt a long-range interaction between two sequences conserved in all RNase P RNAs (Fig. 5, lanes 4 to 6). No mature Rpm1r was detected in this strain (34). The pattern of transcripts was similar to those obtained when *RPM2* was disrupted (lane 5). Additional copies of *RPM2* did not alter the transcript pattern in the original mutant strain (lane 6). In contrast to the result obtained with the synthetic petite mutant carrying the wild-type *RPM1* gene, the status of the *RPM2* gene did not significantly affect the RNA transcripts from the mutant strain. An identical result (data not shown) was observed with the second mutant described by Sulo et al. (34).

DISCUSSION

Rpm2p has previously been reported to be a subunit of mitochondrial RNase P (10). Further work with the *RPM2* gene has revealed it to be multifunctional. Rpm2p has an unknown function essential for normal cell growth (15) and as shown here is required for the maturation of the RNase P RNA subunit of the enzyme, Rpm1r. In prokaryotes, the RNA is the catalytic subunit of the enzyme, with the protein playing a supporting role (12). The accumulation of precursor Rpm1r and the lack of mature Rpm1r in the disruption strain raised the possibility that the precursor tRNA accumulation phenotype (24) was due not to defective Rpm2p but rather to the inability to synthesize a mature RNA subunit which accompanies alteration of Rpm2p. This scenario would require that precursor Rpm1r accumulating in the absence of Rpm2p be inactive. While prokaryotic precursor RNase P RNAs are ac-

tive in vitro (5, 31), no test of their ability to function in vivo has been done.

One potential explanation for Rpm1r biosynthesis being perturbed in *rpm2* mutants was that the assignment of Rpm2p as a subunit of yeast mitochondrial RNase P was erroneous and it really is an RNA-processing enzyme necessary for the synthesis of Rpm1r. Indeed, Rpm2p and prokaryotic RNase P protein subunits have no sequence similarity and Rpm2p is substantially larger (10). Rpm2p is not similar to Pop1p, the only eukaryotic protein known to be required for *S. cerevisiae* nuclear RNase P activity (18). However, Rpm2p is the most abundant protein to purify with mitochondrial RNase P activity (25) and anti-TrpE-Rpm2p precipitates both Rpm1r and RNase P activity in mitochondrial extracts (10). Thus, Rpm2p has the characteristics of a bona fide subunit as opposed to an RNA-processing enzyme predicted to be transiently associated with Rpm1r precursors.

That Rpm1r transcripts and $tRNA^{Pro}$ transcripts remain associated in the absence of RNase P activity raised a second possible explanation for the altered maturation of Rpm1r. If processing of the polycistronic transcript were carried out in an obligatory order, the inability to remove the $tRNA^{Pro}$ at the proper time might compromise all further processing steps and prevent the synthesis of mature Rpm1r. This possibility was addressed directly by repeating the disruption in a different petite mutant which did not contain the $tRNA^{Pro}$ downstream of *RPM1*. Nonetheless, Rpm1r processing was disrupted and no mature Rpm1r was made.

Another explanation consistent with our results is that Rpm2p is both a subunit of the enzyme, carrying out a supporting role analogous to the prokaryotic proteins, and an RNA-processing enzyme required for the biosynthesis of Rpm1r. There are several scenarios that could lead to the accumulation of precursor with both 5′ and 3′ extensions. First of all, the RNase P activity per se could be required for maturation of one or both ends of Rpm1r. However, previous work has demonstrated that preexisting RNase P activity was not required to make mature Rpm1r RNA (34), and our efforts to process a 5′ or 3′ extended precursor in vitro by using active RNase P have failed (data not shown). Another scenario is that one or more additional nuclease activities mature the two ends of Rpm1r and are encoded by RPM2 but function independently of RNase P activity. Alternatively, one processing step could depend on Rpm2p and the other could be carried out by a different enzyme, with processing at one end being obligatory to processing at the other end. A third possibility is that Rpm2p does not itself support any nuclease activity other than RNase P and that its role in the biosynthesis of Rpm1r is as an RNA chaperone (13). If Rpm2p association with precursor Rpm1r were required for the precursor to attain a conformation required for processing, mutations in Rpm2p that inhibit this association would lead to the accumulation of precursor Rpm1r. If mutations in *RPM1* result in defective interaction of Rpm2p with the mutant RNA, subsequent processing of Rpm1r precursors would not occur. A similar mechanism has been proposed to explain the accumulation of precursors to nuclear RNase P RNA in strains in which the protein and RNA subunits are not compatible (32). The accumulation of rRNA precursors is known to occur, for example, in the absence of some ribosomal proteins (37). It is also possible that the COOH end of Rpm2p may serve to recruit other processing enzymes to the RNA. In the truncation strain, this recruitment could be compromised.

