Polynucleotide-protein interactions in the translation system. Identification of proteins interacting with tRNA in the A- and P-sites of *E. coli* ribosomes

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ABSTRACT

Ultraviolet irradiation ($\lambda = 254$ nm) of ternary complexes of *E. coli* 70 S ribosomes with poly(U) and either Phe-tRNA^{Phe} (in the A-site) or NAcPhe-tRNA^{Phe} (in the P-site) effectively induces covalent linking of tRNA with a limited number of ribosomal proteins. The data obtained indicate that in both sites tRNA is in contact with proteins of both 30 S and 50 S subunits (S5, S7, S9, S10, L2, L6 and L16 proteins in the A-site and S7, S9, S11, L2, L4, L7/L12 and L27 proteins in the P-site). Similar sets of proteins are in contact with total aminosacyl-tRNA and N-acetylaminoacyl-tRNA. However, here no contacts of tRNA in the P-site with the S7 and L25/S17 proteins were revealed, whereas in the A-site total aminosacyl-tRNA contacts L7/L12. Proteins S9, L2 and, probably, S7 and L7/L12 are common to both sites.

INTRODUCTION

According to modern concepts of the mechanism of protein synthesis pretranslocated aminosacyl-tRNA's and peptidyl-tRNA's are localized in the A-site of *E. coli* ribosomes, whereas during translocation peptidyl-tRNA is transferred to the P-site /1,2/. The A- and P-sites have up to now been considered mainly from a functional standpoint. In order to elucidate the proteins belonging to the A- and P-sites or contacting with the tRNA in these sites various indirect approaches have been used, such as physico-chemical methods /3/, crosslinking with bifunctional, including photoaffinity agents /4-6/ and the effect of modification or elimination of some of ribosomal proteins /7-11/. However, the results did not permit identification of the proteins contacting tRNA. Moreover, physico-chemical methods and bifunctional agents only allow determination of intergroup distances in proteins and polynucleotid-

es and the modification or removal of individual proteins may indirectly effect the formation of the complexes and their structure and also certain stages of the translation process.

Ultraviolet-induced polynucleotide-protein crosslinking provides the unique possibility of detecting direct contacts between interacting components of native nucleoproteins. Earlier this approach was applied to a number of nucleoproteins, including ribosomes /12-19/. We have used it in an extensive study of the translation system of E.coli. In particular, by means of ultraviolet-induced polynucleotide-protein crosslinking direct contacts were demonstrated between 16 S RNA and a number of split-proteins (S2, S3, S5, S14 and S21) in 30 S subunit /17/. In the present study we have used the ultraviolet-induced crosslinking technique to identify proteins in E.coli ribosomes interacting in the A- and P-sites with tRNA. (A preliminary communication is in /20/).

MATERIALS AND METHODS

70 S ribosomes isolated from E.coli MRE600 according to /21/ displayed a homogeneous peak on centrifugation in a sucrose gradient (20 mM of Mg^{+2} , 200 mM of NH_4Cl , pH 7.2) and contained the total set of proteins, as revealed by two-dimensional electrophoresis in polyacrylamide gel /22/. Over 30% of the ribosomes proved active in the formation of the ternary complexes /23/ with poly(U) and either Phe-tRNA^{Phe} or NAcPhe-tRNA^{Phe}. In the absence of poly(U) ribosome binds 1/10 to 1/20 the amount of tRNA (Table 1). The poly(U) species of molecular weight 30 000 was kindly donated by Dr. C.B.Kirillov (Leningrad).

Total [³²P]-tRNA (2-7x10⁷ cpm per A₂₆₀ unit) was isolated from E.coli MRE 600 according to /24/; and after aminocyclization by [¹⁴C]-phenylalanine (Amersham, 495 mCi/mM) it was enriched to 10-20% tRNA^{Phe} by fractionation on BDAE-cellulose as described in /25/. The Phe-tRNA^{Phe} content in the preparations was determined from the ratio of [³²P] to [¹⁴C] after splitting of phenylalanine in alkaline medium and its separation from desacylated tRNA on FN11 paper in n-butanol-acetic acid-water (4:1:5) /26/. The degree of acetylation of

Phe-tRNA^{Phe} with acetic anhydride /27/ or N-acetoxysuccinimide /26/, determined by a similar method was not less than 95%.

