Nucleotide Sequence of Escherichia coli Tyrosine Transfer Ribonucleic Acid

Abstract. *The nucleotide sequence of one of the* Escherichia coli *tyrosine-transfer ribonucleic acids was determined in order to compare its sequence with that of yeast tyrosine-transfer ribonucleic acid. Forty-four positions of both transfer ribonucleic acids are occupied by the same nucleotides if they are arranged in the manner shown here. The information obtained suggests that the conformation of transfer ribonucleic acid molecules may be a greater contributing factor than a specific nucleotide sequence in the interaction of transfer ribonucleic acid with its corresponding aminoacyl-transfer ribonucleic acid synthetase.*

Since the determination of the structure of yeast $tRNA^{Ala}$ (*1, 2*), the structures of several tRNA's of yeast (*3–6*) and of *Escherichia coli* (*7, 8*) have been elucidated. However, the manner in which the aminoacyl-tRNA synthetases recognize their functional sites on the tRNA remains to be answered. Our previous studies on the species specificity of tyrosine-tRNA's from yeast and *E. coli* showed that although these two tRNA's have similar properties, such as acceptance of tyrosine with homologous synthetases and response to the same synonym trinucleotides in binding to ribosomes, they are different structurally (*9*). In order to explore the possibility that the structural differences in the $tRNA^{Tyr}$ of yeast and *E. coli* cause the observed species specificity, determination of the nucleotide sequence of *E. coli* $tRNA^{Tyr}$ was undertaken. This communication describes the almost complete nucleotide sequence of *E. coli* $tRNA_2^{Tyr}$. Furthermore, comparison of the structure of $tRNA^{Tyr}$ from yeast and *E. coli* is made to study the probable functional sites.

The methods employed in this investigation are essentially the same ones used in other similar investigations (*2–6*). The $tRNA_2^{Tyr}$ from *E. coli* B was purified by countercurrent distribution procedures (*10*) and digested with bovine pancreatic ribonuclease and ribonuclease T_1. Fragments thus obtained were separated first by column chromatography on DEAE-cellulose in 7*M* urea at *p*H 8.0 (*11*) and then further separated by the same procedure at *p*H 3.5 (*12*). The base compositions and the nucleotide sequences of the fragments thus separated were determined. Finally, by limited hydrolysis of tRNA with ribonuclease T_1 at 0°C (*13*) overlapping fragments were obtained. The information gathered from the complete and limited enzymatic digestion products was sufficient to construct the defined sequence.

Comparison of the nucleotide sequences of yeast $tRNA^{Tyr}$ and *E. coli* $tRNA_2^{Tyr}$ is shown in Fig. 1. Nucleotides that are not yet definitely identified are designated as P-, Q-, R-, and S-. From spectral and chromatographic properties, nucleotides P- and Q- appear to be modified pyrimidines. Nucleotide R- is a modified G- which is resistant to ribonuclease T_1 and nucleotide S- is modified A-. In addition, when tRNA was isolated from *E. coli* cells grown on media containing ^{35}S-sulfate, the thio groups of nucleotides P- and S- were fully labeled and nucleotide Q- contained some labeled sulfur. This partial labeling of the thio group may be due to lability of the sulfur-containing residues during isolation, to the extent of thiolation during growth of the *E. coli* cells, or to contamination from other oligonucleotides containing thio groups. Other nucleotides were identified by chromatography and spectroscopy. The data from the two sets of fragments are in good agreement, except that the ribonuclease T_1 fragment P-(U-C-C-C-C-)G- always appeared in low yields and was only partially separated from the A-C-U-R-U-A-S-A-ψ-C-U-G- fragment by the methods used.

In previous investigations (*10*) it was shown that *E. coli* $tRNA_1^{Tyr}$ contains two 4ThU- molecules and no other thionucleotides. Preliminary investigations of mixed $tRNA_{1,2}^{Tyr}$ show that nucleotide P- in $tRNA_2^{Tyr}$ is replaced by 4ThU- in $tRNA_1^{Tyr}$. Another 4ThU- may be located adjacent to the first 4ThU- as indicated above. Furthermore, when ^{35}S-labeled $tRNA_1^{Tyr}$ was hydrolyzed with alkali and the products were analyzed by chromatography, all the radioactivity was located in a single spot that corresponded to the position of 4ThU- under those conditions. It is unlikely then that nucleotides Q- and S- may contain thio groups in the case

