Identification of the Paromomycin-resistance Mutation in the 15 S rRNA Gene of Yeast Mitochondria*

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A fragment of yeast mitochondrial DNA cloned in *Escherichia coli* has been used to sequence the 15 S rRNA gene. The mtDNA insert in the recombinant plasmid was derived from the genome of the wild type strain of *Saccharomyces cerevisiae* D273-10B. Alignment of the experimentally determined 5' and 3' sequences of the mature 15 S rRNA with the DNA sequence indicates the gene to be 1686 nucleotides long. S1 nuclease mapping has failed to reveal the presence of intervening sequences in the gene. The 15 S rRNA gene has been localized to the region between 37.5 and 40.1 units of the wild type mitochondrial map. Based on the orientation of the 5' and 3' ends, the gene is transcribed from the same strand of DNA as the 21 S rRNA and most other mitochondrial genes.

*S. cerevisiae* D273-10B/A21 is isogenic with the D273-10B strain, but carries three antibiotic resistance mutations in the par, olili, and ery loci. This strain was used to isolate cytoplasmic petite mutants whose retained segments of mtDNA contain only the par locus. The mutant designated DS80 was ascertained to have the least complex mitochondrial genome (2600 base pairs) with the complete 15 S rRNA gene. The restriction map of the DS80 genome was identical to that of the comparable region in the cloned fragment with the single exception of a *Tha* I site at 39.8 map units which is absent in DS80. The sequence of the 15 S rRNA gene in DS80 revealed a single nucleotide difference at position 1514 of the gene where a C has been exchanged for a G. This nucleotide substitution accounts for the loss of the *Tha* I site. The mutation in the 15 S rRNA gene of the paromomycin-resistant strain indicates that the par locus is a genetic marker of the gene.

The 15 S rRNA of yeast mitochondria exhibits extensive homology with the 16 S rRNA of *E. coli* both in its primary and secondary structures. The 3' tail of yeast 15 S rRNA, however, does not have the 5'-ACCUCC-3' sequence postulated to be the mRNA binding site of the *E. coli* ribosome (Shine, J. and Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. U. S. A.* 71, 1342-1346). The same region of the yeast 15 S rRNA has the sequence 3'--AAATTCTATA--5' which can base pair with certain sequences found upstream of the initiation codons of yeast mitochondrial genes. It is suggested that this sequence may be functionally analogous to the Shine and Dalgarno sequence of the *E. coli* 16 S rRNA.

The 15 S and 21 S rRNAs of yeast mitochondria have been known for some time to be encoded in the organellar genome (1). Unlike most other ribosomal genes, those of yeast mitochondria are quite widely separated on the genome and have recently been shown to be transcribed individually (2). The 21 S rRNA gene occupies the span of mitochondrial DNA from 94 to 100 map units (3, 4). The gene for the 15 S rRNA has been mapped near the paromomycin-resistance locus (par) at approximately 30–40 map units (5, 6). The two genes are separated minimally by a region of some 25 kbp containing two cytochrome oxidase genes (7, 8) and most of the tRNA genes of the yeast mitochondrial genome (9, 10).

There has been some uncertainty regarding the relationship of the par locus and the 15 S rRNA gene. While earlier studies suggested this mitochondrial marker to be in the 15 S rRNA coding sequence (11), this was placed in doubt by subsequent reports that certain ρ⁻ mutants' genetically verified to retain the par marker, failed to hybridize to the 15 S rRNA (6, 12). The par marker has been variously mapped both on the 3' (12) and 5' (6, 13) side of the 15 S rRNA gene. These results were of considerable interest since they raised the possibility that paromomycin resistance might result from a mutation in some still unidentified mitochondrial gene.

In order to clarify the nature of the paromomycin resistance mutation, we have sequenced the 15 S rRNA genes of a resistant and sensitive strain of *S. cerevisiae*. The DNA sequences obtained from the two strains together with information about the 5' and 3' termini of the gene indicate that resistance to paromomycin arises from a single base change in the 15 S rRNA coding sequence. These results strongly imply that the par locus is a true genetic marker of the 15 S rRNA gene. In addition, the gene sequence has revealed the existence of a specific region near the 3' end of the 15 S rRNA which could have a function similar to the Shine and Dalgarno sequence in the 16 S rRNA of *E. coli*.

