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Determination of tRNA nucleotide residues directly interacting with proteins in the post- and pretranslocated ribosomal complexes

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Nucleotide residues of *E.coli* tRNA interacting directly with proteins in pre- and posttranslocated ribosomal complexes have been identified by analysis of photo-induced tRNA-protein cross-links. A9, G18, A26 and U59 residues of NAcPhePhe-tRNA, located in the A_b-site (pretranslocated complex) have been cross-linked with proteins S10, L27, S7 and L2 respectively. In deacylated tRNA, located in the P_b-site, residues C17, G44, C56 and U60 have been cross-linked with proteins L2, L5, L27 and S9 respectively. The G44-L5 cross-link disappeared after translocation (NAcPhePhe-tRNA located in the P_t-site).

Escherichia coli ribosome; tRNA-binding site; tRNA-protein contacts; UV-induced tRNA-protein cross-links; Localization of cross-linked tRNA residues

1. INTRODUCTION

Direct tRNA-protein interactions in ribosomal complexes have been demonstrated by the technique of UV-induced formation of polynucleotide-protein cross-linkages, and proteins involved in these interactions have been identified [1]. The sets of proteins cross-linkable to tRNA change in the course of its transfer from A- to P-site of elongating ribosome, and in response to changes of the functional state of the ribosomal complex [2]. The identification of proteins cross-linked to tRNA in ribosomal complexes provided a basis for a more precise classification and structural characterization of ribosomal tRNA-binding sites [2]. Identification in the tRNA molecule of nucleotide residues directly interacting with ribosomal proteins has opened fundamentally new possibilities for characterization of tRNA-binding sites and description of tRNA movement in the ribosome during elongation.

The methods, described in this paper, have allowed us to determine nucleotide residues in *E. coli* $tRNA^{Phe}$, bases of which are involved in direct interactions with ribosomal proteins. These data have been obtained for $tRNA^{Phe}$, located in A_b- and P_b-sites of pretranslocated and in P_t-site of posttranslocated ribosomal complexes.

2. MATERIALS AND METHODS

70S ribosomes of *E. coli* MRE 600 (Biolar, Olaine) have been used throughout this work. Monomercurated tRNA has been prepared according to [3] from $tRNA^{Phe}$ (Boehringer, Mannheim). Aminoacylation of $tRNA^{Phe}$ with [^{3}H]phenylalanine (Amersham, England, specific activity 137 Ci/mmol) and acetylation have been carried out according to [4,5]. Polyclonal antibodies against ribosomal proteins (IgG fraction) have been prepared at the Institute of Hematology and Blood Transfusion (Moscow) from the serum of rabbits immunized with a complete mixture of *E. coli* 70S ribosomal proteins according to the standard technique.

Pre- and posttranslocated complexes:
$tRNA^{Phe}$ · 70S · poly(U) · NacPhePhe-tRNA-Hg (I),
$tRNA^{Phe}$-Hg · 70S · poly(U) · NacPhePhe-tRNA (II) and
NacPhe -tRNA-Hg · 70S · poly(U) (III)
(monomercurated tRNA in A_b, P_b and P_t sites, respectively) have been prepared and their composition and functional state determined according to [6]. Homogeneity of the obtained complexes was at least 80%. Irradiation of complexes (0.5-1.0 nmol) was performed under a low pressure mercury lamp (254 nm) in Petri dishes with continuous stirring at 4°C (cf. [1]). The absorbed dose was 10-15 quanta per nucleotide, irradiation time - 10-20 min (calculated according to [1], taking into account optical density of the irradiated layer at 254 nm and the intensity of the incident light). Under these irradiation conditions the extent of the complex degradation leading to tRNA release and deacylation of aminoacyl- and peptidyl-tRNA did not exceed 5% (cf. [2]). Isolation of monomercurated $tRNA^{Phe}$ from A_b, P_b and P_t sites of irradiated complexes (free and cross-linked to proteins) was done as in [2]. After deacylation of isolated $tRNA^{Phe}$ [7] the labeling was performed using [^{32}P]pCp and T4 RNA-ligase [8].

tRNA linked proteins have been identified after incubation of the probe containing free and protein-linked 3'-labeled $tRNA^{Phe}$ with individual immune complexes.

In order to obtain individual immune complexes, ribosomal proteins separated by two-dimensional PAAG-electrophoresis [9] have been transferred electrophoretically to nitrocellulose and incubated with a mixture of polyclonal antibodies against ribosomal proteins. For isolation of individual tRNA-protein cross-linkages, the immune complexes containing [^{32}P]-radioactivity, i.e. the tRNA-linked protein, have been dissociated by the addition of 0.2 M glycine buffer (pH 2.8). Position of the protein-linked nucleotide in tRNA has been determined in the isolated individual cross-linkages according to [7].

