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Nucleotide residues of tRNA, directly interacting with proteins within the complex of the 30 S subunit of *E. coli* ribosome with poly(U) and NAcPhe-tRNA^{Phe}

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With the aid of photoinduced tRNA-protein cross-linking, nucleotide residues A21, U45 and U60 were shown to interact directly with proteins S5, S7 and S9 respectively, in the complex of the 30 S subunit of *E. coli* ribosome with poly(U) and NAcPhe-tRNA^{Phe}. These nucleotide residues are located in the central part of the L-form in the tertiary structure of RNA.

Ribosomal subunit; 30 S·poly(U)·NAcPhe-tRNA^{Phe} complex; Ultraviolet-induced crosslinking; tRNA-protein interaction

1. INTRODUCTION

Protein biosynthesis (translation) is carried out on mRNA by means of the ribosome and a set of aminoacyl-tRNAs. A great deal of information is now available about the main stages of translation, the role and primary structure of the majority of the macromolecules in the system, the architecture of ribosomes and tRNAs' tertiary structure [2–4]. An important role in the correct self-assembly of ribosomal complexes and their rearrangement in the course of translation is played by noncovalent specific interactions between the macromolecules in these complexes. It is evident, for instance, that codon-anticodon interactions alone can provide neither the necessary accuracy in the choice of the proper aminoacyl-tRNA in the elongation process nor the migration of tRNAs and mRNA along the ribosome in the course of translocation. Hence, specific interactions of tRNA with other components of a ribosome (besides mRNA) – ribosomal RNAs [5] or ribosomal proteins [6] – must take place. It is not yet known, however, which nucleotide residues of tRNA molecules are involved in the direct interaction with ribosomal proteins at different stages of translation, mainly due to the methodological difficulties in performing such a study.

The complex of methods used in our work provides a way to answer the questions. Our approach includes photo-induced formation of tRNA-protein cross-linking inside the ribosomal complex, isolation and fractionation of the tRNA molecules, cross-linked to various proteins, identification of the proteins, and the location in tRNA of the nucleotide residue, cross-linked to the respective protein. The results obtained show that nucleotide residues A21, U45 and U60 of the tRNA molecule interact directly with proteins S5, S7 and S9, respectively, within the complex of the 30 S subunit of the *E. coli* ribosome with poly(U) and NAcPhe-tRNA^{Phe}.

2. MATERIALS AND METHODS

30 S subunits of *E. coli* MRE-600 ribosomes were purchased from Biolar (USSR). Their activity in poly(U)-dependent binding of NAcPhe-tRNA^{Phe} was more than 90%. Monomer-

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curated tRNA was prepared according to [7] using $tRNA^{Phe}$, purchased from Boehringer (FRG). It was aminoacylated and acetylated as in [8]. Formation of the ternary 30 S· poly(U)·NAcPhe-$tRNA^{Phe}$ complex, its irradiation (254 nm), isolation of the mercurated NAcPhe-$tRNA^{Phe}$ (both free and cross-linked to proteins) from the irradiated complex on thiopropyl-Sepharose and deacylation were carried out as described [8]. The deacylated preparation was labelled at the 3'-end by using T_4 phage-induced RNA ligase [9] and [^{32}P]pCp, and subjected to electrophoresis in polyacrylamide gel [10% polyacrylamide, 0.5% bisacrylamide, 100 mM Tris-borate (pH 8.3), 1 mM EDTA; voltage gradient, 35 V/cm; 3 h) either immediately or after treatment with proteinase K [10]. The radioactivity distribution in the gel was determined by autoradiography. The results are given in fig.1.

The radioactive regions of the gel were cut off and eluted with 12% $NaClO_4$ solution (4 h under vigorous stirring). The products eluted were precipitated by addition of 5 vols acetone and

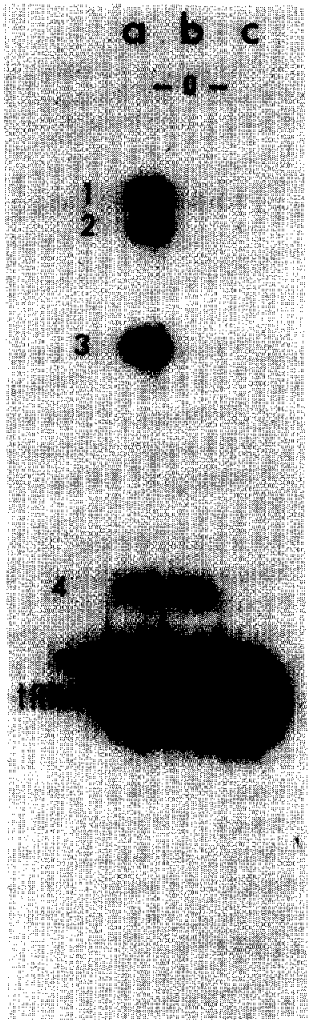

Fig.1. Radioactivity distribution in polyacrylamide gel after electrophoretic separation of 3'-labelled $tRNA^{Phe}$, isolated from irradiated 30 S·poly(U)·NAcPhe-$tRNA^{Phe}$ ternary complex before (a) and after proteinase K treatment (b) and from the non-irradiated complex (c).

