THE CORRECTED NUCLEOTIDE SEQUENCE OF YEAST LEUCINE TRANSFER RIBONUCLEIC ACID

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SUMMARY

The nucleotide sequence of "Renaturable" leucine transfer RNA from Baker's yeast has been re-investigated. The results showed that (i) this tRNA has a sequence of DCD at positions 19-21, (ii) it has an anticodon m^5CAA and (iii) it has a pseudouridine at position 40.

The nucleotide sequence of "Renaturable" leucine transfer RNA ($tRNA^{Leu}_3$) has been reported independently by two laboratories (1, 2). There are, however, three lines of discrepancy between these two sequences, namely (i) the sequence of DDC[1] (1) or DCD (2) at positions 19-21, (ii) the anticodon CAA (1) or m^5CAA (2) and (iii) a pseudouridine (1) or a cytidine (2) at position 40. The nucleotide sequence of this tRNA has been re-analyzed carefully. The results are reported in this communication.

MATERIALS AND METHODS

Baker's yeast tRNA was prepared according to Holley (3). $tRNA^{Leu}_3$ purified as previously described (4) was degraded with T_1 ribonuclease (Sankyo). The products were separated by chromatography in a DEAE-cellulose (Whatman DE-23) column with the conditions described previously (2). Fractions under peaks containing the questioned oligonucleotides were combined, and the urea removed by gel filtration in

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[1] The abbreviations used are: D, 5,6-dihydrouridine; Ψ, pseudouridine; m^5C, 5-methylcytidine; ac^4C, N^4-acetylcytidine; G^m, 2'-O-methylguanosine; m^2G, N^2-methylguanosine; m^2_2G, N^2,N^2-dimethylguanosine; Pan RNase, pancreatic ribonuclease. One A_{260} unit is defined as that amount of material per ml of solution which produces an absorbance of 1 in 1 cm light path at 260 nm.

columns of Biogel P-2 (Bio Rad). Oligonucleotides under each peak were further separated by, first, incubation with bacterial alkaline phosphatase (Worthington BAPF) and then chromatography in isobutyric acid-NH_4OH-H_2O (66:1:33, solvent A) or by high voltage electrophoresis in 7% HCOOH. The nucleotide composition of each oligonucleotide was analyzed by digestion with pancreatic ribonuclease (Worthington) and bacterial alkaline phosphatase, followed by chromatography of the products in isopropanol-NH_4OH-H_2O (7:1:2, solvent B). The 5′ terminal nucleoside of each oligonucelotide was determined by degradation of the 3′ dephosphorylated oligonucleotide with snake venom phosphodiesterase (Worthington) and separation of the products in solvent B. The nucleotide sequences of all three questioned oligonucleotides were determined with a common procedure. The oligonucleotide was incubated with polynucleotide phosphorylase (P-L Biochemicals) in the presence of inorganic phosphate and bacterial alkaline phosphatase. A trinucleotide from the 5′ end was isolated by chromatography of the products in solvent A or n-propanol-NH_4OH-H_2O (55:10:35, solvent C). The trinucleotide was further purified by incubation with bacterial alkaline phosphatase and re-chromatography in solvent A. This purified trinucleotide was analyzed by degradation with pancreatic ribonuclease and chromatography of the products in solvent B. Nucleosides or nucleotides were identified by their ultraviolet spectra at neutral, acidic, and alkaline pH's.

RESULTS

Figure 1 shows the chromatographic pattern of the products obtained by degradation of the tRNA with T_1 RNase. Peak 14 containing two pentanucleotides CΨCAG and UAUCG, peak 16 containing the hexanucleotide DCDAAG and peak 17, the anticodon fragment were analyzed with the procedure described above. The results of these analyses are summarized in Table I. Based on these results and those we reported previously (2) a

