Volume 15 Number 18 1987 Nucleic Acids Research

Transcription initiation and RNA processing of a yeast mitochondrial tRNA gene cluster

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Received June 29, 1987; Revised and Accepted August 24, 1987

ABSTRACT

Expression of 5 yeast mitochondrial tRNA genes (Ala, Ile, Tyr, Asn and Metm), localized upstream from the oxi1 gene has been analyzed by *in vitro* capping using guanylyltransferase, northern hybridization and S1 nuclease mapping in the wild type and a rho$^-$ strain. The 5 tRNA sequences belong to the same transcriptional unit which is initiated 133 bp upstream from the tRNA(Ala) gene at a promoter sequence TTATAAGTA. Furthermore, a truncated tRNA(Tyr) transcript, 2 nucleotides shorter than mature tRNA(Tyr) has been found, only in the rho$^-$ strain. This minor transcript may result from secondary transcription initiation at a variant nonanucleotide sequence, ATATAAGGA, which overlaps the tRNA(Tyr) coding sequence by 3 nucleotides. The polycistronic precursor has proven to be useful in investigation of the mechanisms of tRNA processing. Maturation of this primary transcript proceeds exclusively by precise endonucleolytic cleavages at the 5' and 3'-ends of tRNA sequences.

INTRODUCTION

The mitochondrial (mt) DNA of *Saccharomyces cerevisiae* codes for the RNA components of the mitochondrial translation machinery (2 rRNAs and 24 tRNAs) and for a small number of mRNAs (1). A multicomponent nuclear-encoded RNA polymerase is responsible for the transcription of all the mitochondrial genes (2-5). Unlike the situation in mamalian mitochondria, the yeast mt DNA contains at least 20 unique transcription initiation sites dispersed throughout the genome, which have been identified using the method of *in vitro* capping with guanylyltransferase (6). Such sites are found upstream from polypeptide coding genes, tRNA genes and the two rRNA genes. Transcription starts within a highly conserved sequence, $\begin{matrix}A\\T\end{matrix}$TATAAGTA, with the last A being the site of initiation. *In vitro* transcription using mitochondrial RNA polymerase indicates that this nonanucleotide box is required for the specificity of transcription (7). Furthermore, *in vitro* mutagenesis shows that the essential sequences for promoter recognition coincide with this box (5, 8, 9). This promoter motif has been found 5' to four mt tRNA genes and, in most cases, it serves not only as the transcriptional initiation site for the tRNA gene itself but also for additional tRNA and/or protein genes located downstream (6, 10-13).

Production of mature mt tRNA from the multigenic precursors requires nucleolytic processing at both the 5'- and 3'-ends of the tRNA, as well as addition of the 3'-CCA and modification of nucleosides. Information concerning the synthesis of mt tRNAs in *S. cerevisiae* has been provided by petite mutants which lack large portions of mt DNA and are incapable of mitochondrial protein synthesis. Some petites produce mature tRNAs, whereas others which lack a certain region of the mitochondrial genome (the tRNA synthesis locus), only accumulate tRNA precursors some of which are correctly processed at their 3'-end but retain 5' leader sequences (11, 14-18). The product of the tRNA synthesis locus, a 9S RNA (19), has recently been shown to be the RNA component of the mitochondrial RNase P, which is necessary for 5'-end processing of mt tRNAs (20).

Of the 24 *S. cerevisiae* mt tRNA genes, 16 are organized as several clusters between the 21S rRNA and oxi1 genes. In this region, only one transcriptional initiation site has been characterized so far (17, 18). To gain more information about the expression of tRNA genes from this region, we have analysed transcription and RNA processing of a cluster of 5 tRNA genes ($tRNA^{Ala}$, $tRNA^{Ile}$, $tRNA^{Tyr}$, $tRNA^{Asn}$ and $tRNA^{Met}_{m}$) situated upstream of the oxi1 gene. We present evidence that these 5 tRNAs are cotranscribed from a transcriptional initiation site located upstream from the $tRNA^{Ala}$ gene. In the petite mutant studied here, this primary transcript is subsequently processed by precise endonucleolytic cleavages at the 3'-end of the tRNAs yielding monomeric precursors with 5'-extensions.

MATERIAL AND METHODS

Preparation and analysis of mitochondrial nucleic acids.

Growth conditions and isolation of mt DNA and mt RNA from the *S. cerevisiae* wild type strain SM202 and the rho$^-$ strain 1H1263 have been previously reported (21-23). Electrophoresis of mt RNA on agarose-urea gels, transfer to DBM paper by electroblotting, and RNA-DNA hybridization were as described (24, 25).