The complexes Phe-tRNA^{Phe}·poly(U)·70 S and NAcPhe-tRNA^{Phe}·poly(U)·70 S were formed in buffer A solution (10 mM MgCl₂, 20 mM Tris·HCl, 200 mM NH₄Cl, 2 mM 2-mercaptoethanol, pH 7.2) at 0°C. In a typical experiment 1-3 ml of buffer A contained 10 A₂₆₀ units (225pM) 70 S ribosomes, 1-0.1 A₂₆₀ units (1600-160pM) tRNA and 1 A₂₆₀ unit poly(U) (cf./23,28/). The same method was used to obtain total aminoacyl- and acetyl-aminoacyl-tRNA's and their complexes with 70 S ribosomes, employing as template an oligonucleotide mixture (0.2 A₂₆₀ unit per A₂₆₀ unit of 70 S ribosome) formed on treating yeast tRNA with 0.1M KOH (20 min at 20°C), neutralizing with two volumes of ethanol.

After incubation of the mixtures for 20 min, aliquotes were analyzed by filtration through nitrocellulose filters (Synpore, Czechoslovakia), or by centrifugation in a sucrose gradient. Complexing of tRNA to the ribosome in the absence of template was followed in a similar fashion. Location of tRNA in the ternary complexes was revealed by the puromycin test /29/. The complexes and controls were irradiated in 1.5-4 cm Petri dishes at 0°C under stirring using the full light of a low-pressure mercury lamp at a distance of 7 cm from the dishes. The incident light intensity determined by uridine actinometry /30/ was $2.7-8.4 \times 10^{16}$ quanta per cm² per min. The optical density of the irradiated mixtures at 254 nm normally exceeded 1.0, so that in calculation of the energy absorbed per nucleotide it was assumed that the light was totally absorbed by the reaction mixture.

At given time intervals two aliquotes were taken from the irradiated solutions. One was directly filtered through a millipore filter and washed with A buffer. To the second before filtering EDTA was added up to a concentration of 15 mM, following which it was incubated for 20 min at 24°C (to dissociate the non-crosslinked complex into 30 S and 50 S subunits, poly(U) and Phe-tRNA^{Phe} or NAcPhe-tRNA^{Phe}) and then washed on the filters with B buffer, differing from buffer A

in containing 5 mM EDTA instead of Mg^{+2} . The radioactivity retained on the filter in the first case reflects the overall quantity of tRNA in both the crosslinked and non-crosslinked complexes, and in the second case, the amount of tRNA cross-linked to the ribosome by the irradiation.

To test the functional state of the crosslinked tRNA in the NAcPhe-tRNA^{Phe}·poly(U)·70 S complex determination of the N-acetylphenylalanine transfer to puromycin was made using NAc[¹⁴C]-Phe-tRNA^{Phe}. The irradiated complex, prior to its dissociation by EDTA and filtration, was incubated with puromycin (2 mM, 30 min, 24°C). In this case the radioactivity retained on the filter reflects the quantity of NAcPhe-tRNA^{Phe} crosslinked to the ribosome but incapable of transferring N-acetylphenylalanine to puromycin; it did not exceed 10% of the total crosslinked NAcPhe-tRNA^{Phe}.

The ternary complexes (before and after irradiation) were precipitated with two volumes of ethanol, dissolved in B buffer (see above) or else in 20 mM Tris·HCl (pH 6.8) containing 10 mM of EDTA and 0.1% of sodium dodecylsulfate (buffer C) for complete dissociation of nucleoprotein to free polynucleotides and proteins and the resultant mixtures were centrifuged in a sucrose gradient prepared with the respective buffers. To determine the integrity of the polynucleotide chains the ternary complexes after irradiation were treated

Table 1

Binding of Phe-tRNA^{Phe} and NAcPhe-tRNA^{Phe} with E.coli 70 S ribosome as revealed by retention of [³²P]-tRNA on nitrocellulose filters (radioactivity retained on filters in complete system was equal to 5 000-20 000 counts/min)

Phe- [³² P]-tRNA ^{Phe} :	NAcPhe [³² P]-tRNA ^{Phe} :	Poly(U):	Binding with 70 S ribosome (per cent to the binding in the complete system)
:	:	:	:
:	:	:	:
:	:	:	:
:	:	:	:
+	-	+	100
+	-	-	4.9 ± 0.3
-	+	+	100
-	+	-	8.1 ± 0.25

with pronase (20 μ g of pronase per 10 A₂₆₀ units of 70 S ribosomes, 37°C, 30 min) in C buffer and then centrifuged in a sucrose gradient.