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3. RESULTS AND DISCUSSION

The analyzed ribosomal complexes contain one molecule of monomercurated tRNA located in a tRNA-binding site. Adsorption on thiopropyl-Sepharose after irradiation and dissociation of the respective ribosomal complexes allows the isolation of this tRNA either free or cross-linked to proteins [2]. Introduction of ^{32}P-label in the 3′-end of tRNA^{Phe} followed by the adsorption on immobilized individual immune complexes leads to identification of proteins, cross-linked to this tRNA (Table I). Not all tRNA-protein cross-linkages formerly found in these complexes by iodination of cross-linked proteins [2] may be detected by the technique of immune complexes. Perhaps tRNA has been available for ligation only in a part of cross-linked complexes or the technique of immunodetection is less sensitive than methods used previously.

The material adsorbed on the immune complexes is homogeneous in terms of protein and tRNA location in the initial complexes. This allowed us to determine the position in the tRNA chain corresponding to nucleoside residues cross-linked with various proteins (cf. [7]). Eight nucleoside residues of tRNA are cross-linked to

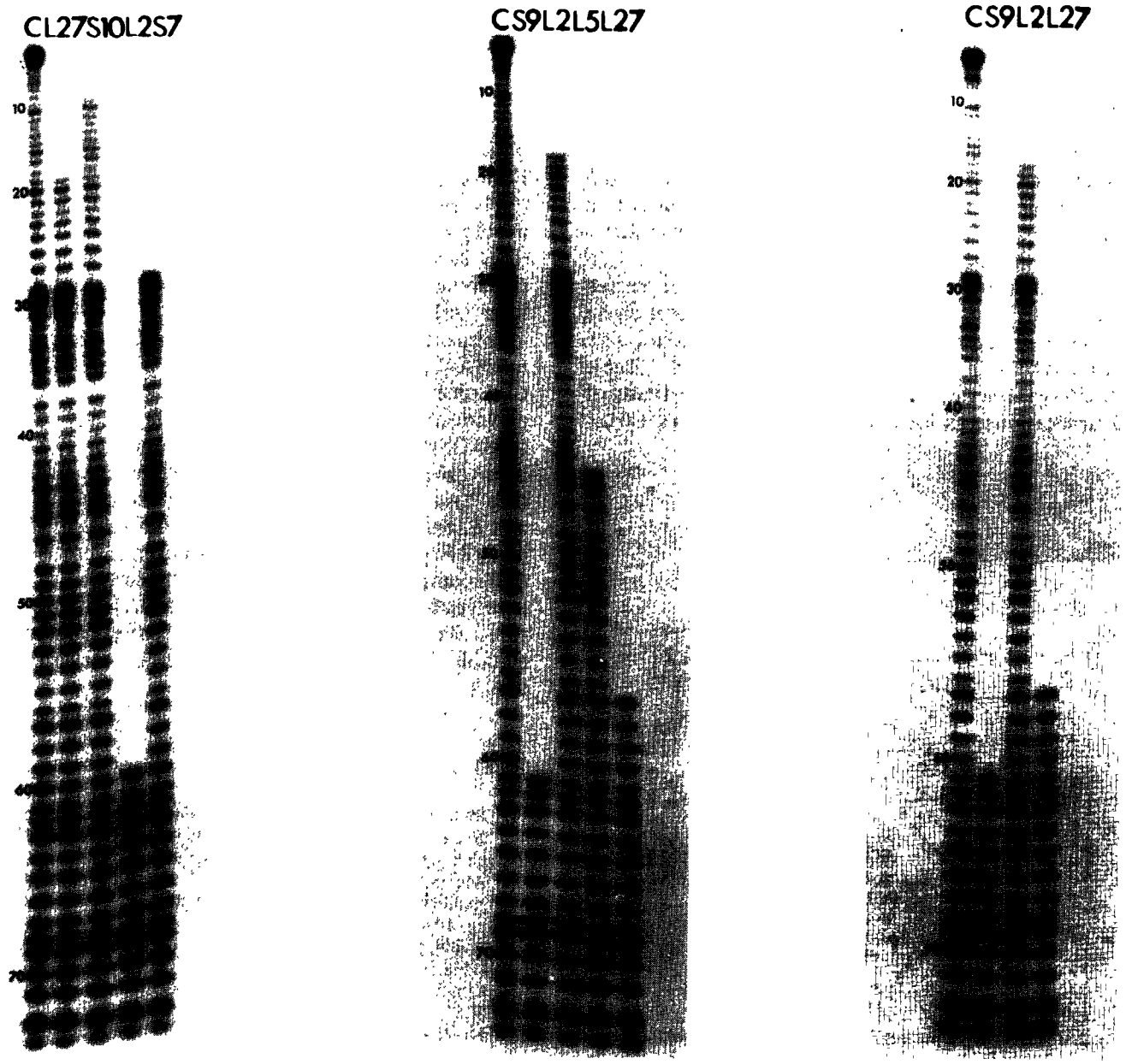

Fig. 1. Distribution of radioactivity in the sequencing PAAG after electrophoresis of oligonucleotides after statistical hydrolysis of [^{32}P]-pCp-ligated native and protein-linked tRNA^{Phe}. A, B and C, tRNA-protein cross-linkages isolated from the complexes I, II and III, respectively. tRNA-cross-linked proteins are marked above the strips; C, control (free tRNA).

TABLE I

The nucleotides of *E. coli* tRNA directly interacting with ribosomal proteins in pre- and posttranslocated ribosomal complexes

Ribosomal complexes	tRNA-binding sites [2]	Ribosomal proteins cross-linked to tRNA					
		S7	S9	S10	L2	L5	L27
1 (pretranslocated complex)	A_b	A26 (800)	-	A9 (670)	U59 (1200)	-	G18 (1420
2 (pretranslocated complex)	P_b	-	U60 (1980)	-	C17 (1020)	G44 (580)	C56 (750)
3 (posttranslocated complex)	P_t	-	U60 (2100)	-	C17 (1900)-		C56 (1050)

