Characterization of tRNA genes in tRNA region II of yeast mitochondrial DNA

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ABSTRACT

We have isolated individual mitochondrial tRNAs from a petite mutant O₁P₂-1 known to contain a limited subset of mitochondrial tRNA genes and have mapped these genes on the wild type genome of the yeast strains MH41-7B and D273-10B. To obtain DNA for fine structure mapping and DNA sequence analysis of these genes, we screened a yeast mitochondrial DNA-pBR322 recombinant bank with the isolated tRNAs. We report here the fine structure mapping of recombinant clones containing the tryptophan, formyl methionine and proline tRNA genes as well as the DNA sequence of the proline tRNA gene. The combination of restriction mapping and DNA sequence analysis has enabled us to locate these genes precisely on the wild type genome and to determine their direction of transcription.

INTRODUCTION

The arrangement of many yeast mitochondrial tRNA genes has been determined by genetic mapping (1,2), physical mapping using bulk labelled tRNAs (3,4) and more recently by fine structure restriction mapping and DNA sequence analysis (5,6,7,8). Physical mapping studies using bulk labelled tRNAs demonstrated four regions (tRNA I-IV) of the wild type genome that contained tRNA genes (9). The tRNA I region contains most of the tRNA genes. The tRNA III region contains the gene for the glutamic acid tRNA (6) and tRNA IV contains a serine tRNA gene (5). The tRNA II region contains genes for formyl methionine tRNA, proline tRNA and tryptophan tRNA. We have previously reported the sequence of the tryptophan tRNA gene (10) and report here the sequence of the proline tRNA gene. The sequence of the formyl methionine tRNA itself has been reported (11). Knowledge of the sequences of these tRNA genes coupled with restriction mapping and hybridization studies have enabled us to precisely locate and determine the direction of transcription of these three genes. The formyl methionine tRNA gene is located at 27.1 units, the proline tRNA gene at 28.6 units and the tryptophan tRNA gene at 41.8 units on the wild type genome of the

strain D273-10B. All of these tRNAs are transcribed from the same strand of DNA in a clockwise direction from the chloramphenicol resistance marker towards the oligomycin II resistance marker.

METHODS

Isolation of nucleic acids

Mitochondrial DNA was isolated from purified mitochondria as described previously (5). Plasmid DNA was isolated from cleared lysates after chloramphenicol amplification. Mitochondrial tRNA was isolated from a petite strain $O_I P_2$ -1 that was derived from the wild type strain MH41-7B. The tRNA was repaired and labeled using *E. coli* nucleotidyl transferase as described previously (5). Individual tRNAs were separated by two dimensional electrophoresis (12) and recovered from the gel by electro-elution. Acylation reactions were done using mitochondrial synthetase (5).

Restriction digests and filter hybridizations

DNA was digested with restriction enzymes according to procedures recommended by the commercial suppliers of the enzymes (New England Biolabs or Bethesda Research Labs). Fragments were resolved on 0.7% agarose gels, transferred to nitrocellulose filters by the method of Southern (13) and hybridized to radiolabelled tRNA for 16 hours at 60°C in Denhardt's buffer (14) containing 6 x SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate).

DNA Sequence Analysis

DNA sequencing was done using the Maxam-Gilbert technique (15). Fragments were separated on 20% and 6% acrylamide gels containing 7 M urea. Fragments containing the formyl methionine tRNA gene were obtained by cutting pYm 233 with Hinc II and labelling with $[\gamma^{32}P]ATP$ using polynucleotide kinase. Fragments containing the proline tRNA gene were obtained by cutting the plasmid pYm 111 with Taq I or Hinf I. The staggered ends were labelled with $[\alpha^{32}P]dCTP$ or $[\alpha^{32}P]dATP$ respectively. To obtain a sequence across the Taq I site the plasmid was cut with Hha I and the resulting fragment containing the majority of the tRNA gene was labelled with $[\gamma^{32}P]ATP$ and polynucleotide kinase.

RESULTS

Isolation, identification and mapping of $O_I P_2$ -1 tRNAs on yeast mitochondrial DNA.

Mitochondrial tRNAs isolated from the petite $O_I P_2$ -1 were labelled with $[\alpha^{32}P]ATP$ and nucleotidyl transferase, and separated by two dimensional polyacrylamide gel electrophoresis. Figure 1 shows an autoradiogram of such a

Figure 1. An autoradiogram of O_1P_2-1 tRNAs isolated from mitochondria, labelled with $[\alpha^{32}P]ATP$ and nucleotidyl transferase and separated on a two dimensional polyacrylamide gel.

gel. The major RNAs, labelled 1-6 were recovered by electro-elution and acylated in a series of experiments to determine their identity. Earlier genetic mapping studies predicted that this petite would contain tRNA genes coding for serine, glutamic acid, valine, threonine, proline, formyl methionine and tryptophan tRNAs. The serine tRNA was not found (see discussion) and each of the six tRNAs did charge with one of the other predicted amino acids (Table I). The only significant contamination between tRNAs was that the valine tRNA contained considerable methionine tRNA. The valine tRNA was purified by electrophoresis a second time before use in hybridization studies.