Molecular cloning and DNA sequencing

The isolation of the recombinant plasmid pYm8C1 carrying a 1.8 kb mt DNA HpaII fragment containing the $tRNA^{Ala}$, $tRNA^{Ile}$ and $tRNA^{Tyr}$ genes has been described previously (23).

For preparation of probes and DNA sequencing, restriction fragments were 5'-end labelled using T4 DNA polynucleotide kinase and γ-^{32}P-ATP or 3'-end labelled using DNA polymerase (Klenow fragment) and the appropriate ^{32}P-dNTP or using terminal transferase and ^{32}P-ddATP (26-28). The labelled fragments were separated on 4% polyacrylamide gels. Sequencing was done by the method of Maxam and Gilbert (27).

In vitro capping of primary transcripts and RNA sequencing

The *in vitro* capping reactions using α-^{32}P-GTP and guanylyltransferase (BRL, Inc.) were as described (29). The reaction was stopped by adding an equal volume of 10 mM EDTA, 1%

SDS. The mixture was extracted twice with phenol and ethanol precipitated. The RNA pellet was resuspended in 10 mM Tris-Cl (pH 7.4), 1mM EDTA and loaded on agarose-urea gels. Total T1 RNase digestion of the labelled transcripts and RNA sequencing reactions were according to (30, 31).

S1 nuclease mapping and primer extension analyses

S1 nuclease mapping was performed as described (19), except that annealing was at 42° C for 4 h. The probes used (fig.1) are the following: probe 1: NsiI-HindIII fragment, 5'-end labelled at the NsiI site located in the tRNA(Ala) coding sequence and cut at the HindIII site in the vector; probe 2: RsaI-NsiI fragment, 5'-end labelled at the RsaI site located in the tRNA(Ile) gene; probe 3: NsiI-AvaII fragment, 3'-end labelled at the NsiI site in the tRNA(Ala) gene; probe 4: DdeI-RsaI fragment, 5'-end labelled at the DdeI site in the tRNA(Tyr) gene.

The 5'-end of the $tRNA^{Ala}$ transcripts was identified by primer extension using a synthetic 17-mer (TGCATGCAACGCAGTCG) complementary to residues 26-42 of the $tRNA^{Ala}$ sequence. The primer was 5'-end-labelled and purified on a 20% polyacrylamide-7M urea gel. After elution, the labelled primer (0.5 10^6 cpm) was ethanol-precipitated together with 30 µg of mt RNA and the primer extension reaction performed as described (19).

RESULTS

Organization and nucleotide sequence of the tRNA gene cluster

The localization of tRNA genes in the oxi1 region of the *S. cerevisiae* mitochondrial genome has been described previously (23, 32, 33). A map illustrating their organization is shown in

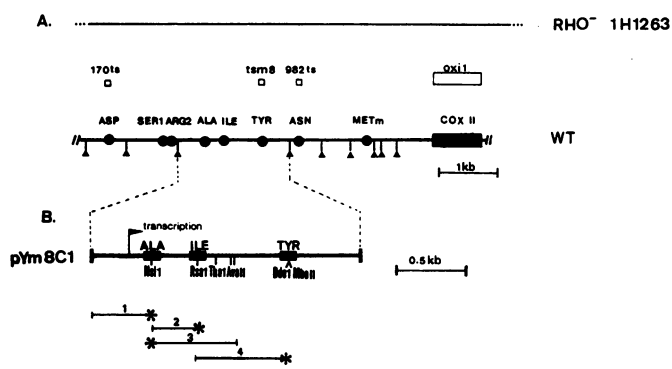

Figure 1 : *Organization of tRNA genes in the oxi1 region of the mitochondrial genome.* (A) The tRNA genes (Asp, Ser1, Arg2, ...) are represented on the portion of wild type mt DNA delimited by the genetic markers 170ts and oxi1. The mt DNA repeat unit retained in the rho⁻ 1H1263 strain is shown in the top lane. The HpaII restriction sites are indicated (▲). (B) Restriction map of the cloned 1.8 kb HpaII fragment (pYm8C1) retaining tRNA(Ala), tRNA(Ile) and tRNA(Tyr) genes. Probes 1 to 4, used in northern hybridization and in S1 nuclease mapping, are indicated and described in Material and Methods. The asterisks represent the labelled termini.

