THE NUCLEOTIDE SEQUENCE OF $tRNA^{Gly}$ FROM YEAST

Mitsuaki Yoshida

Faculty of Pharmaceutical Sciences, University of Tokyo

Hongo, Tokyo, Japan

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Summary. The nucleotide sequence of glycine tRNA from yeast was determined on ^{32}P-labeled material. The molecule is 73 nucleotide long and can be folded into the " clover leaf " model.

Two major and two minor species of glycine tRNAs are found when yeast tRNA is fractionated on benzoylated DEAE-cellulose column (1). One of these $tRNA^{Gly}$ can easily be purified by combination of two different polyacrylamide gel electrophoresis because of its high mobility. I report here the nucleotide sequence of this $tRNA^{Gly}$. The 73 nucleotides sequenced for the tRNA is smallest among tRNAs which have been sequenced so far except the tRNA for cell wall biosynthesis (2).

A culture of Saccharomyces cerevisiae X2180 was grown in 50 ml of low phosphate medium containing 0.1 mCi per ml of ^{32}P-orthophosphate. The medium was prepared as follows: 100 ml of a solution containing 1% yeast extract and 2% polypeptone was mixed with 1 ml of 1 M $MgSO_4$ and 1 ml of conc. NH_4OH. After stirring for 1 hour, the precipitates were filtered off with filter paper and the filtrate was adjusted to pH 5.8 with conc. HCl. After sterilization, 4% glucose was supplemented. RNA was extracted by treatment of cells with phenol in 0.1 M sodium acetate, pH 5.0, and 1% SDS at room temperature. The RNA taken in 150 µl of 20% sucrose and 0.02% bromophenol blue was carefully applied on top of a 10% polyacrylamide gel slab (2 x 100 x 400 mm), which was polymerized as described by Adams et al (3). Electrophoresis was carried out at 4°C in 0.09 M Tris, 0.09 M boric acid, and 0.0027 M EDTA, pH 8.3, for 15 hours at 25 mA (Fig. 1A). A band

