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RNase P RNA in *Candida glabrata* mitochondria is transcribed with substrate tRNAs

Hsiao Hsueh Shu[1] and Nancy C.Martin*
Department of Biochemistry, School of Medicine, University of Louisville, Louisville, KY 40292 and
[1]Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109-1651, USA

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ABSTRACT

The biosynthesis of some mitochondrial enzymes requires contributions of both the mitochondrial and nuclear genomes. The ribonucleoprotein enzyme Ribonuclease P (RNase P) is composed of a mitochondrial encoded RNA and nuclear coded protein in many yeasts, including *C. glabrata*. We have determined that there are at least two sites of transcription initiation that contribute to the expression of the mitochondrial RNase P RNA. A nonanucleotide promoter sequence is located upstream of the initiator tRNA while the other site of initiation of transcription is at an undetermined upstream site. An analysis of the transcripts from the region of the RNase P gene demonstrates directly that the RNase P RNA is present in large primary transcripts and located between the precursors to the initiator $tRNA_f^{Met}$ and $tRNA^{Pro}$ genes. Thus this enzyme subunit is synthesized with some of its substrate tRNAs. An activity with cleavage site specificity like a previously described endonuclease that cleaves near the 3′ end of tRNAs, RNase P activity and one or more additional endonucleases or exonucleases not described previously are required to convert the primary transcript to its final functional RNAs.

INTRODUCTION

The circular mitochondrial genome of *Candida glabrata* is 19,000 bases in length and codes for 2 ribosomal RNAs, 8 polypeptides, 23 tRNAs (6) and an RNase P RNA (15). The protein component of this mitochondrial RNase P is nuclear coded and must be synthesized in the cytoplasm and imported into the mitochondrion for assembly (15). As a first step towards understanding the assembly of this enzyme we have investigated the biosynthetic pathway of the RNA and find that it is made as part of a primary transcript that includes two substrate tRNAs.

DNA sequence analysis of the region around the RNase P gene revealed that it is flanked on the 5′ side by the $tRNA_f^{Met}$ gene and on the 3′ side by the $tRNA^{Pro}$ gene (6). Upstream of the $tRNA_f^{Met}$ gene is a nonanucleotide sequence TATAAGTAA which has been shown to serve as a promoter when found in other locations on this genome. Several primary transcripts from *C. glabrata* mitochondrial DNA have been identified and sequenced and they do initiate at the penultimate adenine of nonanucleotide sequences such as that found upstream of the $tRNA_f^{Met}$ (6). A similar element serves as a promoter in *S. cerevisiae* (3,5). No such sequence is found between the $tRNA_f^{Met}$ and RNase P gene, nor between the RNase P gene and the $tRNA^{Pro}$ gene. The proximity of these three genes and the presence of elements similar to known promoters suggested to us that these genes were transcribed together.

We demonstrate here that the RNase P RNA is initially synthesized in a polycistronic precursor RNA, containing both $tRNA_f^{Met}$ and $tRNA^{Pro}$ sequences. Separation of the $tRNA_f^{Met}$ transcript from the rest of the molecule occurs by an endonucleolytic cleavage. Maturation of the RNase P RNA and its separation from the $tRNA^{Pro}$ occurs in several steps that require activities not heretofore described. Because these processing events occur in both wild type and petite deletion strains unable to carry out mitochondrial protein synthesis, the activities responsible cannot depend on protein products of the mitochondrial genome. The 5′ leader of the $tRNA_f^{Met}$ and the $tRNA^{Pro}$ must be removed by RNase P. The nonanucleotide promoter upstream of the $tRNA_f^{Met}$ gene promotes initiation of transcription at the penultimate A in the conserved TATAA-GTAA sequence. In addition, some transcripts from this region appear to arise from an upstream promoter and continue through the TATAAGTAA promoter sequence identified here.

MATERIALS AND METHODS

Strains and culture conditions

The wild type *C. glabrata* strain CBS 138 and the derivative petite mutants are the same as used in the previous studies (15). Cells were grown in 2% galactose, 0.2% glucose, 1% bactopeptone and 1% yeast extract.

Northern hybridizations

Mitochondria were isolated and lysed as described by Miller and Martin (11). Mitochondrial RNAs were isolated and treated with

* To whom correspondence should be addressed

DNase I as described previously (15). RNAs were separated on a 1.5% agarose-6 M urea gel and transferred to Zeta-probe membrane (Bio Rad). Probes and hybridization conditions were the same as used in Shu *et al.* (15).

