Identification of a new promoter within the tRNA gene cluster of the mitochondrial DNA of *Saccharomyces cerevisiae*

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Received July 27, 1987; Accepted October 8, 1987

ABSTRACT

We have identified a new promoter within the tRNA gene cluster of *Saccharomyces cerevisiae* mitochondrial DNA. It is located upstream of the gene encoding the Leucyl tRNA. It conforms to the consensus sequence of other yeast mitochondrial promoters, ATATAAGTA. It serves as a site for the initiation of transcription in vivo and in vitro.

INTRODUCTION

The mitochondrial genome of bakers' yeast, *Saccharomyces cerevisiae*, includes genes for 8 proteins, a 21S rRNA and 14S rRNA, about 25 tRNAs and a tRNA processing locus (for a review, see 1). When the tRNA processing locus is absent as in some mutant cell lines designated cytoplasmic petites (large deletions of the mitochondrial genome) the 5' ends of tRNA gene transcripts are not processed normally (2-5). However petites that retain the tRNA processing locus are able to correctly process the 5' ends of tRNA gene transcripts (2-5).

There are multiple sites of initiation of transcription in the yeast mitochondrial genome (8, 9). A consensus sequence at the sites of transcriptional initiation has been identified, ATATAAGTA, with the last A being the initiating nucleotide (6-10). Nineteen unique transcriptional initiation sites have been identified throughout the 80 kb yeast mitochondrial genome by labeling the 5' ends of primary transcripts with guanylyltransferase (9). These in vitro labeled primary transcripts were digested with ribonuclease T₁ and the sequence of the labeled limit digests was determined (9). In thirteen of the nineteen instances, the sequence of the limit digests permitted their assignment to previously sequenced regions of the mitochondrial genome (9).

A "700" nucleotide transcript has been found in the cytoplasmic petites IL8-8c/F11 (F11 is the short name) and IL8-8c/D41, suggesting the location of this transcript to the first portion of the tRNA gene cluster of the mito-

Figure 1. A detailed map of the tRNA gene cluster. Symbols: An open flag represents a nonanucleotide sequence with no evidence that it is functional in vivo, or in vitro. A closed flag with a ball under it represents a nonanucleotide sequence that functions both in vivo and in vitro. The slanted lines within the 21S rRNA region, indicates an intron. The cross hatched regions are regions for which the DNA sequence has not been published.

chondrial genome (2). The tRNA gene cluster is a DNA region of about 10kb between the 21S rRNA gene and oxi 1 gene and codes for 16 out of a total of 24-25 tRNAs (Fig. 1). The rest of the tRNA genes are scattered through the remainder of the mitochondrial genome (for a review, see 1). This "700" nucleotide transcript, labeled in vitro with vaccinia virus guanylyltransferase, hybridized to fragment 7 of the petite F11 double digested with Hpa II and Hinc II (8). The sequence of the 24 nucleotides of the labeled ribonuclease T₁ limit digest product was determined (9). In this paper, we report the DNA sequence for the site of initiation of this "700" nucleotide transcript, which identifies it as a promoter upstream of the tRNA^{Leu} gene. Since there is no other nonanucleotide consensus sequence between this promoter site and tRNA^{Arg2} gene (a region which includes the genes for tRNA^{leu}, gln, lys, arg₁, gly, asp, ser₂, and arg₂, Fig. 1), it is probably the promoter for all of these tRNA genes. We will designate this promoter as the tRNA^{Leu} gene promoter. Another new promoter has been described between tRNA^{Arg2} and tRNA^{Ala} genes (13). Our primer extension analysis supports previous S1 nuclease protection studies of this region (14), and suggests that the GC clusters may be used as RNA processing sites. We provide new evidence that there may be readthrough from an upstream promoter (possibly the tRNA^{Cys} gene promoter 14, 15), implying that there is incomplete or no termination between these two promoters.

MATERIALS AND METHODS

Strains.

The Saccharomyces cerevisiae wild type strain IL8-8c and a petite strain derived from it, IL8-8c/F11 (F11 is the short name for this cytoplasmic

Figure 2. Physical maps of the plasmids. The solid thin line represents the vector DNA, and the wide bar represents the mitochondrial DNA insert. pFH 814 and pFH 1003 contain Hpa II fragments 8 and 10 from the petite IL8-8c/F11 (short name F11). pFH 8/b371 is a Bal-31 deletion of pFH 814. Symbols: The flag represents the mitochondrial promoter. III = Hind III; C = Hinc II; and E = EcoR I.

petite strain) were generously provided by Dr. Hiroshi Fukuhara and Dr. Monique Bolotin-Fukuhara. A detailed map of the petite F11 is given in Locker and Rabinowitz (16). The *E. coli* strain used was RR1ΔM15 (17) and the plasmid used for cloning was pUR 250 (18). The clones pYm132b and pTB3'Δ+64, both of which contain the 14S rRNA gene promoter, are described in Wettstein et al. (19) and in Biswas et al. (10), respectively.