Values in brackets show radioactivity (cpm) adsorbed on the immune complexes after addition of mixture of free and cross-linked to proteins monomercurated [^{32}P]-tRNA^{Phe}. The mean values from at least three experiments are given. The background is about 50 cpm.

proteins in complexes I, II and III (Fig. 1, Table I). Four of them interact with proteins in the A_b-site, and the remaining four – in the P_b-site (pretranslocated ribosome), three of these latter residues remain in P_t-site in the posttranslocated ribosome.

Although two proteins (L2 and L27) have interacted with tRNA^{Phe} in all the complexes studied, location on the tRNA chain of nucleoside residues, interacting with these proteins in A and P-sites is different (Fig. 2, Table I). Thus the set of tRNA-protein contacts in terms of their location in tRNA chain appears to be a strict structural characteristic of tRNA-binding site of the ribosome.

The change in the location of contacts of L2 and L27 proteins with tRNA after its transfer from A- to P-site (Fig. 2) is probably a reflection of tRNA turn in the ribosome around the axis, connecting the anticodon loop with the CCA-end of the molecule. This is in agreement with the idea of the existence of a bend in mRNA between A and P codons in the elongating ribosome (cf. [10]).

None of the eight nucleoside residues directly interacting with proteins in complexes I, II and III appears to be involved in the formation of the secondary structure (Fig. 2). If the tertiary structures of yeast tRNA^{Phe} [11] and *E. coli* tRNA^{Phe} are similar, five residues of the *E. coli* tRNA^{Phe}, interacting with ribosomal proteins in these complexes (A9, G18, A26, G44 and C56), may be involved also in stabilization of the tRNA tertiary structure. We propose that such interactions should inevitably induce changes in the tertiary structure of tRNA.

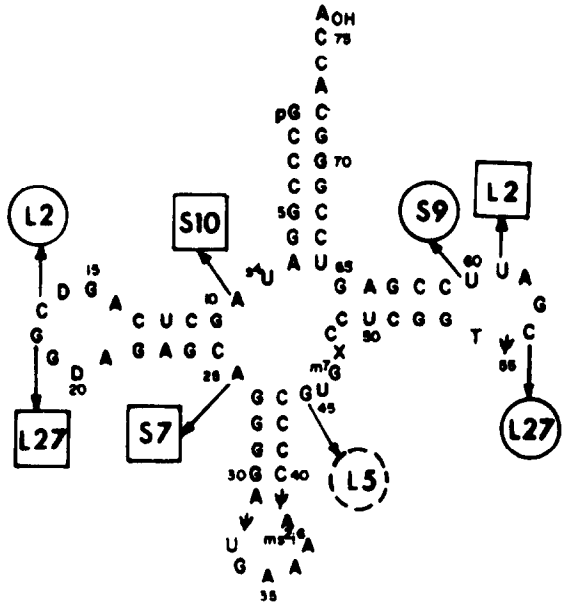

Fig. 2. Distribution of nucleotide residues cross-linked with proteins during UV-irradiation of ribosomal complexes I, II and III on the secondary structure of *E. coli* tRNA^{Phe}. Proteins cross-linked with tRNA^{Phe} in A- and P-sites are marked by squares and circles, respectively. The protein cross-linked with tRNA^{Phe} only in the P_b-site is marked with the dotted circle.

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