In order to identify the ribosomal proteins crosslinked to tRNA following ultraviolet irradiation two volumes of ethanol were added to the complex solution containing 10 A₂₆₀ unit 70 S ribosome and the precipitate dissolved in 100-200 μ l of B buffer was digested with a mixture of ribonucleases A (5-10 μ g) and T₁ (20 a.u.) for 4 hr at 37°C. After adding urea, the hydrolysate was analysed by two-dimensional electrophoresis according to our modification /18/ of Metz and Bogorad's procedure /31/. The gel was stained with a 0.1% solution of Coomassie brilliant blue R250 in 25% *iso*-propanol containing 7.5% acetic acid and autoradiographed. The [³²P]-containing zones of the gel were cut out and counted in a scintillation spectrometer SL30 (Intertechnique, France).

It had earlier been shown /18/, that the crosslinked oli-

Fig. 1. Effect of ultraviolet irradiation ($\lambda = 254$ nm) of the Phe-tRNA^{Phe}·poly(U)·70 S, NAcPhe-tRNA^{Phe}·poly(U)·70 S and Phe-tRNA^{Phe}·poly(U)·70 S complexes on [³²P]-tRNA retention by nitrocellulose filters in the presence of EDTA (curves 1, 2 and 3 respectively). Curve 4 - filtration of the Phe[³²P]-tRNA^{Phe}·poly(U)·70 S complex in the presence of 10 mM of Mg²⁺. To the right - semilogarithmic plot of curve 1 (ordinates - lg (100- α), where α is the percentage of retained [³²P]-tRNA (radioactivity retained on filters in the presence of Mg²⁺ was equal to 10 000-30 000 counts/min).

Fig. 2. Sedimentation patterns of the Phe $[^{32}\text{P}]$ -tRNA^{Phe} poly(U). 70 S ternary complexes in a sucrose gradient; to the left — before irradiation; to the right — after irradiation ($\lambda = 254$ nm, 30 quanta per nucleotide); open circles — optical density at 260nm; closed circles — radioactivity. A and B, sedimentation in the presence of 10 mM of Mg^{+2} ; C and D, sedimentation in the presence of 5 mM of EDTA; E and F, sedimentation in the presence of 5 mM of EDTA and 0.1% dodecylsulfate after dissociation of the complex on adding 15 mM of EDTA and 0.1% dodecylsulfate; G, the same as F, but after the treatment of the irradiated complex with pronase. Sucrose gradient 5–20%, rotor SW-40; A, B, C and D — 39 000 rev/min, 6 hours; E, F and G — 35 000 rev/min, 17 hours.

gonucleotide fragments lower the mobility of proteins somewhat in the first direction (at pH 4.5) but do not effect their mobility in the second direction (in the presence of sodium dodecylsulfate). The position of [32 P]-oligonucleotide-containing proteins determined autoradiographically facilitates ready identification of a considerable portion of the ribosomal proteins crosslinked to tRNA as the result of ult-

Fig. 3. Two-dimensional electrophoretic separation of ribosomal proteins according to /18/ and /31/. C - total mixture of ribosomal proteins; the polyacrylamide gel was stained with Coomassie; A and B - autoradiographs of the gel after separation of proteins crosslinked to tRNA fragments. The complexes Phe- 32 P-tRNA^{Phe}.poly(U).70 S and NAcPhe- 32 P-tRNA^{Phe}.poly(U).70 S (A and B respectively) were irradiated ($\lambda = 254$ nm, 20 quanta per nucleotide), digested with a mixture of RNAses A and T₁ and the resulting hydrolysates were subjected to electrophoresis.

reviolat irradiation of the ternary complexes. However this system does not separate all groups of ribosomal proteins sufficiently so that, for instance, one cannot from the position of the radioactivity on the electropherogram discriminate between proteins S9 and S11, L25 and S17, L27, S13 and S14 (Fig. 3). The L25/S17 spot showed very little radioactivity and was not treated further, while the other two spots were subjected to additional electrophoresis in 7.5% polyacrylamide gel at pH 4.5 according to /32/.

Here these proteins had noticeably different mobilities (Fig.4). However, the presence of an oligonucleotide fragment strongly influenced the protein mobility in this system. Therefore, the final identification of the crosslinked proteins was performed according to /33/. The $[^{32}\text{P}]$ -containing zones of the gel were iodinated with $[^{125}\text{I}]$ and subjected to tryptic hydrolysis. After separation of the resultant peptides by electrophoresis followed by thin layer chromatography on cellulose and autoradiography the peptide map was compared with

Fig. 4. Electrophoretic separation of ribosomal proteins according to /32/. Above - total mixture of 30 S subunit proteins stained with Coomassie; below - distribution of $[^{32}\text{P}]$ radioactivity after additional electrophoretic separation of the contents from the spot labelled on Fig. 3B as S9, S11.

the maps obtained in the same manner from known ribosomal proteins. This procedure permitted reliable and unequivocal identification of almost all the crosslinked proteins.