Primer extensions

50 μg of mitochondrial RNA were annealed at 70°C for 5 minutes with 10 pmoles of 5′ end radio-labelled oligonucleotide complementary to the $tRNA^{Pro}$ gene (5′ GCGCCTGACC-TTTTGGCTTC) and the mixture cooled to room temperature gradually over 30 minutes. Isolated precursor RNAs were annealed with an oligonucleotide complementary to nucleotides 325−349 in region II (6). Reverse transcription was performed in 100 mM NaCl, 20 mM Tris-HCl, pH 8.3, 6 mM $MgCl_2$, 20 mM DTT and 10−20 units of AMV reverse transcriptase (Life Science) at 42°C. The extension products were separated on an 8% acrylamide-8.3 M urea sequencing gel.

Capping reactions

60 μg of mitochondrial RNAs were incubated with 25−30 μM of [α-^{32}P] GTP (New England Nuclear) and 5 units of guanylyl transferase (BRL) under the conditions recommended by the manufacturer.

RNA sequencing

Capped RNAs were digested with 100 units of ribonuclease T1 at 37°C for 30 minutes. The labelled 11 nucleotide long RNA was recovered from a gel slice. pCp labeled precursor RNAs were recovered as described previously (15) but we were unable to obtain clear 3′ sequences due to some heterogeneity of the ends. To enable an identification of the most abundant 3′ ends we produced short RNAs by RNase H digestion (Boehringer Mannheim Biochemicals) directed by an oligonucleotide complementary to nucleotides 356 to 359 in region II (6). The digestion mix was separated on a 20% acrylamide−8.3 M urea sequencing gel. Two products from the 3′ end were recovered and sequenced as described previously (15).

RESULTS

The RNase P RNA gene in *C. glabrata* mitochondria is flanked by the $tRNA_f^{Met}$ and $tRNA^{Pro}$ genes. These genes, in turn, are flanked by the cytochrome b (cob) gene on the 5′ side and the cytochrome oxidase 1 (cox1) gene on the 3′ side (Fig. 1, bottom). From our previous sequencing studies of the mature RNase P RNA (15) we know that 14 nucleotides separate the 3′ end of the $tRNA_f^{Met}$ gene and the 5′ end of the mature RNase P and 19 nucleotides separate the 3′ end of the mature RNase P RNA and the 5′ end of the $tRNA^{Pro}$. A potential nonanucleotide promoter sequence is located 8 nucleotides upstream of the $tRNA_f^{Met}$ coding sequence but not elsewhere within the area. This arrangement predicts that initiation of transcription will occur upstream of the $tRNA_f^{Met}$ gene and continue through the downstream RNase P and $tRNA^{Pro}$ genes. To determine