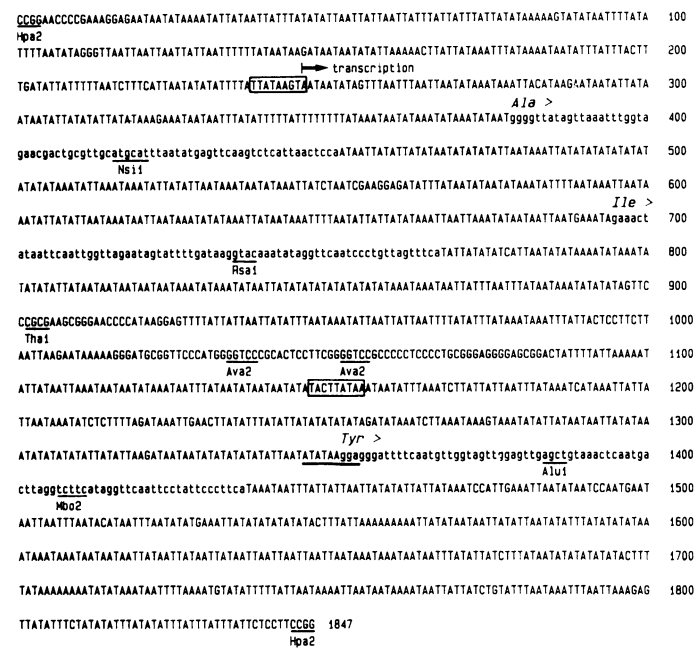

Figure 2: *DNA sequence of the wild type 1.8 kb HpaII fragment.* The nonanucleotide sequences TTATAAGTA (positions 238-246 and on the opposite strand, positions 1147-1155) are in boxes and the variant motif ATATAAGGA (positions 1346-1354) is underlined. Lower case letters represent the structural genes for tRNA(Ala), tRNA(Ile) and tRNA(Tyr). The site of transcriptional initiation is indicated by an arrow.

fig.1. Although the DNA sequence of the major part of this region has already been reported (23, 34-37), several gaps remained between the $tRNA_2^{Arg}$ and $tRNA^{Asn}$ genes. To provide a basis for studies of the expression of tRNA genes in this region, we have completed the sequence using a 1.8 kb HpaII fragment (pYm8C1) encoding $tRNA^{Ala}$, $tRNA^{Ile}$ and $tRNA^{Tyr}$ in the wild type strain SM202 (fig. 2). The 3 tRNA coding sequences can be folded into conventional cloverleafs. The $tRNA^{Ile}$ and $tRNA^{Tyr}$ gene sequences are colinear with their respective tRNA sequences as determined from another wild type strain (38, 39). However, the $tRNA^{Ala}$ gene sequence differs from the one published by Bonitz and Tzagoloff (34) at positions 22 and 23 (residues 400 and 401 in fig. 2). Our sequence has A22-G23 instead of the previously reported G22-A23. In this corrected sequence, A is located at it's standard position in the generalized cloverleaf (40).

Analysis of primary transcripts

Transcriptional initiation sites in the tRNA gene cluster situated upstream from the oxi1 gene, were identified by *in vitro* capping experiments and confirmed by S1 nuclease mapping and