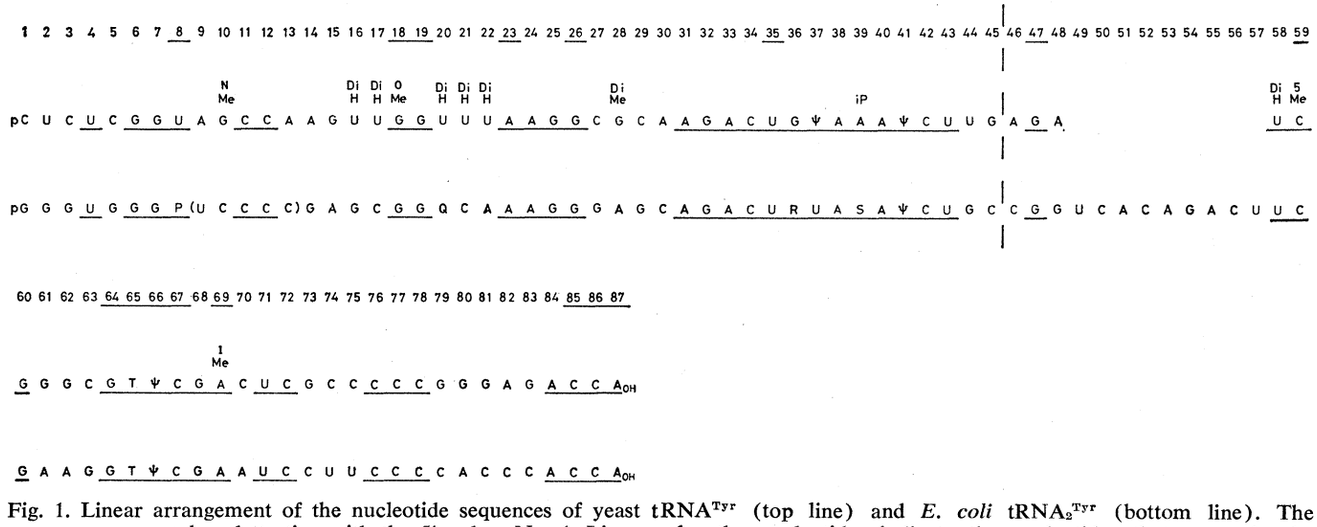

Fig. 1. Linear arrangement of the nucleotide sequences of yeast $tRNA^{Tyr}$ (top line) and *E. coli* $tRNA_2^{Tyr}$ (bottom line). The sequences are numbered starting with the 5′-end as No. 1. Lines under the nucleotides indicate the nucleotides that occur in the same position in both tyrosine tRNA's. Lines under the numbers indicate the positions at which the nucleotide shown in the arrangement occurs in all known tRNA's.

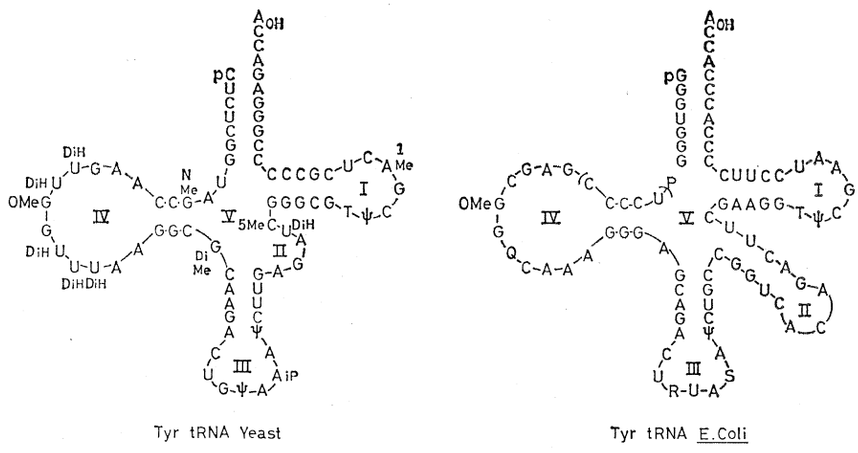

Fig. 2. Cloverleaf arrangement of the nucleotide sequences of yeast $tRNA^{Tyr}$ and *E. coli* $tRNA_2^{Tyr}$.

of *E. coli* $tRNA_1^{Tyr}$. As a matter of fact, in *E. coli* $tRNA_1^{Tyr}$, the nucleotide corresponding to Q- is tentatively identified as C-. Identification of nucleotide S- from both *E. coli* $tRNA_{1,2}^{Tyr}$ is at present being pursued. Our investigations show that *E. coli* $tRNA_2^{Tyr}$ does not contain 4ThU- as such, whereas *E. coli* $tRNA_1^{Tyr}$ contains two 4ThU-molecules. This fact is further substantiated from the comparison of absorption at 335 nm to that at 260 nm ($tRNA_1^{Tyr}$, 4.03; $tRNA_2^{Tyr}$, 0.35). It is quite possible that nucleotide designated P- is a modification product of 4ThU-.