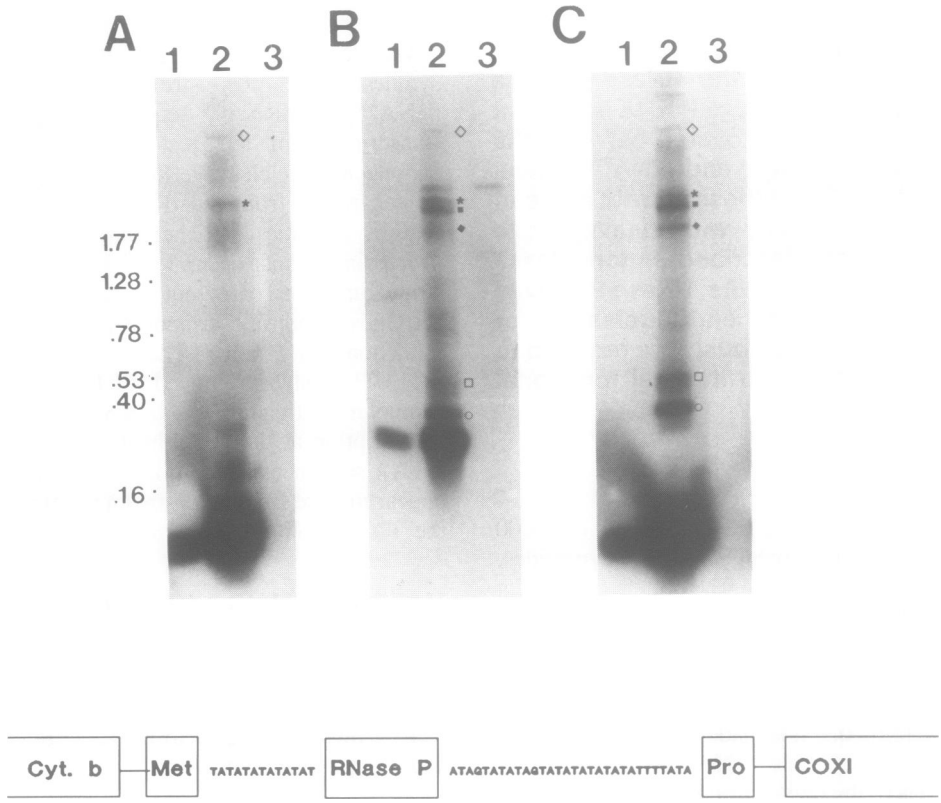

Figure 1. Large transcripts in petite 8 retain $tRNA_f^{Met}$, RNase P RNA and $tRNA^{Pro}$. Mitochondrial RNAs were separated on a 1.5% agarose-6M urea gel, transferred to Zeta-probe membrane and hybridized to radiolabelled oligonucleotide complementary to $tRNA_f^{Met}$ (Panel A), $tRNA^{Pro}$ (Panel C), or radiolabelled RNA complementary to RNase P RNA (Panel B). Lane 1, wild type mitochondrial RNA, lane 2, petite 8 mitochondrial RNA, and lane 3, petite 103 mitochondrial RNA. 5.4 kb RNA (□), 2.9 kb RNA (*), 2.5 kb RNA (■), 2.0 kb RNA (◆), 500 nucleotide RNA (◇) and 310 nucleotide RNA (○). Migration of RNA size standards are indicated at the left side of Panel A. Numbers are in kb. A schematic diagram of this region of the DNA is given below the Northerns as is the sequence between the tRNA and RNaseP coding regions.

whether the RNase P RNA is transcribed as a single unit or transcribed with other genes to form a polycistronic precursor, transcript mapping was performed. No transcripts containing all three RNAs were detected when mitochondrial RNAs from wild type cells were hybridized with probes complementary to $tRNA_f^{Met}$, RNase P and $tRNA^{Pro}$. Rather, mature tRNAs and RNA from the RNase P gene (see below) were present (Figure 1, lanes 1).

We then added RNAs from two petite deletion mutants to the analysis because previous studies with petites in *S. cerevisiae* demonstrated that petites do accumulate precursor RNAs relative to wild type strains. One petite, petite 8, contains all three genes plus partial genes for cob and cox1, while the other, petite 103, contains none of them (15). RNA from the latter serves as a hybridization specificity control.

Many larger RNAs were identified using the same probes against the mitochondrial RNAs of petite 8 (Figure 1, lanes 2). All three probes hybridized to transcripts of 5.4 kb and 2.9 kb in length suggesting that the three genes are indeed transcribed together. Four transcripts, about 2500 nucleotides, 2000 nucleotides, 500 nucleotides and 310 nucleotides long, hybridized to both the RNase P RNA probe and the $tRNA^{Pro}$ probe but failed to hybridize to the $tRNA_f^{Met}$ probe. These transcripts could arise after separation of the $tRNA_f^{Met}$ from the primary transcript or from initiation events between the $tRNA_f^{Met}$ and RNase P RNA. A 3.15 kb transcript recognized by the RNase P RNA probe but not the tRNA probes is present in both petite RNAs even though petite 103 does not contain the RNase P RNA gene. Ethidium bromide staining of the gel indicates that the 3.15 kb signal co-migrates with the large subunit cytoplasmic ribosomal rRNA. We assume that this probe cross-hybridizes to this abundant cellular RNA. The fact that this RNA is present in petite but not wild type RNA is likely due to the increased amount of cytoplasmic RNA contamination that occurs during preparation of mitochondria from petites. All the results from the Northern hybridization experiments with petite 8 indicate that the RNase P RNA is co-transcribed with $tRNA_f^{Met}$ and $tRNA^{Pro}$ in this strain.