Rpm1r varies in size in different yeasts (38) as well as in different strains of *S. cerevisiae* (22), and the gene is usually located between the $tRNA_f^{Met}$ and $tRNA^{Pro}$ genes (Fig. 2A). The mature RNA is derived from a longer transcript. Our previous characterization of the 5′ end of Rpm1r from D273-10B by primer extension indicated two 5′ ends, terminating with a G and differing by 6 nucleotides (22). The mitochondrial DNA in W3031A was derived by cytoduction transfer of mitochondrial DNA from D273-10B (28). The mature 5′ end of Rpm1r in the strain used here should be the same. The 5′ end matches the most abundant 5′ end found in the previous studies. The 3′ end of mature RNA includes the 3′ conserved sequence element and the mature Rpm1r is 418 or 419 nucleotides long. Northern analyses revealed several RNA transcripts from the *RPM1* gene that are longer than the mature RNAs (19, 22), but their identities were never established. The disruption of *RPM2* led to the accumulation of longer transcripts containing Rpm1r. Maturation of both the 5′ and 3′ ends is blocked completely.

It is less clear that all accumulation of high-molecular-weight transcripts from *RPM1* is due to the lack of Rpm2p per se. Petite deletion mutants are known to accumulate many more precursor RNAs than do wild-type cells, and *rpm2*::*LEU2#6* is a petite mutant. One approach to address this issue would be to determine if a decrease in *RPM2* expression correlated with an increase in precursor accumulation. However, the rate of production of petite mutants under these conditions is not known, and it would be difficult to ascertain which transcripts arise from remaining wild-type genomes and which arise from newly created petite mutant genomes. An alternate approach would be to seek conditions under which the maintenance of a wild-type genome can be forced to occur in the disruption strain. Preliminary results with this latter approach show promise and will be reported elsewhere.

While the long RNAs in this region could arise either from multiple sites of transcription or from RNA processing events, a consensus sequence for transcription initiation in yeast mitochondria is known (30). One consensus sequence lies 28 nucleotides upstream from the $tRNA_f^{Met}$ (20), while a second sequence, which deviates slightly, lies between the $tRNA_f^{Met}$ and *RPM1* genes (2). The former (Fig. 2A) was shown by Christianson and Rabinowitz (9) to function as a promoter sequence in vivo. Of the 17 promoters identified, only 1 was located in the region of interest, leading to the proposal that $tRNA_f^{Met}$, Rpm1r, and $tRNA^{Pro}$ were derived from the same primary transcript with subsequent RNA processing to yield the three mature RNAs. This transcript has never been identified by Northern blot with probes specific for $tRNA_f^{Met}$, Rpm1r, and $tRNA^{Pro}$. RNAs hybridizing with both Rpm1r and $tRNA^{Pro}$ probes were found, but no RNAs which also hybridized to $tRNA_f^{Met}$ were detected (19, 22). Despite the accumulation of putative precursors in *rpm2*::*LEU2#6*, no transcripts containing all three RNAs were detected by Northern analysis. However, primer extension experiments with the wild type and *rpm2*::*LEU2#6* reveal RNAs containing both Rpm1r and $tRNA_f^{Met}$ that extend to the promoter sequence upstream of $tRNA_f^{Met}$ (9). Thus, $tRNA_f^{Met}$ and Rpm1r are transcribed together. Another extension product falls precisely at the 3′ end of the $tRNA_f^{Met}$, indicating a separation of the tRNA from downstream transcripts by a previously identified endonuclease (7) that cleaves at the 3′ end of the tRNA (step 1 in Fig. 2A).