**MATERIALS AND METHODS**

**Yeast Strains**—The strains of *S. cerevisiae* used in the present study are listed in Table I. *S. cerevisiae* D273-10B is a respiratory competent haploid strain with the sensitive alleles at all the known

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The abbreviations used are: ρ⁻ mutants, cytoplasmic petite mutants of *S. cerevisiae* with long deletions in mtDNA, kbp, kilobase pairs.
antibiotic resistance loci of mitochondrial DNA. This strain was used to derive the triple resistant mutant D273-10B/A21 (14). Resistance to oligomycin, paromomycin, and erythromycin was introduced by sequential selection of spontaneous resistant mutants on media containing one of the three antibiotics (14). The oligomycin resistance mutation in D273-10B/A21 has previously been shown to be at the oli1 locus and to involve a single base change in the sequence of the gene for subunit 9 of the ATPase (17, 18). The erythromycin resistance mutation has been mapped in the 21 S rRNA gene but has not been sequenced.

Isolation of the r' Mutant DS80—The paromomycin-resistant strain D273-10B/A21 was mutagenized with ethidium bromide under previously described conditions (19). Approximately 1000 independent r' mutants were screened for the retention of the paromycin marker in the following way. The mutants were crossed to the paromycin sensitive haploid strain D22. Following prototrophic selection on minimal glucose medium and growth scored after 2 days of incubation at 30 °C. In such crosses, r' mutants containing the par locus give rise to respiratory competent recombinants capable of growth on paromycin sensitive medium. Mutant clones ascertained to have retained the paromycin resistance marker were further tested for the presence or absence of other mitochondrial genetic loci by crossing to appropriate mit- tester^. The details of these genetic tests have been described (19). Of the original 1000 mutants screened, 4 had genotypes consistent with the retention of only the par locus. These were analyzed for the complexity of their mtDNAs. The clone DS80 had the least complex mitochondrial genome with a repeat length of 2.6 kb.

Selection of the Recombinant Clone pYm132—The construction of hybrid plasmids containing Eco RI fragments of mtDNA from S. cerevisiae D273-10B cloned into pBR322 has been reported (20). The recombinant clone pYm132 was chosen for sequencing the 15 S rRNA gene because one of the insert of the Hind I site in the D273-10B mtDNA previously mapped at 38 and 38.5 units, respectively, of the D273-10B mitochondrial genome (21). The mitochondrial DNA insert in pYm132 has been shown to contain the region of the mitochondrial wild type genome from 37 to 42 map units (22) and the 15 S rRNA and tryptophan tRNA genes.

Restriction Mapping of Mitochondrial and Plasmid DNA—Mitochondria were prepared from DS80 by the glusulase procedure of Faye et al. (23). The mitochondria were lysed with 2% Sarkosyl, extracted with water-saturated phenol, and the aqueous phase dialyzed overnight adjusting the CsCl concentration of the dialyzed extract to a refractive index of 1.3970-1.3975.

The recombinant clone pYm132 was grown in L broth in the presence of 20 μg of ampicillin/ml. Following chloramphenicol amplification, cells were harvested and plasmid DNA purified by the method of Davis et al. (24).

Restriction Analysis—Analytical digestions were carried out on 0.2-0.5-μg samples of DNA in a buffer containing 10 mM Tris-acetate, pH 7.5, 6 mM MgCl2 and 6 mM mercaptoethanol. The digestion products were separated electrophoretically on 1% or 1.5% agarose gels in the Tris-borate buffer system described by Peacock and Dingman (25). The gels were calibrated with internal standards consisting of a 3-kbp HindIII digest of PFX174RF and a HindIII digest of λ DNA (26, 27).

### Table I

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<th>Name</th>
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* R. Berlani, unpublished studies.
* Mit mutants have genetic lesions in genes coding for subunits of cytochrome oxidase, ATPase, and apocytochrome b. Ox1/2 codes for subunit 2, ox2 for subunit 3, and ox3 for subunit 1 of cytochrome oxidase. The cob1 locus is located in the last exon of the apocytochrome b gene.

### Yeast Mitochondrial 15 S rRNA Gene

**Table I:** Genotypes of S. cerevisiae strains

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**Hae III digest of PFX174RF and a HindIII digest of λ DNA (26, 27).**

**DNA Sequencing—** Restriction digest of DNA were 5' end labeled with [γ-32P]ATP (specific activity of 2000-3000 Ci/mmol, New England Nuclear) in the presence of T4 polynucleotide kinase. The labeled mixtures were denatured in formamide and the single strands separated electrophoretically on 6 or 8% polyacrylamide gels (28). The labeled single strands were recovered from the gels and used for sequencing by the method of Maxam and Gilbert (29).