To determine whether the three genes are co-transcribed in wild type cells, a more sensitive primer extension experiment was performed (Figure 2). A primer complementary to the 5′ end of the $tRNA^{Pro}$ was annealed to DNase I treated wild type mitochondrial RNAs, and incubated with four nucleotides and AMV reverse transcriptase. The products of the extension reaction were separated on an 8% sequencing gel along side a DNA sequencing ladder. Several different sized cDNAs were synthesized. The smallest one (1 in Figure 2) terminates 13 nucleotides from the 3′ end of the $tRNA_f^{Met}$ sequence. This site is at the mature 5′ end of the RNase P RNA (15). This termination product probably represents the end of a processing intermediate in which the 5′ end of the RNase P RNA is mature and the 3′ end is still connected to the $tRNA^{Pro}$. Alternatively there could be a strong secondary structure formed near the processing site on the precursor transcript which causes premature termination. The second, and major, extension product (2 in Figure 2), seems to terminate at the 3′ end of the $tRNA_f^{Met}$ sequence. This is the product predicted if the RNase P RNA is still connected to the $tRNA^{Pro}$ sequence but has had the $tRNA_f^{Met}$ released. The third extension product (3 in Figure 2) ends in the middle of the $tRNA_f^{Met}$ sequence where the D-loop stem forms in this tRNA. Reverse transcriptase is known to stop at regions of secondary structure. Thus, this cDNA is likely to represent a premature termination rather than an end of an RNA. The fourth termination product (4 in Figure 2) falls at the penultimate A of the sequence TATAAGTAA, the putative promoter sequence. In addition, there is a product (5 in Figure 2) that extends past the nonanucleotide sequence and terminates near the 3′ end of the coding sequence of the cytochrome b gene. These results demonstrate that cDNAs which include RNase P RNA and the $tRNA_f^{Met}$ can be synthesized by priming from the 5′ end of the $tRNA^{Pro}$. Therefore, we conclude that the RNase P RNA is co-transcribed with $tRNA_f^{Met}$ and $tRNA^{Pro}$ in wild type cells. That there was an extension product extending past the nonanucleotides sequence upstream of the $tRNA_f^{Met}$ suggests that this sequence is not the only candidate for a site for initiation of transcription of the RNase P RNA precursor. In fact, the data are consistent

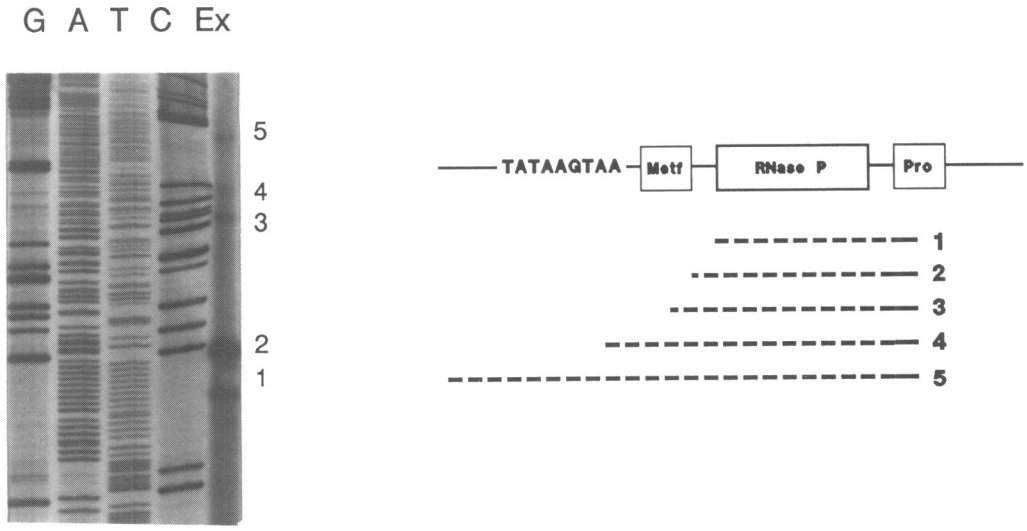

Figure 2. The RNase P RNA is co-transcribed with $tRNA_f^{Met}$ and $tRNA^{Pro}$ in wild type cells. A. A primer complementary to the 5′ end of the $tRNA^{Pro}$ was annealed with wild type mitochondrial RNAs and extended by incubating with reverse transcriptase. No products were seen if the sample was first treated with RNase P or if reverse transcriptase was absent (data not shown). The extension products were separated on 8% sequencing gel along side a DNA sequencing ladder. Lanes G,A,T,C, dideoxy sequencing reactions, lane 1, extension products. B. Schematic diagram of results.

with the hypothesis that transcription initiates far upstream and the nonanucleotide sequence just upstream of the $tRNA_f^{Met}$ is a processing site, not an initiation site of transcription. To differentiate between these two possibilities we determined whether the nonanucleotide sequence upstream of the $tRNA_f^{Met}$ gene served as a promoter by defining the primary transcripts of mitochondrial DNA in this region.