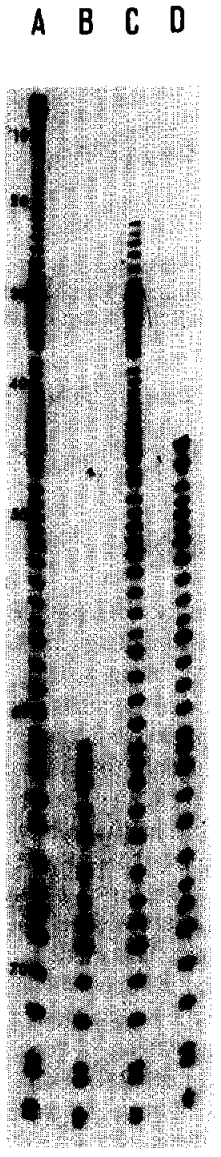

Fig.2. Radioactivity distribution in polyacrylamide gel after electrophoresis of oligonucleotide mixture produced by random hydrolysis of 3'-end-labelled (ligated with [^{32}P]pCp) initial $tRNA^{Phe}$ (A) and $tRNA^{Phe}$, cross-linked to proteins S9 (B), S5 (C) and S7 (D) in the 30 S·poly(U)·NAcPhe-$tRNA^{Phe}$ complex. Oligonucleotides cross-linked to proteins are eliminated by phenol extraction before electrophoresis.

0.01% dextran (M_r 20000). The product isolated from each band was divided into two parts.

One part was dissolved in 10 μl of 10 mM Tris-HCl (pH 7.2), radioiodinated (^{125}I) in the presence of chloramine T [11] and after addition of 20 μl of an RNase mixture [10 mg/ml of RNase A and 100000 AU/ml of RNase T_1 in 20 mM EDTA and 10 mM Tris-HCl (pH 7.4)] was incubated for 12 h at 37°C. The iodinated proteins, containing short cross-linked oligonucleotides, were identified by their position in polyacrylamide gels after two-dimensional electrophoresis [12].

The other part of each product was dissolved in 20 μl of 50 mM NaOH, containing 1 mM EDTA, incubated for 5–10 s at 100°C in the presence of 5 μg tRNA carrier, cooled, neutralised with 50 mM HCl, extracted with phenol and precipitated by addition of 3 vols alcohol. The set of oligonucleotides obtained was separated in polyacrylamide gels under the conditions described above for tRNA-protein cross-link fractionation. The results are given in fig.2.

3. RESULTS AND DISCUSSION

The 30 S·poly(U)·NAcPhe-tRNA^{Phe} complexes, containing ordinary and monomercurated tRNA, are practically identical both in composition and properties and in the ratio of proteins cross-linked to tRNA under UV irradiation [8]. The use of monomercurated tRNA enables simple separation of tRNA (free or cross-linked to proteins) from other components of the complex after its irradiation and dissociation [8]. Therefore, we have used the 30 S·poly(U)·NAcPhe-tRNA^{Phe} complex containing monomercurated tRNA. The molar ratio of NAcPhe-tRNA^{Phe} to 30 S subunit in the complex formed was 0.9. After addition of 50 S subunits to this complex more than 90% of NAcPhe was transferred to puromycin (see [13]).

Absorption of 10 photons per nucleotide (irradiation time 10–30 min, depending on the absorbance of the irradiated layer) is not accompanied by noticeable deacylation of NAcPhe-tRNA^{Phe}, and about 10% of tRNA is cross-linked to 30 S subunits within the 30 S·poly(U)·NAcPhe-tRNA^{Phe} complex.

Four main radioactive products, less mobile than the initial tRNA, ligated with pCp, are clearly detected after electrophoresis of tRNA, isolated from the irradiated complex (fig.1, cf. a and c). The mobility of the compounds in bands 1–3 after exhaustive proteolysis with proteinase K increases and becomes equal to that of initial ligated tRNA (fig.1b). Proteolysis does not affect the mobility of the substance in band 4. It should be noted that the only substance with the same mobility in this system is a product of intramolecular cross-linking formed by UV irradiation of free tRNA^{Phe} (not shown) (cf. [14]).

