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Transfer RNA genes in the cap-oxil region of yeast mitochondrial DNA

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ABSTRACT

A cytoplasmic "petite" (ρ^-) clone of Saccharomyces cerevisiae has been isolated and found through DNA sequencing to contain the genes for cysteine, histidine, leucine, glutamine, lysine, arginine, and glycine tRNAs. This clone, designated DS502, has a tandemly repeated 3.5 kb segment of the wild type genome from 0.7 to 5.6 units. All the tRNA genes are transcribed from the same strand of DNA in the direction cap to oxil. The mitochondrial DNA segment of DS502 fills a sequence gap that existed between the histidine and lysine tRNAs. The new sequence data has made it possible to assign accurate map positions to all the tRNA genes in the cap-oxil span of the yeast mitochondrial genome. A detailed restriction map of the region from 0 to 17 map units along with the locations of 16 tRNA genes have been determined. The secondary structures of the leucine and glutamine tRNAs have been deduced from their gene sequences. The leucine tRNA exhibits 64% sequence homology to an E. coli leucine tRNA.

INTRODUCTION

Most of the mitochondrial tRNAs of S. cerevisiae are encoded in the region of mitochondrial DNA (mtDNA) between the cap and oxil loci. These genetic loci have been shown to be markers of the 21S rRNA (1) and the gene of subunit 2 of cytochrome oxidase (2,3). The results of "petite deletion" mapping and hybridization of tRNAs to restriction fragments of mtDNA indicate the presence of some 15-16 tRNA genes in the cap-oxil span (4,5).

During the past several years, a substantial number of tRNA genes, both from the cap-oxil and other regions of the genome have been sequenced. The gene sequences were determined in mtDNA segments of ρ^- clones selected for the retention of specific tRNA markers (6-8). A number of tRNA genes were also sequenced in fragments of mtDNA cloned in E. coli (9,10). Despite the considerable progress made in defining the number of yeast mitochondrial tRNA genes, some of the genes previously mapped between the cap and oxil loci have not been found due to gaps in the sequence of this region.

In order to complete the sequence of the cap-oxil region, additional ρ^- mutants have been isolated and their genomes sequenced. In this communication we report the DNA sequence of the clone DS502 whose segment of mtDNA has seven tRNA genes including the leucine and glutamine tRNAs that had not been

sequenced. The new sequence together with previous data obtained from other ρ^- clones provides a complete restriction map of the principal tRNA region of yeast mtDNA in which 16 tRNA genes have been identified and accurately placed on the physical map.

MATERIALS AND METHODS

Strains and media: The wild type, antibiotic resistant, mit$^-$ and syn$^-$ strains of yeast used in this study are listed in Table I. The solid media had the following compositions. YPD (2% glucose, 1% yeast extract, 2% peptone, 2% agar), YPEG (2% ethanol, 3% glycerol, 1% yeast extract, 2% peptone, 2% agar), WO (2% glucose, Wickerham's minimal salts and cofactors, 2% agar).

Isolation and characterization of DS502: The respiratory competent strain of S. cerevisiae D273-10B/A21 was mutagenized with ethidium bromide and ρ^- clones containing the cysteine tRNA marker were isolated. For the genetic characterization, the ρ^- mutants were cross-replicated on lawns of the mit$^-$ and syn$^-$ testers spread on WO plates. After two days of prototrophic selection, the diploid colonies issued from the crosses were replicated on YPEG plates and growth scored after an additional 2 days of incubation at 30°. The details of these procedures have been described elsewhere (14).

Purification of mtDNA: Yeast was grown at 30° to early stationary phase in medium containing 2% glucose, 1% yeast extract and 1% peptone. Mitochondria were prepared by the glusulase procedure (15) and the DNA extracted with 2% Sarkosyl and purified by centrifugation on CsCl gradients (16).

Table I

Genotypes of S. cerevisiae Strains

Strain	Genotype	Locus	Reference
D273-10B/A21	α,met,ρ^+,ω^+,$0^R_{625}P^R_{626}E^R_{624}$	oli1,par,ery	(11)
M9-94/A1	α,met,ρ^+,ω^+,mit$^-$	oxi1	(12)
aM9-94-4B	a,adel,ρ^+,ω^+,mit$^-$	oxi1	(12)
G76-26	α,met,ρ^+,ω^+,syn$^-$	cysteine tRNA	(6)
aG76-26-8A	a,adel,ρ^+,ω^+,syn$^-$	cysteine tRNA	(6)
G76-35	α,met,ρ^+,ω^+,syn$^-$	histidine tRNA	(6)
aG76-35	a,adel,ρ^+,ω^+,syn$^-$	histidine tRNA	(6)
G8-41	α,met,ρ^+,ω^+,syn$^-$	glutamine tRNA	this study
aG8-41	a,adel,ρ^+,ω^+,syn$^-$	glutamine tRNA	this study
M7-37	α,ρ^+,ω^+,syn$^-$	aspartic tRNA	(13)
aM7-37-70C	a,adel,ρ^+,ω^+,syn$^-$	aspartic tRNA	(13)
aG116-40-7A	a,adel,ρ^+,ω^+,syn$^-$	tyrosine tRNA	(6)

