RECOGNITION OF tRNA BY THE RIBOSOME
A possible role of 5 S RNA

S.K. DUBE
Nachwuchsabteilung, Max-Planck-Institut für Experimentelle Medizin, 34 Göttingen, W. Germany

Received 23 July 1973

1. Introduction

Transfer RNA's (tRNA's) play a key role in protein biosynthesis and are ideal molecules for studying nucleic acid – protein and nucleic acid – nucleic acid interactions. This is so because a tRNA molecule interacts with a number of components of the translational process viz. amino acyl-tRNA-synthetase, ribosome, various translational factors and messenger RNA's.

The highly specific interaction of tRNA with its cognate synthetase indicates that the tRNA('s) for each amino acid possesses certain unique structural features. On the other hand the interaction of tRNA with ribosomal binding sites, translational factors and messenger RNA's argues that each tRNA also has structural features which it shares with all other tRNA's. Of all the tRNA's in *E. coli*, the two methionine tRNA's (formylatable methionine tRNA, $tRNA_f^{Met}$ [1] and nonformylatable methionine tRNA, $tRNA_m^{Met}$ [2]) provide a very intriguing example of specificity. Both tRNA's are aminoacylated by the same synthetase [3, 4] but only $tRNA_f^{Met}$ interacts with transformylase [5], and with initiation factors to form initiation complex on the ribosome [6] while $tRNA_m^{Met}$ is like the rest of the tRNA's in these respects. The methionine tRNA's, therefore, are very useful for studying the unique and common structural features of tRNA's.

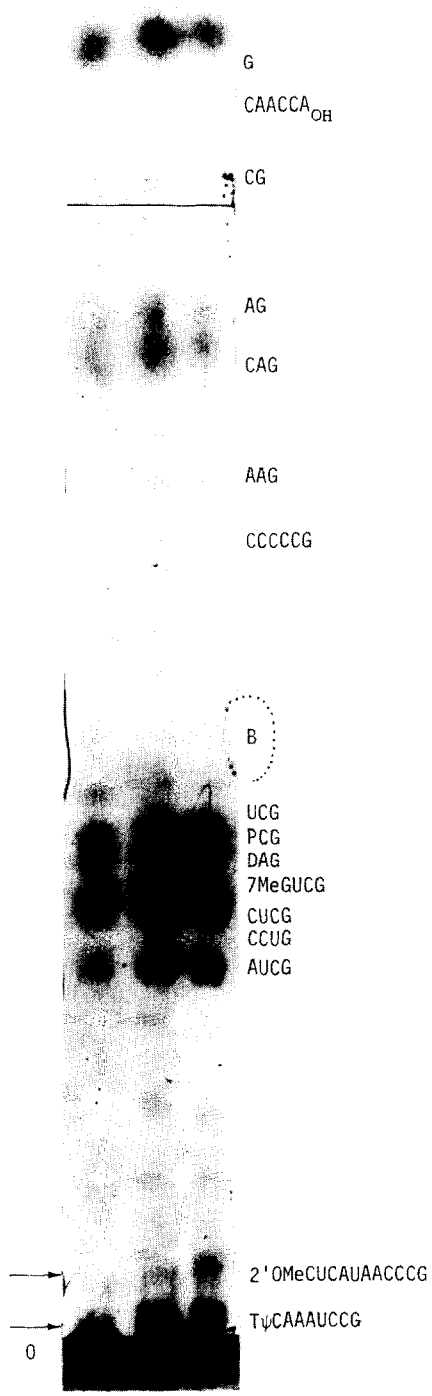
Recently [7] it was shown that *E. coli* methionyl-tRNA-synthetase protects the anticodon loop, the 3′ stem and the extra loop of $tRNA_f^{Met}$ against digestion by T_1 ribonuclease. The data suggested that the tRNA-synthetase interacting area in the molecule is arc shaped with at least three contact points. This paper presents results of RNAase T_1 digestion of ribosome-formylmethionyl-tRNA initiation complex showing that the 70 S ribosome protects the T Ψ C loop and the anticodon loop of the tRNA, whereas, the 30 S ribosomal subunit protects only the anticodon loop.