Goodman *et al.* (*7*) have determined the nucleotide sequences of both suppressor $tRNA^{Tyr}$ and the normal $tRNA^{Tyr}$ isolated from the same mutant of *E. coli*. Our data obtained from the preliminary investigation of $tRNA_1^{Tyr}$ is in good agreement with theirs, except for thionucleotide content. However, comparison of their data with our findings in *E. coli* B $tRNA_2^{Tyr}$ shows that the 5′-end fragment obtained by pancreatic ribonuclease hydrolysis differs by one G- residue. (Also there may be a corresponding translocation of A- and C- in the 3′-end fragment obtained by ribonuclease T_1 hydrolysis.) This then may be another difference in the sequences of *E. coli* $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$. However, in consideration of the difficulty in determining the sequence of the 3′-end fragment that contains the 19 nucleotides obtained by hydrolysis with ribonuclease T_1, the sequence of the fragments in this region of the molecule needs to be reexamined.

As compared to 78 nucleotides in yeast $tRNA^{Tyr}$, *E. coli* $tRNA_2^{Tyr}$ contains 87 nucleotides. Comparison of these two tRNA's in the cloverleaf arrangement is shown in Fig. 2. For ease of description various regions of the molecules are designated by Roman numerals.

The additional nine nucleotides in *E. coli* $tRNA_2^{Tyr}$ can be placed in region II (Fig. 2). Arrangement of the nucleotide sequence of *E. coli* $tRNA_2^{Tyr}$ appears similar to that of yeast $tRNA^{Ser}$ in that both have an additional hydrogen-bonded region with a loop of three unpaired nucleotides. Yeast $tRNA^{Tyr}$ by comparison has five nucleotides in region II. This region in both these tRNA's is very different.

Another region of the tRNA molecule that appears to be different is region IV. This is the region which is generally known as the "dihydro U-loop," since all the tRNA's, except *E. coli* $tRNA_2^{Tyr}$, for which the structure has been elucidated contain DiHU- in this region. The *E. coli* $tRNA_2^{Tyr}$ has no DiHU-, whereas yeast $tRNA^{Tyr}$ has five DiHU- molecules in this region. The DiHU-'s are replaced by two C-'s, one modified C-, one G-, and one A-. Two dinucleotides, OMeG-G- and A-A-, occur in the same place in both tRNA's.

In region V of *E. coli* $tRNA_2^{Tyr}$ the DiMeG- which occurs in yeast $tRNA^{Tyr}$ (*3*) and is found also in other yeast tRNA's (*2*, *4*, *5*) is replaced by A-. The 5MeC- in yeast $tRNA^{Tyr}$ is replaced by C- in *E. coli* $tRNA_2^{Tyr}$.

The remaining two regions show striking similarity. Region I contains G-T-Ψ-C-, a tetranucleotide found in all the known tRNA's in the same relative position. Of the seven nucleotides present in the nonhydrogen-bonded region, only one nucleotide is different. Also 1MeA- in yeast is replaced by unmodified A- in *E. coli*. In the hydrogen-bonded region, yeast $tRNA^{Tyr}$ has five G:C base pairs, whereas *E. coli* has three G:C and two A:U pairs. Another region which shows striking similarity is region III. With the exception of differing modifications of three nucleotides (G-, Ψ-, and iPA- in yeast, and R-, U-, and S- in *E. coli*, respectively), all seven nucleotides in the nonhydrogen-bonded region and six of the ten nucleotides in the hydrogen-bonded region are identical in both tyrosine-tRNA's. If one recalls that response of these two tRNA's to synonym trinucleotides UAU and UAC in ribosomal binding is identical (*9*), it is significant that there is no sequential difference between the two tRNA's in this region.

The regions that remain to be examined are the 5′- and 3′-ends. In yeast $tRNA^{Tyr}$ the 5′-end is pC-, whereas in *E. coli* it is pG-. In both the tRNA's the last four nucleotides at the 3′-end are identical. Base pairing with the 5′-end can occur from the 5th to 11th nucleotides (from 3′-end), forming seven base pairs. In yeast $tRNA^{Tyr}$ there are five G:C, one G:U, and one A:U base pairs, whereas in *E. coli* $tRNA_2^{Tyr}$ the G:U pair is replaced by a conventional G:C base pair. Even though the base pairing in this region appears to be fairly similar in both tRNA's, the location of G- and C- is altered.