Restriction endonuclease analysis: Restriction enzymes were purchased from New England BioLabs, Mass. Digestions of the mtDNA were carried out in a buffer containing 10 mM Tris-Cl, pH 7.5, 10 mM $MgCl_2$ and 10 mM 2-mercaptoethanol. DNA fragments resulting from the digestions were separated on 1% or 1.2% agarose gels in a Tris-borate buffer system (17). The sizes of the fragments were estimated with a calibration mixture consisting of a HaeIII digest of φX174RF (18) and a HindIII digest of λ DNA (19).

DNA sequencing: Restriction fragments were treated with bacterial alkaline phosphatase (Worthington Biochem., N.J.) and labeled at the 5' ends with (γ-^{32}P)ATP (New England Nuclear Corp., Mass., 2,000-3,000 Ci/mmol) in the presence of T4 polynucleotide kinase (20). The labeled fragments were separated into single strands on 4%, 6%, or 8% polyacrylamide gels and sequenced by the chemical modification method of Maxam and Gilbert (20).

Characterization of tRNA mutation in G8-41: The mutation of the syn$^-$ strain G8-41 has been localized by "petite deletion" mapping to a region of mtDNA with the leucine and glutamine tRNA genes. To identify the defective tRNA, G8-41 was grown in 2% galactose, 1% yeast extract and 1% peptone. Total mitochondiral tRNAs were prepared, charged singly with different ^{3}H-amino acids, and separated by reverse-phase chromatography on RPC-5 (21). Wild type tRNAs were acylated and chromatographed under identical conditions. The ^{3}H-amino acids used were leucine, glutamic acid and aspartic acid (the specific activities ranged from 40-100 mCi/mmol, New England Nuclear Corp., Mass.). Both glutamic acid and glutamine tRNAs have been shown to be acylated with glutamic acid (22). The results of the acylation assays indicated that G8-41 has a lesion in the glutamine tRNA. Of the amino acids tested, only the glutamine tRNA failed to be charged in the mutant (data not shown).

RESULTS

Genetic and physical properties of the DS502 genome: In a previous study, we found that ρ^- clones selected for the retention of markers in tRNA genes have mtDNA segments enriched in sequences from the cap-oxi1 region (6). This approach was used to isolate the clone DS502. The genotype of DS502 indicated the presence of genetic markers in the histidine, cysteine and glutamine tRNA genes (Table II). The retention of the glutamine tRNA marker suggested that the mitochondrial genome of DS502 extended into a region of mtDNA for which no sequence data had been available. This was also supported by the restriction map of the DNA.

DS502 was ascertained to have a circular genome with a tandemly repeated segment of mtDNA. The unit length of the segment was estimated to be 3.5 kb from the sizes of the fragments produced with HinfI, TaqI and other restriction endonucleases (Fig. 1).

The restriction map of the DS502 mtDNA was derived from an analysis of the

Table II

Genetic and Molecular Complexity of mtDNA in the ρ^- Clones

ρ^- Clone	Genetic Markers								Repeat length of mtDNA (kb)
	ery	cap	cys	his	gln	asp	tyr	oxil	
DS504	-	-	+	+	-	-	-	-	1.8
DS502	-	-	+	+	+	-	-	-	3.5
DS200/A5	-	-	-	-	-	+	+	-	6.4
DS200/A1	-	-	-	-	-	-	-	+	4.5
DS200/M6	-	-	-	-	-	-	+	-	2.4
DS513	+	+	+	+	-	-	-	-	16

The isolation and characterization of the DS504, DS200/A5, DS200/A1, DS513, and DS200/M6 clones has been described (2,6,7).

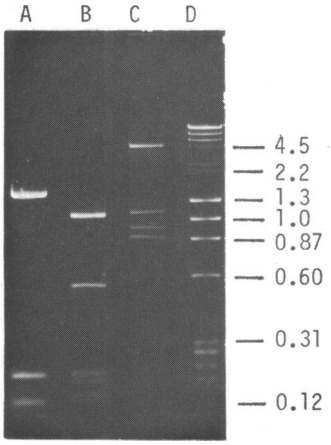

Fig. 1. HinfI digestion products of DS504, DS502 and DS200/A5 mtDNAs. The fragments were separated on a 1.5% agarose gel and stained with ethidium bromide. A: DS504, B: DS502, C: DS200/A5, D: HindIII digest of λ and a HaeIII digest of ϕX174RF DNA. The sizes of the standards are indicated in the right hand margin in kilobases.

fragments generated by various combinations of restriction enzymes. The location of the restriction sites on the circular map of DS502 is shown in Fig. 2. The segment contains 9 HinfI, 6 HpaII and HaeIII, 5 TaqI, 3 AluI and MboII and 2 HphI and SacII sites. None of these sites have previously been mapped on the wild type mtDNA of the parental D273-10B strain (23).