2. Experimental

^{32}P-labelled charged, formylated $tRNA_f^{Met}$ was complexed with 70 S ribosome in the presence of AUG, GTP and initiation factors [8, 9]. fMet-$tRNA_f^{Met}$ was similarly complexed with the 30 S ribosomal subunit. For control, fMet-$tRNA_f^{Met}$ was mixed with 70 S ribosome in the absence of AUG, GTP and initiation factors. The preparations were digested with T_1 ribonuclease under identical conditions for varying lengths of time and the products fractionated by one dimensional electrophoresis on DEAE paper [10]. This fractionation procedure was used because it enabled many analyses to be performed in a relatively short time. It might, however, be pointed out that the oligonucleotides do not resolve as well in the one dimensional fractionating system as in the standard two dimensional system [10]. Nevertheless, the resolution was good enough to identify the products without much difficulty and in cases of doubt further analysis [11] served to eliminate any ambiguities.

3. Results

Fig. 1 shows the results of a typical experiment. Two points in the figure are noteworthy, namely,



the relatively low yields of TΨCAAAUCCG and 2′OMeCUCAUAACCCG in the digestion products of fMet-tRNA$_f^{Met}$ – 70 S complex and of 2′OMeCUCAUAACCCG in the digestion products of fMet-tRNA$_f^{Met}$ – 30 S complex. These oligonucleotides come respectively from the TΨC and the anticodon region of tRNA$_f^{Met}$ [12], (fig. 2). The radioactivity at the origin represents incomplete digestion of tRNA and consists of a mixture of large oligonucleotide fragments including GTΨCAAAUCCG, GTΨCAAAUCCGG, GG2′-OMeCUCAUAACCCG and UCGGG2′OMeCUCAUAACCCG.

4. Discussion

Since the anticodon loop is known to bind to ribosomes [13–15], the protection by the ribosome of oligonucleotide from the anticodon region of the tRNA is not surprising. However, the protection of

Fig. 1. RNAse T_1 digestion of formylmethionyl-tRNA$_f^{Met}$ complex with 70 S ribosome, fMet-tRNA$_f^{Met}$ complex with 30 S subunit and fMet-tRNA$_f^{Met}$ plus 70 S. ^{32}P-labelled tRNA$_f^{Met}$ from *E. coli* CA 265 was prepared as described [11, 23]. The tRNA was about 90% pure, tRNA was charged with methionine under formylating conditions [24]. *E. coli* MRE600 salt-washed ribosomes and crude initiation factors were prepared as described [25]. The ribosomal subunits were prepared from these 70 S ribosomes essentially according to the method of Monro [26] as described [14]. For initiation complex formation the reaction mixture (50 μl) contained 0.1 M Tris–HCl, pH 7.2; 0.05 M KCl; 6 A_{260} units of salt-washed 70 S ribosomes or 3 A_{260} units of 30 S subunits; 0.15 A_{260} units of A_pU_pG; 0.0002 M GTP; 0.005 M magnesium acetate, or 0.01 M in case of reaction mix containing 30 S subunits; 50 μg of initiation factors, 1.0 A_{260} units of fMet-tRNA$_f^{Met}$. The reaction mixture was incubated at 20°C for 20 min, then chilled and 1 μg RNAse T_1 added. At 5 min intervals 5 μl aliquots were withdrawn and applied on to DEAE paper. The control reaction mixture was prepared under identical conditions and contained all components used in the initiation complex formation with 70 S ribosomes with the omission of AUG, GTP and initiation factors. The digests were fractionated in a one dimensional system [10] using 7% formic acid and the electrophoretogram autoradiographed. The products were identified by further analysis [11]. The figure shows digestion pattern at one time point. 70, 30, C refer respectively to T_1 digestion products of fMet-tRNA$_f^{Met}$ – 70 S initiation complex, fMet-tRNA$_f^{Met}$ – 30 S initiation complex and fMet-tRNA$_f^{Met}$ plus 70 S control. B is the blue marker. 0 is the origin.