Isolation of DNA.

The isolation of mtDNA from strain F11 employed the method of Locker et al. (20), except that spheroplasts were obtained with zymolyase (Miles Laboratories) rather than with glucylase treatment. Isolation of plasmid DNA from *E. Coli* was as described in Maniatis et al. (21).

Enzymes and Reagents.

Restriction enzymes were purchased from Bethesda Research Lab., International Biotechnologies Inc. (IBI), and New England Biolabs. Assay conditions used were those described by the supplier. The assay conditions for EcoR I* were as described by IBI. Ssp I was purchased from New England Biolabs; however, with their reaction conditions, and over digestion, an "Ssp I*" or a contaminating nuclease activity was observed. This will be described later. ³²P nucleotides were purchased from Amersham.

Construction of plasmids.

Mitochondrial DNA was isolated from the petite F11 and digested with Hpa II. Fragments 8 and 10 were purified by elution after agarose gel electrophoresis and cloned into pUR 250 digested with Acc I, to yield clones pFH 814 and pFH 1003, respectively (see Fig. 2). pFH 814 was reduced in size by Bal-31 digestion, and subcloned as follows: 10 µg of pFH 814 was digested with Eco RI and then with 1.5 U of Bal-31 (New England Biolabs) for intervals between 10 and 40 seconds. The DNA was extracted with phenol-chloroform twice, precipitated with ethanol, and the ends blunted by reaction with the Klenow fragment of *E. coli* DNA polymerase I enzyme, as described in Maniatis et al. (21). The DNA was then digested with Hind III. DNA which was smaller than the original cloned Hpa II fragment 8 was isolated by elution from the agarose gel and cloned into pUR 250 which was digested with Hind III and Hinc II. This generated the Bal-31 deleted clone pFH 8/b371 (see Fig. 2 and 5), which was chosen because the size of the insert indicated that it would overlap with the previously sequenced region (11) and also facilitate sequencing of the unsequenced region.

DNA sequencing.

The appropriate 5' [³²P] end labeled restriction fragments were sequenced by the method of Maxam and Gilbert (22). Sequencing began at the EcoR I site in pFH 814 and pFH 8/b371 and at the EcoR I* site in pFH 814 (see Fig. 4B and 5).

Isolation of RNA for reverse transcription.

Mitochondria were isolated as described in Locker et al. (20), except that spheroplasts were produced by digestion with zymolyase (Miles Laboratories) rather than with glucanase. The mitochondrial pellet was resuspended in lysis buffer (20), extracted twice with phenol-chloroform, and the nucleic acids were precipitated with ethanol. 450 µg of mtRNA from the petite F11 and 1000 µg of mtRNA from IL8-8c were then digested with RNase free DNase I (13 ng) for 45 min at 37°C. The mtRNA was then treated with 40 µg/ml proteinase K for 30 min and extracted with phenol, phenol-chloroform and chloroform, precipitated with ethanol, washed with 70% ethanol, resuspended in H₂O and stored in aliquots at -80°C in 67% ethanol, 0.3M NaAcetate. There was no difference in the quality and integrity of the RNA before or after DNase I digestion as determined by agarose urea gel electrophoresis (23).

Isolation of primers for reverse transcription.

Two primers were isolated for reverse transcription. Primer 2 was isolated from clone pFH 1003 and was obtained by end labeling Hinf I fragments