Nucleotide sequence of DS502 mtDNA: The DS502 genome was sequenced by the chemical derivatization method of Maxam and Gilbert (20). The sequences were obtained on 5'-end labeled restriction fragments following separation of the single strands by electrophoresis on polyacrylamide gels. The fragments used to sequence the DNA are shown in Fig. 2. Since some of the regions of the

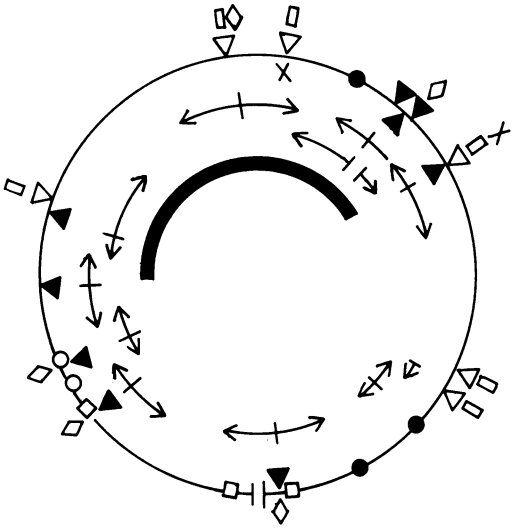

Fig. 2. Restriction map of the DS502 genome. The circle shows the locations of the HinfI (▲), MboII (□), TaqI (◇), HphI (○), HpaII (△), HaeIII (▯), AluI (●) and SacII (x) sites. The juncture of the DNA is indicated by the break in the circle. The arrows denote the direction and approximate length of the sequences obtained. The solid bar corresponds to the region of the DNA whose sequence is reported in Fig. 3.

DS502 genome have already been sequenced in other ρ^- clones (6,7), we did not attempt to obtain the complete sequence of the DNA.

The sequence of a 1.5 kb region between the HinfI sites at 1.7 and 4 map units is presented in Fig. 3. This region is unique to DS502 and is not present in any of the other ρ^- clones studied previously. Partial sequences of the regions flanking the HinfI sites, however, confirmed the sequences obtained in earlier studies from the clones DS504 and DS200/A5 (6,7). The sequence reported here is complete except for a stretch of approximately 190 nucleotides that was not sequenced due to the absence of restriction sites for end-labeling. Although nucleotides 447-636 could not be read accurately, the sequencing gels indicated that this region consists of an almost pure A+T sequence unlikely to to have a coding function.

The sequence of the DS502 region reported here has an A+T-rich character. There are two short sequences with a sufficiently high G+C content to be candidates for tRNA genes. These occur at nucleotides 1171-1252 and 1286-1357. Each sequence can be folded into a cloverleaf structure with the appropriate bases at the invariant positions of standard procaryotic and eucaryotic tRNAs. The two genes have been identified to code for $tRNA^{Leu}_{UAA}$ and $tRNA^{Gln}_{UUG}$ based on the anticodons in the deduced secondary structures. Although neither tRNA has been sequenced, Martin et al. (24) have determined the base composition of the leucine tRNA. The reported composition is in excellent agreement with that derived from the gene sequence (Table III).