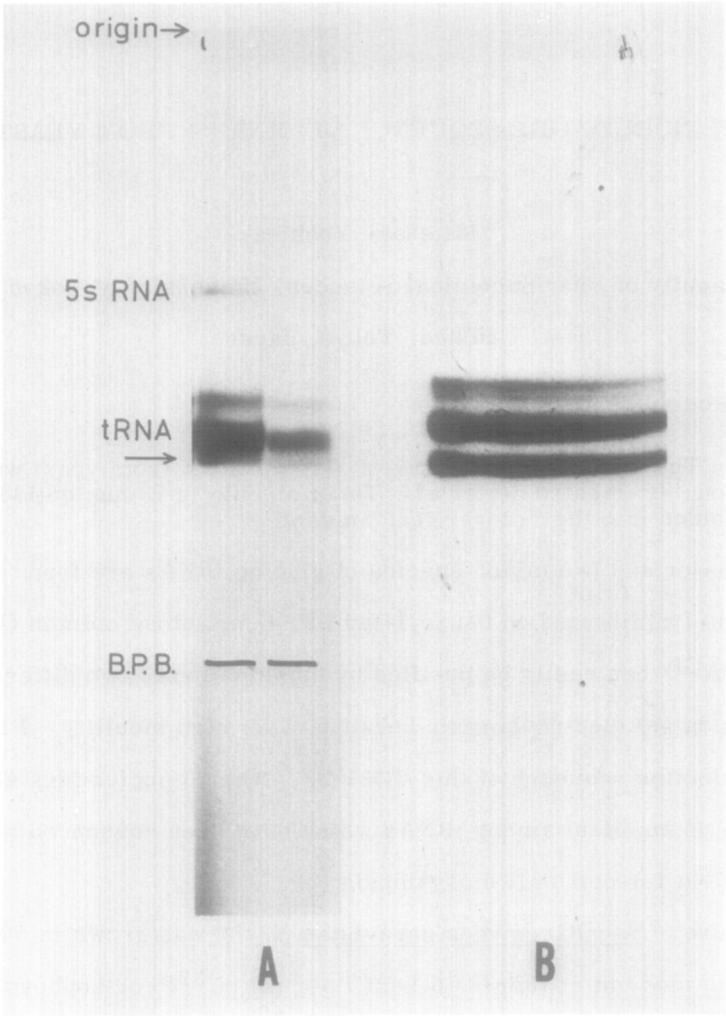

Fig. 1. Polyacrylamide gel electrophoresis of crude RNA extracted from yeast and partially purified tRNA. (A); Crude RNA preparation was separated on 10% polyacrylamide gel in 0.09 M Tris, 0.09 M boric acid and 0.0027 M ETDA, pH 8.3. Electrophoresis was carried out at 25 mA for 15 hours at 4°C until bromophenol blue traveled more than 30 cm. (B); RNA extracted from the fastest-moving band on the gel shown in Fig. 1A was fractionated on 12.5% gel in 30 mM Tris acetate, pH 8.0, and 5 M urea at room temperature. Electrophoresis was similar to the conditions for Fig. 1A.

run just ahead of major tRNA band (shown by arrow in Fig. 1A) was cut out, RNA was extracted with 0.5 M NaCl-20 mM Tris-HCl, pH 8.5, and precipitated with 2 volumes of ethanol. The RNA was further fractionated by another polyacrylamide gel electrophoresis in 5 M urea and 30 mM Tris acetate, pH 8.0, at room temperature at 25 mA for 20 hours (Fig. 1B). RNA was

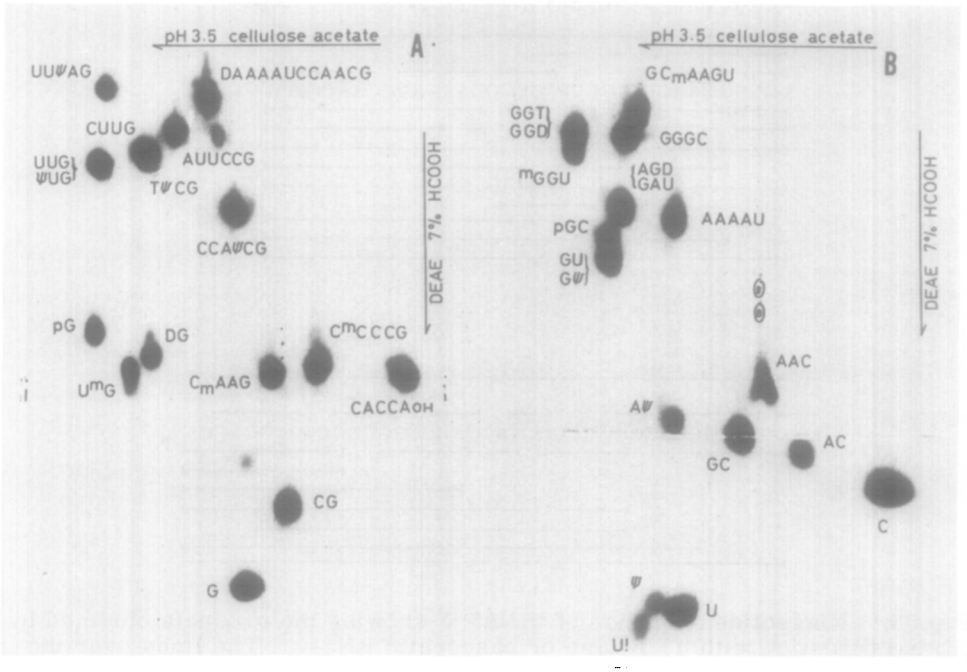

Fig. 2. Two dimensional fractionation of T1 (A) or pancreatic RNase (B) digest of $tRNA^{Gly}$. B is blue marker. Radioautographs showing the sequences of the nucleotides.

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extracted from a band which moved fastest and was proved to be > 95% pure, as judged from the presence of contaminating oligonucleotides in nuclease digests.

Sequence studies on the ^{32}P-tRNA were carried out according to the methods developed by Sanger et al (4, 5). Finger prints of complete T1 or pancreatic RNase digests are shown in Fig. 2. Larger oligonucleotides produced by partial T1 and pancreatic RNase digestion were isolated by thin layer homochromatography (6) and identified on the basis of the products of their complete digests with T1 or pancreatic RNase. The results are summarized in Fig. 3.

