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Nucleotide sequences of three proline tRNAs from *Salmonella typhimurium*

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ABSTRACT

The nucleotide sequences of three proline tRNAs from Salmonella typhimurium were determined by post-labeling procedures. The three proline tRNAs had almost identical sequences in the D-arm and TΨC-arm, and all contained 1-methylguanosine next to the 3'-end of the anticodon. The anticodon sequences of $tRNA_1^{Pro}$, $tRNA_2^{Pro}$ and $tRNA_3^{Pro}$ were 5'-CGG-3', 5'-GGG-3' and 5'-VGG-3', respectively. The nucleotide sequence homologies of $tRNA_2^{Pro}$ to $tRNA_1^{Pro}$ and $tRNA_3^{Pro}$ were 68% and 78%, respectively.

INTRODUCTION

A number of frameshift suppressor mutants, which suppress frameshift mutations in the histidine operon of Salmonella typhimurium, have been isolated by Riddle and Roth (1). These workers examined the chromatographic profiles of tRNAs from frameshift suppressor and control strains by BD-cellulose column chromatography, and found that suppressor mutations in sufA and sufB genes produced alteration of the chromatographic profile of proline tRNA (2). This suggested that the sufA and sufB genes of S. typhimurium are structural genes for proline tRNAs. It also suggested that in the frameshift suppressor strains, altered proline tRNAs suppress frameshift mutations by reading codons consisting of four bases with repeated C.

Cummins et al. recently reported that the SUF2 gene product of Saccharomyces cerevisiae is a proline tRNA, and that this mutation in the SUF2 gene can suppress a +1 G-C insertion mutation into the histidine operon (3). The tRNA gene of SUF2-1, which is a frameshift suppressor mutant of SUF2 contains an extra G-C base pair at a position corresponding to the anticodon loop of the proline tRNA. Since the anticodon sequence of wild type SUF2+ proline tRNA inferred from the DNA sequence is 3'-GGA-5', the anticodon sequence of SUF2-1 tRNA that can suppress the four base codon 5'-CCCU-3', might be 5'-AGGG-3'. These data clearly indicate that both in Salmonella and in yeast, altered tRNA in suppressor strains is involved in correction of frameshift mutation. To obtain clearer information on the

mechanism of tRNA-mRNA interactions in frameshift suppression, it is necessary to determine the nucleotide sequences of suppressor tRNAs as well as of all isoacceptor species of proline tRNAs from the wild type strain and from which suppressor tRNA is derived.

In this communication, we report the nucleotide sequences of three proline tRNAs from wild type S. typhimurium determined by post-labeling techniques and discuss the implications of codon-anticodon interactions of proline tRNAs with mRNA in frameshift suppressor mutation.

MATERIALS AND METHODS

Unfractionated tRNA from S. typhimurium was prepared by the procedure described by Zubay (4). Salmonella proline tRNAs were fractionated by BD-cellulose column chromatography by the procedures described by Riddle and Roth (2). The proline tRNAs were designated as $tRNA_1^{Pro}$, $tRNA_2^{Pro}$ and $tRNA_3^{Pro}$ according to their order of elution from the column. Each $tRNA^{Pro}$ was purified by successive DEAE-Sephadex A-50 and RPC-5 column chromatographies, and finally by two dimensional polyacrylamide gel electrophoresis. The materials and procedures used for sequence analysis of $tRNA_s^{Pro}$ by post-labeling techniques were as described previously (5).

RESULTS AND DISCUSSION

S. typhimurium contains three isoacceptor species of proline tRNA, as reported by Riddle and Roth (2). The amount of $tRNA_2^{Pro}$ was less than 5% of that of total proline tRNAs judging from their results on the chromatographic profile on BD-cellulose column chromatography. The nucleotide sequences of all species of proline tRNAs were determined by the partial formamide·degradation method described previously (5). To obtain clearer resolution of 5'-end labeled oligo-nucleotides on polyacrylamide gel, we used a long polyacrylamide gel of 90 cm instead of 40 cm length. With this long gel the nucleotide sequence from residue 2 to residue 73 of tRNA could be easily determined by a single electrophoretic run. The 5'-terminal nucleotide of a proline tRNA was determined by two-dimensional cellulose thin-layer chromatography of a nuclease P_1 digest of 5'-terminal labeled tRNA. The nucleotide sequence near the 3'-end of the tRNA was determined and confirmed by the chemical degradation method using 3'-terminal labeled tRNA (6). By combination of the data obtained by limited formamide hydrolysis with the data obtained by the two other procedures, the total nucleotide sequences of the three proline tRNAs from S. typhimurium were