In addition to the two tRNA genes, the sequence contains 4 clusters of nucleotides with a very high G+C content. Each cluster has several HpaII/HaeIII sites and in some cases SacII sites. Such HpaII/HaeIII site clusters

```
   1
5'-GAGTCTTTTTATTGAATATTAAATTCTATTATAAC TTATTAATTAATTAATTAATTATAATAATAATAAT
   HinfI

  71 AATATTTATTATTAATTATTAAATATTTATTATTA TATATAGGATTTAATTTTAAATATTAATAAAAAAA

 141 GAATAAAATAAAATAAAATGAATAATATTTCTTTA TTCCTTCGGTCGGANTCCTTCGGGGTCCGCCCCGC
       HaeIII                                          HinfI
 211 AGGGCGGCCGGACTATTTATTATTATAATATAATA TTTAATCAATAGAGTTTATAATTTATTTAATGAAT
         HpaII

 281 ATTTTATAAATATGTAAAACAATTCCTTTTTATTA TTATAAATTTTTCATTATTTATTTATTTATTTATT

 351 TATTTATTTATTCAATATATAAAAATAATTATAAA AAAGATTATTAAAAATAATAATTTTAATGATAAAT

 421 ATATATTATATATATTTAATATAAAA---- ( A+T-rich sequence of approximately 190

     nucleotides ) ------ TTTAAATTTTATTA TAGAAGTTTATACTTAATATAAATTATATTTCCTT

 686 TATAAATTATTAATATATCCTTTTTAATTAAATAA AATAAAAATATTATAAATATTAATAATTAATTTTT

 756 TATTTATATTTATATATATATTAAAGATTAAATAT ATTATTAATACTAGTTTATAATTTATTATTAATAA
       HaeIII                          TaqI
 826 ATAGTCCGGCCGCCCTGCGGGGCGGACCCCGGGTC GACTTAAATTATAATTTAATAATTTTTATTTATTA
     HpaII                         HpaII
 896 ATAGTTTTATTATTAATATAAAAAGAGTAAGGATA ATAATAAATTCTTTTAATTTATTTTTTAATAAAAT
                                 HaeIII
 966 ATAATTTTAAAATAGTTTTTATAGTCCGGCCCGCC CCGCGGCGCCCGAAGGAGGTTCGGTCTGGCATTAA
                           HpaII       SacII
1036 TTATAATAATTATATTAATATTATTATTATTTAAT ATATTATAATATATTTATTTATATTTTATAATATT
                                                                    Leu →
1106 AATAATTATTTTATATTTAATAAATATAATATATA TTTTATATTTTAATAACTATCTAATTAATAgctat
                                                                    AluI
1176 tttggtggaattggtagacacgatactcttaagat gtattactttacagtatgaaggttcaagtccttta
                                                  Gln →
1246 aatagcaATAAATATATATAATATATATAATATAT ATAAAtgagtcgtagacaataggtaagttaccaaa
                                                  HinfI
                           TaqI
1316 atttgagtttggagtttgtttgttcgaatcaaacc gattcaaTATTATAATATATATATTATTTATATAT
                           HinfI       HinfI

1386 AAATATATAATTATACTCCTATTTTTATATTAATT AATTAATTAATTAATAATATATGATAATATAAAAA
                                                  HpaII
1456 TTATTGAATTATTAACTCCTTCGGGGTCCGCCCCG CGGGGGCGGGCCGGACTC-3'
                                    SacII     HaeIII HinfI
```

Fig. 3. Nucleotide sequence of the DS502 genome. The sequence shown is that of the non-transcribed strand in the region indicated in Fig. 2. The tRNA genes have been typed in lower-case.

Table III

Base Composition of Yeast Mitochondrial Leucine tRNA

Leucine tRNA[a]		Leucine tRNA gene[b]	
A	29.9%	A	30.6%
U	33.3%	T	34.1%
G	19.7%	G	20%
C	17%	C	15.3%

[a] From Martin et al (24). The values for G and U include modified bases.

[b] These values include the 3'-CCA not encoded in the gene

have previously been shown to be scattered in many other regions of the yeast genome, their function still being a subject of speculation (25-27). Several of the G+C clusters have inverted homologous sequences capable of fairly extensive base pairing. Some examples of such inverted repeats are shown in Fig. 4.

Structures of the yeast mitochondrial leucine and glutamine tRNAs: The secondary structures of the leucine and glutamine tRNAs are shown in Fig. 5. The mitochondrial leucine tRNA has the long variable loop noted in all eucaryotic and procaryotic leucine tRNAs (28). The TΨC and D loops of the leucine and glutamine tRNAs have the usual conserved bases at the appropriate positions. The two tRNAs also have normal size stems with seven base pairs in the acceptor and 5 base pairs in the TΨC and anticodon stems. The anticodons are bracketed by a U and a purine on the 3' and 5' sides, respectively.

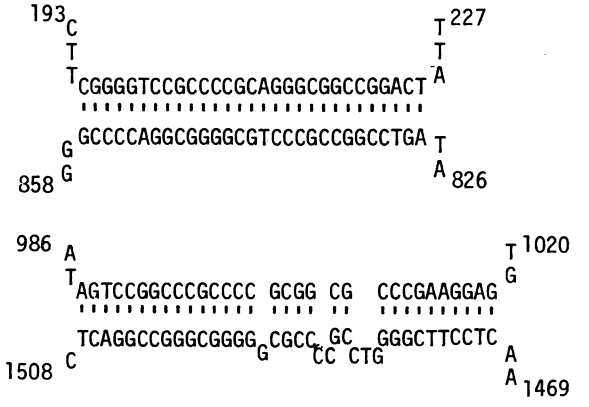

Fig. 4. Base pairing of the inverted repeated sequences in DS502. The nucleotides have been numbered according to the convention of Fig. 3.