Region II in yeast $tRNA^{Tyr}$ consists of nucleotide Nos. 46–50, whereas in *E. coli* $tRNA_2^{Tyr}$ this region is represented by nucleotides Nos. 46–59 (Fig. 1). If the modified bases are considered the same as normal bases, 44 nucleotides occur at the same position in both molecules; in region II, three nucleotides occur in the same position. Of these 44 positions at which the same nucleotide occurs in both tRNA's, 16 positions are also occupied by the same nucleotide in yeast alanine-, phenylalanine-, serine-, and valine-tRNA's. The modified bases in *E. coli* $tRNA_2^{Tyr}$, as in all other known tRNA's, appear to be located in nonhydrogen-bonded regions. The nature of these modified bases appears to vary from one tRNA to another.

From the results presented here, it appears that regions I and III in *E. coli* $tRNA_2^{Tyr}$ are similar to regions I and III in yeast $tRNA^{Tyr}$. Regions II and IV in *E. coli* are different from regions II and IV in yeast; in other known tRNA's, regions II and IV are also variable (*2–6*).

Molinaro *et al.* (*14*) have shown that the modification of DiHU- in yeast alanine-, serine-, and valine-tRNA's

does not appear to alter their amino acid–acceptor activity. If this observation is extended to other tRNA's, it appears that DiHU-'s in region IV may not be involved in tRNA-synthetase interaction. Also, the results of Zachau *et al.* (*4*) show that the two yeast serine-tRNA's have different sequences in the nonhydrogen-bonded parts of region II. These two serine-tRNA's still have the same amino acid–acceptor activity.

The interaction of tRNA and aminoacyl-tRNA synthetase can occur in several ways: (i) the enzyme can recognize a specific nucleotide sequence or sequences in more than one part of the molecule; (ii) the enzyme can recognize the conformation of a certain region or regions of the tRNA molecule; and (iii) the enzyme-tRNA interaction can be dependent upon the conformation of the entire tRNA molecule. These and other observations (*15*), coupled with the results presented here, make it very unlikely that the specific sequence of nucleotide is primarily involved in the enzyme-tRNA interaction. It is then likely that the conformation of the tRNA plays an important role in conferring the required specificity for the interaction with specific synthetase. The nonhydrogen-bonded regions of tRNA must contribute in the formation of specific conformation of tRNA molecules, since hydrogen-bonded regions are deemed unlikely to be involved in this type of interaction by various studies. Indirect evidence for the involvement of conformation of tRNA in interaction with synthetase has been shown in two cases. Penswick (*16*) demonstrated that yeast $tRNA^{Ala}$ retains its acceptor activity after being cleaved by ribonuclease T_1 within the anticodon, as long as the two halves remain bonded together. Second, Cramer *et al.* (*17*) showed that inactivation of yeast serine-tRNA synthetase occurs at a higher temperature than that at which serine is incorporated into seryl-$tRNA^{Ser}$. (We have observed that such is also the case with $tRNA^{Tyr}$ in *E. coli.*) They have also indicated that this may be due to partial loss of the tRNA conformation and the tRNA is therefore incapable of interacting with synthetase.

Since yeast $tRNA^{Tyr}$ and *E. coli* $tRNA^{Tyr}$ do not interact with the corresponding heterologous synthetases, it is possible that they have different conformations in addition to the shown differences in their primary structures. The major differences in conformation between these two tRNA's may be contributed by the additional nucleotides in region II. It would be interesting to determine the contribution of the conformation of region II in a given tRNA in conferring that tRNA with its observed species specificity.