An additional sequence, called the SP promoter, does function in vitro as a promoter (2, 3). If it is a promoter in vivo, transcripts initiating from the FP site extend through the SP promoter into the downstream genes (Fig. 5). Thus, transcripts initiating from upstream promoters are not always terminated before the polymerase reaches a downstream promoter and transcription units overlap in yeast mitochondrial DNA. In

fact, there is no clear evidence for transcription termination signals in yeast mitochondria. Evidence for similar overlapping transcription units has also been demonstrated with *Neurospora* mitochondria (17).

Regardless of the sites of initiation of transcription, maturation of both 5′ and 3′ ends of Rpm1r occurs and neither step happens in *rpm2*::*LEU2#6* (steps 5 and 4 in Fig. 2A). The most abundant precursor that accumulates in the absence of full-length Rpm2p has an extended 5′ end and still contains $tRNA^{Pro}$ sequences at its 3′ end. Its size is consistent with a 3′ end falling exactly at the 3′ end of the $tRNA^{Pro}$. Although transcription does continue downstream of the $tRNA^{Pro}$, we infer that the 3′ end of $tRNA^{Pro}$ is formed by the 3′ endonuclease (7). Another less abundant *RPM1* transcript, extended about 80 nucleotides downstream of the mature 3′ end of Rpm1r, was also found in *rpm2*::*LEU2#6* (Fig. 3). After longer exposure the same end was also detected in wild-type cells (data not shown). Perhaps maturation of the 3′ end of Rpm1r is a two-step process (steps 3 and 4 in Fig. 5) in which the latter process cannot proceed in the absence of full-length Rpm2p.

Establishing a role of Rpm2p in the biosynthesis of Rpm1r and characterizing RNA transcription and processing in this region of mitochondrial DNA have raised new questions as to the role of Rpm2p. Further work will be required to determine if Rpm2p plays a direct role as a nuclease or a more indirect role as a chaperone in the biosynthesis of Rpm1r. Since the absence of an intact *RPM2* gene in *rpm2*::*LEU2#6* precludes the synthesis of mature Rpm1r, we cannot be sure whether the lack of mature Rpm1r or the absence of full-length Rpm2p or both lead to compromised mitochondrial RNase P activity. To differentiate between these possibilities, genes that can make mature Rpm1r must be synthesized, introduced into mitochondria, and function or reconstitution of an active enzyme in vitro must be achieved so that different RNAs can be tested for their ability to support RNase P activity.

ACKNOWLEDGMENT

This work was supported by NIH grant GM27597.