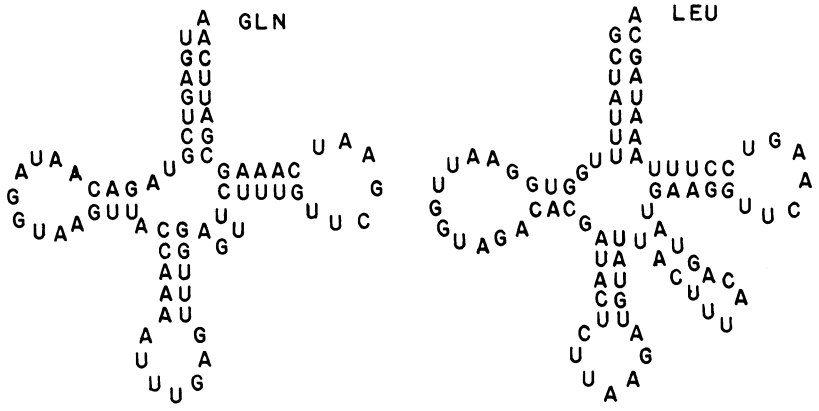

Fig. 5. Secondary structures of the $tRNA^{Gln}_{UUG}$ and $tRNA^{Leu}_{UAA}$ deduced from the DNA sequences of the genes.

Most of the mitochondrial tRNAs of yeast have been found to be 35-50% homologous to bacterial and yeast cytoplasmic tRNAs (6-10). The extent of sequence homology between the yeast mitochondrial and cytoplasmic leucine tRNAs (29, 30) also falls in this range. Somewhat higher homology, however, is seen with the $tRNA^{Leu}_{GAG}$ of E. coli K12 (31). The mitochondrial and E. coli tRNAs have identical D stems and loops. The TΨC loops and distal two base pairs in the stem also have identical sequences (Fig. 6).

Physical map and positions of the tRNA genes in the cap-oxil span: The physical locations of the tRNA genes shown in Fig. 7. have been determined from the DNA sequences and restriction maps of a series of ρ^- clones with over-

	Acc. stem	D stem	D loop	D stem	Anti-codon stem	Anti-codon loop	Anti-codon stem
Yeast mit.	GCUAUUU	UG	GUGG	AAUUGGUA	G ACAC	G AUACU	CU UAA GA UGUAU
E. coli K12	GC.....	UG	GUGG	AADDGGDA	G ACAC	G .UAC.	.U .A. G. .GUA.
Yeast cyt.	G.U..UU	UG	G..G	A...GGD.	. A..C	G	.U .AA G. Ψ....
Yeast cyt.	G..A.UU	UG	G..G	A.D.GGD.	. A..C	G ..A..	.U UA. G. Ψ.C..

	Variable loop	TΨC stem	TΨC loop	TΨC stem	Acc. stem		Homology
Yeast mit.	UACUUUACAGUA	U	GAAGG	UUCAAGU	CCUUU	AAAUAGC	ACCA
E. coli K12	U.C...A.AG..	U	...GG	TΨCAAGU	CC..U	.....G.	ACCA 64%
Yeast cyt.	UA...UA...U.	.	.A..G	TΨC.A.U	C..UU	A...A.C	ACCA 49%
Yeast cyt.	UA..UU....U.	.	.A.GG	TΨC.A.U	CC.UU	A..U..C	ACCA 55%

Fig. 6. Comparison of the yeast mitochondrial to yeast cytoplasmic and E. coli leucine tRNAs. Only the homologous bases are shown in the E. coli K12 (31) and the two yeast cytoplasmic (29,30) tRNAs.