of the plasmid (21) and then digesting with Alu I. The appropriate fragment, which represents nucleotide 1172 to 1287 in the petite D273-10b/DS502 (11) and spans tRNA^{Leu} to tRNA^{Gln} genes (Fig. 4C), was purified by size on a 12% nondenaturing polyacrylamide gel. Even though only one strand was labeled, the primer was further purified on a strand separating gel (21). Primer 1 was isolated from clone pFH 814. The clone was end labeled at the EcoR I site (21) and then digested with Hind III. The ³²P-labeled EcoR I-Hind III fragment, encompassing Hpa II fragment 8, was purified by elution after agarose gel electrophoresis. After digestion with Ssp I, it was purified a second time on a strand separating gel (21). When the ³²P-labeled EcoR I-Hind III fragment was digested with Ssp I lot no2, in 50mM NaCl, it yielded more than one "limit" digest product. The expected ³²P labeled Ssp I-EcoR I primer and one of the fragments generated from a "Ssp I*" like activity (or a contaminating nuclease activity) were isolated as described above and mapped to within the DNA sequence (Fig. 5). Both yielded the same result in a reverse transcription assay (data not shown). Primer 1 maps from bp 422 to the EcoR I site in pFH 814 (Fig. 5), and from bp 463 to the EcoR I site (Fig. 5), the latter being one of the fragments generated by an "Ssp I*" like activity.

Hybridization of purified mtRNA to primers.

The primers 1 and 2 were hybridized to 160 µg F11 mtRNA or to 260-300 µg of IL8-8c mtRNA in 8% formamide, 0.4 M NaCl, 40mM Pipes pH 6.4, 1mM EDTA. Primer 1 and 2 were hybridized overnight at 37°C and 43°C, respectively, (approximately T_m -25°C).

Reverse Transcription.

After overnight hybridization, the nucleic acids in the reaction mixture were precipitated with ethanol, washed with 70% ethanol, and resuspended in assay buffer for reverse transcription (as described by Life Sciences Co.). The reaction was for one hour at 41°C with 20 U of Avian Myeloblastosis Virus (AMV) reverse transcriptase (James Beard, Life Sciences Co.), including 30 U of RNasin (Promega Biotech), and when indicated, including 1.0 µg/ml actinomycin D.

Promoter strength analysis.

Equal molar amounts of the 14S rRNA promoter (pYm132b digested with Cla I, or pTB3'Δ+64 digested with EcoR I) and the tRNA^{Leu} gene promoter (pFH 814 digested with EcoR I) were transcribed in vitro as described in Wettstein et al., (19), except for the addition of 12U of RNasin/reaction, and 100 µg/ml rabbit serum albumin. The mitochondrial RNA polymerase was gener-

Figure 3. In vitro transcription of pFH 814. pFH 814 was cut with EcoR I and transcribed in vitro as described in Materials and Methods. M1 = pBR322 digested with Sau 3A as marker. M2 = Phi X 174 digested with Hae III as marker. 814 = in vitro transcription from template pFH 814 digested with EcoR I.

ously provided by Dr. Baruch Ticho. The transcription reaction products were separated by electrophoresis on a 5% denaturing polyacrylamide gel and the intensity of the bands representing specific runoff transcripts for each promoter was determined in one of three ways: by eluting the reduced silver grains from the autoradiograph and measuring the absorbance as described by Suissa (24); by scanning the autoradiograph with an LKB laser densitometer

Figure 4. A partial map of the petite F11. (A) A restriction map of the region around the promoter (derived in part from 11). Symbols: * = EcoR I*; A = Alu I; F = Hinf I; II = Hpa II, and B = the extent of Bal-31 deletion in pFH 8/b371. (B) The region sequenced in pFH 814 and pFH 8/b371. (C) Primers used in reverse transcription experiments. Primer 1 is Ssp I or Ssp I"" to EcoR I of pFH 814. Primer 2 is Alu I to Hinf I of pFH 1003. (D) Primer extension products from primer 2 (see Fig. 6). (E) Primer extension products from primer 1 (see Fig. 7 and 8).

and the area in mV was calculated by an IBM LC9540 chromatography integrator; and by eluting the specific runoff products from the acrylamide with 0.5ml of 90% v/v NCS tissue solubilizer for 20h, and resuspending in 6ml budget solve scintillation fluid, for radioactivity measurement.

RESULTS

DNA sequencing of Hpa II fragment 8 from the petite F11.

Hpa II fragment 8 from the petite IL8-8c/F11 (F11 is the short name) was cloned into pUR 250 (Fig. 2). Using purified mitochondrial RNA polymerase for in vitro transcription, this fragment was shown to contain a specific site of initiation of transcription about 320 nucleotides from the EcoR I site in clone pFH 814 (see Fig. 3). Furthermore, when this in vitro transcript was labeled with [γ - 32 P] ATP and digested with ribonuclease T₁, it generated a limit digest product 24 nucleotides in length (data not shown). This indicates that the clone pFH 814 has the site of initiation for the "700" nucleotide transcript, previously assigned to fragment 7 of the petite F11 double digested with Hpa II and Hinc II (8).