REFERENCES

1. **Attardi, G., and G. Schatz.** 1988. Biogenesis of mitochondria. Annu. Rev. Cell Biol. **4:**289–333.
2. **Biswas, T. K.** 1991. *In vitro* transcription analysis of the region of *Saccharomyces cerevisiae* mitochondrial DNA containing the $tRNA_f^{Met}$ gene. Nucleic Acids Res. **19:**5937–5942.
3. **Biswas, T. K.** 1996. Expression of mitochondrial RNase P RNA subunit-encoding gene from a variant promoter sequence in *Saccharomyces cerevisiae*. Gene **170:**23–30.
4. **Biswas, T. K., and G. S. Getz.** 1986. Nucleotides flanking the promoter sequence influence the transcription of the yeast mitochondrial gene coding for ATPase subunit 9. Proc. Natl. Acad. Sci. USA **83:**270–274.
5. **Brown, J. W., E. S. Haas, B. D. James, D. A. Hunt, and N. R. Pace.** 1991. Phylogenetic analysis and evolution of RNase P RNA in proteobacteria. J. Bacteriol. **173:**3855–3863.
6. **Brown, J. W., and N. R. Pace.** 1992. Ribonuclease P RNA and protein subunits from bacteria. Nucleic Acids Res. **20:**1451–1456.
7. **Chen, J.-Y., and N. C. Martin.** 1988. Biosynthesis of tRNA in yeast mitochondria. J. Biol. Chem. **263:**13677–13682.
8. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
9. **Christianson, T., and M. Rabinowitz.** 1983. Identification of multiple transcriptional initiation sites on the yeast mitochondrial genome by *in vitro* capping with guanylyltransferase. J. Biol. Chem. **258:**14025–14033.
10. **Dang, Y. L., and N. C. Martin.** 1993. Yeast mitochondrial RNase P. Sequence of the *RPM2* gene and demonstration that its product is a protein subunit of the enzyme. J. Biol. Chem. **269:**19791–19796.
11. **Dieckmann, C. L., and R. R. Staples.** 1994. Regulation of mitochondrial gene expression in *Saccharomyces cerevisiae*. Int. Rev. Cytol. **152:**145–181.
12. **Guerrier-Takada, C., and S. Altman.** 1984. Catalytic activity of an RNA molecule prepared by transcription *in vitro*. Science **233:**285–286.
13. **Herschlag, D.** 1995. RNA chaperones and the RNA folding problem. J. Biol. Chem. **270:**20871–20874.
14. **Hollingsworth, M. J., and N. C. Martin.** 1986. RNase P activity in the mitochondria of *Saccharomyces cerevisiae* depends on both mitochondrial and nucleus-encoded components. Mol. Cell. Biol. **6:**1058–1064.
15. **Kassenbrock, C. K., G.-J. Gao, K. R. Groom, P. Sulo, M. D. Douglas, and N. C. Martin.** 1995. *RPM2*, independent of its mitochondrial RNase P function, suppresses an ISP42 mutant defective in mitochondrial import and is essential for normal growth. Mol. Cell. Biol. **15:**4763–4770.
16. **Kohrer, K., and H. Domdey.** 1991. Preparation of high molecular weight RNA. Methods Enzymol. **194:**398–405.
17. **Kubelik, A. R., J. C. Kennell, R. A. Akins, and A. M. Lambowitz.** 1990. Identification of *Neurospora* mitochondrial promoters and analysis of synthesis of the mitochondrial small rRNA in wild-type and the promoter mutant [*poky*]. J. Biol. Chem. **265:**4515–4526.
18. **Lygerou, Z., P. Mitchell, E. Petfalski, B. Seraphin, and D. Tollervey.** 1994. The *POP1* gene encodes a protein component common to the RNase MRP and RNase P ribonucleoproteins. Genes Dev. **8:**1423–1433.
19. **Martin, N. C., M. J. Hollingsworth, H.-H. Shu, and D. R. Najarian.** 1985. Expression and function of the tRNA synthesis locus in *Saccharomyces cerevisiae*, p. 193–202. *In* E. Quagliariello et al. (ed.), Achievements and perspectives of mitochondrial research, vol. II. Biogenesis. Elsevier Science Publishers, Amsterdam.
20. **Martin, N. C., D. L. Miller, K. Underbrink, and X. Ming.** 1985. Structure of a precursor to the yeast mitochondrial $tRNA^{fMet}$. Implications for the function of the tRNA synthesis locus. J. Biol. Chem. **260:**1479–1483.