Each of the bands 1–3 contains a single protein, which was identified according to [13] as S7, S5 and S9, respectively. Therefore, each of these bands contains the product of tRNA cross-linking to a particular protein molecule, while the products of tRNA cross-linking with two or more pro-

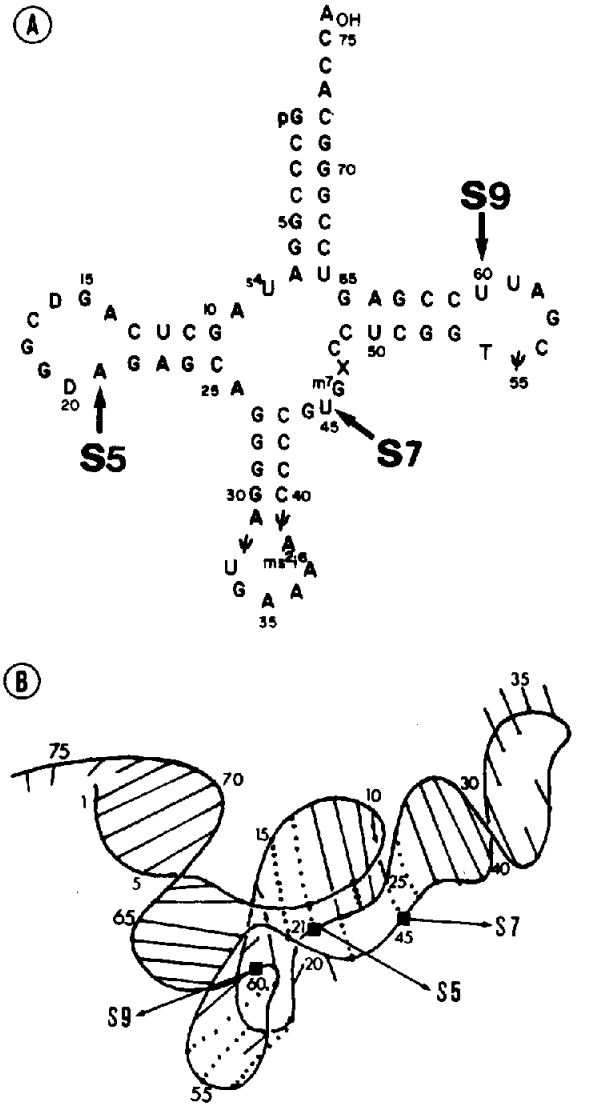

Fig.3. Location on the tRNA^{Phe} secondary structure (A) [16] and its three-dimensional model (B) of nucleotides directly interacting with proteins S5, S7 and S9 in the 30 S·poly(U)·NAcPhe-tRNA^{Phe} complex.

tein molecules are practically absent. This result could be naturally expected on the basis of the low total extent of cross-linking.

Only tRNA fragments free of covalently bound protein remain in the aqueous phase after limited random cleavage of internucleotide bonds in tRNA-protein complexes and the subsequent extraction with phenol. The length of the longest fragment, containing radioactive label, corresponds to the distance from the ligated tRNA 3′-end to the nucleotide immediately preceding the residue cross-linked to protein. As can be seen in fig.2, the ladders for tRNAs, cross-linked to proteins S5, S7 and S9, after limited cleavage of internucleotide bonds and removal of the oligonucleotides cross-linked to proteins, broke off on residues 22, 46 and 61 respectively (nucleotide residues in tRNA are numbered from the 5′-end according to [15]).

It should be noted that radioactivity above these nucleotides is virtually absent, thus demonstrating the stability of the cross-links under the given conditions of limited cleavage of internucleotide bonds.

Thus, in the 30 S·poly(U)·NAcPhe-tRNA^{Phe} complex, proteins S5, S7 and S9 are cross-linked and, hence, interact directly with A21 in the dihydrouridine loop, U45 in the variable loop and U60 in the TΨC loop of the tRNA molecule (fig.3A). Comparison of the known *E. coli* tRNA primary structures [15] shows that they all have adenine residue in position 21 and virtually pyrimidines only in positions 45 and 60.

It is noteworthy that the nucleotide residues which are cross-linked to (or interact directly with) proteins S5, S7 and S9 lie close together and are located in the central part of the three-dimensional model of tRNA (fig.3B).

One can suppose that interaction of this region of tRNA with ribosome components together with anticodon loop fixation in the decoding region of the 30 S subunit is important for correct tRNA location in the ribosome.

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