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1-----+-----+-----+-----+-----+-----+
CAATTCCTTTTATTATTATAAAATTTTCATTATTATTATTATTATTCATATATA
EcoR I*
61-----+-----+-----+-----+-----+-----+
AAAATAATTATAAAAAAGATTATTAAAAATAAATTTAATGATAAATATATATTATATA

121-----+-----+-----+-----+-----+-----+
TATTAATATAAAAAATAAATAAATAAATATATTATGAAATATTATATAAAATTTGTATAT
              (+1)          Ssp I          (+24)
181-----+-----+-----+-----+-----+-----+
GTATATATTATAATAATGTTATATAAGTAATAATATAATAAAATATTTTATGTAATTTAT
              PROMOTER          Ssp I
241-----+-----+-----+-----+-----+-----+
ATATATTTATAATTATAAAAAATAAATATTATAAATAATAAAATTAATAATAATAAAT
              Ssp I
301-----+-----+-----+-----+-----+-----+
TTTAATAAAATAAATTATATATTTAATTTATTATGAAGTTTATACTTAATATAAATTAT
(B)
361---/-+-----+-----+-----+-----+-----+
ATTCCTTTATAAATTATTAATATATCCTTTTAAATTAATAAAATAAAATATTATAAA
              Ssp I
421-----+-----+-----+-----+-----+-----+
TATTAATAATTAATTTTTTATTATATTTATATATATTAAGATTAAATATATTATTA
Ssp I          "Ssp I**7"
481-----+-----+-----+-----+-----+517
ATACTAGTTTATAATTATTATTAATAAATAGTCCGG
              Hpa II
              acggatccggggaattc
              EcoR I

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Figure 5. DNA sequence of the region of the promoter upstream of tRNA^{Leu} gene. The relevant restriction sites and the promoter are underlined and labeled. The sequence of the capped T₁ transcript (9) is from the first nucleotide of the transcript (+1) to the first G of the transcript (+24). The sequence of the plasmid pFH 814 from Acc 1 insert site to EcoR 1 site is designated in lower case letters. Symbol: B = the first nucleotide retained in the Bal-31 deletion clone pFH 8/b371.

Portions of this region of the mitochondrial genome have been sequenced from another strain of yeast (a petite DS502, which was derived from the wild type strain of yeast D273-10B, 11). However, the 320 nucleotide *in vitro* transcript locates the promoter within the unsequenced A-T rich region. In order to define the nature and position of this promoter, this region of the genome has been sequenced. The subcloning and sequencing strategy is given in Fig. 4B. The DNA sequence is given in Fig. 5. The consensus nucleotide sequence for the promoter is present at position 201-209 with nucleotide 209 being the initiating nucleotide. The sequence from position 209-232 matches the previously determined 24 nucleotide sequence of the T₁ ribonuclease digest of the capped "700" nucleotide transcript, except that we find a "T" at position 231 instead of an "A" (9). The number of bases from the promoter to the 3' end of the tRNA^{Leu} gene is 728 nucleotides (combined data from the

Figure 6. Primer extension with primer 2. Primer 2 was hybridized to 300 μ g of mtRNA from IL8-8c or to 160 μ g of mtRNA from petite F11 at 43°C overnight. Reaction conditions for reverse transcription are described in the methods section. In the experiment illustrated the reaction mixture did not contain RNasin or actinomycin D, but identical results were obtained when these reagents are included (data not shown). Symbols: P = primer 2; M = molecular weight markers, Phi X 174 digested with Hae III (the 271 and 281 nucleotide bands travel anomalously probably related to their secondary structure); IL8 illustrates primer extension products with primer 2 and mtRNA from IL8-8c; F11 illustrates primer extension products with primer 2 and with RNA from the petite F11. Primer extension products 2a, 2b, 2c, and 2d are those so designated in the summary in Fig. 4D.

petite F11, and the petite DS502, 11). Computer analysis of the region from tRNA^{Cys} to tRNA^{Leu} genes, reveals no long open reading frames, as expected for an AT rich region.

Primer extension analysis of petite F11 and Wild type IL8-8c mtRNA.