Each of these tRNAs was hybridized to restriction enzyme digests of MH41-7B (the parental strain of O_1P_2-1) and to D273-10B (the strain from which our recombinant plasmids were constructed). Figure 2 shows the pattern of DNA fragments obtained by digestion of D273-10B DNA with the restriction enzyme Hha I as well as the autoradiogram obtained when each tRNA was hybridized to identical digests that had been transferred to nitrocellulose paper. Using the restriction maps constructed by Morimoto and Rabinowitz (9) for these strains we were able to place the physical boundaries on the location of these tRNA genes. The results for D273-10B are summarized in Table II, and the results obtained for MH41-7B are shown in Figure 3.

TABLE I

ACYLATION OF INDIVIDUAL tRNAs WITH [3 H]-AMINO ACIDS

tRNA	Glu	Trp	Pro	Met	Val	Thr
1	358	735	459	0	45	606
2	213	44,057	459	-	-	-
3	7,773	-	-	-	-	-
4	371	489	-	39,400	1,641	-
5	557	0	411	35,300	248	173
6	153	0	8,827	230	206	169

Transfer RNAs were charged with mitochondrial synthetase in a reaction containing 100 mM Tris, pH 7.5, 15 mM MgCl₂, 6 mM ATP, 0.2 mM dithiothreitol and 5 μ Ci [3 H]-amino acids (specific activity 11-60 Ci/mmol). TCA precipitable counts were determined and blank values have been subtracted.

Figure 2. Mitochondrial DNA from the wild type strain D273-10B was digested with the restriction enzyme Hha I, run on a 0.7% agarose gel and transferred to nitrocellulose filters for hybridization with: a) tRNA^{Glu}, b) tRNA^{Trp}, c) tRNA^{Pro}, d) tRNA^{Met}, e) tRNA^{Val}, f) tRNA^{Thr}. Lanes d-f show hybridization that trails from Hha I band 1 because yeast mitochondrial DNA is not isolated as an intact molecule. Random shearing produces submolar amounts of large restriction fragments and a size range of degradation products from these fragments.

The valine (7,8), threonine (8), glutamic acid (6) and tryptophan (10) tRNA genes that code for the tRNAs isolated from O₂P₂-1 have been sequenced and the precise location and direction of transcription of all of them except the tryptophan tRNA gene have been reported. In order to precisely locate

TABLE II
HYBRIDIZATION OF INDIVIDUAL tRNAs TO RESTRICTION ENZYME
DIGESTS OF D273-10B DNA

tRNA	Digest and Fragment Number		
	Hha I	Eco RI	Hinc II
Glu	8	3	2
Trp	3	2	3
Pro	6	2	4
Met	1	2	4
Val	1	1	1
Thr	1	1	1

Figure 3. A map showing the location of the individual tRNAs with respect to the restriction enzyme sites as mapped on the DNA of the strain MH41-7B by Morimoto and Rabinowitz (9).

the tryptophan tRNA gene on the genome and to determine its direction of transcription, we obtained a fine structure map of the recombinant plasmid pYm 132 (Figure 4). This plasmid contains the Hha I site located at 37.8 units, the Hinc II site located at 38 units, the Bam HI site located at 38.5 units on the D273-10B wild type map derived by Morimoto and Rabinowitz (9), and the small ribosomal RNA gene. The Hinf I site located 2670 base pairs from the Hinc II site on pYm 132 is in the T ψ CG arm of the tRNA with the majority of the structural gene sequence located to the left of this site (10). Therefore, the direction of transcription of this gene is clockwise from the chloramphenicol resistance marker to the oligomycin II resistance marker. The tryptophan tRNA gene is located at 41.8 units on the map of the wild type strain D273-10B.

We also used hybridization of the purified formyl methionine tRNA to locate mitochondrial DNA-pBR322 recombinants containing this tRNA gene. Figure 5 shows a restriction map that we derived for the plasmid pYm 233, which contains the formyl methionine tRNA gene. All of the recombinant plasmids that contained the formyl methionine tRNA gene could be cut in the mitochondrial portion of the plasmid with Hinc II and Hpa I, therefore,

Figure 4. Restriction enzyme site map of the mitochondrial DNA insert contained in pYm 132. Each unit corresponds to 700 base pairs on the map of Morimoto and Rabinowitz (9). The tryptophan tRNA gene is located at the Hinf I site at 41.8 units. Alu I (A), Bam HI (B), Cla I (C), Hae III (Ha), Hinc II (Hc), Hinf I (Hf), Hha I (Hh). Cap and oli 2 refer to the chloramphenicol and oligomycin II resistance markers on the wild type genome.