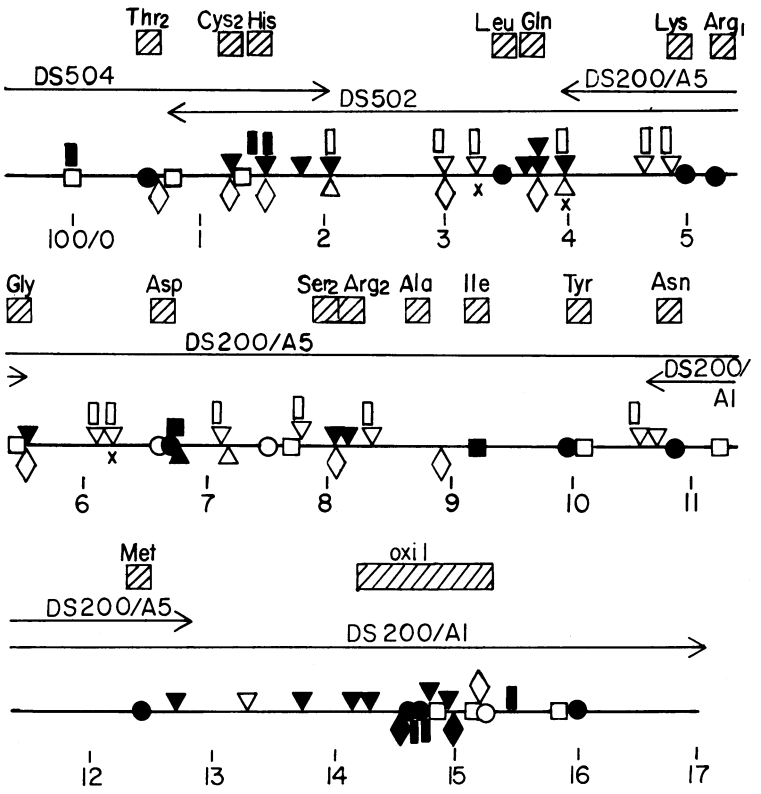

Fig. 7. Physical map of mtDNA in the cap-oxi1 span. The symbols used to denote the restriction sites are: HinfI (▲), TaqI (◇), MboI (○), MboII (□), HaeIII (▯), HpaII (△), AluI (●), HphI (▮), RsaI (■), SacII (✕), PvuII (◆). The locations of the tRNA genes are shown by the hatched boxes. The segments of the wild type genome retained in the ρ^- clones are indicated by the double headed arrows. The map units are marked off below the map. Each map unit corresponds to 700 base pairs.

lapping segments of mtDNA. The genotypes of the clones are listed in Table II. The sequences and tRNA genes found in most of the clones have already been reported (6,7). The deletion endpoints or physical limits of the mtDNA segments of the ρ^- clones were established by comparing the nucleotide sequences in the regions of overlap. The DS502 segment has an MboII site at 0.8 units in common with the segment of DS504. The absence in DS502 of the neighboring AluI and TaqI sites at 0.6 units indicates that the deletion in DS502 occurred between 0.6 and 0.8 map units. The exact deletion endpoints were determined from the sequence of a TaqI fragment which crossed the junction of the DS502 segment. A comparison of the sequences of DS502, DS504 and DS200/A5 showed that

the deletions in DS502 were initiated 79±2 nucleotides to the left of the MboII site at 0.8 units and 47±2 nucleotides to the right of the TaqI site at 5.5 units. The mtDNA segment, therefore, bridges a gap between the genes of the threonine and aspartic acid tRNAs.

The complete sequence of the cap-oxi1 span has revealed the presence of 16 tRNA genes between the 21S rRNA and the cytochrome oxidase subunit 2 genes. No other coding sequences have been found in this region. The tRNA genes account for only 10% of the total DNA, the rest being composed of A+T-rich sequences and G+C clusters. The positions of the genes on the physical map have been adjusted to the right-most PvuII site in the oxi1 gene. This site has been placed at 15 units of the D273-10B map (23) and has been adopted as the reference for the entire region.

The locations of the tRNA genes are listed in Table IV. The genes are scattered from 0.6 ($tRNA^{Thr}_{UGU}$) to 12.4 ($tRNA^{Met}_{CAU}$) units. Although most of the genes are separated by fairly long A+T-rich sequences, in some instances the gene spacers are very short. For example, the 5' end of the glutamine tRNA is only 33 nucleotides away from the 3' end of the leucine tRNA. Even more striking are the serine and arginine tRNAs that are separated by only 3 nucleotides (7,9). The alignment of the ρ^- genomes relative to the wild type map indicate that all the tRNA genes are encoded in one DNA strand with the same 5' to 3' orientation. The direction of transcription in all cases is cap → oxi1.

Table IV

Map Positions of tRNA Genes

tRNA	Map Units
Thr (UGU)	0.6
Cys (GCA)	1.3
His (GUG)	1.4
Leu (UAA)	3.5
Gln (UUG)	3.7
Lys (UUU)	4.9
Arg (UCU)	5.3
Gly (UCC)	5.4
Asp (GUC)	6.6
Ser (GCU)	8.0
Arg (ACG)	8.1
Ala (UGC)	8.7
Ile (GAU)	9.3
Tyr (GUA)	10.1
Asn (GUU)	10.9
Met (CAU)	12.4

DISCUSSION

The deletion mutant DS502 reported here was selected for the retention of genetic markers in the cysteine, histidine and glutamine tRNA genes. The DS502 mtDNA segment has provided the sequences of two new tRNA genes and has helped to bridge a sequence gap in the cap-oxi1 region for which no data had been available. The two genes unique to the DS502 genome have been identified as the $tRNA^{Leu}_{UAA}$ and $tRNA^{Gln}_{UUG}$. Although the mtDNA segment of DS502 includes five other tRNA genes, their sequences had already been determined from other ρ^- clones whose segments partially overlapped with DS502 (6,7).

The leucine and glutamine tRNAs have conventional secondary structures. The leucine tRNA possesses a long variable loop (17 nucleotides), a feature observed in all other known eucaryotic and procaryotic leucine tRNAs. Both tRNAs also exhibit the correct bases at the invariant positions. Their D loops contain the two guanosines which base pair with the cytosine and pseudouridine of the TΨC loop (32). The TΨC loops consist of 7 nucleotides with 5 base pairs in their stems. The anticodon loops and stems are also regular with the appropriate bases bracketing the three nucleotides of the anticodons.

