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Structure and function of tryptophan tRNA from wheat germ

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ABSTRACT

The coding properties of $tRNA^{Trp}$ from yeast and wheat germ were studied. Unlike E. coli $tRNA^{Trp}$ or mitochondrial $tRNA^{Trp}$, eukaryotic $tRNA^{Trp}$ did not recognize the UGA codon in vitro. The sequence of wheat germ $tRNA^{Trp}$ as determined by [^{32}P] post-labelling techniques is:

pGGAU*CCGU m^1Gm^2GCGCAADDGmGDAGCGCm^2_2GψCUGACmUCmCAm^1GAψmCAGAAGGDU

GCGUGTψCG m^1A(A)UUCACGψCGGGUCCACCA$_{OH}$

The interesting features are: (i) Presence of a C_{11}:G_{24} base pair in contrast to the U_{11}:G_{24} in E. coli Su$^-$ $tRNA^{Trp}$. (ii) The anticodon sequence is -CmCA- compared to -CCA- in E. coli $tRNA^{Trp}$. (iii) Lack of a hyper-modified base i^6A adjacent to the 3'-end of the anticodon. (iv) Presence of -TψCG- sequence instead of -ψψCG- sequence present in mammalian $tRNA^{Trp}$.

INTRODUCTION

Tryptophan is usually coded for by a single codon UGG in both prokaryotic and eukaryotic system (1-4) except that in mitochondria the codon UGA also codes for tryptophan (5). But $tRNA^{Trp}$ Su$^+$UGA obtained from E. coli Su$^+$UGA strain can translate the nonsense codon UGA and cysteine codon UGU as well as its normal codon UGG (1). The anticodon sequence of the normal and suppressing species of $tRNA^{Trp}$ from the corresponding strains of E. coli was, however, identical (1). The only change detected in the supressing species of $tRNA^{Trp}$ was the replacement of G_{24} with A_{24}. The non-supressing form of $tRNA^{Trp}$ could also translate the UGA codon as tryptophan in polypeptide synthesis in vitro, but with a much lower efficiency than the supressor species (1). Single UGA terminator codon present in natural mRNA was also recognized by the non-supressor $tRNA^{Trp}$ with a very low efficiency to produce a "fused protein" (6).

Studies involving polypeptide synthesis in vitro (2), polypeptide chain termination assays in vitro (3), genetic analysis of yeast mutants (4) and

recent analysis of eukaryotic mRNA sequences suggest that in eukaryotes UGA is a nonsense codon.

In order to study if eukaryotic $tRNA^{Trp}$ species could also recognize UGA like E. coli $tRNA^{Trp}$ species the coding properties of wheat germ and yeast $tRNA^{Trp}$ species was studied. Unlike prokaryotic (Su^-) $tRNA^{Trp}$ species wheat germ or yeast $tRNA^{Trp}$ species did not recognize UGA codon in vitro. The nucleotide sequences of wheat germ $tRNA^{Trp}$ was also determined.

MATERIALS AND METHODS

Purification of Wheat Germ $tRNA^{Trp}$ - Tryptophan tRNA was purified from unfractionated wheat germ tRNA by chromatographic separation on a BD-cellulose column followed by separation on a DEAE sephadex column (15). A final purification of 36-fold was achieved by a RPC-5 chromatography system (specific activity of purified $tRNA^{Trp}$, 1,820 pmoles per A260 units, yield 20%). In vitro protein synthesis using synthetic polynucleotide was carried out as described earlier (9). The procedures used for sequence analysis were (a) the rapid print-out method of Gupta and Randerath (7) and its modification by Kuchino et al. (10) and (b) the rapid read-off gel sequencing method using partial enzymatic digestion of 5'-[^{32}P] labelled tRNA (8,11).

RESULTS

Recognition of Codons UGG and UGA by Tryptophan tRNA Species

Polypeptide synthesis in vitro as directed by poly [r-(UGA)] and poly r (U,G), which contained the codons UGA and UGG respectively, was carried out using the purified $tRNA^{Trp}$ species. Poly [r (UGA)] was synthesized, by the action of RNA polymerase on DNA-like template, poly [d(T-G-A):d(T-C-A)]. For codon UGG, the commercially obtained polynucleotide poly r (U,G) was used. The results are shown in Table 1. It can be seen that codon UGG was recognized by all the $tRNA^{Trp}$ species. As expected, the nonsense triplet UGA was recognized by E. coli Su^+UGA $tRNA^{Trp}$ and a 7-8 fold stimulation of the incorporation of tryptophan was observed in the presence of poly [r(UGA)]. Under the same condition, E. coli Su^- $tRNA^{Trp}$ showed only a slight stimulation. Previous heating of the Su^- $tRNA^{Trp}$ from E. coli stimulated the recognition of UGA codon. Hirsh and Gold (12) has also reported earlier that both E. coli Su^- and Su^+UGA $tRNA^{Trp}$ recognize UGA codon in vitro. Both of the eukaryotic $tRNA^{Trp}$ species tested, however, did not recognize UGA codon, even after heating.