A primer which spans the tRNA^{Leu} and tRNA^{Gln} genes (primer 2, Fig. 4C) was used to identify the in vivo transcriptional products of both the petite F11 and wild type mitochondrial genomes in this region. Four AMV reverse transcriptase products are noted. Their lengths are about 260-270 nucleotides (2a), 390-420 nucleotides (2b), 400-440 nucleotides (2c), and 770 nucleotides (2d) (Fig. 4D and Fig. 6). The first three products probably result either from processing or pauses at intermediate sites in reverse transcription. These sites are very near the GC rich clusters at positions 230-259 and 394-421 from the 5' end of primer 2 (11). The products of primer extension with petite F11 RNA, 2a, 2b, and 2c, are obtained in approximately equal amounts, but with the RNA of the wild type strain, the primer extension product 2a is much more prominent (on longer exposures one can see primer extension to site 2b with wild type IL8-8c, data not shown). In view of these differences in the proportion of primer extension products with the mtRNA of the two strains we favor the interpretation that these results reflect different levels of in vivo processing in the wild type strain compared to the petite, rather than artifacts of the reverse transcription. Primer extension product 2d is about 770 nucleotides in length, which is in good agreement with the 763 nucleotides predicted for the site of initiation at the consensus promoter.

To more accurately map the promoter another primer was isolated which was closer to the promoter (Primer 1, Fig. 4C). In the experiment illustrated in Fig. 7, one can align the primer extension product (320 nucleotides) obtained with both the petite F11 and the wild type IL8-8c mtRNA with the initiation nucleotide of the promoter, designated as the tRNA^{Leu} gene promoter. In addition to the 320 nucleotide product there are lower levels of larger primer extension products with both the petite F11 and the wild type IL8-8c mtRNA (see Fig. 8 and summary in Fig. 4 E). This suggests that there is read-through from an upstream promoter (i.e. there is incomplete termination between the upstream promoter and the tRNA^{Leu} promoter). The larger primer extension products are about 600 nucleotides and 1000 nucleotides in length. This would place them near the GC cluster just upstream of the tRNA^{Leu} gene promoter and at the 3' end of tRNA^{His} gene respectively (25).

Figure 7. Mapping the promoter by primer extension with primer 1. Primer 1 was hybridized overnight to 260 μ g of mRNA from IL8-8c or to 160 μ g of mRNA from the petite F11, at 37°C. Reaction conditions for reverse transcription are described in the materials and methods section. In the experiment illustrated the reverse transcription mixture contained RNasin and actinomycin D. A 32 P end labeled EcoR I-HindIII fragment from pFH 814 was purified and sequenced by the Maxam-Gilbert method (22). Symbols: G = G; A = A + G; T = T + C; C = C. IL8 is primer extension with primer 1 and mRNA from IL8-8c. F11 is primer extension with primer 1 and mRNA from the petite F11. Primer extension product 1a is the same as in the summary Fig. 4E.

Promoter strength analysis.

If the tRNA^{Leu} gene promoter is equal in strength to the 14S rRNA gene promoter, then in an in vitro transcription assay they should incorporate

Figure 8. Primer extension with Primer 1. The primer extension experiment is identical to that illustrated in Figure 6, except ^{32}P Phi X 174 Hae III markers were used to identify the large primer extension products on longer exposures. Symbols: P = primer 1; M = molecular weight markers, Phi X 174 digested with Hae III; F11 illustrates primer extension products with primer 1 and mtRNA from the petite F11; IL8 illustrates primer extension products with primer 1 and mtRNA from IL8-8c, the dots mark the faint bands. Panels 8A and 8B represent 20h and 11 day exposures respectively. Primer extension products 1a, 1b, and 1c are those so designated in summary form in Fig. 4E.

[α ^{32}P] UTP equally, and the intensity on an autoradiogram should be proportional to the number of UMP's/transcript. Although the apparent relative strength of the tRNA^{Leu} gene promoter varies somewhat with the method employed for transcript quantitation the results indicate that the tRNA^{Leu} gene

TABLE 1 promoter strength analysis

	pFH814(p _{leu})	pYm132b(p _{14S})	p _{leu} /p _{14S}	obs/exp
<u>experiment 1</u>				
LKB abs	8.9x10 ⁶	5.1x10 ⁶	1.7	1.5
Silver grain elution	0.085	0.076	1.1	1.0
Scintillation counting	526	389	1.4	1.3
UMP's per transcript	144	134	1.1	---
	pFH814(p _{leu})	pTB3' Δ +64(p _{14s})	p _{leu} /p _{14S}	obs/exp
<u>experiment 2</u>				
LKB abs	33.8x10 ⁶	3.2x10 ⁶	10.6	2.1
Silver grain elution	0.488	0.110	4.4	0.9
Scintillation counting	1252	162	7.7	1.5
UMP's per transcript	144	29	5.0	---

