NUCLEOTIDE SEQUENCE OF "RENATURABLE" LEUCINE TRANSFER RIBONUCLEIC ACID*

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"Renaturable" leucine transfer ribonucleic acid from baker's yeast was found by Lindahl et al. [1] to be able to exist in a relatively stable denatured configuration which has only low amino acid acceptor activity. Incubation at 60° for 5 min, followed by slow cooling in the presence of magnesium ion, was found to disrupt the incorrect comformation and allow renaturation to the native, biologically active structure. Although the physiological significance of the reversible denaturation process, if any, is not known, it is of interest to try to determine if there is a unique feature of the primary or secondary structure that would account for the stability of the second, inactive conformation. The "renaturable' leucine tRNA ($tRNA_a^{Leu}$)** was purified in our laboratory by sucessive chromatography on benzoylated DEAE cellulose and Sephadex G-100 columns [2]. We determined the nucleotide sequence of this tRNA [3] by Holley's method [4]. In this paper, we would like to report our results and compare them with those recently reported by Kowalski et al. [5].

Purified $tRNA_a^{Leu}$ was digested with pancreatic ribonuclease [2]. The identity of the products from this digestion are shown in table 1. Sequence analyses on these fragments were described in [2]. The nucleotides, including those of minor bases, were identified by their spectra at neutral, acidic and basic pH.

The tRNA was also digested with T_1 ribonuclease [6]. To determine their nucleotide sequences, the purified T_1 fragments were further digested with pancreatic RNase, alkaline phosphatase, snake venom phosphodiesterase, or polynucleotide phosphorylase (singly or in combinations), and the products were isolated by paper or column chromatography and identified by their spectra. Details of this procedure were given in [6]. The identity of the T_1 RNase digestion products are shown in table 2.

Large oligonucleotides were obtained from partial digestion of $tRNA_a^{Leu}$ with T_1 RNase. After the first digestion, described in fig. 1, fragments 1 (from the 5'-end to the dihydrouridine loop) and 2 (the remainder of the molecule) were obtained. Fragment 2 was further digested as described in fig. 1 to obtain the three oligonucleotides shown. This result indicates that in this tRNA the diHU loop is more susceptible to T_1 RNase attack than is the anticodon loop. This resistance of the anticodon stem and loop to T_1 Rnase digestion was also evident from the "complete" digestion of this tRNA with T_1 RNase. As shown in table 2 the longest fragment was a product of incomplete action of the T_1 RNase and was found to contain the entire anticodon stem and loop. Since there are unsubstituted guanosines in this region which should be susceptible to T_1 RNase, it would seem that this region has a very compact tertiary structure in the "denatured" configuration that the tRNA was in at the time of digestion.

From the results of the complete pancreatic RNase

* Part III in a series, "Studies on yeast leucine transfer ribonucleic acid"; part II, ref. [6].

** Abbreviations used are tRNA, transfer ribonucleic acid; $tRNA_a^{Leu}$, "renaturable" leucine tRNA; standard abbreviations are used for the four major nucleosides; AcC, N^4-acetylcytidine; diHU, 5,6-dihydrouridine, 5mC, 5-methylcytidine; T, ribothymidine; 2mG, N^2-methylguanosine; 2'OmG, 2'-*O*-methylguanosine; dimG, N^2-dimethylguanosine; 1mG, N^1-methylguanosine; RNase, ribonuclease.

Table 1

Products formed by complete degradation of $tRNA_a^{Leu}$ with pancreatic ribonuclease

COH	ApGpCp
8–9 Cp + 7–8 Up	ApApCp
AcCp + 5mCp + ψp + diHUp	Gp2mGpCp
dimGpCp	GpApApUp
2GpUp	ApAp1mGpCp
Gp5mCp	GpApGpCp
ApUp	ApGpGpCp
ApCp	pGpGpUp
2'-OmGpGpdiHUp	ApApGpGpCp
GpApψp	ApApGpApUp
	ApApGpApGpTp