[α-^{32}P] GTP and guanylyl transferase can be used to label the triphosphate 5′ ends of primary transcripts from petite 8 mitochondria. Two RNAs, one around 2.9 kb and the other about 1.7 kb, were clearly labeled above the background smear (Figure 3). The 2.9 kb RNA is the same size as the transcript detected in Northern hybridizations which retains the $tRNA_f^{Met}$, RNase P RNA, and $tRNA^{Pro}$ sequences (Figure 1). A 1.7 kb RNA was never detected in those Northern experiments and must either be an RNA transcript of petite 8 mitochondria with unknown gene sequences, or a contaminating RNA from the cytoplasm. On an ethidium bromide stained gel, the 1.7 kb RNA migrates close to the small cytoplasmic ribosomal rRNA and could be its 20S rRNA precursor (16). The labelled RNAs were digested with ribonuclease T1 and a resulting 11 base oligonucleotide separated from other, smaller labelled RNA fragments on a 20% sequencing gel (Figure 3,B). The 11 nucleotide RNA was isolated and sequenced (Figure 3,C). The RNA sequence matches the sequence upstream of the $tRNA_f^{Met}$ gene (6). It initiates at the penultimate A of the sequence TATAA-GTAA and extends to the second nucleotide within $tRNA_f^{Met}$ sequence which is known to be a G residue. As this is the only nonanucleotide sequence known to be present in petite 8 (6) and the 11 nucleotide RNA matches perfectly, these results demonstrate that this particular nonanucleotide sequence in *C. glabrata* can be used as a promoter for transcription.

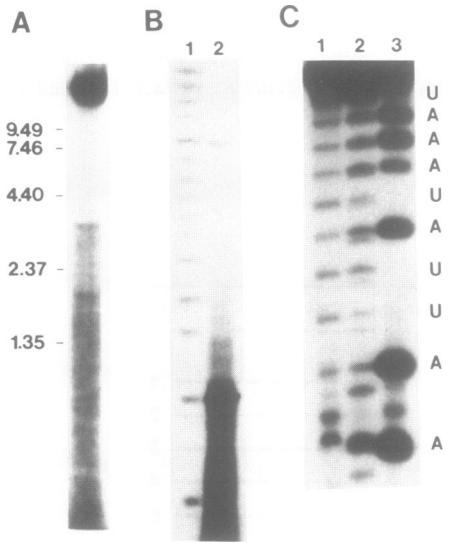

Figure 3. The nonanucleotide sequence upstream of $tRNA_f^{Met}$ is a promoter. The guanylyl transferase and [α-^{32}P] GTP labelled RNAs in petite 8 were separated on a 1.5% agarose-6 M urea gel along side RNA size standards (Panel A). The labelled RNAs were subsequently digested with ribonuclease T_1 and separated on a 20% acrylamide-8.3 M urea sequencing gel (lane 2, panel B) along side an alkaline ladder (lane 1, Panel B). The 11 nucleotide product was recovered from the gel and sequenced (Panel C). Lane 1, alkaline hydrolysis of capped $tRNA_f^{Met}$ precursor, lane 2, Phy M digested (U+A), and lane 3, U2 digested (A).