RESULTS AND DISCUSSION

Earlier it was shown that with a 10 mM concentration of Mg^{+2} in their ternary complexes with 70 S ribosomes and template aminoacyl-tRNA binds predominantly in the ribosomal A-site /23/, whereas N-acetylaminoacyl-tRNA binds predominantly in the P-site /1,23,34/.

As revealed by the ratio of $[^{32}P]$ -tRNA to A_{260} , the 70 S ribosome preparations used in our work form ternary complexes with poly(U) and either Phe-tRNA^{Phe} or NAcPhe-tRNA^{Phe} to the extent of about 30%. This is sufficient for detecting the ribosomal proteins crosslinked to tRNA. The presence of even a large amount of the non-complexed ribosomes has no effect on the results of the present study since we estimate only the proteins crosslinked to tRNA. According to the ratios of $[^{32}P]$ -tRNA to $[^{14}C]$ -Phe and/or NAc $[^{14}C]$ -Phe (determined after chromatographic separation of the alkaline hydrolysates), our routine ternary complex preparations contained less than 3% deacylated tRNA and the NAcPhe-tRNA^{Phe}. poly(U)·70 S complex, less than 3% Phe-tRNA^{Phe}. Hence the complexes are of a highly homogeneous composition.

Fig. 5. Tryptic peptide maps of the iodinated (^{125}I) proteins. A - protein comigrating with the left radioactivity peak (Fig. 4, below); B and C - known proteins S9 and S11 respectively.

In our routine preparations of $\text{NACPhe-tRNA}^{\text{Phe}} \cdot \text{poly(U)} \cdot 70\text{ S}$ about $78 \pm 2\%$ of N-acetylphenylalanine was transferred to puromycin, whereas in the $\text{Phe-tRNA}^{\text{Phe}} \cdot \text{poly(U)} \cdot 70\text{ S}$ complex such transfer underwent only $2.9 \pm 0.5\%$ of phenylalanine. Bearing in mind the amounts of $\text{Phe-tRNA}^{\text{Phe}}$ and $\text{NACPhe-tRNA}^{\text{Phe}}$ binding to the ribosome in the absence of template (Table 1) it may be concluded that in the first complex almost 90% of tRNA is located in the P-site whereas in the second complex over 90% of tRNA is located in the A-site.

Addition of $\text{Phe-tRNA}^{\text{Phe}}$ to the $\text{NACPhe-tRNA} \cdot \text{poly(U)} \cdot 70\text{ S}$ complex and of $\text{NACPhe-tRNA}^{\text{Phe}}$ to the $\text{Phe-tRNA} \cdot \text{poly(U)} \cdot 70\text{ S}$ complex results in the formation of more than 70% of dipeptide (with reference to the complex), showing that the complexes were of high functional activity and homogeneity (will be published separately).

One can see from Fig. 1 that absorption up to 55 quanta per nucleotide has practically no effect on the quantity of $[^{32}\text{P}]$ -tRNA retained on millipore filters in the presence of 10 mM of Mg^{+2} . Addition of EDTA to the complexes liberates the tRNA which readily passes through the filters. However when EDTA is added to irradiated ternary complexes less tRNA is released, such that after absorption of 55 quanta per nucleotide over 30% of tRNA is retained on the filter in the case of $\text{Phe-tRNA}^{\text{Phe}} \cdot \text{poly(U)} \cdot 70\text{ S}$ complex and over 20% in the case of $\text{NACPhe-tRNA}^{\text{Phe}} \cdot \text{poly(U)} \cdot 70\text{ S}$. It should be stressed that even after irradiation tRNA bound to the 70 S ribosome in the absence of template is not retained on the filters in the presence of EDTA (Fig. 1).

