# THE NUCLEOTIDE SEQUENCE OF YEAST MITOCHONDRIAL HISTIDINE-tRNA

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## 1. Introduction

In yeast mitochondria, tRNAs corresponding to the 20 amino acids are specified by the mitochondrial genome [1,2]. The protein biosynthetic machinery of this organelle appears to be autonomous with respect to its tRNA complement, since the only mitochondria-associated cytoplasmic tRNA probably does not participate in the transfer of amino acids in mitochondrial DNA-coded proteins [3].

The mitochondrial tRNAs (mt tRNAs) have a special base composition, with a very low G + C content; they also show less posttranscriptional modifications than the corresponding cytoplasmic tRNAs [4]. The nucleotide sequence determinations of mt tRNAs from yeast and from *Neurospora crassa* showed unique structural features [5,6]. Furthermore mt tRNAs lack some structural features [6,7] which have been conserved throughout evolution of tRNAs from procaryotes or from eucaryotes. Further sequence information on other mt tRNAs will show whether there is a strong selective pressure for conservation of organelle tRNA sequences as is the case for procaryotic or eucaryotic tRNAs. To gain a further insight into the particulars of mt tRNAs we have purified and determined the nucleotide sequence of yeast mt $tRNA^{His}$.

## 2. Materials and methods

The preparation of yeast mt tRNAs from purified mitochondria has been described [5]. The isolation of mt $tRNA^{His}$ has been performed using the two-dimensional polyacrylamide gel electrophoretic system [8], the application of which to yeast mt tRNA fractionation has been reported [2,5].

The procedures for complete ribonuclease digestion of tRNA, 5′-postlabelling and fingerprinting of the digestion products as well as the methods employed for sequencing each oligonucleotide were identical to those reported for mt $tRNA^{Phe}$ [5]. mt $tRNA^{His}$ was postlabelled either on its 5′-end with $T_4$ polynucleotide kinase (gift from Dr G. Keith) after removal of the 5′-terminal phosphate with calf intestine alkaline phosphatase (Boehringer/Mannheim) or on its 3′-end with yeast tRNA-nucleotidyl transferase (gift from Dr R. Giége) after degradation of the terminal $C-C-A_{OH}$ by snake venom phosphodiesterase. The procedures used were essentially those in [9]. [$\gamma$-$^{32}$P]ATP (2000–3000 Ci/mmol) and [$\alpha$-$^{32}$P]ATP (400–600 Ci/mmol) were from Amersham/Searle. After partial digestions with ribonucleases [10,11] or with bidistilled water ('ladder') the fragments were resolved on 0.5 mm thick polyacrylamide gels [12]. In addition, the method developed in [13], modified as in the legend to fig.2, was used essentially for determination of the minor nucleosides. The mononucleosides 5′-phosphate were analysed by thin-layer chromatography (TLC) on cellulose plates using the solvent systems A, B and C in [14].

## 3. Results

### 3.1. *Purification of mt $tRNA^{His}$*

Two-dimensional polyacrylamide gel electrophoresis of total yeast mitochondrial tRNA revealed only one

Table 1
Sequence of 5′-postlabelled oligonucleotides longer than trinucleotides present in fingerprints of ribonuclease digests of yeast mt $tRNA^{His}$

| $T_1$ RNase digestion products | Pancreatic RNase digestion products |
|---|---|
| $t_1$: pA–A–U–A–U–A–U–U–U–C–A–A–U*–G | p1: pA–G–A–A–A–A–U* |
| $t_2$: pU–A–U–U–C–A–C–C–C–C–A | p2: pG–A–G–U* |
| $t_3$: pA–A–A–A–U*–A–C–G | p3: pG–A–A–U |
| $t_4$: pU–U–A–A–A–U–C–U–G | p4: pA–A–A–U |
| $t_5$: pA–U–U–C–U–C–A–G | |
| $t_{6a}$: pT–U*–C–G | |
| $t_{6b}$: pC–U*–U–G | |

U* are modified uridines

spot corresponding to histidine-tRNA which hybridizes with mt DNA [2]. Aminoacylation with the 20 amino acids of the tRNA extracted from this spot showed accepting capacity only for histidine.

### 3.2. *Sequence analyses*

The 5′-$^{32}$P-labelled oligonucleotides, longer than trinucleotides, present in complete $T_1$ RNase and pancreatic RNase digests of mt $tRNA^{His}$, have been sequenced by partial digestion with $P_1$ nuclease and two-dimensional homochromatography. Their sequences are listed in table 1.