Most of the yeast mitochondrial tRNAs average 35-50% homology with either procaryotic or eucaryotic tRNAs (6-10, 33-35). The leucine tRNA, however, is significantly more homologous to the E. coli tRNAs than to its cytoplasmic counterpart. The homology with the E. coli K12 $tRNA^{Leu}_{GAG}$ is particularly evident in the D and TΨC loops. The observed homology implies a closer evolutionary relationship of the mitochondrial species to procaryotic leucine tRNAs. The similarity in the primary sequences may also explain the fact that E. coli synthetase will aminoacylate yeast mitochondrial but not cytoplasmic leucine tRNA (36).

The DS502 clone has been useful in completing the sequence analysis of the cap-oxi1 span in S. cerevisiae D273-10B. Based on the present and earlier studies (2,6,7), this region codes for 16 different tRNAs. The tRNA genes and the gene of subunit 2 of cytochrome oxidase are encoded in the same DNA strand and are transcribed in the direction cap → oxi1. This situation is to be contrasted with mitochondrial genomes of higher eucaryotes in which tRNA genes are found in both DNA strands (37,38).

Many of the tRNA genes located in the cap-oxi1 span have already been mapped by hybridization of specific aminoacylated tRNAs to the mtDNA segments of ρ^- clones originating from the first quadrant of the wild type genome (4,5,39). These studies provided a relative order of the tRNA genes, but did not indicate their exact map positions. The identity and locations of the tRNA genes derived from the nucleotide sequence of the cap-oxi1 region are in general agreement with the genetic evidence. Some discrepancies, however, exist between the two sets of data. For example, the order of the glycine, aspartic acid and alanine tRNA genes are different from that deduced by "petite deletion" mapping

(4). This may be due either to strain-specific differences in the gene organization of yeast mtDNA or to errors in the mapping method. Despite these minor differences, it is significant that the number of tRNA genes found in the cap-oxi1 region comes very close to what had been predicted by hybridization of tRNAs to the genomic DNAs of ρ^- clones. In another communication we have presented evidence for the ability of yeast mitochondria to recognize all the amino acid codons with only 24 tRNAs (40). This number is in good agreement with the mapping data of Wesolowski and Fukuhara (4) and is supported by the genes found in the DNA sequence of the principal tRNA coding region of the yeast mitochondrial genome reported here.

The mode of transcription and processing of the mitochondrial tRNAs of yeast is not known presently. The sequences upstream of the tRNA genes do not appear to have common sequences that can be ascribed promoter functions, although it cannot be excluded that the promoters may reside within the structural sequences themselves. Alternatively, the genes may be co-transcribed from an extended region of the DNA. The primary transcripts could be further processed by mitochondrial RNases capable of recognizing the secondary structures of the tRNAs in the large transcripts. Some initial processing of primary transcripts from the cap-oxi1 region could also involve cleavage of the RNA at the HpaII/HaeIII site clusters. The ability of such G+C clusters to base pair is suggested by their sequences which frequently exhibit inverted homologies. The complementary regions in the clusters often have a common 10-12 nucleotide sequence that has been proposed to serve as a recognition site for a double strand specific RNase similar to RNase III (27). The presence of complementary G+C clusters in the tRNA region tends to support this type of processing model. The leucine and glutamine tRNA genes, for example, are flanked by two inverted repeats with the common twelve nucleotide sequence. Cleavage of the RNA at these sequences would have the effect of releasing the two genes in a single smaller transcript that could be processed to the final size by a P3 type of RNase (41).

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REFERENCES

Abbreviations: ρ^- refers to cytoplasmic "petite" mutants resulting from long deletions in mtDNA. Mit loci are markers in mitochondrial genes that code for subunit polypeptides of cytochrome oxidase (oxi), cytochrome b (cob), and the oligomycin-sensitive ATPase (pho). Syn loci are markers in mitochondrial tRNA and rRNA genes. The antibiotic resistance loci referred to in this paper are paromomycin (par), erythromycin (ery), chloramphenicol (cap) and oligomycin (oli).