TABLE 1

Recognition of Codons UGG and UGA by Tryptophan tRNA Species

tRNA Used	Heat Treatment	[^{3}H] Trp Polymerized (pmoles/ml) -Template	+ poly [r (U-G-A)]	+ poly r (U,G)
E. coli CAJ 64 (Su^+)	-	48	320	1094
(5 A_{260} unit/ml)	+	37	285	703
E. coli B (su^-)	-	43	58	1098
(18 A_{260} unit/ml)	+	41	85	842
Yeast	-	54	51	620
(10 A_{260} unit/ml)	+	52	55	580
Wheat Embryo	-	59	63	810
(7 A_{260} units/ml)	-	61	65	740

The reaction mixture for protein synthesis contained per ml, 22 A_{260} units of E. coli MRE 600 ribosomes washed with NH_4Cl, 1.5 mg of DEAE-cellulose treated S-100 proteins free of termination factor R_1 and R_2, 72 nmoles of poly [r(U-G-A)] as synthesized by transcription of poly [d(T-G-A)]. [d(T-C-A)] or 5 A_{260} units of poly r (U,G) (Miles Laboratories), indicated amounts of tRNA samples, 5 µCi of [^{3}H] tryptophan (specific activity 1100 µCi/µmole) and in the case of poly r (U,G) 20 nmoles of each of nonradioactive phenylalanine, leucine, cysteine, valine and glycine. Mg^{2+} concentration used was 0.02 M; after 60 min at 37° aliquots were assayed for hot 5% Cl_3CCOOH insoluble radioactivity. Since yeast and wheat embryo $tRNA^{Trp}$ were partially charged by the E. coli synthetase, tRNA free yeast or wheat embryo S-100 proteins (0.25 mg/ml) were also added for homologous charging.

Determination of the Sequence of $tRNA^{Trp}$

Modified Nucleotides:

The following modified nucleotides were detected in $tRNA^{Trp}$ using (i) a high pressure ion-exchange chromatographic method (13) and (ii) in vitro ^{32}P-labelling technique (14): T, ψ, D, Cm, m^1G, m^2G, m^2_2G, m^1A, ψm and Gm.

Analysis of Oligonucleotides Produced by T_1 and Pancreatic RNase Digestion:

Non-radioactive oligonucleotides produced by digestion of $tRNA^{Trp}$ with either T_1 RNase or pancreatic RNase were characterized as described (15). The sequences of the oligonucleotides were found to be fully consistent with the sequence presented.

Overlapping Sequences

Rapid Print-Out Method

Figure 1 shows the chromatographic analysis of 5'-terminal nucleotides of the labelled fragments between position 1-69 of $tRNA^{Trp}$ in the ammonium