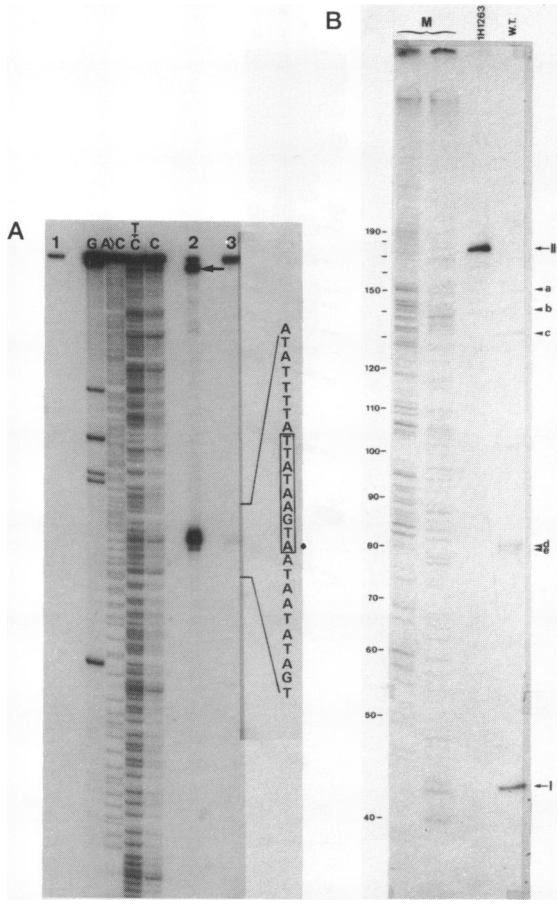

Figure 3: *Determination of the 5'-end of tRNA(Ala) transcripts.* **(A)** S1 nuclease mapping: probe 1 (see fig. 1) was hybridized with mt RNA from rho⁻ strain 1H1263 and wild type SM202. After digestion, the protected fragments were run on a polyacrylamide gel using the sequencing reactions products G, A>C, T+C and C of the probe as references. Probe 1 was digested with S1 nuclease without hybridization (lane 1) or after hybridization with mt RNA from strain 1H1263 (lane 2) or from wild type (lane 3). The non-coding strand sequence is reported. The promoter sequence is boxed and the dot indicates the initiating nucleotide. In addition to mt DNA sequences, probe 1 contains 12 bp of vector sequences. In lane 2, a S1 protected fragment (marked with an arrow) slightly shorter than the probe, is observed with the mt RNA from rho⁻ strain 1H1263. This fragment results from a transcript initiated at the oxi 1 promotor and is present due to the repetitive structure of the mt DNA in the petite used.
(B) Primer extension analysis: the primer complementary to the tRNA(Ala) sequence (see Material and Methods) was hybridized to mt RNA of wild type and rho⁻ strains. Run-off products were electrophoresed on a 6% acrylamide-7M urea gel next to a dideoxy-chain termination sequencing ladder of M13 (M). The length in nucleotides of the sequencing products is given at the left. (I): 42 nucleotides run-off product corresponding to the mature 5'-end of tRNA(Ala); (II): 175 nucleotides run-off product corresponding to the transcriptional initiation site. The additional signals (a to e) observed with mt RNA from the wild type result from degradation of the primary transcript.

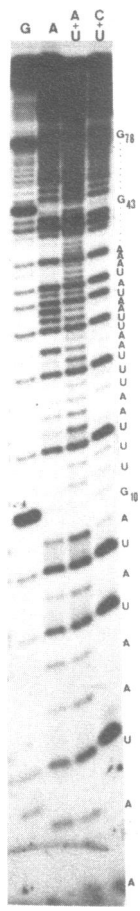

Figure 4: *RNA sequencing*. The 200 nucleotide labelled fragment obtained by *in vitro* capping of rho⁻ 1H1263 mt RNA was subjected to enzymatic RNA sequencing reactions. Lanes labelled G, A, A+U, C+U represent the products of partial digestion with the base specific ribonucleases T1, U2, PhyM and B. cereus, respectively .

primer extension. For this purpose, we have used the rho⁻ strain 1H1263 spanning the region between $tRNA^{Asp}$ and oxi1 genes (see fig. 1). This petite lacks the tRNA synthesis locus and therefore accumulates tRNA precursors.

A nonanucleotide sequence, TTATAAGTA, which is known to act as a promoter element for mitochondrial gene transcription (6), is found in the 5'-region of the $tRNA^{Ala}$ gene (positions 238-246; fig. 2). The 5' termini of $tRNA^{Ala}$ transcripts were identified by S1 protection experiments after annealing of probe 1 (see fig. 1) to mt RNA from wild type and rho⁻ 1H1263. A protected fragment terminating at this nonanucleotide, is obtained for both the mutant and wild

type (fig. 3A). This result was confirmed by primer extension using a synthetic 17-mer complementary to residues 26-42 of the $tRNA^{Ala}$. An extension product of 42 bases corresponding to the 5'-end of the mature tRNA, is observed in the wild type, whereas it is absent in the rho^- strain and replaced by an extension product of 175 nucleotides (fig. 3B). The 5'-end of this product abuts the nonanucleotide box located 133 bp upstream of the $tRNA^{Ala}$ gene.