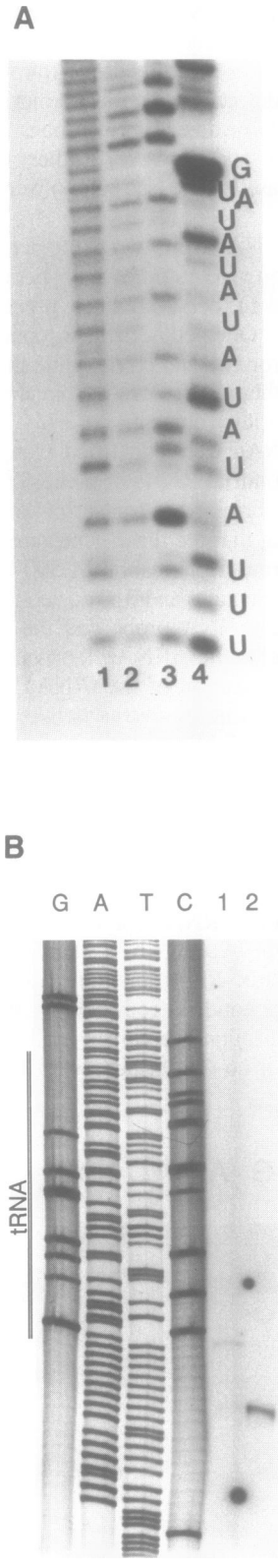

Figure 4. End determination of precursor RNAs. Panel A. Sequence of a fragment produced by RNase H cleavage from precursor RNAs labeled at the 3′ end with pCp. Lane 1, alkaline hydrolysis ladder; 2, A + U by digestion with Phy M; 3, A by digestion with U_2, 4, G by digestion with T_1. Panel B, Primer extension analysis of precursor RNAs. Lane 1, extension products from precursor RNAs, lane 2 extension product from mature RNA. GATC sequencing ladder generated from the same primer. The extension product resulting from the mature RNA end is barely visible in this photograph but clearly present in the original film.

Having established that the $tRNA_f^{Met}$, RNase P RNA and $tRNA^{Pro}$ genes are transcribed together and that the nonanucleotide sequence upstream of the $tRNA_f^{Met}$ does serve as a promoter we turned to an examination of the RNAs that are liberated from this transcript in wild type cells. Three RNAs, including the mature RNase P RNA, arise from the region between the two tRNA genes (15). The ends of the mature RNA were reported previously (15). We isolated the two larger RNAs, labeled them with pCp and used enzymes to try and determine a sequence from their 3′ ends. The sequence was unclear as the RNAs had ragged ends. Since it is easier to separate short RNAs differing by one nucleotide from each other than it is to separate 200 nucleotide plus RNAs differing by one nucleotide from each other we reasoned that RNase H directed digestion (see methods) could produce 3′ end labeled fragments that we could sequence. The digestion by RNase H was partial but both RNAs yielded the same digestion pattern indicating that they have the same ends (data not shown). The process was repeated on a mixture of the two larger RNAs and two products of about 30 nucleotides differing by one nucleotide were recovered and sequenced. The predominant product RNA yielded the sequence shown in Figure 4a. As can be seen in this figure, there are 16 RNA fragments in the alkali digest ladder starting with the RNA corresponding to the G residue. A residues should have a signal in lane 3 as dark or darker than the signal in lane 2 and U residues should have a signal in lane 2 but not in lane 3. While this generally holds, the Us in positions 1, 3, and 7 from the 3′ end had to be assigned by comparison to the DNA sequence. The data indicate that this RNA ends 4 nucleotides upstream of the beginning of the $tRNA^{Pro}$ gene. The minor component ends 5 nucleotides upstream (data not shown). Primer extension analysis of this same RNA sample (Figure 4B) revealed two ends, one that was identical to the 5′ end of the mature RNaseP RNA and one that appears to extend to the 3′ end of the $tRNA_f^{Met}$. Thus, both of the larger RNAs have the same 3′ end but differ at their 5′ ends. A schematic diagram of the primary transcript and processing products inferred from the experiments in Figure 4 is given in Figure 5.

DISCUSSION

The Northern hybridizations and primer extension results clearly demonstrate that RNase P RNA in *C. glabrata* mitochondria is transcribed with $tRNA_f^{Met}$ and $tRNA^{Pro}$. We have not demonstrated by pulse chase experiments that this polycistronic transcript is actually converted to the tRNAs and RNase P RNA but infer from their structures that they are. Thus, for ease, we refer to subsequent events as processing events and the intermediate we identify as precursors.

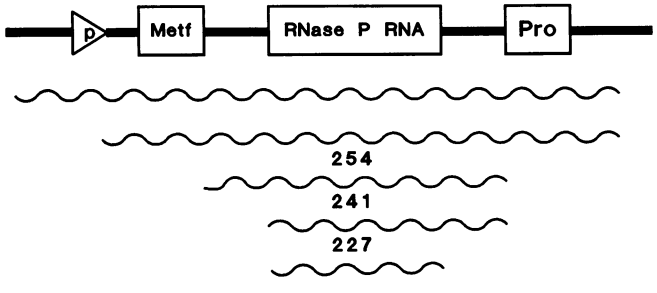

Figure 5. Schematic diagram showing RNAs identified by primer extension and sequencing. The first two wavy lines are the two primary transcripts that initiate either upstream of or at the nonanucleotide promoter sequence and extend past the $tRNA^{Pro}$ gene. The 254 and 241 base RNAs appear to be intermediates in the maturation of *C. glabrata* RNase P RNA.