The $T_1$- and pancreatic RNase digestion products were aligned into a unique sequence by polyacrylamide gel electrophoretic analysis of partial enzymatic digests of 5′- or 3′-$^{32}$P-labelled tRNA. Figure 1 shows such an analysis of 3′-labelled mt $tRNA^{His}$ which gives the sequence of residues 34–70. Overlap of the U–A–U–U–C–A sequence (residues 65–70) with

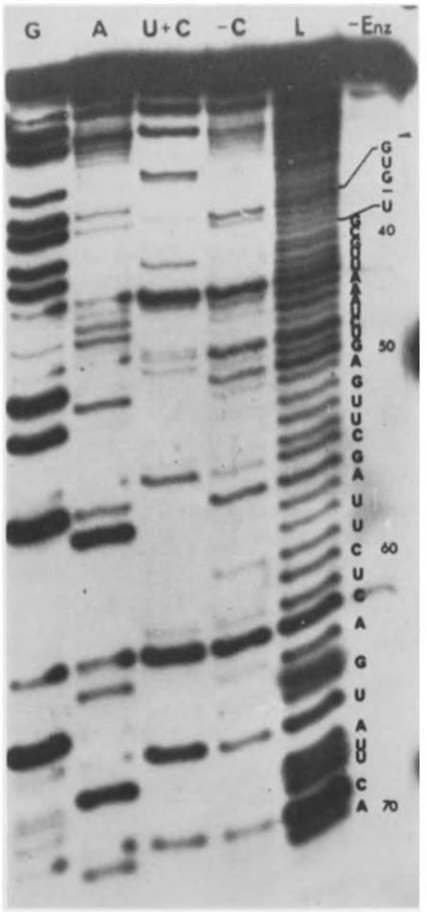
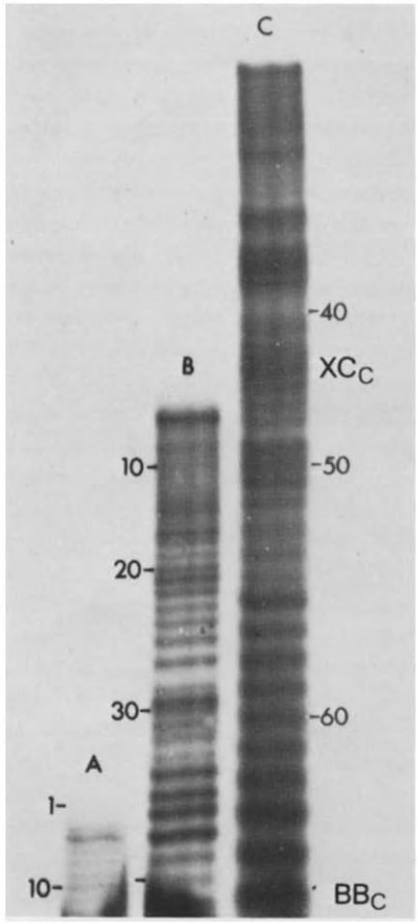



Fig.1. Autoradiogram of partial enzymatic digests of 3′-$^{32}$P-labelled yeast mt $tRNA^{His}$ fractionated on a 20% polyacrylamide gel. From left to right: (G) $T_1$ RNase ($2 \times 10^{-3}$ and $2 \times 10^{-2}$ U/μg); (A) $U_2$ RNase (0.04 and 0.2 U/μg); (C + U) pancreatic RNase ($10^{-3}$ and $4 \times 10^{-3}$ μg/μg); (–C) Phy I RNase ($2 \times 10^{-4}$ U/μg); (L) 5 μg tRNA incubated at 100°C in 10 μl bidistilled water for 5 and 15 min; (–Enz) no enzyme. Pyrimidines. $C_{49}$, $U_{58}$, $C_{62}$, $U_{67}$, $U_{68}$ and $C_{69}$ could not be derived from this sequencing gel but could be deduced from the results listed in table 1 (oligonucleotides $t_2$, $t_4$, $t_5$ and $t_{6a}$) as well as from those reported in fig.2b. $U_{53}$, $U_{59}$ and $C_{60}$ which are faint on the radioautogram are not visible on the photograph.

oligonucleotide $t_2$: U–A–U–U–C–A–C–C–C–C–$A_{OH}$ allowed us to extend this sequence to nucleotide 75. Analysis of 5′-$^{32}$P-labelled mt tRNA gave the sequence of nucleotides 1–45 (result not shown) and provided a large overlap with the sequence 34–75. Some pyrimidines which were difficult to derive from the sequencing gel (see fig.1) could be deduced from the oligonucleotide sequences listed in table 1 and also from the RNA sequencing method developed in [13]. Furthermore, this method allowed the direct determination of all minor nucleosides located within the whole sequence. Figures 2 show some of the results obtained by this method. The gel fractionation of the 5′-labelled fragments resulting from partial hot water hydrolysis of unlabelled mt tRNA$^{His}$ is shown in fig.2a. Figure 2b shows the identification by thin-layer chromatography of the 5′-terminal mononucleoside 5′-phosphate corresponding to fragments 1–70 of fig.2a. The sequence of residues 1–8 which is G–G–U–G–A–A–U–A can basepair with the sequence U–A–U–U–C–A–C–C of oligonucleotide $t_2$ (table 1) leading to an acceptor stem of 8 basepairs. Residues 17 and 20 correspond to pD, residues 27, 32, 38 and 54 to pψ and residue 53 to pT. Residue 37 was identified as pm$^1$G by two-dimensional TLC using the solvent systems A + B and C + B quoted in section 2 (results not shown).