1. Dujon, B. (1980) Cell 20, 185-197.
2. Coruzzi, G. and Tzagoloff, A. (1979) J. Biol. Chem. 254, 9324-9330.

3. Fox, T.D. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6534-6538.
4. Wesolowski, M. and Fukuhara, H. (1979) Molec. Gen. Genet. 170, 261-275.
5. Van Ommen, G.J.B., Groot, G.S.P. and Borst, P. (1977) Molec. Gen. Genet. 154, 255-262.
6. Berlani, R.E., Pentella, C., Macino, G. and Tzagoloff, A. (1980) J. Bacteriol. 141, 1086-1097.
7. Bonitz, S.G. and Tzagoloff, A. (1980) J. Biol. Chem. in press.
8. Bos, J.L., Osinga, K.A., Van der Horst, G., and Borst, P. (1979) Nucl. Acids Res. 6, 3255-3266.
9. Martin, N.C., Miller, D., Hartley, J., Moynihan, P. and Donelson, J.E. (1980) Cell 19, 339-343.
10. Miller, D.L., Martin, N.C., Pham, H.D. and Donelson, J.E. (1979) J. Biol. Chem. 254, 11735-11740.
11. Tzagoloff, A., Akai, A. and Foury, F. (1979) FEBS Lett. 65, 391-396.
12. Tzagoloff, A., Akai, A., Needleman, R.B. and Zulch, G. (1975) J. Biol. Chem. 250, 8236-8242.
13. Trembath, M.K., Macino, G. and Tzagoloff, A. (1978) Molec. Gen. Genet. 158, 35-45.
14. Slonimski, P.P. and Tzagoloff, A. (1976) Eur. J. Biochem. 61, 27-41.
15. Faye, G., Kujawa, C. and Fukuhara, H. (1974) J. Mol. Biol. 88, 185-203.
16. Sanders, J.P.M., Borst, P. and Weijers, P.J. (1975) Molec. Gen. Genet. 143, 53-64.
17. Peacock, A.D. and Dingman, C.W. (1968) Biochem. 7, 668-674.
18. Sanger, F., Air, G.M., Barrell, B.G., Brown, N.L., Coulson, A.R., Fiddles, J.C., Hutchison, C.A., Slocombe, P.M. and Smith, M. (1977) Nature 265, 687-695.
19. Murray, K. and Murray, N.E. (1975) J. Mol. Biol. 98, 551-564.
20. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 560-564.
21. Macino, G. and Tzagoloff, A. (1979) Molec. Gen. Genet. 169, 183-188.
22. Martin, N.C. and Rabinowitz, M. (1978) Biochem. 17, 1628-1634.
23. Morimoto, R. and Rabinowitz, M. (1979) Molec. Gen. Genet. 170, 25-48.
24. Martin, R.P., Schneller, J.-M., Stahl, A.J.C. and Dirheimer, G. (1977) Nucl. Acids Res. 4, 3497-3510.
25. Prunell, A., Kopecka, H., Strauss, F. and Bernardi, G. (1977) J. Mol. Biol. 110, 53-74.
26. Cosson, J. and Tzagoloff, A. (1979) J. Biol. Chem. 254, 42-43.
27. Tzagoloff, A., Akai, A., Nobrega, M. and Macino, G. (1980) Current Genet. in press.
28. Gauss, D.H., Gruter, F. and Sprinzl, M. (1979) Nucl. Acids. Res. 6, 1-44.
29. Chang, S.H., Kuo, S., Hawkins, E. and Miller, N.R. (1973) Biochem. Biophys. Res. Commun. 51, 951-955.
30. Randerath, K., Chia, L.S.Y., Gupta, R.C., Randerath, E., Hawkins, E.R., Brum, C.K. and Chang, S.H. (1975) Biochem. Biophys. Res. Commun. 63, 157-163.
31. Blank, H.U. and Soll, D. (1971) Biochem. Biophys. Res. Commun. 43, 1192-1197.
32. Kim, S.-H., Suddath, F.L., Quigley, G.J., McPherson, A., Sussman, J.L., Wang, A., Seeman, N.C. and Rich, A. (1974) Science 185, 435-440.
33. Martin, R.P., Sibler, A.P., Schneller, J.M., Keith, G. Stahl, A.J.C. and Dirheimer, G. (1978) Nucl. Acids. Res. 5, 4579-4592.
34. Sibler, A.-P., Bordonne, R., Dirheimer, G. and Martin, R.P. (1980) C.R. Acad. Sc. Paris, t.290, 695-698.
35. Sibler, A.-P., Martin, R.P. and Dirheimer, G. (1979) FEBS Lett. 107, 182-186.
36. Casey, J.W., Hsu, H.J., Getz, G.S., Rabinowitz, M. and Fukuhara, H. (1974) J. Mol. Biol. 88, 735-747,

37. Angerer, L., Davidson, N., Murphy, W., Lynch, D. and Attardi, G. (1976) Cell 9, 81-90.
38. Ohi, S., Ramirez, J.L., Upholt, W.B. and Dawid, I.B. (1978) J. Mol. Biol. 121, 299-310.
39. Colletti, E., Frontali, L., Palleschi, C., Wesolowski, M., and Fukuhara, H. (1979) Molec. Gen. Genet. 175, 1-4.
40. Bonitz, S.G., Berlani, R., Coruzzi, G., Li, M., Macino, G., Nobrega, F.G., Nobrega, M., Thalenfeld, B.E. and Tzagoloff, A. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, in press.
41. Altman, S. (1978) in "Transfer RNA" (S. Altman, ed.) MIT Press, Cambridge, Mass., pp. 48-77.