Table 2

Products formed by complete degradation of $tRNA_a^{Leu}$ with T_1 ribonuclease

2mGp	UpUpUpGp
2Gp	UpApApGp
2ApGp	5mCpApApGp
CpAcCpGp	CpApApCpCpApCpCOH
UpUpGp	diHUpCpdiHUpApApGp
pGp	ApApUpCpUpCpUpUpApGp
Cp2'-OmGpGp	CpdimGpCpCpUpGpApψpUp5mCpApAp-
ApUpGp	1mGpCpCpCpApGpGpUpApUpCpGp
Tpψp CpGp	

digestion, the complete T_1 and partial T_1 RNase digestions, the primary nucleotide sequence of $tRNA_a^{Leu}$ was derived and arranged in the cloverleaf secondary structure shown in fig. 2. The only ambiguity in this sequence is the order of two T_1 oligonucleotides UUG and UUUG. The order shown was derived from two considerations: (i) All known tRNA's have a uridine at position 8, and (ii) this sequence allows maximum base pairing in the amino-acid-acceptor stem.

This tRNA is 85 nucleotides in length and has 5mC–A–A as the anticodon. The anticodon stem of this tRNA contains only four base pairs while all other tRNA's with established structure have five in the same region. This leaves nine unpaired bases in the anticodon loop, as opposed to the usual seven. As in the case of *E. coli* $tRNA^{Leu}$ and yeast $tRNA^{Asp}$, N^1-methylguanosine is adjacent to the 3′ side of the anticodon of this tRNA.

Kowalski et al. recently reported the nucleotide sequence of the "denaturable" leucine tRNA [5]. This sequence, shown in fig. 3, was determined using

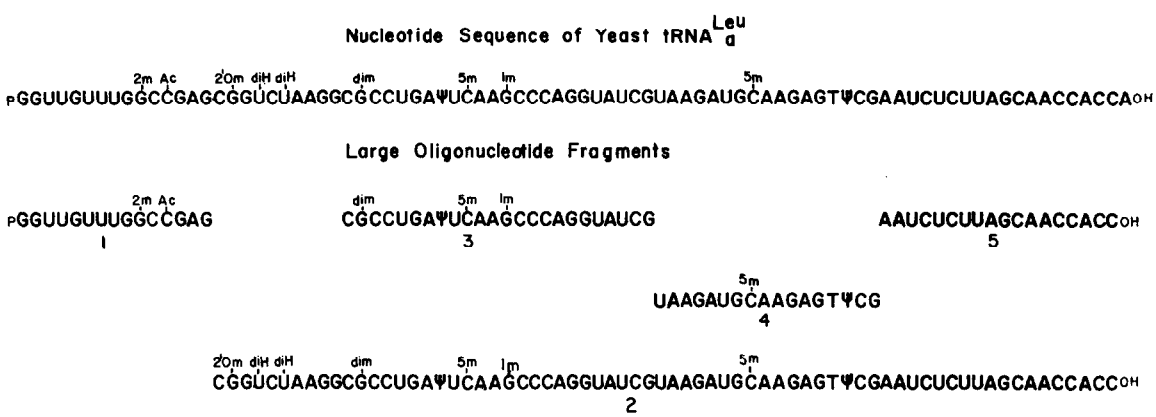

Fig. 1. Nucleotide sequences of oligonucleotides obtained from partial degradation of $tRNA_a^{Leu}$ with T_1 RNase. Approximately 1000 absorbance units* of the purified tRNA was incubated with 15,000 units of T_1 RNase at 0° for 20 min. Chromatography of the products on a DEAE cellulose in the presence of 7 M urea gave fragments 1 and 2. Further digestion of fragment 2 (300 absorbance units with 100,000 units of T_1 RNase at 0° for 10 min and separation of the products on a DEAE cellulose column at 55° in the presence of 7 M urea gave fragments 3, 4 and 5.

* One absorbance unit is defined as that amount of material per ml of solution which produces an absorbance of 1 in a 1 cm light path at 260 nm.