The use of this box as a transcriptional initiation site was determined by *in vitro* capping of primary transcripts. Labelling of mt RNA from strain 1H1263 with guanylyltransferase followed by separation on an agarose-urea gel, yielded four major capped transcripts of 2500, 850, 450 and 200 nucleotides, respectively (result not shown). The origins of these transcripts were determined by direct RNA sequencing of their 5' regions. The 2500 and 850 nucleotide transcripts are both generated by processing of a RNA precursor transcribed from a promoter, TTAAAAGTA, situated upstream from the oxi1 gene (Bordonné et al., in preparation). The 5' sequence of the 200 nucleotide long transcript (fig. 4) exactly matches the DNA sequence immediatly downstream of the nonanucleotide box TTATAAGTA, situated 133 bp upstream from the $tRNA^{Ala}$ gene. The 3'-terminal adenosine of this motif coincides with the transcriptional initiation site. The transcripts of 450 and 200 nucleotides have identical 5' terminal sequences, demonstrating that they are initiated at the same site. Therefore, the 200 nucleotide transcript corresponds to a monomeric $tRNA^{Ala}$ precursor and the 450 nucleotide transcript to a $tRNA^{Ala}$ - $tRNA^{Ile}$ dimeric precursor. Further evidence that these two genes are cotranscribed are provided by Northern hybridization and S1 mapping studies (see below).

In addition to the nonanucleotide TTATAAGTA located upstream from the $tRNA^{Ala}$ gene, a second copy of this sequence is also found between the genes for $tRNA^{Ile}$ and $tRNA^{Tyr}$, but on the opposite DNA strand (positions 1147-1155; fig.2). Since none of the 4 major capped transcripts corresponds to initiation at this second TTATAAGTA motif, we have searched for a minor transcript arising from this site. If transcription began at this site, we would expect to detect a 61 nucleotide-long fragment after digestion of capped mt RNA with T1 RNase (see fig. 2). As no such 61-mer was found, we conclude that this nonanucleotide is not or very inefficiently used for transcription initiation or that the resulting transcript is very unstable.

Characterization of multigenic tRNA precursors and identification of RNA processing sites

The above results support the idea that the nonanucleotide sequence TTATAAGTA upstream of the $tRNA^{Ala}$ gene serves as a transcriptional initiation site, not only for this tRNA but also for the additional tRNA genes located downstream (Ile, Tyr, Asn, Metm). To investigate this possibility, we have analyzed the transcripts present in both wild type SM202 and rho^- strain

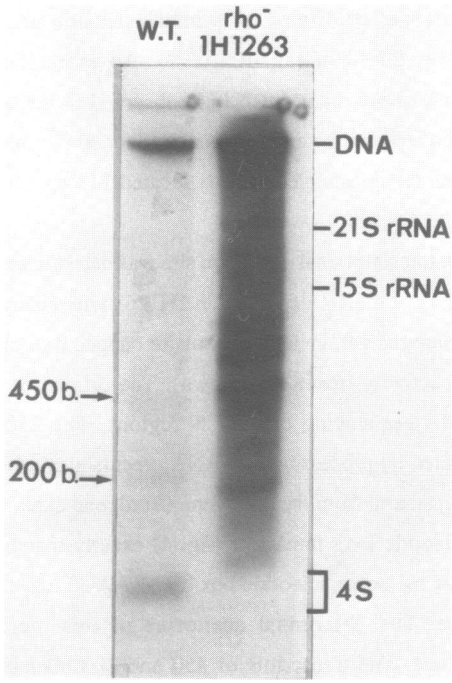

Figure 5: *Northern blot analysis.* Total mt RNA from wild type SM202 and rho⁻ strain 1H1263 was separated on a 1% agarose-urea gel, transferred to DBM paper and hybridized with probe 2 (fig. 1). Two tRNA precursors (200 and 450 nucleotides) observed in the wild type strain, are indicated by an arrow.

1H1263 by Northern hybridization and S1 nuclease mapping. Mitochondrial RNAs from both strains were separated on a 1% agarose-urea gel, transferred to DBM paper and hybridized with a $tRNA^{Ala}$ - $tRNA^{Ile}$ gene probe (probe 2 in fig. 1). Multiple transcripts, up to 3000 nucleotides in size, were detected in the rho⁻ strain (fig. 5). Based on the distance separating the $tRNA^{Ala}$ promoter from the $tRNA^{Met}_{m}$ gene (ie. about 2900 bp), the presence of a 3000 nucleotide transcript suggests that the 5 tRNA genes (Ala, Ile, Tyr, Asn, Metm) are cotranscribed. The shorter transcripts hybridizing to the tRNA gene probe, would then correspond to RNA processing intermediates. Even in the wild type strain, the monomeric $tRNA^{Ala}$ precursor (200 nucleotides) and a dimeric $tRNA^{Ala}$ - $tRNA^{Ile}$ precursor (450 nucleotides), are revealed in addition to mature-sized 4S RNA (see fig. 5).