Several TATAAGTAA sequences have already been shown to be promoters in *C. glabrata* (6) and the TATAAGTAA sequence upstream of $tRNA_f^{Met}$ certainly is a promoter in petites. The low abundance of primary transcripts in wild type cells prevented a direct demonstration that the sequence is a promoter in these cells but the primer extension data is consistent with this proposition. In addition, there must be an upstream promoter in wild type cells as indicated by the existence of primer extension products starting from the $tRNA^{Pro}$ and extending through the nonanucleotide sequence. This suggests that transcription which initiates at an upstream promoter can continue through another, downstream promoter. Thus, RNase P RNA can be transcribed from the promoter just upstream of $tRNA_f^{Met}$, as well as from one (or more) upstream promoters. Similar observations have been made in studies of *Neurospora* mitochondrial gene expression. There are at least two promoters used to transcribe the 19S rRNA gene in *Neurospora*. One abuts the rRNA gene while the other is 3 kb upstream (9).

In contrast to the situation in wild type cells, there appears to be only one promoter that provides RNase P RNA in petite 8, i.e. only one transcript of the same size as an RNase P RNA containing transcript can be labelled by guanylyl transferase and [α-^{32}P] GTP. If other initiation sites leading to RNase P RNA synthesis exist, they must be too weak to be detected by this methodology.

This gene arrangement is also present in a number of other yeasts (13,18) including *S. cerevisiae*. Although it has been postulated that the $tRNA_f^{Met}$ and RNase P RNA are also transcribed together in *S. cerevisiae* (12,17) there has been no direct demonstration that this is the case.

RNase P RNA genes are not flanked by tRNA genes in *E. coli* (14), *S. typhimurium* (1), yeast cytoplasm (10) nor in humans (2). In humans, the RNA does not appear to be derived from a larger precursor RNA (2) but the *E. coli* RNase P RNA is synthesized with both 5′ and 3′ extensions (7,14). Thus, like the maturation of the *C. glabrata* RNase P, *E. coli* RNase P requires additional, as yet, uncharacterized RNA processing activities. Unlike *C. glabrata*, none of the processing events necessary to yield mature RNase P RNA in *E. coli* include tRNA processing. Primer extension experiments with wild type RNA are consistent with the idea that $tRNA_f^{Met}$ is separated from the rest of the transcript by an endonuclease activity that cleaves at the 3′ end of the tRNA. Such an activity has been identified in mitochondrial extracts of *S. cerevisiae* (4). We did not sequence the 5′ ends of the precursor RNAs from wild type cells, but the structure of the largest precursor from the P8 petite has been determined directly. It starts with the U directly 3′ of the tRNA sequence and ends exactly as do the wild type RNAs described here (19). We could not identify an RNA that ended at −1 relative to $tRNA^{Pro}$ nor a $tRNA^{Pro}$ precursor that contained a 4 base leader. Therefore, the data as to whether RNase P RNA is required for the initial separation of $tRNA^{Pro}$ from the polycistronic transcript is ambiguous. The RNA that ends with nucleotide −5 relative to the 5′ end of the $tRNA^{Pro}$ could arise if the nucleotides were removed after the $tRNA^{Pro}$ was liberated by RNase P. Alternatively, the cleavage after −5 relative to the $tRNA^{Pro}$ could occur first to separate RNase P RNAs from $tRNA^{Pro}$ and then the 5 base 5′ leader on the $tRNA^{Pro}$ would be removed by

RNase P. In either case, the processing event that results in the accumulation of the transcript ending with the −5 nucleotide relative to the $tRNA^{Pro}$ is an event not heretofore described. The subsequent processing activities that convert the longest tRNA free RNase P RNA precursor to yield the mature RNase P are of the same category. We cannot tell from our data whether the shortening of the 254 base RNA at the 5′ end which results in the 241 base RNA occurs by endonucleolytic or exonucleolytic cleavage. The same is true for the shortening of the 254 base RNA which yields the mature 3′ end of the RNase P RNA. Additional experiments to address this issue will be needed.

