Nucleotide sequence of the mitochondrial structural genes for cysteine-tRNA and histidine-tRNA of yeast

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ABSTRACT

We have determined the nucleotide sequence of a segment of Saccharomyces cerevisiae mtDNA that contains the structural genes for a cysteine-tRNA and a histidine-tRNA. The genes are approximately 85 bp apart, they do not contain intervening sequences or sequences coding for the 3'-CCA terminus and they are surrounded by nearly pure AT segments. The tRNAs deduced are very AT-rich, 74 and 75 nucleotides long, respectively, and contain one or more unusual features not found in tRNAs from other sources.

INTRODUCTION

Mitochondria are known to contain a separate genetic system with DNA, ribosomes and tRNAs distinct from those present in the nucleus or the cytoplasm [1]. In the yeast Saccharomyces cerevisiae, mtDNA is a 25-μm circle containing genes for rRNAs, tRNAs and several proteins (see Fig. 1) [2]. As part of an investigation into the structure and organization of these mitochondrial genes, we have determined the nucleotide sequence of a region downstream from the 21S rRNA gene. The sequence of two tRNA genes, present in this region, is presented here. Our results have been presented in preliminary form at a recent Symposium [3].

MATERIALS AND METHODS

Yeast strains and isolation of mtDNA and mitochondrial tRNAs

Petite mutants 22 and 23 of Saccharomyces cerevisiae strain JS1-3D were isolated by L.A. Grivell in this laboratory and are described in refs 4 and 5. Retention of the C321 marker...
Fig. 1. Physical map of the mtDNA of *S. cerevisiae*, strain JSI-3D. The physical map was constructed by Sanders et al. [34] and shows recognition sites for HindII, HindIII and EcoRI. The outer ring gives the approximate positions of 4S RNA genes (■) [8]. The position of the transcripts of the rRNA genes is shown outside the outer ring. The open block in the 21S rRNA represents an intervening sequence.

in this mutant was checked by the replica-cross technique of Deutsch et al. [6]. The mtDNA was prepared as described by Moorman et al. [7]. After the NaI gradient the DNA was further purified using a Sepharose-CL-2B (Pharmacia) column. Mitochondrial tRNA was purified as described [8] and was a gift of Mr. P.M.Van Beroen en Henegouwen of this laboratory.

Hybridization

Mitochondrial tRNA was labelled in vitro using $\gamma^{32}$P]ATP (The Radiochemical Centre, Amersham, UK) as described [9] and hybridized to separated restriction fragments immobilized on nitrocellulose filters [10] for 16 h in 3 x SSC, 0.1% sodium dodecyl sulphate at 59°C.

DNA sequence analysis

Petite 23 mtDNA was digested with endonuclease HapII and
end-labelled with [γ-32P]ATP and polynucleotide kinase as described [11]. The fragments were purified by centrifugation through columns of Sephadex-G50 (Pharmacia) [12], digested with endonuclease HhaI and separated in 3.5% polyacrylamide slab gels (50 mM Tris-citrate (pH 8.0), 2.5 mM sodium EDTA). Fragments were eluted from crushed gel segments in 0.5 M NaCl, 10 mM Tris-HCl, 10 mM EDTA (pH 7.9) (2 vols) and water-saturated phenol (1 vol). DNA fragments labelled at one 5'-end, were sequenced by the Maxam-Gilbert procedure [13] and by a modified chain-termination method [14]. The four reaction mixtures were analysed on 10% polyacrylamide gels (90 x 30 x 0.05 cm). To avoid smearing in the T and C lanes after chemical modification, the fragments were purified from unreacted hydrazine by centrifugation through columns of Sephadex-G50.

**Enzymes**

Restriction endonuclease HhaI was purchased from New England Biolabs, Beverly, Mass. (USA). Bacterial Alkaline Phosphatase (BAPF) from Worthington, Freehold, N.J. (USA). DNA polymerase I of *Escherichia coli* and pancreatic deoxyribonuclease (grade I) from Boehringer, Mannheim (Germany). Polynucleotide kinase from *E. coli* infected with phage T4 was a gift from Dr. H. Van Ormondt (The State University Leiden) and endonuclease HapII was a gift of Mr. M.J. Hillebrand of this laboratory.