The complete nucleotide sequence of yeast mt tRNA$^{His}$ deduced from these analyses is shown in fig.3.

a




## 4. Discussion

Yeast mt tRNA$^{His}$ is 75 nucleotides long and has a G + C content of 38.7%. It contains all the invariant or semi-invariant residues found in tRNAs active in elongation of protein synthesis [15]. This organellar tRNA has two interesting structural features:

1. Its acceptor stem is one basepair longer than usual, as is the case for tRNA$^{His}$ from *S. typhimurium* or *E. coli* [16]. Such an acceptor stem of 8 basepairs, leaving only 3 nucleotides (C–C–$A_{OH}$) unpaired on the 3′-end, seems therefore to be a specific structural feature of histidine tRNAs. Whether it is a 'procaryotic-type' feature is still an open question, since no eucaryotic tRNA$^{His}$ has been sequenced until now.
2. Yeast mt tRNA$^{His}$, unlike the two bacterial tRNAs$^{His}$, has only 4 nucleotides basepaired in its anticodon stem. This unusual feature is also found in *N. crassa* mt tRNA$^{Val}$ (U. L. RajBhandary, personal communication) but is not specific to mitochondrial tRNAs, since it has already been found in some eucaryotic tRNAs.

Yeast mt tRNA$^{His}$ contains 8 rare nucleosides. Unlike yeast mt tRNA$^{Phe}$ [5], it has a ribothymidilic residue in position 53 as expected from our earlier

Fig.2. (a) Fractionation pattern obtained after incubation of 5 μg unlabelled mt tRNA$^{His}$ at 80°C for 5 min in 10 μl bidistilled water. The digestion products were 5′-postlabelled and 3 identical aliquots were loaded on a 15% polyacrylamide gel (0.5 × 200 × 400 mm) at time 0 (A), 4 h (B), 8 h (C) and electrophoresed at 1500 V for 11 h. Fragments are numbered in the 5′ to 3′ direction. $XC_C$ and $BB_C$ (lane C) indicate the tracking dyes, xylene cyanol FF and bromophenol blue, respectively.

b



Fig.2. (b) Analysis of the 5'-terminal nucleotides of fragments 1–70 shown in fig.2 (a). The different fragments were eluted from the gel and ethanol precipitated with carrier tRNA as in [5]. They were degraded to mononucleosides [5'-$^{32}$P]phosphate with $P_1$ nuclease and analysed by TLC using solvent B: HCl/isopropanol/$H_2O$ (17.6:68:14.4, by vol.) (◀) indicate the positions of minor nucleotides.

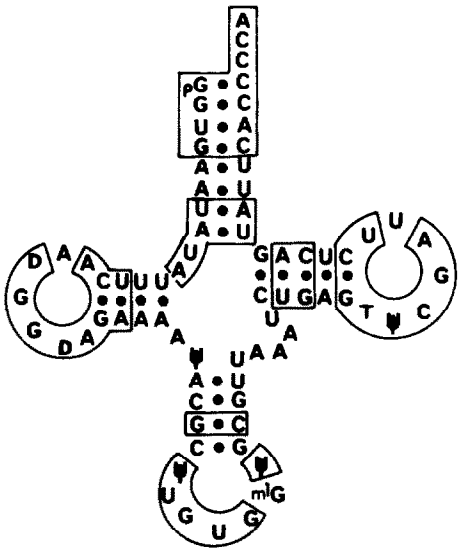


Fig.3. Nucleotide sequence of yeast mt $tRNA^{His}$ arranged in cloverleaf form. Nucleotides which are common with *S. thyphimurium* or *E. coli* $tRNA^{His}$ are in boxes.

results [4] showing that total yeast mt tRNA contains almost one residue of ribothymidine per molecule. mt $tRNA^{His}$ contains a $m^1G$ residue in the 3'-position next to the anticodon, as is the case for yeast mt $tRNA^{Phe}$ [5], $tRNA_f^{Met}$ and $tRNA_2^{Ser}$ (our unpublished results). The high frequency of such a modification in this position in yeast mt tRNAs is possibly necessary for adequate codon–anticodon recognition in this peculiar system, which uses a reduced number of isoaccepting tRNAs for mitochondrial protein synthesis [1,2].

Histidine-tRNAs from yeast mitochondria and *S. typhimurium* or *E. coli* [16] have 47 nucleotides in common, which are essentially located in the acceptor stem and in the three loops of the cloverleaf (see fig.3). The percentage of sequence homologies (63%) is rather high for a mitochondrial tRNA. Other sequenced yeast mt tRNAs: $tRNA^{Phe}$ [5], $tRNA_f^{Met}$ and $tRNA_2^{Ser}$ (our unpublished results) show fewer similarities to the corresponding procaryotic or eucaryotic tRNAs.

Finally, the DNA sequence of the histidine tRNA gene from yeast 'petite' mutants, independently determined [17], is in total agreement with our tRNA sequence. Since both sequences are colinear there is no intervening sequence as has been found in some yeast nuclear tRNA genes [18,19].

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