**Sequencing of the 3' End of the 15 S rRNA—** Total yeast mitochondrial RNA was prepared essentially as described by Bonitz et al. (29). The RNA was separated on a preparative slab gel containing 1% low-melting agarose (Bethesda Research Laboratories, Rockville, MD). The gel was stained with ethidium bromide to visualize the ribosomal RNAs. The band corresponding to the mitochondrial 15 S RNA was cut out of the gel and eluted by dissolving the agarose at 65 °C. The agarose was removed by two consecutive extractions with water-saturated phenol. The water phase was extracted with ether to remove the phenol and the RNA recovered by precipitation with 70% ethanol from a 0.3 M solution of sodium acetate. As judged by electrophoretic analysis, the 15 S rRNA was intact following the procedure.

The purified 15 S rRNA (5 μg) was labeled at the 3' end as described by Bruce and Uhlenbeck (30) using T4 RNA ligase (P-L Biochemicals) and 3',5' cytosine biophosphate. Very little intact 15 S rRNA was recovered after labeling. Rather smaller but discrete RNAs were detected upon gel electrophoresis. Three RNA fragments of approximately 25, 30, and 120 bases were isolated from a 12% polyacrylamide gel. These fragments were sequenced by the chemical procedure of Pearse (31).

**RESULTS**

Restriction Maps of DS80 mtDNA and of the pYm132 Insert—DS80 was determined to have a mitochondrial genome consisting of a tandemly repeated segment of mtDNA 2.6 kb in length. Digestion of the DNA with HincII, Bam H1, Hha I, Mbo II, Hph I, or Sac II generated a single fragment corresponding to the unit length repeat of the genome (Fig. 1). In addition, the DS80 mtDNA contains multiple sites for Hae III, Tag I, Mbo I, Alu I, Hinf I, and Hpa II. The restriction map of the unit circle deduced from the sizes of fragments produced in various digestions is shown in Fig. 2. There is a fairly random distribution of restriction sites over the length of the DNA except for a region of approximately 1 kb which has only a cluster of Sac II, Ava II, and Mnl I sites.

The recombinant plasmid pYm132 contains an inserted segment of mtDNA from the paromomycin-sensitive strain D273-10B. This plasmid was used previously to sequence the tryptophan tRNA gene located at 41.8 map units (22). In the present study, the pYm132 insert has allowed us to obtain the sequence of the 15 S rRNA gene in a paromomycin-sensitive background. The pYm132 insert and the DS80 genome were oriented on the wild type map by virtue of the Hha I, HincII, and Bam H1 sites which have been assigned between 37 and 39 units of the D273-10B map by Morimoto and Rabinowitz (21). As shown in Fig. 3, the pYm132 insert has the region of wild type mtDNA extending from 36.7 to 42.5 map units and encompasses the entire sequence retained in DS80. The DS80 mtDNA is cleaved into two nearly identical size fragments of 1.3 kb by Hae III (Fig. 1). The Hae III fragment with the internal Bam H1 site is present in the pYm132 insert. The restriction sites in this region coincide exactly in both DS80 and pYm132 except for a Tha I site which is only present in pYm132. The significance of this single difference will be discussed in a later section.

The second 1.3 kb Hae III fragment of the DS80 genome is absent in the pYm132 insert. This fragment must therefore have been generated as a result of a recombination between the deletion endpoints of the DS80 mtDNA segment. Based on the location of the Sac II site in pYm132, the deletions in DS80 can be estimated to have occurred at 37.1 units on the
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Fig. 1. Restriction digests of DS80 mtDNA. Purified DS80 mtDNA was digested with Hae III (lane 2), Hpa II (lane 3), HincII (lane 4), and Mbo I (lane 5). The digestion products were separated on a 1% agarose gel and stained with ethidium bromide. Lane 1 was loaded with a mixture of \( \Phi X_{174} \)RF DNA digested with Hae III and A DNA digested with HindIII. The sizes of the standards are shown in the left hand margin.

left side and 40.8 units on the right side of the map presented in Fig. 3.