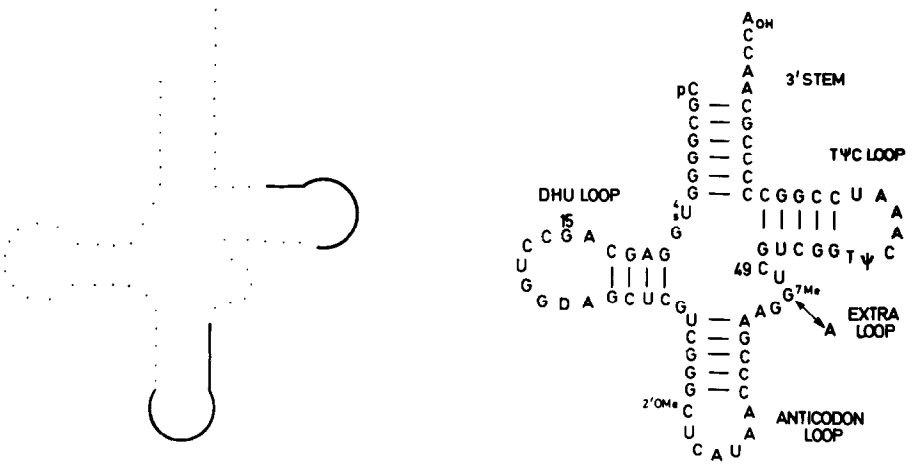


Fig. 2. The nucleotide sequence of $tRNA_f^{Met}$ from *E. coli* CA 265 [1]. Standard abbreviations are used for the four usual nucleotides. Other abbreviations are: Ψ, pseudouridylic acid; T, thymidylic acid; 7MeG, 7-methylguanylic acid; 2'O MeC, 2'O-methylcytidylic acid; D, dihydrouridylic acid; $^{S}_{4}$U, 4 thiouridylic acid. The regions protected by the 70 S ribosome are marked by a solid line, fig. 2a.

the TΨC loop coupled with the fact that this protection is afforded by the 70 S ribosome but not by the 30 S ribosomal subunit is very interesting. This indicates that the TΨC loop is recognized by some component of the ribosome not present in the 30 S subunit but present in the 50 S subunit. This component could be a ribosomal RNA or a ribosomal protein or both. It is interesting to note that the 5 S RNA present in the 50 S subunit [16] has a GAAC sequence [17] which is complementary to the GTΨC sequence in $tRNA_f^{Met}$ and in all prokaryotic tRNA's. However, the fact that the isolated 5 S RNA does not protect the TΨC loop against digestion by T_1 ribonuclease (SKD unpublished observations) suggests that if the protection of TΨC loop is due to tRNA–5 S RNA interaction, other ribosomal components must also be involved in stabilizing this interaction.

Since the GAAC sequence occurs twice in *E. coli* 5 S RNA [17] and two tRNA's bind to ribosome during peptide bond formation the question arises whether the TΨC loop of both ribosome-bound tRNA's could be simultaneously protected against digestion by T_1 ribonuclease. The experiments [18] showing inhibitory effect of TΨCG on the binding of phe-tRNA to the ribosome at both the peptidyl-tRNA and the aminoacyl-tRNA binding sites would argue in favour of such a protection. Were this the case it would not be difficult to define the geometrical distance between the two GAAC sequences of 5 S RNA on the ribosome.

While the GAAC sequence occurs twice in prokaryotic 5 S RNA [17, 19] it occurs only once in eukaryotic 5 S RNA [20]. The absence of GTΨC sequence in the eukaryotic initiator tRNA, $tRNA_f^{Met}$ [21, 22] but its presence in other eukaryotic tRNA's may be of relevance in this context. In the eukaryotic $tRNA_f^{Met}$ the GTΨC sequence is replaced by GAUC sequence. Also it is interesting to note that the *E. coli* 5 S RNA does not contain a sequence complementary to the GAUC sequence but the eukaryotic 5 S RNA does. If the GAUC sequence in eukaryotic 5 S RNA plays a vital role in determining the binding specificity of the initiator tRNA, then, the two GAAC sequences in prokaryotic 5 S RNA must be functionally non-equivalent. On the other hand, the GAAC sequence which is common to both prokaryotic and eukaryotic 5 S RNA's must be functionally identical in the two systems and involved in the binding of the incoming aminoacyl-tRNA.