To precisely determine the 5'- and 3'-ends of tRNA precursors, probes 2 to 4 (see fig. 1) were prepared from the recombinant clone pYm8C1. The 5'-termini of $tRNA^{Ile}$ transcripts were localized using probe 2 (see fig.1). S1 mapping results (fig.6A) show a signal corresponding to

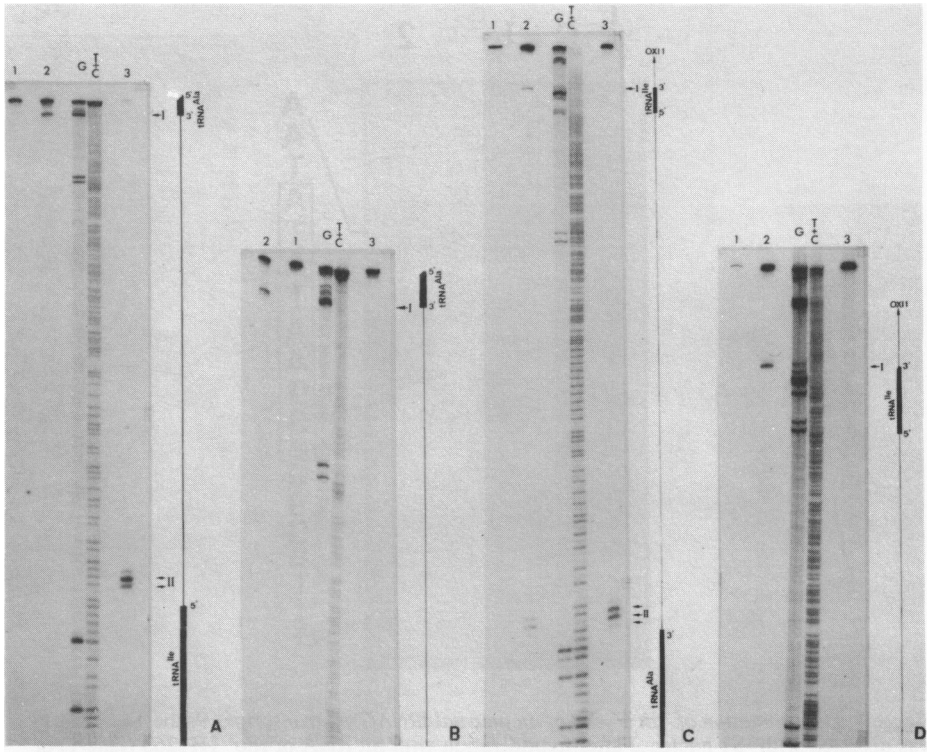

Figure 6: *Localization of the 5' and 3'-termini of tRNA(Ala) and tRNA(Ile) transcripts by S1 nuclease mapping.* **(A)** and **(B)**, probe 2 (fig.1) was hybridized with mt RNA, digested with S1 nuclease, and the protected fragments sized on polyacrylamide-urea gels relative to the products of G and T+C chemical modification reactions of the probe. **(C)** and **(D)**, probe 3 (fig.1) was analysed in a same manner. Lanes are labelled as follows: (1) probe treated with S1 nuclease without hybridization; (2) probe hybridized to mt RNA of petite strain 1H1263 and treated with S1 nuclease; (3) probe hybridized with mt RNA of wild type and treated with S1 nuclease. The protected fragments are indicated by I and II. tRNA sequences are represented by heavy black lines.

the 5'-end of $tRNA^{Ile}$ in the wild type. In the rho$^-$ strain however, this signal is absent and instead, a different protected fragment is found (indicated by I in lane 2; fig. 6B), which corresponds to the 3'-end of the $tRNA^{Ala}$ coding sequence. This experiment shows that, in the petite strain, there is a transcript carrying $tRNA^{Ile}$ plus a leader sequence extending to the 3'-end of the preceding tRNA gene.

The 3'-termini of $tRNA^{Ala}$ and $tRNA^{Ile}$ transcripts were determined using probe 3 (see fig.1). A fragment terminating at the 3'-end of the $tRNA^{Ala}$ coding sequence, is found for both the wild type and the rho$^-$ mutant (fig. 6C). This transcript corresponds to the 200