Nucleotide Sequences of the 15 S rRNA Genes in DS80 and pYm132—In order to obtain the sequence of the yeast mitochondrial 15 S rRNA gene and to ascertain whether the paromomycin resistance locus is included in the structural gene, the same regions of mtDNA were analyzed in the paromomycin-sensitive parental strain D273-10B (pYm132) and the paromomycin-resistant mutant D273-10B/A21 (DS80). The DS80 genome was subjected to different digestions with restriction endonucleases followed by 5'-end labeling and separation of the single strands for chemical derivatizations by the method of Maxam and Gilbert (28). The same approach was used to obtain the sequence of the gene in pYm132 except that in certain cases the appropriate Hae III fragments were first isolated preparatively from the plasmid prior to secondary cleavages and labeling. The restriction sites used for 5'-end labeling and the lengths of the sequences read on the gels are summarized in Fig. 4.

Both mtDNAs were sequenced from approximately 37.5 to 40.1 map units. The sequence of the non-transcribed strand is presented in Fig. 5. As will be shown later, the region sequenced contains the entire 15 S rRNA gene (nucleotides +1 to +1686). The 15 S rRNA gene has an average G + C content of only 20%, a value considerably lower than that found in other eucaryotic and procaryotic rRNAs. As with most other yeast mitochondrial genes, the 15 S rRNA gene is bordered by A + T-rich sequences. It is of interest that the gene contains a G + C-rich cluster with several Hpa II and a Hae III sites. This sequence is similar to other Hpa II/Hae III site clusters that occur in numerous noncoding regions of the yeast mitochondrial genome. S1 mapping data (see below) indicate the G + C cluster to be part of the mature 15 S rRNA. The nucleotide sequences obtained from DS80 and pYm132 were identical over the entire region analyzed with the sole exception of nucleotide position +1514 (Fig. 6). In pYm132.

Fig. 2. Restriction map of DS80 mtDNA. The DS80 genome is represented as a unit length circle. The location of the deletion endpoints is denoted by the broken line in the circle. The following symbols are used for the restriction sites: Sac II (○), Hha I (⊗), HincII (△), Alu I (◇), Mbo I (●), Taq I (◆), HincII (◇), Mnl I (●), Hpa II (△), Hae III (◇), Tha I (◇), Bam H1 (○), Mbo II (○), Rsa I (◇), Hph I (△), Ava II (◇).

Fig. 3. Physical limits of the DS80 mtDNA segment and of the pYm132 insert. The \( \rho \) genome and the cloned fragment have been aligned and oriented on the \( S. ceresvisiae \) D273-10B map through the Hha I and HincII sites previously mapped at 37.6 and 38 map units (21). Only the Sac II (Sac), Hha I (Hh), HincII (C), Bam H1 (B), and Hae III (Ha) sites are shown. The location of the 15 S rRNA gene is depicted by the bar on the upper part of the figure. The direction of transcription is indicated by the arrow.

Fig. 4. Restriction fragments used to sequence the 15 S rRNA gene in DS80 and pYm132. The symbols used for the restriction sites are the same as in Fig. 2. The restriction sites used for 5'-end labeling and the extent of the sequences read on the sequencing gels are indicated by the open (DS80) and solid (pYm132) arrows.
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Definition of the 5' and 3' Termini of the Mature 15 S rRNA—The single base substitution in the sequence of the paromomycin-resistant strain occurred within a region which exhibited sufficient homology with the 16 S rRNA of E. coli to suggest that the mutated allele is in the structural gene. This was confirmed by determining the 5' and 3' ends of the 15S rRNA. The 5' end of the RNA was established by S1 mapping. The single stranded Hindl-Ava II restriction fragment containing the sequence from 37.2 to 38.1 map units (cf. Fig. 4) labeled at the 5' end of the Hindl site was hybridized to the 15 S rRNA purified on a preparative agarose gel. The hybrid was treated with S1 nuclease to digest the single stranded regions. The DNA-RNA hybrid remaining after the S1 digestion was derivatized by the Maxam and Gilbert method (28). The sequencing gel of Fig. 7 shows the A + G and T + C ladders obtained in this experiment. The results indicate the presence of two RNA transcripts differing by some 80 nucleotides at their 5' termini. The most abundant transcript corresponding to the 15 S rRNA has a heterogeneous 5' end whose sequence starts at nucleotides −3 to +6 of the DNA sequence (Fig. 5). The observed heterogeneity of the 5' end may be due to an artifact of the S1 digestion, although we cannot exclude that the RNA itself is really heterogeneous. The second transcript representing only a small percentage of the total hybrid has a 5' terminus approximately 80 nucleotides upstream of the mature 15 S rRNA. This species probably represents a small amount of an incompletely processed precursor of the 15 S rRNA which, because of the small difference in size, co-purifies with the mature ribosomal RNA on agarose gels. The 5' terminus of the precursor agrees with the recently reported start of transcription of the 15 S rRNA gene determined by an entirely different method (33).