Figure 5. Restriction enzyme site map of the plasmid pYm 233. Refer to Figure 4 for details. Hpa I (HI), Hpa II (HII). The formyl methionine tRNA is at 27.1 units. The Cla I site is at nucleotide 23 in the plasmid pBR 322 (16).

we were able to locate this gene at 27.1 units on the wild type map of D273-10B (9). An examination of the formyl methionine tRNA sequence determined by Canaday *et al.* (11) shows that these two enzymes should cut in the D loop of the tRNA. Sequence analysis around the Hinc II site of pYm 233 enabled us to confirm that this gene codes for the formyl methionine tRNA sequenced by Canaday *et al.* (11). Since the 3' end-labelled, purified tRNA hybridized to Hinc II band 4 in both wild type strains, we predicted that most of the structural gene would be located in that fragment. To confirm this, we identified a Hinc II-Hpa II fragment in pYm 233 of 250 base pairs. This fragment contained the tRNA gene sequence from the Hinc II site in the D loop to the 3' end, 14 additional base pairs of the mitochondrial DNA, the AT tails used to join the mitochondrial DNA to the plasmid DNA, and finally a portion of the plasmid DNA. We nick-translated this fragment and hybridized it to a Hinc II digest of D273-10B. The probe hybridized to Hinc II band 4 (data not shown). This allowed us to orient the plasmid restriction map with respect to the wild type map and to deduce that this tRNA gene is transcribed in a clockwise direction from the chloramphenicol resistant marker to the oligomycin II resistance marker.

Figure 6. Restriction enzyme site map of the mitochondrial DNA portion of the plasmid pYm 111. The proline tRNA is located at 28.6 units. Refer to Figure 4 for details. Sau 3 A (S).

Figure 7. The sequence of the yeast mitochondrial proline tRNA gene. Only the non-coding strand is presented. The arrows indicate the directions of sequencing away from the labelled ends. The region corresponding to the tRNA is underlined.

Finally, we identified plasmids that contained the gene for the proline tRNA. Figure 6 shows a restriction map that we derived for the plasmid pYm 111. Hybridization of the proline tRNA to restriction digests of plasmid DNA located the tRNA gene in the Hha I, Taq I, Hinf I cluster and sequence analysis demonstrated that the Hha I site was in the D stem of the tRNA. This allows the exact localization of the tRNA gene at 28.6 units on the map of the wild type strain D273-10B. Figure 7 summarizes the results of DNA sequencing around these sites. When the portion of the DNA sequence that corresponds to the tRNA gene is folded into a cloverleaf, it has the anticodon 5' UGG 3' (Figure 8).

To establish the direction of transcription of the proline tRNA gene, we isolated the Hinf I - Hpa II fragment located from 28.8-29.3 units and hybridized it to a Hha I digest of D273-10B DNA (data not shown). It hybridized to Hha I fragment 6 thereby allowing us to orient the plasmid restriction map with respect to the wild type map. A consideration of the map and the sequence allowed us to establish the direction of transcription as being clockwise from the chloramphenicol resistance marker to the oligomycin II resistance marker.

Figure 8. Cloverleaf structure of yeast mitochondrial proline tRNA as deduced from the DNA sequence. The CCA sequence which is not encoded has also been added.

DISCUSSION

The work reported here centers on the tRNA genes located in tRNA region II of the yeast mitochondrial genome. Although intensive sequencing of yeast mitochondrial tRNAs and their genes has been accomplished in the past year, the sequence of the proline tRNA or its gene has not been published. As with the other tRNA genes sequenced so far, there is no intervening sequence, the CCA end is not encoded and the structural gene is surrounded by AT rich DNA. The proline tRNA structure deduced from the DNA sequence is 52% A + T. It has all the invariant nucleotides as elaborated by Rich and RajBhandary (17). The D loop is somewhat unusual in that, following the numbering rules in Sprinzl *et al.* (18), nucleotides are missing from position 17 and 22. The semi-invariant positions are, with the exception of an A in position 48, like that found in all other tRNAs. Most unusual is the U-U pair of the TΨCG stem. The only other reported proline tRNA sequence (18) is from the phage T₄ and this tRNA is 52% homologous with the yeast mitochondrial tRNA.

Petite mutants have been convenient for genetic mapping studies and DNA sequence analysis because they retain limited, discrete regions of mitochondrial DNA. It is now clear that petites are a convenient source of a discrete subset of tRNAs. It has been known for some time that some petites

produce functional tRNAs while others do not (19) but it had not been established that those petites which do produce functional tRNAs produce all of the tRNAs from the genes they retain. The original petite $O_I P_2$ contained seven tRNA genes (1) but the mutant used in these studies produced only six tRNAs. We have hybridized a purified serine tRNA gene probe to restriction digests of the mutant used here and did not find the serine tRNA gene. We presume that this gene has been deleted as a secondary event, and for that reason, designate the mutant used here as $O_I P_2$ -1.

Wesolowski and Monnerot (20) have constructed physical maps of a number of petite mutants whose tRNA gene complement had been determined by hybridization of 3H -aminoacyl tRNAs to the petite DNAs. Although the map locations of some tRNA genes could be narrowed down considerably by these correlations, others such as those coding for threonine, valine, formyl-methionine and tryptophan tRNAs were consistent with more than one location. Mapping studies, hybridization of restriction fragments and sequence analysis of cloned fragments now enable the precise location of the tRNAs genes which code for the tRNAs produced by $O_I P_2$ -1. In addition, these studies have established the direction of transcription of three more mitochondrial tRNA genes. All of these genes are transcribed in the same direction as the majority of yeast mitochondrial genes.

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