**RESULTS**

Heyting et al. [4] have previously constructed a detailed map of the region of yeast mtDNA that contains the gene for 21S rRNA. Map construction utilized a series of cytoplasmic petite mutants that had retained overlapping mtDNA segments from this region [4,5]. The mtDNA segments retained in two of these mutants - petites 22 and 23 - are shown in the map in Fig. 2. More detailed analysis of these mutants has shown that these two mutants contain an inverted duplication but, contrary to our previous interpretation [5], this duplication is not present in wild-type mtDNA (unpublished observations). Both mutants contain mtDNA segments on which several 45 RNA genes
Fig. 2. Physical map of the region of the 21S rRNA gene in S. cerevisiae strain JS1-3D containing the genes for cysteine (cys)-tRNA and histidine (his)-tRNA (small black boxes). The dashed area represents the intervening sequence in the 21S rRNA gene (black areas). The repeating units in the mtDNAs of petite mutants 23 and 22 are indicated, both containing an inverted duplication [5]. The map units refer to the physical map presented in Fig. 1. Nomenclature of the HapII fragments of petite 23 mtDNA is from ref. 5 and the horizontal arrows show how the nucleotide sequence was determined from the HapII site inward in 23YY3 (tRNA^His_ gene) and 23YY1 (tRNA^Cys_ gene) after secondary digestion with HhaI (see Materials and Methods). Have been localized [8]. This is verified for petite 23 mtDNA by the blotting experiment in Fig. 3. All HapII fragments present in petite 23 mtDNA hybridize with mitochondrial 4S RNA.

To obtain the nucleotide sequence of some of these 4S RNA genes, petite 23 mtDNA was sequenced from a HapII site as indicated in Fig. 2 (see also Materials and Methods). Since only one strand was sequenced, two independent methods [13,14] were used to minimize errors. Both gave the same sequence and this is shown in Figs 4 and 5. From a comparison of this sequence with known tRNA sequences we infer the presence of genes for cysteine-tRNA and histidine-tRNA in this segment of mtDNA.

In view of the unusual structure of the putative cysteine-tRNA gene (see Discussion), most of the sequence shown in Fig. 5 was also determined in mtDNA from petite 22 (see legend to
DISCUSSION

Our sequencing of a mtDNA segment that hybridizes with mitochondrial 4S RNA has uncovered putative structural genes for histidine-tRNAs and cysteine-tRNAs. Only a single gene that hybridizes with histidine-tRNA has been found in yeast mtDNA and it was roughly mapped at the position where we now find it [15]. Moreover, the sequence of our histidine-tRNA gene is identical to the preliminary sequence of histidine-tRNA, determined by R.P.Martin, A.P.Sibler and G.Dirheimer (personal communication). This indicates that this tRNA\textsuperscript{his} gene is used to make tRNA\textsuperscript{his} in vivo. Whether the cysteine-tRNA gene that we have detected is also functional, is less clear. There are
Fig. 4. Autoradiograms of DNA sequencing gels of petite 23 mtDNA. The sequences were obtained by the modified chain-stopper technique [14] after 5'-specific labelling of the HapII site at map position 3.3% (see Fig. 2). A: Sequence gel of the cysteine-tRNA gene. B: Sequence gels of the histidine-tRNA gene. The numbers refer to the sequence in Fig. 5.
Fig. 5. Nucleotide sequence of the cysteine-tRNA and histidine-tRNA genes (underlined) with flanking regions. The sequence -40 to 90 and 150 to 265 was determined by the modified chain-stopper technique [14] in petite mutant 23; the sequence -10 to 240 by the chemical cleavage technique [13] in petite mutant 23; the sequence -57 to 46 and 65 to 206 by the modified chain-stopper technique in petite mutant 22.

probably two cysteine-tRNA genes in yeast mtDNA [15,16] and one might not be used to make tRNA\textsuperscript{CYS} used in protein synthesis, although both can be loaded. We assume that it is functional for the remainder of the discussion.

Both mitochondrial tRNA genes lack an intervening sequence as found in some of the nuclear genes for tRNA in yeast [17, 18]. No intervening sequences have been found either in the structural gene for subunit 9 of the yeast mitochondrial ATPase complex [19] or in the 15S rRNA gene [3], whereas intervening sequences are present in the 21S rRNA gene [20] and in the
structural gene for apo-cytochrome b [3,21]. Both tRNA genes also lack sequences coding for the CCA present at the 3'-end of all mature tRNAs, including mitochondrial tRNAs [22-25]. The same holds for nuclear tRNA genes in yeast [17,18] and some tRNA genes in bacteriophage T-even DNAs [26], but not for the tRNA genes of Escherichia coli [27,28]. Both mitochondrial tRNA genes are AT-rich, the histidine-tRNA gene containing 63 mole percent A+T, the cysteine-tRNA gene 71%. This is in line with the high mole percent A+T of total yeast mitochondrial tRNA (65% [29]) and phenylalanine-tRNA (67% [23]).