The chain length of 73 nucleotides is shortest in tRNAs which have been sequenced except $tRNA^{Gly}$ from Staphylococcus epidermis (2) for cell wall synthesis, and the highest mobility of this $tRNA^{Gly}$ in gel electrophoresis is consistent with the relatively short chain length of this tRNA. The sequence

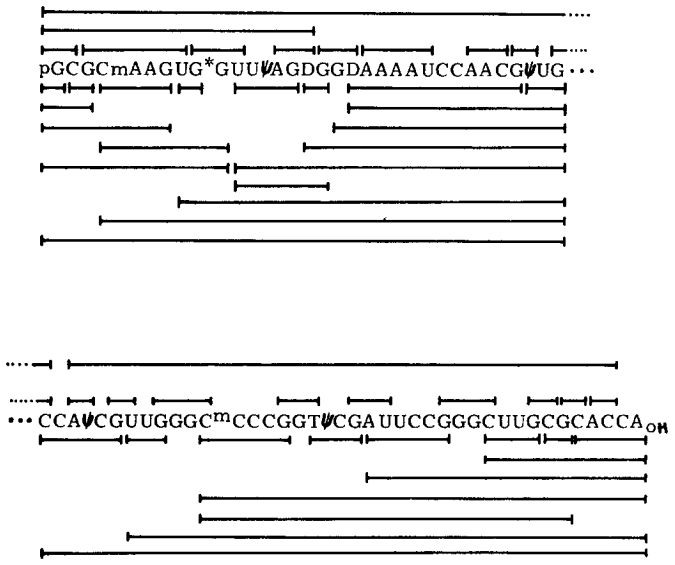

Fig. 3. Nucleotide sequence of $tRNA^{Gly}$ showing the products obtained by partial digestion with T1 RNase or pancreatic RNase. The lines over the sequence represent the pancreatic RNase products and ones under the sequence are T1 RNase products. G* is probably N1-methyl-G.

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is folded into a typical "clover leaf" form with the common features of other tRNAs (Fig. 4). Although clover leaf structure of almost all other tRNAs has purine nucleotide at junction of anticodon stem and dihydrouracil stem, the structure has cytidylic acid at this position. $tRNA^{His}$ from Salmonella typhimurium (7) and E. coli (8) also have been reported to have cytidylic acid at this position. Dihydrouracil loop contains 7 residues like $tRNA^{Gly}$ from S. epidermis (2) and $tRNA^{Trp}$ from yeast (9), while most of the tRNAs from various sources have 8 to 10 nucleotides in this loop. Another point of interest is the presence of 2'-O-methylcytidylic acid in the aminoacyl stem, which is base paired. No tRNA has been found which contains methylated nucleotide in the aminoacyl stem.

The sequence GCC is in the anticodon position. Since GGX (X = U, C, A, and G) is the code for glycine, the anticodon sequence suggests that this tRNA is for glycine and corresponds the code GGU and GGC. The nonradioactive tRNA was fractionated by the same procedures as described above and the

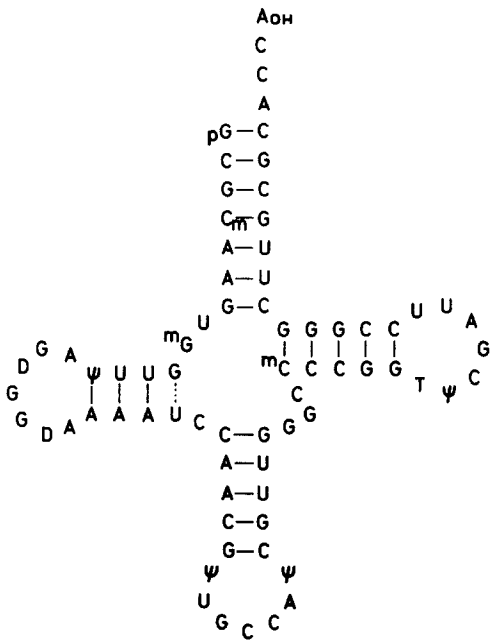

Fig. 4. The nucleotide sequence of $tRNA^{Gly}$ arranged in the "clover leaf" pattern of other tRNAs. Cm is tentatively identified as 2'-0-methyl-C, ^{m}G as N1-methyl-G and ^{m}C as 5-methyl-C.

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purified tRNA was charged with a mixture of ^{14}C-amino acids using crude aminoacyl-tRNA synthetase preparation. Glycine was identified as a main amino acid in the hydrolysate of the aminoacyl-tRNA. Actually the purified preparation accepts 950 μμmoles of ^{14}C-glycine per one A_{260} unit. Almost no significant homology was found by comparing the sequence with $tRNA^{Gly}$ from S. epidermis (2) and E. coli (10).

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