Addition of EDTA dissociates the starting ternary complexes to poly(U) , tRNA, 30 S and 50 S subunits (Fig. 2C), but after absorption of ~ 30 quanta per nucleotide the complexes sediment in a single peak with a high sedimentation constant (Fig. 2D). Complete dissociation of the irradiated complexes is not achieved even by EDTA in combination with sodium dodecylsulfate, which normally breaks down the original complex to proteins and polynucleotides (Figs. 2E and 2F). These data evidenced that ultraviolet ($\lambda = 254\text{ nm}$) irradiation of the

Table 2

Proteins of *E. coli* 70 S ribosome covalently crosslinked to tRNA after ultraviolet irradiation ($\lambda = 254$ nm, 20-30 quanta per nucleotide) of the ternary complexes of 70 S ribosome, template and either aminoacyl-tRNA or N-acetylaminoacyl-tRNA.

Relative activity (mean values of three experiments) is shown as the percentage of total radioactivity in the two-dimensional gel portions containing proteins to which are crosslinked fragments of [32 P]-tRNA. The average total radioactivity present in the gel was equal to 20 000 - 40 000 counts/min; background - 200 counts/min/square cm of the gel.

Ribosomal proteins	Complexes of 70 S ribosome, template and aminoacyl-tRNA (tRNA located in A-site)		Complexes of 70 S ribosome, template and N-acetylaminoacyl-tRNA (tRNA located in P-site)	
	Phe - [32 P]-tRNA ^{Phe}	total aminoacyl- [32 P]-tRNA	N-AcPhe- [32 P]-tRNA ^{Phe}	total N-acetyl-aminoacyl- [32 P]-tRNA
S5	19.5	12.5	-	-
S7	2.8	4.5	2.2	-
S9	16.2	18.5	18.4*	20
S10	14.9	15.0	-	-
S11	-	-	*	*
L2	12.8	13.0	20.1	18.0
L4	-	-	18.7	15.0
L6	18.8	14.0	-	-
L7/L12	-	9.5	15.6	12.0
L16	15.0	11.0	-	-
L25/S17	-	-	3.6**	-
L27	-	-	21.4	35

* The radioactivity was approximately equally distributed between proteins S9 and S11.

** Proteins not finally identified.

ternary complexes result in formation of strong, most probably covalent, crosslinks between the nucleoprotein components. From the fact that treatment of the irradiated complexes with pronase yielded no RNA with sedimentation constant higher than 23 S and between 16 S and 23 S (Fig. 2G), the irradiation must have caused no crosslinking between polynucleotides in the complexes. Since ultraviolet irradiation does not result in formation of the protein-protein crosslinks /17/, the crosslinking in the ternary ribosomal complexes must have occurred between the RNA's and the ribosomal proteins.

Ultraviolet irradiation induces a variety of photochemical reactions in components of polynucleotides and proteins /35, 36/, that can induce changes in the higher structure of the macromolecules themselves and of their complexes. The ultraviolet-induced polynucleotide-protein crosslinking can therefore give reliable information on the interaction between RNA's and proteins only when the radiation doses are sufficiently low so as not to change the quaternary structure of the nucleoproteins. Intensive tRNA-protein crosslinking in the ternary ribosomal complexes can be observed when a dose

as low as 50 quanta per nucleotide is absorbed (Fig. 1), which is accompanied by not more than 30% loss in ability of 70 S ribosome to form the Phe-tRNA^{Phe}.poly(U).70 S complex and caused no changes in the sedimentation pattern of the complexes in the presence of Mg⁺² (Figs. 2A and 2B), nor cleavage of the RNA's chains as revealed by gel electrophoresis in the presence of urea (data not shown). At such doses N-acetylphenylalanine in NAcPhe-tRNA^{Phe}.poly(U).70 S retained the same ability to transfer to puromycin as in the non-irradiated complex. However the only vigorous criterion for the formation of crosslinks in the intact (in terms of quaternary structure) complex, is the single-hit kinetics of crosslinking /17/.

Fig. 1 shows that the crosslinking of tRNA in complexes follows an exponential law up to absorption of ~ 25 quanta per nucleotide but even at higher doses (up to 60 quanta per nucleotide) only insignificant deviation from the exponential curve is observed. Consequently, the crosslinking must have occurred between the polynucleotides and ribosomal proteins, the process taking place at doses below 50 quanta per nucleotide in the native ternary complexes and serving as a probe of the actual contacts between RNA's and these proteins and as a means for study of interacting components. When the radiation dose was significantly increased, RNA degradation sets in and much multi-hit crosslinking of the proteins to RNA occurs /37, 38/, reflecting changes in the polynucleotide-protein contacts due to the radiation-induced structural changes in the complex.

It has been mentioned above that in the Phe-tRNA^{Phe}.poly(U).70 S and in NAcPhe-tRNA^{Phe}.poly(U).70 S complexes, the tRNA molecules are located predominantly in the A- and P-sites, respectively. One can see from Fig. 3 that the sets of proteins containing the crosslinked tRNA fragments differ considerably from one complex to the other.