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References and Notes

1. Abbreviations: tRNA, transfer ribonucleic acid; Tyr, tyrosine; Ala, alanine; Ser, serine; p and - indicate a phosphate group, on the left side they represent a 5′-phosphate, and on the right side a 3′-phosphate; A, adenosine; C, cytidine; G, guanosine; U, uridine; Ψ, pseudouridine; T, ribothymidine; DiHU, 4,5-dihydrouridine; 4ThU, 4-thiouridine; OMeG, 2′-*O*-methylguanosine; *N*-MeG, N^2-methylguanosine; DiMeG, N^2-dimethylguanosine; 5MeC, 5-methylcytidine; 1MeA, 1-methyladenosine; iPA, N^6-(Δ^2-isopentenyl)adenosine; the subscript OH is used to emphasize the presence of a 3′-hydroxyl group; DEAE, diethylaminoethyl.
2. R. W. Holley, J. Apgar, G. A. Everett, G. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, A. Zamir, *Science* **147**, 1462 (1965).
3. J. T. Madison, G. A. Everett, H. Kung, *ibid.* **153**, 531 (1966).
4. H. G. Zachau, D. Dutting, H. Feldman, *Angew. Chem.* **78**, 392 (1966).
5. U. L. RajBhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, H. G. Khorana, *Proc. Nat. Acad. Sci. U.S.* **57**, 751 (1967).
6. A. A. Bayev, T. V. Venkstern, A. D. Mirsabekov, A. I. Krutilina, L. Li, V. D. Akselrod, *Mol. Biol.* **1**, 754 (1967).
7. H. M. Goodman, J. Abelson, A. Landy, S. Brenner, J. D. Smith, *Nature* **217**, 1019 (1968).
8. S. K. Dube, K. A. Marcker, B. F. C. Clark, S. Cory, *ibid.* **218**, 232 (1968).
9. B. P. Doctor, J. E. Loebel, D. A. Kellogg, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 543 (1966).
10. M. N. Lipsett and B. P. Doctor, *J. Biol. Chem.* **242**, 4072 (1967).
11. R. V. Tomlinson and G. M. Tener, *Biochemistry* **2**, 697 (1963).
12. G. W. Rushizky and H. A. Sober, *Biochem. Biophys. Res. Commun.* **14**, 276 (1964).
13. J. R. Penswick and R. W. Holley, *Proc. Nat. Acad. Sci. U.S.* **53**, 543 (1965).
14. M. Molinaro, B. Sheiner, F. A. Neelon, G. L. Cantoni, *J. Biol. Chem.* **243**, 1277 (1968).
15. T. H. Kuo and E. B. Keller, *Fed. Proc.* **27**, 341 (1968).
16. J. R. Penswick, thesis, Cornell University (1966).
17. F. Cramer, personal communication.

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Gametogony of Eimeria tenella (Coccidia) in Cell Cultures

Abstract. *Mature male and female gametocytes of* Eimeria tenella, *a coccidium of the chicken ceca, developed in primary cultures of chick embryonic kidney cells inoculated with sporozoites. Immature gametocytes appeared in the cultures after approximately 144 hours of incubation at 41°C. Mature micro- and macrogametes were present from 160 to 190 hours after inoculation.*

We have obtained gametogony in cultured cells inoculated with motile *Eimeria tenella* (coccidia) sporozoites. Until now, only limited asexual development in vitro after inoculation with sporozoites has been reported (*1*), although Bedrnik (*2*) demonstrated development of sexual stages and oocysts after inoculation of cell cultures with second generation *E. tenella* merozoites from the ceca of chickens.

Young male and female gametocytes appeared in the cultures after approximately 144 hours of incubation. Until then it was not possible to differentiate between immature macrogametocytes, immature microgametocytes, and immature schizonts. We identified the young microgametocytes by the numerous football-shaped clumps of nuclear material that stained dark pink with Giemsa's stain against a blue staining residual cytoplasmic mass. As the gametocyte matured, the nuclear material elongated, as though being pulled from either end, and then curled into comma-shaped microgametes, usually first seen at the periphery of the cytoplasmic mass. After 160 to 190 hours of incubation, microgametes too numerous to count filled the entire gametocyte (Fig. 1A). The mature microgametocyte, averaging 17.6 by 20.0 μ (range: 11.5 by 15.4 μ to 26.9 by 35.5 μ) had indistinct walls, and free microgametes protruded into the surrounding vacuole. The presence of large, cytoplasmic plastic granules that stained positively with periodic acid–Schiff reagent (PAS) and bromphenol blue identified the macrogametocytes, which averaged 16.5 by 22.8 μ (range: 15.3 by 19.2 μ to 18.2 by 28.8 μ). These plastic granules did not stain with methyl green–toluidine blue but appeared as vacuoles at the periphery of the cytoplasmic mass (Fig. 1B). Another identifying characteristic of the maturing macrogametocyte was the basophilic karyosome that became prominent with toluidine blue or Giemsa's stain.

The schizogonic cycle in vitro closely paralleled that in vivo. Within an hour after inoculation many sporozoites had invaded cells, although penetration of the cells continued for 24 hours. By 18 hours we saw trophozoites. Many first generation schizonts were mature at 48 hours, and second generation troph-