To identify the 3' terminus, the 15 S rRNA was sequenced directly by the chemical method of Peattie (31). The sequencing gel shown in Fig. 8 indicates that the 3'-end labeling of the 15 S rRNA using pCp yielded a unique sequence which lines up unambiguously with the DNA sequence ending with nucleotide +1686. The rRNA was partially degraded during the labeling reaction and the fact that discrete fragments were observed both on agarose and polyacrylamide gels suggests certain regions of the RNA to be more susceptible to degradation than others. The source of the degradative activity is not known. Theoretically, since most ribonucleases leave 3' phosphates, the 3' end of fragments derived internally will not be substrates for labeling in the pCp reaction. In practice, all the fragments originating from the RNA labeled at a unique 3' site which we therefore conclude corresponds to the 3' terminus of the 15 S rRNA.

Based on the experimentally identified termini of the mature 15 S rRNA, the single nucleotide difference observed in the DNA sequences of the paromomycin-sensitive and resistant strains lies within the gene, exactly 172 nucleotides upstream of the 3' terminus.

Absence of an Intervening Sequence in the 15 S rRNA Gene—The 21 S rRNA gene of certain strains of yeast has been shown to contain a 1-kbp intron which is excised during maturation of the RNA (3, 4). The possible presence of an intervening sequence in the 15 S rRNA gene in the D273-10B strain has been examined by S1 mapping. The 15 S rRNA purified on agarose was hybridized to DS80 mtDNA cleaved at the unique Sac II site. The resultant hybrid was treated with S1 nuclease at 15 °C to remove any single stranded DNA.

* Dr. Murray Rabinowitz of the University of Chicago has recently informed us that direct sequence analysis of the 5' end of the 15 S rRNA indicates five different termini corresponding to nucleotides +1, +2, +3, +4, and +5 of the sequence presented in Fig. 5.
S1 treatment is done at the higher temperature, the presence of intervening sequences in the 15 S rRNA is unlikely. Using the S1 mapping procedure of Berk and Sharp (32), Tabak et al. (35) have also failed to find an intervening sequence in the 15 S rRNA gene of another yeast strain.

**Homology of Yeast Mitochondrial 15 S and E. coli 16 S rRNAs**—The 3' terminal region of the small subunit rRNAs exhibits a considerable degree of sequence homology among both eucaryotic and procaryotic organisms. As can be seen from the sequences shown in Fig. 10, the 15 S rRNA of yeast mitochondria is also highly conserved in this region when compared to the E. coli and other mitochondrial rRNAs (36-41).

Based on sequence analysis, chemical modification, and enzymatic data, Noller and Woese (42) have recently proposed a secondary structure model of the E. coli 16 S rRNA. According to this model, the 3' region as defined by nucleotides 926-1542 of the E. coli sequence, is divided into a major (962-1391) and a minor domain (1392-1542). The secondary structure of the minor domain shown in Fig. 11 has the following features. (a) It starts with a highly conserved 17 nucleotide sequence. (b) This is followed by a variable sequence of approximately 100 nucleotides that forms a long imperfect helix, (c) on the 3' side of the helix is a second imperfect helix, and (d) the third helix is a long perfect helix. This model is consistent with both the DNA and RNA sequences. The 3' terminal region of the small subunit rRNAs of both eucaryotic and procaryotic organisms has the secondary structure shown in Fig. 11. This region is called the major domain (962-1391). The secondary structure of the minor domain (1392-1542) is not as well conserved among the different rRNAs.