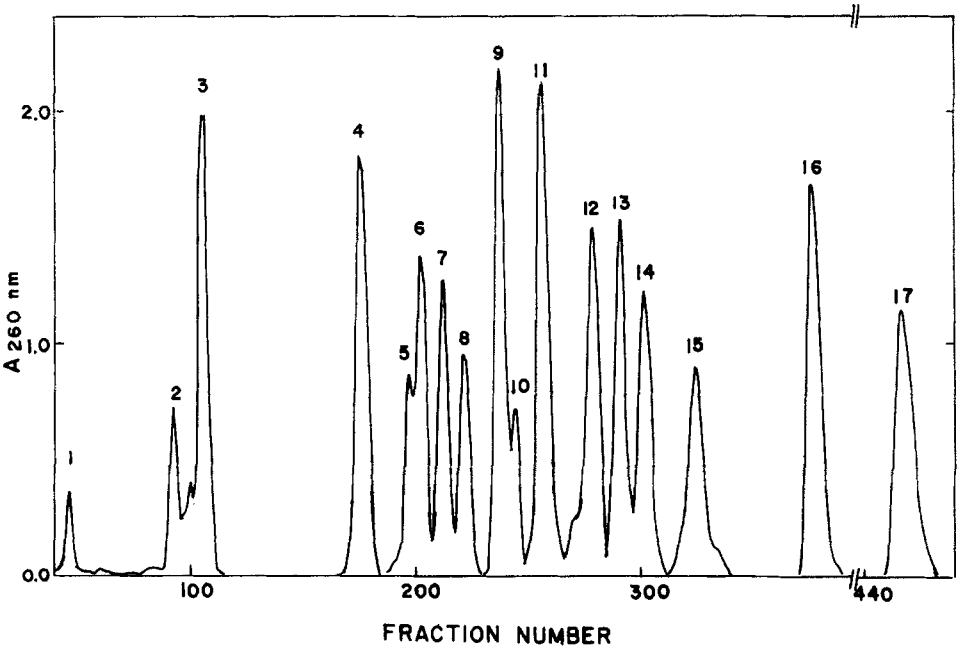

Fig. 1. Chromatography of T_1 RNase digest of $tRNA^{Leu}_3$. 315 A_{260} units of $tRNA^{Leu}_3$ were incubated with 1000 units of T_1 RNase at 37° for 10 hours. The mixture was then applied on a column (1 x 60 cm) of DEAE cellulose. The column was eluted with a linear gradient (0 to 0.4 M) of NaCl in 7 M urea buffered with 0.02 M Tris-HCl, pH 7.5. Total volume of the gradient was 2 liters. Fractions of 3 ml were collected every 10 min.

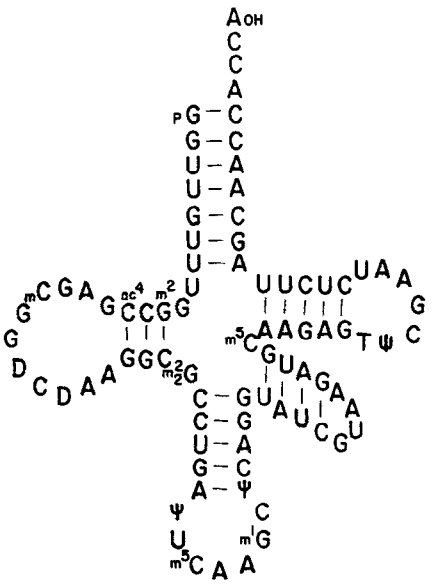

Fig. 2. The corrected nucleotide sequence of yeast $tRNA^{Leu}_3$ in the clover-leaf form.

TABLE I

Nucleotide sequences of three fragments obtained by degradation of $tRNA^{Leu}_{3}$ with T_1 ribonuclease.

Peak No.	Products from Pan RNase + alkaline phosphatase degradation	Nucleoside from snake venom phosphodiesterase degradation	5′ trinucleotide from polynucleotide phosphorylase degradation	Nucleotide sequence
12*	2C, ψ, AG	C	CψC (C)**	CψCAG
14	2D, C, AAG	D	DCD (D)**	DCDAAG
15	U, m^5C, Aψ, AAm^1G	A	AψU (U)**	$A\psi Um^5CAAm^1G$

* The slower moving band from high voltage electrophoresis in 7% HCOOH.

** Nucleoside obtained by degradation of the trinucleotide with Pan RNase.

corrected nucleotide sequence for yeast $tRNA^{Leu}_{3}$ is shown in Fig. 2 in its clover-leaf secondary structure.

DISCUSSION

The three important points shown in Table I and Fig. 2 are (i) $tRNA^{Leu}_{3}$ has a sequence of DCD at positions 19-21, (ii) it has an anticodon m^5CAA and (iii) it has a pseudouridine at position 40. (i) and (ii) are in agreement with the results we reported previously (2) and (iii) agrees with the findings of Kowalski and coworkers (1). The corrections described in this communication plus the fact that the rest of this tRNA structure is agreed upon by the results from two different techniques (1, 2) indicate that the final sequence shown in Fig. 2 must represent the correct structure of yeast $tRNA^{Leu}_{3}$.

It is worth noting that in the degradation of oligonucleotides with polynucleotide phosphorylase, the tri- or tetranucleotides isolated are usually contaminated with nucleoside 5′ diphosphates since all have a similar number of negative charges. The removal of these nucleoside diphosphates can be achieved by dephosphorylation with bacterial alkaline phosphatase and re-chromatography in solvent A (5). Tri- or tetranucleotides so purified provide clear cut information for sequence analysis.

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