Table 1. Promoter strength analysis in *in vitro* transcription experiments. The ratio of the absorbance of the runoff transcript of the tRNA^{Leu} gene promoter to the runoff transcript of the 14S rRNA gene promoter is given. p_{leu} = tRNA^{Leu} promoter and p_{14S} = 14S rRNA promoter. LKB abs = area of specific runoff transcript in mV as determined by LKB densitometer scanning and an IBM LC9540 chromatography integrator. Silver grain elution is the absorbance at 500nm of autoradiographic bands obtained from specific runoff products using the method described by Suissa (23). Scintillation counting = cpm of specific runoff products eluted from acrylamide gel with NCS.

promoter is at least as strong as the 14S rRNA gene promoter (Table 1), and much stronger than the nearest known upstream promoter, the tRNA^{Cys} gene promoter, which is about 25 times weaker than the 14S rRNA gene promoter (19).

DISCUSSION

A new promoter, the tRNA^{Leu} gene promoter, has been identified within the cluster of mitochondrial tRNA genes (Fig. 2). An *in vivo* transcript originating from this region has been capped (8), indicating that a promoter would be found. The sequence of the 5' end of this *in vivo* transcript (9) matches for 23 of 24 nucleotides the sequence of the DNA immediately downstream of the tRNA^{Leu} gene promoter characterized in this report. It has been previously

suggested that the tRNA^{Cys} gene promoter functions as the transcriptional initiation site for most or all of the tRNA gene cluster (15). We present evidence that there may indeed be readthrough from an upstream promoter, possibly the tRNA^{Cys} gene promoter. However, our evidence suggests that the transcription of tRNAs originating at the tRNA^{Cys} gene promoter contributes less than does transcription initiating at the tRNA^{Leu} gene promoter. In vitro transcription from the tRNA^{Cys} gene promoter indicates that it is about 25 times weaker than the 14S rRNA gene promoter (19). On the other hand the tRNA^{Leu} gene promoter is at least as strong as the 14S rRNA gene promoter.

Our primer extension analysis reconfirms that mtRNA from wild type IL8-8C and the petite F11 includes RNA species initiating at the tRNA^{Leu} gene promoter and suggests that they may be processed at or near the GC clusters, even in a petite which lacks the tRNA processing locus and the ability to process the 5' end of transcripts of tRNA genes (2-5). Using S1 nuclease protection studies Palleschi et al. (14) showed that there is processing at GC cluster site 2b and 2c, but observed none at the GC cluster site 2a. They also noticed a larger S1 protected fragment which though not precisely mapped, could reflect a transcript originating from the tRNA^{Leu} gene promoter.

The promoter for tRNA^{Leu} gene conforms to the consensus sequence found at all other sites of mitochondrial initiation (6-10). The sequence of this promoter as well as the bases at the +2 and +3 positions, is identical to the sequence of strong promoters such as the 14S rRNA gene promoter, the tRNA^{Phe} gene promoter, and the tRNA^{F-Met} gene promoter (19, 26). It is the only promoter like sequence identified in the known DNA sequence encompassing the genes for tRNA^{Leu} to tRNA^{Arg2} (11,12). So, it is probably used to transcribe all eight of these tRNA genes. It is interesting to note that the last two of these eight tRNA genes, tRNA^{Ser2} and tRNA^{Arg2}, are amongst the least frequently used in decoding the mitochondrial genome (1), especially tRNA^{Arg2} whose codon is only found four times and that within the intron reading frames (1). We expect that there might be transcriptional attenuation between the genes of the more commonly used tRNAs upstream and tRNA^{Ser2} and tRNA^{Arg2}. We have shown that attenuation does occur during transcription of clusters of genes (27). Between the genes for tRNA^{Arg2} and tRNA^{Ala} Bordonne et al. (13) reported a consensus sequence which functions as a promoter for in vitro transcription and they also showed that the in vivo transcript initiating at this sequence can be capped in vitro. It is not established whether transcription from the tRNA^{Leu} gene promoter terminates before the tRNA^{Ala} gene promoter.

ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Dr. Murray Rabinowitz. We thank Dr. Thomas Christianson for many helpful discussions, and Dr. Baruch Ticho and Dr. Tapan Biswas who provided the mtRNA polymerase and the 14S rRNA promoter containing plasmid, pTB3' Δ +64, respectively. This work was supported by NIH training grant GM07543 and by research grant HL04442 grant from the National Institutes of Health.

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