**Fig. 7.** Sequencing gels of the 15 S rRNA-DNA hybrid before and after S1 nuclease treatment. An Aca II-HinfI digest of D880 mtDNA (10 μg) was labeled with [γ-32P]ATP in the presence of T4 polynucleotide kinase. The mixture was separated on a 6% polyacrylamide gel and the 400 nucleotide long single stranded Aca II-HinfI fragment (37.2-37.7 units in Fig. 5) labeled at the 5' end of the HinfI site was recovered from the gel. The labeled fragment was hybridized to 15 S rRNA (5 μg) purified on agarose. The hybridization mixture was divided into two equal halves, one of which was treated with 20 units of S1 nuclease at 37 °C for 30 min as described by Berk and Sharp (32). To each sample was added carrier yeast tRNA and the nucleic acids precipitated from 2.4 m ammonium acetate, 50 mM EDTA with 3 volumes of ethanol. The precipitates were washed 2 times with 80% ethanol, derivatized by the method of Maxam and Gilbert (28) for the A + G and C + T reactions, and separated on a 10% polyacrylamide sequencing gel. The last nucleotide in the untreated samples (left lanes for each set of channels) corresponds to the 3' end of the Aca II-HinfI fragment. In the S1 treated sample, two clearly separated ends are evident. The penultimate nucleotide in the predominant lower band corresponds to nucleotide -3 in Fig. 5. The second band ends at nucleotide -82. The sequences shown are complementary to those presented in Fig. 5 and are numbered by the same convention. The four lanes on the left are longer channels and separately at 45 °C to further cleave the RNA in regions of mismatch that might arise from the presence of intervening sequences in the DNA (34). The results of Fig. 9 show that at 15 °C, S1 digestion reduces the hybrid to an estimated size of 1.6-1.7 kbp. Since a similar size hybrid is observed when the

**Fig. 8.** RNA sequence of the 3' end of the 15 S rRNA. The sequence shown is of an approximately 120 nucleotide long fragment obtained after 3' end labeling of the purified 15 S rRNA. The fragment was derivatized by the method of Peattie (31) and the products separated on a 12% polyacrylamide gel. The ambiguities between positions 6 and 7, 13 and 16, and 55 and 56 are due to partial degradation of the RNA during the elution and sequencing procedures. The sequence from nucleotides 1 through 22 was unambiguous in gels of the smaller fragments (not shown) and the bases at positions 55 and 56 were confirmed by the DNA sequence.
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![Diagram of yeast mitochondrial 15 S rRNA](image)

**Fig. 9.** Sizing of the 15 S rRNA by S1 mapping. The 15 S rRNA (8 pg) was purified from total yeast mtRNA on low-melting agarose. It was hybridized to 1 pg of DS80 mtDNA under the conditions of Berk and Sharp (32). Prior to hybridization the DS80 mtDNA was linearized by cleavage at the Sac I1 site. Samples of the hybridization mixture were treated with S1 nuclease (5 units) at 15 °C and at 45 °C for 30 min. To each sample was added carrier yeast tRNA and the nucleic acids precipitated from 2.4 M ammonium acetate, 50 mM EDTA with 3 volumes of ethanol. The precipitates were washed two times with 80% ethanol, dried, and separated on a 1.5% agarose gel. Lane I, Sac I1 digested DS80 mtDNA. Lane 2, products after S1 treatment at 15 °C. Lane 3, Hae III digest of QX174RF and a HindIII digest of h DNA. Lane 4, products after S1 treatment at 45 °C. The sizes of the molecular weight standards are shown in the left hand margin. The heavy stained material seen in the lower part of lanes 2 and 4 are mostly due to the carrier tRNA added to the samples.

conserved sequence of 14 nucleotides which has the colicin E3 cleavage site. (d) Immediately downstream is a 10 base pair hairpin with 2 dimethyladenosines in a 4 nucleotide loop. (e) The last important feature of the minor domain is the Shine-Dalgarno hexanucleotide 5'-ACCUCC-3' almost at the very 3' end of the molecule (43).

It is possible to construct a secondary structure from the yeast mitochondrial 15 S rRNA sequence exhibiting most of the features described above for the *E. coli* 16 S rRNA (Fig. 11). The most significant differences in the structure of the yeast rRNA is the presence of a longer and less stable imperfect helix and a longer 3' tail lacking the Shine and Dalgarno sequence (43). In all other respects, the two secondary structures are identical.