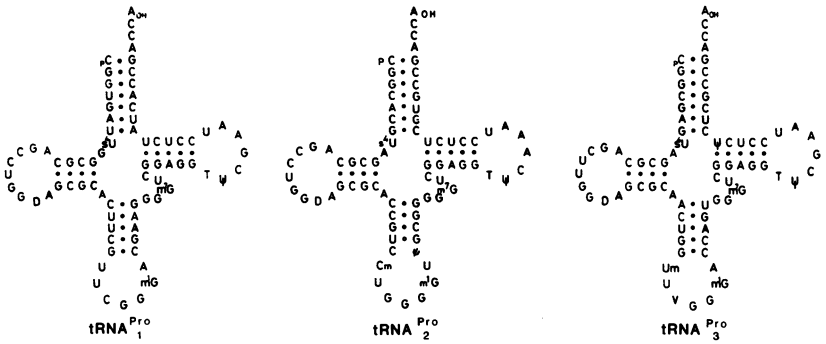

Fig. 1. Nucleotide sequences of proline tRNAs from S. typhimurium arranged in a clover leaf model.

determined to be as shown in Fig. 1.

A common nucleotide sequence with three consecutive G-C base pairs was found in the CCA-stem of all three proline tRNAs. In addition, the three proline tRNAs had almost identical sequences in the D- and TψC-regions, and contained 1-methylguanosine in the position next to the anticodon, as found in other proline tRNAs sequenced so far (7). A part of Salmonella $tRNA_1^{Pro}$ contained 2'-O-methyluridine instead of normal uridine at residue 32. Salmonella $tRNA_3^{Pro}$ contained partly unmodified uridine at residue 32 and 64 in place of 2'-O-methyluridine and pseudouridine, respectively. The overall sequence homologies of $tRNA_1^{Pro}$ to $tRNA_2^{Pro}$, $tRNA_1^{Pro}$ to $tRNA_3^{Pro}$, and of $tRNA_2^{Pro}$ to $tRNA_3^{Pro}$ were 68%, 83% and 78%, respectively.

We (Y. Kuchino, F. Mori, Y. Yabusaki and S. Nishimura, unpublished results) and Reid et al. (personal communication) recently obtained the nucleotide sequences of E. coli proline tRNAs. Our data indicate that E. coli also contains three isoaccepting species of proline tRNAs, with identical nucleotide sequences to their counterparts in S. typhimurium, except for differences in the extents of post-transcriptional modification.

Riddle and Roth reported that the sufA and sufB mutations in S. typhimurium are dominant suppressor mutations affecting the productions of $tRNA_1^{Pro}$ and $tRNA_2^{Pro}$, respectively (2). Moreover, they found that suppressor mutations in the sufA and sufB genes can suppress the same frame-shift mutations in the message (1). In addition, Yourno et al. showed that an mRNA produced in these suppressor strains contained an extra C in a sequence of repeated C residues (8). Therefore, if the suppressor proline tRNAs transcribed from the sufA or sufB gene contain an extra G residue in

their anticodons, as found in the suppressor proline tRNA of S. cerevisiae (3), they should be able to suppress such frameshift mutations in mRNA by standard base pairing with the altered codon sequence. On the other hand, Atkins et al. (9) and Weiss and Gallant (10) reported that normal tRNAs can promote ribosomal frameshifting and suppress the alternative reading frame. Thus it is also possible that the sufA or sufB mutation causes increase in the amount of one proline tRNA species, but not necessarily alteration of structural genes for proline tRNAs.

To determine the molecular mechanism of frameshift suppression in sufA and sufB mutations, we are now determining the nucleotide sequences of suppressor proline tRNAs.

Abbreviations used were: s^4U, 4-thiouridine; D, dihydrouridine; Cm, 2'-O-methylcytidine; Um, 2'-O-methyluridine; V, uridine-5-oxyacetic acid; m^1G, 1-methylguanosine; m^7G, 7-methylguanosine; T, 5-methyluridine; ψ, pseudouridine

REFERENCES

1. Riddle, D.L. and Roth, J.R., J. Mol. Biol. 54, 131-144 (1970).
2. Riddle, D.L. and Roth, J.R., J. Mol. Biol. 66, 495-506 (1972).
3. Cummins, C.M., Donahue, T.F. and Culbertson, M.R., Proc. Natl. Acad. Sci. USA 79, 3565-3569 (1982).
4. Zubay, G., J. Mol. Biol. 4, 347-356 (1962).
5. Nishimura, S. and Kuchino, Y., in RNA-DNA Sequencing in Analysis (Ed. Weissman, S.M.), Pareger Scientific, New York, N.Y., in press.
6. Peattie, D.A., Proc. Natl. Acad. Sci. USA 76, 1760-1764 (1979).
7. Gauss, D.H. and Sprinzl, M., Nucl. Acids Res. 11, rl-r53 (1983).
8. Yourno, J., Ino, I. and Khono, T., J. Mol. Biol. 62, 233-240 (1971).
9. Atkins, J.F., Gesteland, R.F., Reid, B.R. and Anderson, C.W., Cell 18, 1119-1131 (1979).
10. Weiss, R. and Gallant, J., Nature 302, 389-393 (1983).