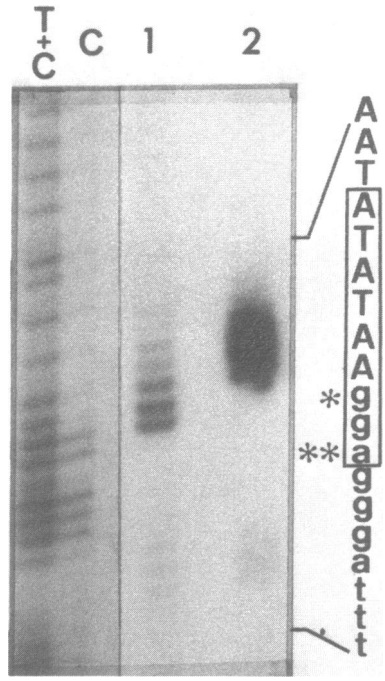

Figure 7: *Determination of the 5'-end of an unusual tRNA(Tyr) transcript.* Probe 4 (see fig.1) was digested with S1 nuclease after hybridization with mt RNA of rho⁻ 1H1263 (lane 1) or wild type (lane 2) and separated relative to the T+C and C sequencing reactions of the probe. The sequence of the non-coding strand (nucleotides 1343-1361 in fig.2) is shown at the right. The lower case letters correspond to tRNA(Tyr) coding sequences. The variant nonanucleotide sequence is boxed. (*) indicates the 5'-end of the mature tRNA(Tyr) in wild type; (**) indicates the 5'-end of the tRNA(Tyr) transcript observed in the petite.

nucleotide-long $tRNA^{Ala}$ precursor. Furthermore, a signal corresponding to a transcript terminating at the 3'-end of $tRNA^{Ile}$ is detected only in the rho⁻ strain (band I lane 2 in fig. 6D). Since the 5'-end of the $tRNA^{Ala}$ precursor corresponds to the initiation site, this result shows the existence of a dimeric precursor carrying $tRNA^{Ala}$ and $tRNA^{Ile}$ sequences, which has been properly processed at the 3'-end of the $tRNA^{Ile}$ sequence. This dimeric product corresponds to the 450 nucleotide transcript found by *in vitro* capping and Northern analysis.

Characterization of an unusual $tRNA^{Tyr}$ transcript

S1 nuclease mapping of the 5'-end of the $tRNA^{Tyr}$ transcripts was performed using probe 4 (see fig.1). A major protected $tRNA^{Tyr}$ transcript, whose leader sequence extends up to the 3'-end of the $tRNA^{Ile}$ gene, was detected when the probe was hybridized to mtRNA from strain 1H1263 (data not shown). This suggests that the $tRNA^{Tyr}$ gene is cotranscribed with the two

upstream tRNA genes. Surprisingly, an additional minor $tRNA^{Tyr}$ transcript, shorter than wild type $tRNA^{Tyr}$ by 2 nucleotides, was also found (fig. 7). This result was confirmed by primer extension analysis (not shown). The truncated $tRNA^{Tyr}$ transcript may result from secondary transcriptional initiation at a variant nonanucleotide motif, ATATAAGGA, which overlaps the 5'-end of the $tRNA^{Tyr}$ coding sequence by 3 nucleotides (see below).

DISCUSSION

The major goal of this study was to analyze the expression of a mitochondrial tRNA gene cluster located in the region delimited by the 21S rRNA and oxi1 genes. The first tRNA gene ($tRNA^{Thr}_2$) from this region is cotranscribed with the 21S rRNA gene (41), and it has been suggested that a promoter situated upstream from the $tRNA^{Cys}$ gene, is used for transcription of 4 to 5 further downstream tRNA genes (17, 18). Up to now, no other initiation site for transcription of the additional tRNA genes and for the oxi1 gene has been identified. We show here that a nonanucleotide sequence, TTATAAGTA, located 133 bp upstream of the $tRNA^{Ala}$ gene acts as a major transcriptional initiation site in the rho$^-$ strain 1H1263. Transcription from this site gives rise to a polycistronic precursor containing 5 tRNA sequences (Ala, Ile, Tyr, Asn and Metm). Analysis of S1 mapping results shows that this site is also used for transcription initiation in the wild strain SM202. In addition, a capped oligonucleotide corresponding to the 10 first nucleotides of the $tRNA^{Ala}$ precursor identified here, was detected in a wild type strain by Christianson and Rabinowitz (6). The oxi1 gene, located downstream of the $tRNA^{Met}_m$ gene, is independently transcribed from another major initiation site (Bordonné et al., in preparation).