If these correlations are not fortuitous, they may be very significant not only in establishing a role of 5 S RNA in protein synthesis but also in showing a

fundamental difference in the initiator tRNA binding sites between prokaryotic (70 S) and eukaryotic (80 S) ribosomes. Furthermore, this difference could also guarantee the required autonomy of the cytoplasmic (80 S) and mitochondrial or chloroplastic (70 S) protein synthesizing systems in the eukaryotic cell.

Acknowledgements

This work was done while I was a Wellcome Fellow at the MRC Laboratory of Molecular Biology, Cambridge. I thank Dr. P.S. Rudland for the gift of initiation factors and AUG, Drs. F. Sanger and F.H.C. Crick for their interest and encouragement and Dr. H. Tsai for many enjoyable discussions during the preparation of the manuscript.

References

[1] Dube, S.K., Marcker, K.A., Clark, B.F.C. and Cory, S. (1968) Nature 218, 232.
[2] Cory, S., Marcker, K.A., Dube, S.K. and Clark, B.F.C. (1968) Nature 220, 1039.
[3] Bruton, C.J. and Hartley, B.S. (1968) Biochem. J. 108, 281
[4] Cassio, D. and Waller, J.P. (1968) European J. Biochem. 5, 33.
[5] Marcker, K.A. (1965) J. Mol. Biol. 14, 63.
[6] Kondo, M., Eggerston, G., Eisenstadt, J. and Langyel, P. (1968) Nature 220, 368.
[7] Dube, S.K. (1973) Nature New Biol. 243, 103.
[8] Anderson, J.S., Bretscher, M.S., Clark, B.F.C. and Marcker, K.A. (1967) Nature 215, 490.
[9] Iwasaki, K., Sabol, S., Wahba, A.J. and Ochoa, S. (1968) Arch. Biochem. Biophys. 125, 542.
[10] Sanger, F., Brownlee, G.G. and Barrell, B.G. (1965) J. Mol. Biol. 13, 373.
[11] Dube, S.K., Marcker, K.A., Clark, B.F.C. and Cory, S. (1969) European J. Biochem. 8, 244.
[12] Dube, S.K. and Marcker, K.A. (1969) European J. Biochem. 8, 256.
[13] Clark, B.F.C., Dube, S.K. and Marcker, K.A. (1968) Nature 219, 484.
[14] Rudland, P.S. and Dube, S.K. (1969) J. Mol. Biol. 43, 272.
[15] Dube, S.K., Rudland, P.S., Clark, B.F.C. and Marcker, K.A. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 161.
[16] Rosset, R., Monier, R. and Julien, J. (1964) Bull. Soc. Chim. Biol. 46, 87.
[17] Brownlee, G.G., Sanger, F. and Barrell, B.G. (1968) J. Mol. Biol. 34, 379.
[18] Ofengand, J. and Henes, C. (1969) J. Biol. Chem. 244, 6241.
[19] Dubuy, B. and Weissman, S.M. (1971) J. Biol. Chem. 246, 747.
[20] Forget, B.G. and Weissman, S.M. (1967) Science 158, 1695.
[21] Simsek, M. and RajBhandary, U.L. (1972) Biochem. Biophys. Res. Commun. 49, 508.
[22] Piper, P.W. and Clark, B.F.C. (1973) FEBS Letters 30, 265.
[23] Marcker, K.A., Dube, S.K. and Clark, B.F.C. (1968) Structure and Function of tRNA and 5 S RNA, pp. 53–64, (Fröholm, L.O. and Laland, S.G., eds.), Academic Press.
[24] Clark, B.F.C. and Marcker, K.A. (1966) J. Mol. Biol. 17, 394.
[25] Anderson, J.S., Bretscher, M.S., Clark, B.F.C. and Marcker, K.A. (1967) Nature 215, 490.
[26] Monro, R.E. (1967) J. Mol. Biol. 25, 347.