The RNA synthesis events described here are only part of the story concerning the biogenesis of mitochondrial RNase P. The components necessary to the synthesis of the mature RNase P RNA include an activity like the 3′ endonuclease that removes 3′ leaders from tRNA precursors as well as endonucleases or exonucleases not described previously. There is a protein(s) necessary for mitochondrial RNaseP activity in vitro (8) and presumably in vivo as well. In addition, it is likely that the assembly of a mitochondrial RNA-protein complex will require additional factors. All of these components must be made in the cytoplasm and imported into the mitochondria. Glycerol gradient analysis of mitochondrial extracts (15) argues that RNA processing and assembly into active enzyme is a complex process. The work reported here is only the first step in our efforts to understand the biogenesis of an RNA-protein complex in mitochondria.

ACKNOWLEDGEMENTS

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REFERENCES

1. Baer, M., and S. Altman. 1985. A catalytic RNA and its gene from *Salmonella typhimurium*. Science **228**:999−1002.
2. Baer, M., T.W. Nilsen, C. Costigan, and S. Altman. 1990. Structure and transcription of a human gene for H1 RNA, the RNA component of human RNase P. Nucleic Acids Res. **18**:97−103.
3. Biswas, T.K., J.C. Edwards, M. Rabinowitz, and G.S. Getz. (1985). Characterization of a yeast mitochondrial promoter by deletion mutagenesis. Proc. Natl. Acad. Sci. USA **82**:1954−1958.
4. Chen, J.Y., and N.C. Martin. 1988. Biosynthesis of tRNA in yeast mitochondria. An endonuclease is responsible for the 3′-processing of tRNA precursors. J. Biol. Chem. **263**:13677−13682.
5. Christianson, T.W., 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.
6. Clark-Walker, G.D., C.R. McArthur, and K.S. Sriprakash. 1985. Location of transcription control signals and transfer RNA sequence in *Torulopsis glabrata* mitochondrial DNA. EMBO J. **4**:465−473.
7. Gurevitz, M., K.J. Swatantra, and D. Apirion. 1983. Identification of a precursor molecule for the RNA moiety of the processing enzyme RNase P. Proc. Natl. Acad. Sci. USA **80**:4450−4454.
8. Hollingsworth, M.J., and N.C. Martin. 1986. RNase P activity in the mitochondria of *Saccharomyces cerevisiae* depends on both mitochondrion- and nucleus-encoded components. Mol. Cell. Biol. **6**:1058−1064.
9. 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.
10. Lee, J.-Y., C.E. Rohlman, L.A. Molony, and D.R. Engelke. 1991. Characterization of *RPR1*, an essential gene encoding the RNA component of *Saccharomyces cerevisiae* nuclear RNase P. Mol. Cell. Biol. **11**:721−730.
11. 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.
12. Mueller, D.M., and G.S. Getz. 1986. The transcriptional regulation of the mitochondrial genome of yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **261**:11756−11764.
13. Ragnini, A., P. Grisanti, T. Rinaldi, L. Frontali, and C. Palleschi. 1991. Mitochondrial genome of *Saccharomyces douglasii*: genes coding for components of the protein synthetic apparatus. Curr. Genet. **19**:169−174.
14. Reed, R.E., and S. Altman. 1983. Repeated sequences and open reading frames in the 3′ flanking region of the gene for the RNA subunit of *Escherichia coli* ribonuclease P. Proc. Natl. Acad. Sci. USA **80**:5359−5363.
15. Shu, H.-H., C.A. Wise, G. D. Clark-Walker, and N.C. Martin. 1991. A gene required for RNase P activity in *Candida (Torulopsis) glabrata* mitochondria codes for a 227-nucleotide RNA with homology to bacterial RNase P RNA. Mol. Cell. Biol. **11**:1661−1667.
16. Udem, S.A., and J. R. Warner. 1972. Ribosomal RNA synthesis in *Saccharomyces cerevisiae*. J. Mol. Biol. **65**:227−241.
17. Wettstein, J., B.S. Ticho, D. Najarian, N.C. Martin, and G.S. Getz. (1986) *In vitro* transcription and promoter strength analysis of five mitochondrial tRNA promoters in yeast. J. Biol. Chem. **261**:2905−2911.
18. Wilson, C., A. Ragnini, and H. Fukuhara. 1989. Analysis of the regions coding for transfer RNAs in *Kluyveromyces lactis* mitochondrial DNA. Nucleic Acids Res. **17**:4485−4491.
19. Wise, C.A. (1991) Structural and functional studies of the yeast mitochondrial RNaseP RNA. Ph.D. Thesis, University of Texas Southwestern Medical School.