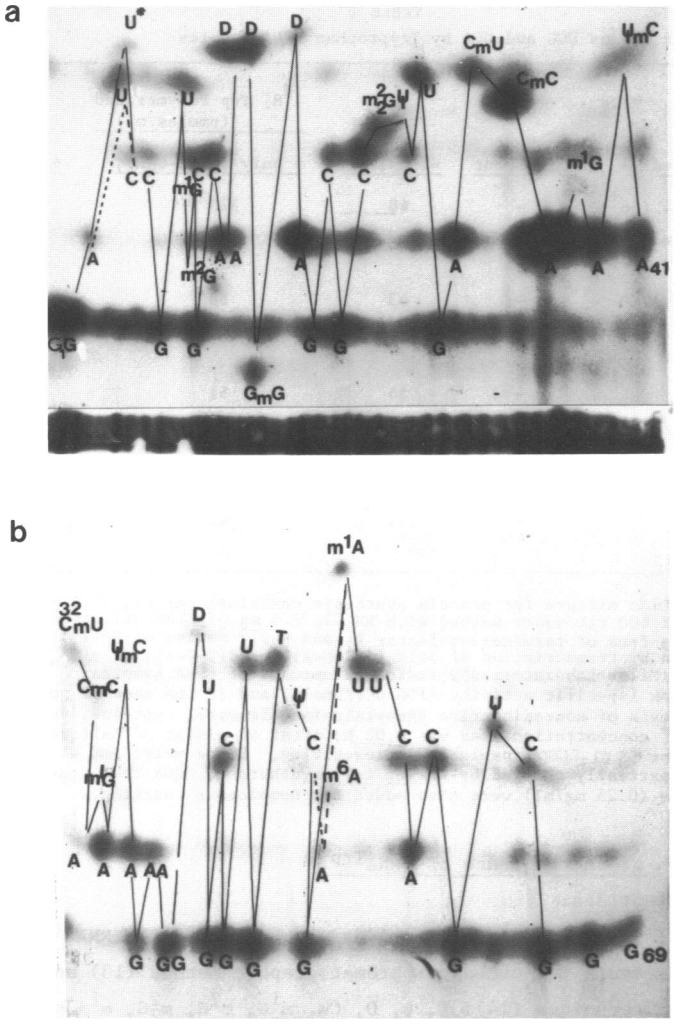

Figure 1

5'-Terminal analysis of oligonucleotides containing (a) bases 1 to 41 and (b) bases 32 to 69 of wheat germ $tRNA^{Trp}$. After RNase T_2 treatment in situ, the released 5'-terminal nucleotides from the contact-transfered PEI-cellulose plate were chromatographed in 0.55 M ammonium sulfate. Positions were numbered 5'- to 3'-. Spots m^1G_{38} and ψm_{39} C_{40} are not clearly resolved here, but longer exposure (not shown) indicates the corresponding spots.

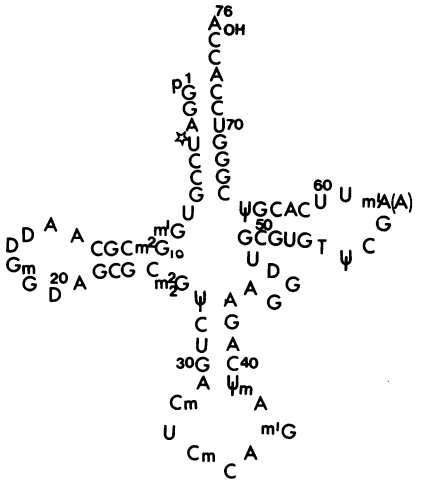

Figure 2
The nucleotide sequence of wheat germ $tRNA^{Trp}$ in the cloverleaf arrangement.

sulfate system. Identical result was obtained by using the ammonium formate solvent system.

The nucleotide sequence from position 4 to 43 of $tRNA^{Trp}$ was also determined by the rapid read-off gel sequencing method (8,11) which agreed with the sequence obtained by the print-out method.

Total Sequence of Wheat Germ $tRNA^{Trp}$

The sequences obtained from the print-out sequencing techniques and the rapid read-off sequencing method, as well as the sequences of the oligonucleotides present in RNase T_1 and pancreatic RNase digests can be arranged in the following nucleotide sequence of wheat germ $tRNA^{Trp}$ present in cloverleaf form common to all tRNA molecules (Figure 2).

DISCUSSION

The inability of eukaryotic $tRNA^{Trp}$ to recognize UGA could be due to the modification of C_{34} to Cm and the lack of hypermodification of base 37.

Structural Features of Wheat Germ $tRNA^{Trp}$

The $tRNA^{Trp}$ from wheat germ contains 76 nucleotides in contrast to the presence of 75 nucleotides in other eukaryotic $tRNA^{Trp}$ species. The stem region contains a modified but uncharacterized U at position 4. The presence of a ribose-methylated pyrimidine has also been reported in position 4 of eukaryotic $tRNA^{Gly}$ species (17). The modified base m^7G present in position 46 and m^5C present in positions 48 and 49 in other eukaryotic tRNAs are absent in $tRNA^{Trp}$. Unlike the U_{11}:G_{24} or U_{11}:A_{24} base pairing observed in

the case of E. coli Su^- and Su^+_{UGA} $tRNA^{Trp}$, respectively, wheat germ $tRNA^{Trp}$ contains the base pair C_{11}:G_{24} in the loop I stem.