The absence of the Shine and Dalgarno sequence prompted us to examine whether the yeast mitochondrial 15 S rRNA might contain an alternate mRNA recognition sequence. Possible base complementarity was sought between the 3' terminal 25 nucleotides of the 15 S mRNA and the 5' regions preceding the initiation codons of six yeast mitochondrial genes coding for the messenger RNAs of apocytochrome b and various subunits of the ATPase and of cytochrome oxidase. As shown in Fig. 12, four of the genes (subunits 1, 2, and 3 of cytochrome oxidase and subunit 6 of the ATPase) had sequences upstream of their initiation codons capable of base pairing with at least seven consecutive nucleotides of the 15 S rRNA sequence 3'-AAATTCTATA-5' (nucleotides +1667 to +1676). In the case of the other two genes, the complementary sequences extended only over 5 and 4 nucleotides. The putative ribosomal binding sequences are present within the known 5' leaders of the messengers for subunit 2 of cytochrome oxidase (47), subunit 3 of cytochrome oxidase, subunit 9 of the ATPase, and the apocytochrome b. At present, there is no information about the leaders of the cytochrome oxidase subunit 1 and ATPase subunit 6.


**Fig. 10.** Homology of the 3' sequence of yeast 15 S rRNA with other RNAs of the small ribosomal subunit. The sequences of *E. coli* 16 S (36), yeast 18 S (37), silkworm 18 S (38), hamster mt 13 S (39), mouse mt 12 S (40), and human mt 12 S (41) ribosomal RNAs have been aligned for optimal homology. The yeast 18 S, silkworm 18 S, and hamster 12 S sequences are not numbered since the complete sequences are not available. The variable helix regions are not shown. Positions with identical nucleotides in all seven RNAs are underlined. * marks the nucleotide identified as the paromomycin resistance allele in the yeast 15 S rRNA.

**Fig. 11.** Secondary structure models of the *E. coli* 16 S and yeast mitochondrial 15 S rRNAs. The *E. coli* structure (left) is based on the model of Noller and Woese (42). The yeast mitochondrial 15 S rRNA (right) has been formed to allow for maximal base pairing. The structural features of the two RNAs are discussed in the text.
Fig. 12. Base complementarity of the putative mRNA-binding sequence in the 15 S rRNA and of sequences upstream of yeast mitochondrial initiation codons. The complete sequence of the 3' tail in the 15 S rRNA is shown on the top line. Sequences upstream of the genes for cytochrome oxidase subunit 2 (7, 44), subunit 3 (8), subunit 1 (29), ATPase subunit 6 (45), subunit 9 (18), and of apocytochrome b (46), complementary to the 15 S rRNA are shown below. The number of nucleotides intervening between the initiation codons and the complementary sequences are indicated for each gene. The 5' leaders have been determined in four yeast mitochondrial messengers (see also Ref. 47). The leader of the apocytochrome b message is 940 nucleotides long, subunit 2 of cytochrome oxidase 54 nucleotides, subunit 3 of cytochrome oxidase 483 nucleotides, and subunit 9 of the ATPase 628 nucleotides.

DISCUSSION

One of the principal aims of the present study was to determine whether the paromomycin resistance locus of yeast mtDNA is part of the 15 S rRNA gene. To answer this question, the gene was sequenced in a wild type sensitive strain and in a paromomycin resistant mutant. The mutation conferring resistance to paromomycin was verified by genetic crosses to be either allelic or very closely linked to the paromomycin resistance allele Pm, originally reported by Wolf et al. (48). In crosses of D273-1OB/A21 to the paromomycin resistant strain KL14-4A with the Pm allele, no sensitive recombinants were noted in more than 300 diploid segregants scored.

Two different sources of mtDNA were used to obtain the sequences of the 15 S rRNAs. To enrich for the 15 S rRNA gene of the paromomycin resistant strain, S. cerevisiae D273-1OB/A21 was mutagenized with ethidium bromide and resistant ρ− clones were selected for the retention of the por locus and loss of other mitochondrial markers. The mutant DS80 was chosen for sequence analysis because of its simple genome which consisted of a tandemly repeated segment of mtDNA only 2.6 kbp long. Since there are no convenient genetic tests for the presence or absence of the paromomycin sensitive allele in ρ− mutants, the 15 S rRNA gene sequence of the paromomycin sensitive strain was obtained from the recombinant clone pYm132 (22). This plasmid was shown in prior studies to contain an insert of the D273-1OB mtDNA with the 15 S rRNA gene. The use of two isogenic strains insured that any differences found in the sequences of the gene would be due to a specific mutation rather than sequence polymorphism which is known to exist among laboratory strains of yeast (49, 50).