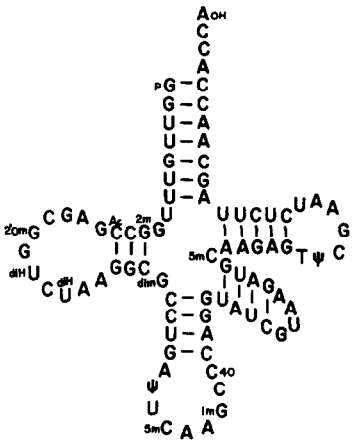

Fig. 2. Nucleotide sequence of "renaturable" leucine tRNA from baker's yeast.

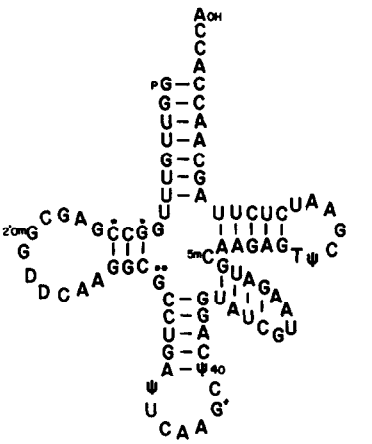

Fig. 3. Nucleotide sequence determined by Kowalski et al., for "denaturable" leucine tRNA from baker's yeast (from [5]).

the Sanger method [7] of ^{32}P labeling and two-dimensional electrophoresis. Assuming that the two sequences are of the same tRNA, this is the first time the nucleotide sequence of a tRNA has been simultaneously and independently determined by both the Holley and Sanger methods. Although the sequences are not identical, it can be seen that there is good agreement between the results obtained by the two methods. While conclusive identification of the minor bases was not made in the Kowalski sequence, their tentative identification [5] agrees with the minor bases which we found [2, 3].

It can be seen by comparison of the two sequences that there are three points of disagreement: (i) Our sequence has diHU-C-diHU in the dihydrouridine loop, while theirs has diHU–diHU–C. (ii) Our sequence has a 5mC–A–A anticodon, and theirs has an unsubstituted C–A–A anticodon. (iii) We found a cytidine at position 40, while they found a pseudouridine at this position.

We have found that the difference in the nucleotide at position 40 did not result from differences in sensitivity of detection and identification in the two methods used. In a subsequent purification of "renaturable" leucine tRNA (unpublished results), we obtained a "renaturable" leucine tRNA that was the major leucine-accepting peak but which had a much different mobility on the BD-cellulose column under the same conditions used in isolation of $tRNA_a^{Leu}$. Preliminary studies of the T_1 RNase digestion products of this second "renaturable" leucine tRNA indicated that it has nearly the same base composition as the first, including the anticodon 5mC–A–A. We found, however, that this tRNA contains a pentanucleotide which corresponds to our C–C–C–A–G sequence, but which contains C,A–G, and ψ. It would, therefore, seem that it has a pseudouridine instead of a cytidine at position 40. This observation suggests that there are two types of "renaturable" leucine tRNA in yeast with respect to the nucleotide at position 40 (designated as 40C $tRNA^{Leu}$ and 40ψ $tRNA^{Leu}$ respectively). The relationship between these two types of leucine tRNA is not known. It would seem logical to suggest that 40C $tRNA^{Leu}$ is a precursor to 40ψ $tRNA^{Leu}$, with the C being converted into a ψ. It is, however, generally believed that ψ is derived from U, not C [8–11], leading to the possibility that 40C $tRNA^{Leu}$ is a mutant form of 40ψ $tRNA^{Leu}$. The $\psi \rightarrow$ C substitution at position 40 reduces the number of base pairs from five to four in the anticodon stem. As found in the mutant species of *E. coli* suppressor tryptophane tRNA [12], the loss of a base pair in the double helical region probably converts the tRNA into a less stable structure. However, since the leucine tRNA reported by Kowalski is also "renaturable" and has five base pairs in the anticodon stem, it is uncertain whether or not the presence of only four base pairs in the anticodon stem of our "renaturable" leucine tRNA is responsible for the stability of its denatured form.

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