Studies with tryptophan tRNA from both avian and bovine tissues have shown that they act as specific primers for reverse transcriptase and bind to a specific site near the 5'-terminus of the viral genome (18,19). Since wheat germ $tRNA^{Trp}$ possesses an extensive sequence homology with both avian and bovine $tRNA^{Trp}$, it is of interest to examine if the wheat germ $tRNA^{Trp}$ can act as a primer for and can also bind to reverse transcriptase. Furthermore, the presence of the sequences -ψψCG- in loop IV of the two reverse transcriptase primer tRNAs, namely $tRNA^{Trp}$ for avian system (20) and $tRNA^{Pro}$ from murine system (21) suggests a possible implication of this sequence in initiation of DNA synthesis. The sequences in this region of $tRNA^{Trp}$ and $tRNA^{Pro}$ are: $C_{48}GUG\psi\psi CGm^1AA_{57}$ for avian $tRNA^{Trp}$ and $C_{48}GGG\psi\psi CAm^1AA_{57}$ for murine $tRNA^{Pro}$. In comparison the sequence of the same region in wheat germ $tRNA^{Trp}$ is $C_{48}GUGT\psi CGm^1AU_{58}$ which differs from the vertebrate sequence by two bases, ψ_{52} and A_{57} being changed to T_{52} and U_{57} in wheat germ.

Conservation of Sequence of Tryptophan tRNA

Wheat germ $tRNA^{Trp}$ shows the highest degree of homology (84%) with vertebrate $tRNA^{Trp}$ species; the extent of homology between $tRNA^{Trp}$ species from wheat germ and yeast or bacteria being 68% and 54%, respectively. It may be mentioned that wheat germ initiator $tRNA^{Met}$ also shows the highest degree of homology (87%) with vertebrate initiator $tRNA_i^{Met}$. The primary sequence between wheat germ and vertebrate $tRNA^{Trp}$ is identical between residues 6 to 76 with the exception of 7 residues. This conservation of the sequences present in all three loops and in the two stems between the higher plant and vertebrate tryptophan tRNA shows that the $tRNA^{Trp}$ evolved by conserving these regions and ultimately in the vertebrate species the sequence is totally preserved.

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REFERENCES

1. Hirsh, D. (1971) J. Mol. Biol. 58, 439.
2. Gupta, N.K. (1968) J. Biol. Chem. 243, 4959.
3. Beudet, A.L. and Caskey, C.T. (1971) Proc. Natl. Acad. Sci. USA 68, 619.

4. Stewart, J.W., Sherman, F., Jackson, M., Thomas, F.L.X. and Shipman, N. (1972) J. Mol. Biol. 68, 83.
5. Heckman, J.E., Sarnoff, J., Alzner-DeWeerd, B., Yin, S. and RajBhandary, U.L. (1980) Proc. Natl. Acad. Sci. USA 77, 3159.
6. Weiner, A.M. and Weber, K. (1971) Nature New Biol. 234, 206.
7. Gupta, R.C. and Randerath, K. (1979) Nucleic Acids Res. 6, 3443.
8. Lockard, R.E., Alzner-DeWeerd, B., Heckman, J.E., Macgee, J.M.W. and RajBhandary, U.L. (1978) Nucleic Acids Res. 5, 37.
9. Ghosh, K. and Ghosh, H.P. (1972) J. Biol. Chem. 247, 3369.
10. Kuchino, Y., Kato, M., Sugisaka, H. and Nishimura, S. (1979) Nucleic Acids Res. 6, 3459.
11. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527.
12. Hirsh, D. and Gold, L. (1971) J. Mol. Biol. 58, 459.
13. Sen, G.C. and Ghosh, H.P. (1974) Anal. Biochem. 58, 578.
14. Silberklang, M., Gillum, A. and RajBhandary, U.L. (1976) Methods in Enzymology, Vol. LIX, p. 58.
15. Ghosh, K., Ghosh, H.P., Simsek, M., RajBhandary, U.L. (1974) J. Biol. Chem. 249, 4720-4729.
16. Barrell, B.G., Anderson, S., Bankier, A.T., de Bruijn, M.H.L., Chen, E., Coulson, A.R., Brouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R. and Young, I.G. (1980) Proc. Natl. Acad. Sci. USA 77, 3164.
17. Gupta, R.C., Roe, B.A. and Randerath, K. (1979) Nucleic Acids Res. 7, 959.
18. Cordell, B., Swanstrom, R., Goodman, H.M. and Bishop, J.M. (1979) J. Biol. Chem. 254, 1866.
19. Baroudy, B.M. and Chirikijian, J.G. (1980) Nucleic Acids Res. 8, 57.
20. Harada, F., Sawyer, R.C. and Dahlberg, J.E. (1975) J. Biol. Chem. 250, 3487.
21. Peters, G., Harada, F., Dahlberg, J.E., Panet, A., Haseltine, W.A. and Baltimore, D. (1977) J. Virol. 21, 1031.