The sequences of the 15 S rRNA genes of the paromomycin-resistant and of the paromomycin-resistant mutant have revealed a single nucleotide change near the 3' end of the gene. The mutation has been localized to nucleotide position 1514 of the 15 S rRNA where a G has been substituted for a C in the mutant. There are several reasons for believing that the single base difference is responsible for the resistant phenotype. The paromomycin resistance marker can be rescued by genetic means from the DS80 mutant. This fact indicates that the mutation conferring resistance must be inherent to the mtDNA sequence retained in DS80. Although some 800 nucleotides of the DS80 genome have not been sequenced, they span the 5' and 3' gene flanking regions consisting of A + T-rich sequences which are highly unlikely to have coding functions. Secondly, Sor and Fukuhara (51) have recently reported the sequence of the 15 S rRNA gene of another paromomycin-resistant yeast strain. Their sequence also reveals the presence of a G at nucleotide 1514.

It is of interest that the substitution of a G for a C occurs at a position corresponding to the first base pair of the imperfect helix in the secondary structure model of the E. coli 16 S rRNA proposed by Noller and Woese (42). This model predicts that the G at nucleotide 1514 (1409 in E. coli) should be base paired with the G at nucleotide 1620 (1490 in E. coli). Paromomycin is known to cause misreading by binding to the small ribosomal subunit of E. coli (52), although the mode of interaction with the drug is not known. It is possible that paromomycin binds near the 3' terminus of the RNA which is known to be fairly accessible at the surface of the 30 S ribosomal subunit (53). The present finding that paromomycin resistance is associated with a point mutation leading to the loss of a single base pair in the imperfect helix suggests this secondary structure feature to be important for the interaction with paromomycin.

The identification of the 5' and 3' termini of the yeast mitochondrial 15 S rRNA together with the gene sequence indicate it to be 144 nucleotides longer than the 16 S rRNA of E. coli. Although the difference in size could be due to the absence of an intervening sequence in the gene, this is not supported by the S1 mapping data reported here as well as those of Tabak et al. (36). Despite its greater length, the yeast 15 S rRNA is homologous to the E. coli 16 S rRNA over its entire length suggesting that the two probably have very similar secondary structures. Similar conclusions have been reached by Sor and Fukuhara (51). The homology is especially evident at the 3' ends of the two rRNAs. The terminal 200 nucleotides of the yeast sequence can be folded to yield a secondary structure exhibiting all the features described by Noller and Woese (42) in their model of the E. coli 16 S rRNA. These include the 10 base pair hairpin with 2 adenosines and 2 guanines in the loop and the long imperfect helix with an internal loop. The almost perfect 25 base pairs in the latter tend to lend credibility to its existence. In addition to the two helices, certain sequences in the 3' region proposed to be important in the stability and function of the 30 S subunit are also conserved in the yeast sequence. For example, the E. coli sequence defined by nucleotides 1392-1410 has been implicated in the binding of tRNAs during protein synthesis. A homologous sequence is also present in the yeast mitochondrial 15 S rRNA.

The most interesting difference seen between the bacterial and yeast mitochondrial rRNAs is the absence in the latter of the Shine and Dalgarno sequence postulated to function in the attachment of the ribosome to mRNA (43). Even though the 15 S rRNA of yeast mitochondria lacks the Shine and Dalgarno sequence, it has in its stead a 10 nucleotide sequence which can base pair to different extents with sequences upstream from all the known yeast mitochondrial genes. The
fact that the complementary sequences range from 4–7 nucleotides and in four known cases occur within the 5' leaders of the messengers, strongly argues that they are involved in ribosomal binding. The 12 S rRNA of mammalian mitochondria have only 7–8 nucleotides following the 10 base pair hairpin. The 3' terminal sequences in these rRNAs lack the Shine and Dalgarno sequence or the ribosomal binding sequence proposed here for the yeast 15 S rRNA. It may be significant that the mRNAs of human mitochondria have recently been shown to either lack a 5' leader or to have only a few nucleotides preceding the AUG initiation codon (54). Presumably this is also true of other mammalian mitochondrial messengers. The absence of leader sequences in the mammalian mRNAs would obviate the need for a Shine and Dalgarno type of binding sequence in the mitochondrial ribosomes.

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REFERENCES

Identification of the paromomycin-resistance mutation in the 15 S rRNA gene of yeast mitochondria.

M Li, A Tzagoloff, K Underbrink-Lyon and N C Martin