Like the gene for subunit 9 of the ATPase complex [19], both tRNA genes are surrounded by very AT-rich sequences. Both tRNA genes are preceded by the sequence 5' -TAATAAA- 3' and followed by the sequence 5' -ATAAATAATA- 3'. The size of the primary transcript of this region is not known and these sequences (if not fortuitous) could, therefore, either play a role in transcription or in the processing of a longer primary transcript. It is of interest that transcription of both tRNA genes is clock-wise on the map in Fig. 1. The same has been found for all genes studied thusfar, i.e. the 21S rRNA gene [3], the 15S rRNA gene (Tabak, H.P. and Menke, H.H., personal communication), the structural gene for subunit 9 of the ATPase complex [19] and apo-cytochrome b [3]. It remains possible, therefore, that all transcripts from yeast mtDNA are processing products derived from very large transcripts of one strand.

All mitochondrial tRNAs sequenced thusfar have unusual structural features not found in tRNAs from other sources [22] and the two tRNAs deduced here are no exception. The tRNA\textsuperscript{his} can be folded in a clover-leaf structure, as shown in Fig. 6. The unusual feature of this tRNA is the U-U couple at the base of the anticodon stem. This tRNA could have an extra G residue at the 5'-end, like tRNA\textsuperscript{his} from E. coli [30].

The secondary structure of tRNA\textsuperscript{CYS} is difficult to deduce from the sequence. The clover-leaf structure with maximal base pairing, shown in Fig. 6B, contains an anticodon stem with six nucleotides (5 bp) rather than the usual five. If the base pair at the bottom of the anticodon stem does not form, shortening the stem, the tRNA has two nucleotides between the D-stem and
Fig. 6. Deduced structures of yeast mitochondrial histidine-tRNA and cysteine-tRNA. A: Histidine-tRNA. B: Cysteine-tRNA (maximal base pairing). C: Cysteine-tRNA (alternative structure).

The anticodon stem, a feature not observed in any tRNA. An alternative structure is presented in Fig. 6C. The unusual features of this structure include a presumably unstable D-stem which lacks the invariant pyrimidine-purine base pairing (numbering follows ref. 22); a U-C couple at the base of the anticodon stem; and the replacement of the invariant A residue, which normally interacts with U₈ [31,32], by a U
TABLE I

HOMOLOGY BETWEEN MITOCHONDRIAL CYSTEINE-tRNAs AND HISTIDINE-tRNAs AND THE CORRESPONDING tRNAs FROM E. coli AND YEAST CELL SAP

From ref. 22.

<table>
<thead>
<tr>
<th>Percentage homology</th>
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<tr>
<td>Yeast mt tRNA\textsuperscript{Cys} - yeast cyt tRNA\textsuperscript{Cys}</td>
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<tr>
<td>Yeast mt tRNA\textsuperscript{Cys} - E. coli tRNA\textsuperscript{Cys}</td>
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<tr>
<td>Yeast cyt tRNA\textsuperscript{Cys} - E. coli tRNA\textsuperscript{Cys}</td>
</tr>
<tr>
<td>Yeast mt tRNA\textsuperscript{His} - E. coli tRNA\textsuperscript{His}</td>
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* Percentage homologous nucleotides in all three cysteine-tRNAs.

residue. The same replacement has also recently been found in tRNA\textsuperscript{TYR} from \textit{Neurospora crassa} mitochondria [25]. Whether the unusual structure, shown in Fig. 6C actually exists and functions in protein synthesis, will have to be verified by studies on the tRNA\textsuperscript{CYS} present in yeast mitochondria.

The mitochondrial cysteine tRNA shows the same sequence homology of 45% to yeast cell sap and \textit{E. coli} cysteine-tRNAs (Table I). The homology of mitochondrial and \textit{E. coli} histidine-tRNAs is higher (57%) but in this case no corresponding cell-sap tRNA is available for comparison. A similar lack of clear-cut prokaryotic structural features has been observed for several other mitochondrial tRNAs [23,24] with tyrosine-tRNA from \textit{N. crassa} as the only exception [25].

After this work was completed, Martin et al. [33] reported the sequence of six other tRNA genes in yeast mtDNA. The tRNA\textsubscript{serII} also has two nucleotides between the D-stem and the anticodon stem, like one of the possible structures for our cysteine-tRNA gene. All tRNA genes sequences by Martin et al. [33] are surrounded by different AT segments, they lack an intervening sequence and they also do not code for the terminal CCA sequence of the tRNA.
Abbreviations: tRNA, transfer RNA; rRNA, ribosomal RNA; SSC, 0.15 M NaCl, 0.015 M sodium citrate (pH 7.0); bp, base pair(s).

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