21. **Martin, N. C., and K. Underbrink-Lyon.** 1981. A mitochondrial locus is necessary for the synthesis of mitochondrial tRNA in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **78:**4743–4747.
22. **Miller, D. L., J. L. Krupp, H.-H. Shu, and N. C. Martin.** 1985. Polymorphism in the structure of the yeast mitochondrial tRNA synthesis focus. Nucleic Acids Res. **13:**859–872.
23. **Miller, D. L., and N. C. Martin.** 1983. Characterization of the yeast mitochondrial locus necessary for tRNA biosynthesis: DNA sequence analysis and identification of a new transcript. Cell **34:**911–917.
24. **Morales, M. J., Y. L. Dang, Y. C. Lou, P. Sulo, and N. C. Martin.** 1992. A 105-kDa protein is required for yeast mitochondrial RNase P activity. Proc. Natl. Acad. Sci. USA **89:**9875–9879.
25. **Morales, M. J., C. A. Wise, M. J. Holingsworth, and N. C. Martin.** 1989. Characterization of yeast mitochondrial RNase P: an intact RNA subunit is not essential for activity *in vitro*. Nucleic Acids Res. **17:**6865–6881.
26. **Mueller, D. M., and G. S. Getz.** 1986. Transcriptional regulation of the mitochondrial genome of yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **261:**11756–11764.
27. **Mueller, D. M., and G. S. Getz.** 1986. Steady state analysis of mitochondrial RNA after growth of yeast *Saccharomyces cerevisiae* under catabolite repression and derepression. J. Biol. Chem. **261:**11816–11822.
28. **Myers, A. M., L. K. Pape, and A. Tzagoloff.** 1985. Mitochondrial protein synthesis is required for maintenance of intact mitochondrial genomes in *Saccharomyces cerevisiae*. EMBO J. **4:**2087–2092.
29. **Osinga, K. A., E. De Vries, G. Van der Horst, and H. F. Tabak.** 1984. Processing of yeast mitochondrial messenger RNAs at a conserved dodecamer sequence. EMBO J. **3:**829–834.
30. **Osinga, K. A., and H. F. Tabak.** 1982. Initiation of transcription of genes for mitochondrial ribosomal RNA in yeast: comparison of the nucleotide sequence around the 5′-ends of both genes reveals a homologous stretch of 17 nucleotides. Nucleic Acids Res. **10:**3617–3626.
31. **Pace, N. R., and D. Smith.** 1990. Ribonuclease P: function and variation. J. Biol. Chem. **265:**3587–3590.
32. **Pagan-Ramos, E., A. J. Tranguch, D. W. Kindelberger, and D. R. Engelke.** 1994. Replacement of the *Saccharomyces cerevisiae RPR1* gene with heterologous RNase P RNA genes. Nucleic Acids Res. **22:**200–207.
33. **Schinkel, A. H., and H. F. Tabak.** 1989. Mitochondrial RNA polymerase: dual role in transcription and replication. Trends Genet. **5:**149–154.
34. **Sulo, P., K. R. Groom, C. Wise, M. Steffen, and N. C. Martin.** 1994. Successful transformation of yeast mitochondria with *RPM1*: an approach for in vivo studies of mitochondrial RNase P RNA structure, function and biosynthesis. Nucleic Acids Res. **23:**856–860.
35. **Ulery, T. L., S. H. Jang, and J. A. Jaehning.** 1994. Glucose repression of yeast mitochondrial transcription: kinetics of derepression and role of nuclear genes. Mol. Cell. Biol. **14:**1160–1170.
36. **Underbrink-Lyon, K., D. L. Miller, N. A. Ross, H. Fukuhara, and N. C. Martin.** 1983. Characterization of a yeast mitochondrial locus necessary for tRNA biosynthesis. Mol. Gen. Genet. **191:**512–518.
37. **Warner, J. R.** 1989. Synthesis of ribosomes in *Saccharomyces cerevisiae*. Microbiol. Rev. **53:**256–271.
38. **Wise, C. A., and N. C. Martin.** 1991. Dramatic size variation of yeast mitochondrial RNAs suggests that RNase P RNAs can be quite small. J. Biol. Chem. **266:**19154–19157.