The nonanucleotide motif, TTATAAGTA, is used for transcription of several yeast mt tRNA genes (6,10-12). However, the same sequence is also found between the $tRNA^{Ile}$ and $tRNA^{Tyr}$ genes, (positions 1147-1155 on the opposite DNA strand; fig. 2) and at other locations on the mitochondrial genome (6), and no corresponding capped transcripts are found. These observations suggest that elements in addition to the nonanucleotide box are important for transcription initiation. Site-specific mutagenesis results show that the efficiency of transcription depends on the nucleotides at position +2 and +3 from the initiation site: a purine at +2 and a pyrimidine at +3 specify a strong promoter, whereas a pyrimidine at +2 allows no or weak transcription (42, 43). This is in agreement with our results since the active $tRNA^{Ala}$ promoter has A and T at positions +2 and +3, whereas the inactive (or weak) promoter motif (between the $tRNA^{Ile}$ and $tRNA^{Tyr}$ genes) has a T and an A instead.

Among the rho$^-$ 1H1263 transcripts analyzed, we have found a $tRNA^{Tyr}$ transcript 2 nucleotides shorter on its 5'-end than mature $tRNA^{Tyr}$. This truncated transcript has also been

detected in another rho$^-$ strain by Nobrega and Nobrega (37). To explain its origin, these authors have suggested inaccurate 5'-end processing of a longer transcript. However, both petites lack the tRNA synthesis locus encoding the 9S RNA, which is part of the mitochondrial RNase P (16, 19, 20). The shortened RNA could also be a degradation product of the primary transcript. Rather than RNA processing or degradation, we propose that the truncated transcript results from secondary transcription initiation at a variant nonanucleotide box, ATATAAGGA, which overlaps the 5'-end of the $tRNA^{Tyr}$ coding sequence by 3 bases. This motif differs only at position -1 from the mitochondrial consensus promoter, ATATAAGTA. Additional support of this hypothesis comes from the fact that the 5'-terminus of the truncated $tRNA^{Tyr}$ transcript coincides with the last adenine of the ATATAAGGA sequence, which is always the initiation site of the promoter boxes described so far. This truncated transcript has not been detected in our *in vitro* capping experiments for the following reasons: (i) since the labelled RNAs were separated on an agarose urea gel, the isolation of a minor capped product synthesized from the ATATAAGGA motif would be difficult; (ii) the digestion of capped mt RNA with T1 RNase should generate a labelled dinucleotide which is not separated from non-incorporated radiolabelled GTP by polyacrylamide-urea gel electrophoresis. The use of this variant promoter motif as a transcriptional initiation site in both petite and wild type strains, deserves further investigations.

The multigenic tRNA precursor described in this study is a good tool for the investigation of tRNA processing. Our results show that conversion of the primary transcript into mature-sized tRNAs proceeds exclusively by precise endonucleolytic cleavages at the 5'- and 3'-ends of the tRNA sequences. In the wild type strain, although the tRNA usually have mature 5'- and 3'-ends, a $tRNA^{Ala}$ precursor with 5'-end corresponding to the nonanucleotide sequence has been found. In the rho$^-$ 1H1263 strain, which is deficient in mt RNAse P, tRNA transcripts are processed at their 3'-ends but have 5' leaders extending either to the preceding tRNA gene or to the transcriptional initiation site. The mitochondrial 3' processing activity must be an endonuclease since the 5'-end of the tRNA precursor abuts the 3'-end of the preceding tRNA (shown by S1 nuclease mapping using both 5' and 3'-end-labelled probes). Endonucleolytic cleavages also occur for the removal of 3'-trailer sequences from eucaryotic nuclear tRNA precursors, such as *Drosophila* (44) and *Xenopus* (45). In contrast, exonucleolytic trimming is used for 3'-processing of yeast nuclear and procaryotic tRNA precursors (46, 47). The yeast mitochondrial 3'-tRNA processing activity is present in petite strains which lack mitochondrial protein synthesis, and therefore, we can suppose that it is nuclear-encoded.

After 3'-processing, tRNA precursors are substrates for the mt tRNA nucleotidyltransferase

(which adds the 3' CCA) and for aminoacyl-tRNA synthetases (32, 48 and our unpublished results). Another processing activity necessary for mt tRNA maturation is the removal of the 5'-leader sequences. This processing step requires the expression of the mitochondrial tRNA synthesis locus (16, 19, 20). Whether the 9S RNA, encoded by this locus, represents the catalytic center of the mitochondrial RNAse P, like the M1 RNA of *E. coli* (49) and the P RNA of *B. subtilis* (50), remains to be demonstrated.

Acknowledgments. We thank Drs. J. Canaday and B.F. Lang for critical reading of the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique (ATP 2080 and 4256).

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