The sets of proteins crosslinked to tRNA differ only insignificantly when Phe-tRNA^{Phe} or total aminocyl-tRNA are inserted into A-site; the same holds for NAcPhe-tRNA^{Phe} or total N-acetylaminocyl-tRNA in the P-site. Differences are

observed, however, in the A-site for protein L7/L12 which crosslinks only to the total aminoacyl-tRNA and in the P-site where proteins S7 and L25/S17 are crosslinked only to NacPhe-tRNA^{Phe} (Table 2).

We thus see that tRNA is apparently rigidly held in the A- and P-sites and does not migrate from one site to the other during the irradiation process. This is the first experimental determination of the proteins contacting (directly interacting) with the tRNA located in A- and P-sites of E.coli ribosomes .

The difference between the sets of proteins crosslinking to tRNA in the A- and P-sites is large enough for it to serve as a means for determining the amount of tRNA in the A-site as well as in the P-site and also for following changes in the tRNA location in the course of translation.

Since not all the different polynucleotide-protein contacts form ultraviolet-induced crosslinks it cannot be maintained that all the proteins contacting with tRNA can thus be determined. However, taking into account the dimensions of tRNA and its ability to crosslink to a sufficiently wide variety of proteins (seven in the A-site and from eight to ten in the P-site) it could be assumed that the ultraviolet-induced crosslinking permits the pinpointing of the majority of the proteins contacting tRNA. It should be stressed that among them can be proteins that under certain conditions are not necessary for the binding and functioning of the tRNA.

Assuming that formation of ribosomal complexes proceeds largely with retention of the tRNA tertiary structure and bearing in mind the small size of a tRNA molecule (with respect to ribosome), one can assume that the proteins in each of the sites, contacting the tRNA, are grouped close together in the ribosome. Since the molecules of at least two proteins (S9 and L2) simultaneously belong to both sites, it is evident that the A-site protein cluster should be close to the P-site protein cluster. This is in agreement with the location of the tRNA-contacting proteins according to the models of Stoffler /39/ and Lake /40, 41/. Despite the highly divergent data, proteins contacting tRNA in the proposed models

cluster on the "head" of the 30S and the adjacent 50 S subunit parts of the 70 S ribosome.

Table 3

Ribosomal proteins crosslinked to tRNA under ultraviolet irradiation of ternary complexes and contiguous with chloramphenicol, puromycin or with tRNA in the peptidyl-transferase center.

Ribosomal proteins	Crosslinked with: tRNA under UV irradiation of ternary complexes with 30S:subunit /45/	Peptidyl-transferase center /1,42/ A- site (see Table 2)	Chloramphenicol /1, 42/	Puromycin /1, 42/
S3/S4	+			
S5	+	+		
S6	+			
S7	+	+	+	
S8	+			
S9	+	+	+	
S10	+	+		
S11	+		+	
S13/S14	+	-		
S15/S16/S17	+			
S19/S20	+			
L2		+	+	+
L4			+	
L6		+	+	+
L17/L12		+		
L11			+	+
L16		+	+	+
L18			+	
L25		+		+
L26			+	
L27		+	+	+

Transfer of the peptidyl residue from peptidyl-tRNA to aminoacyl-tRNA occurs in the peptidyl transferase center located on the 50 S subunit /1, 42/. Therefore one could expect that interaction of tRNA with the proteins of this subunit would take place in both the A- and P-sites. As can be seen from Table 2, experimental data are in agreement with this expectation. Although in the presence of a template aminoacyl-tRNA specifically binds to the 30 S subunit /1,2/, there are no grounds for assuming that the translocated tRNA must necessarily interact with its proteins. However, as one can see from Table 2, tRNA contacts proteins of 30 S subunit before and after translocation. In other words, the A- and P-sites both contain proteins of the two subunits and cross-linking of these proteins with the tRNA may be one of the factors preventing the dissociation of the irradiated complexes (Fig. 2D).

The results presented here are in agreement with reported data on the functional location of the ribosomal proteins. The most complete and reliable is the information on the proteins clustering about the ACC-termini of the tRNA's and involved in formation of the A'- and P'-sites of the peptidyl transferase center (see /1, 7, 42/ and references therein). As can be seen from Table 3 only the proteins participating in the A'-site of the peptidyl transferase center are cross-linked to, i.e. are in contact with, the tRNA molecule located in the ribosomal A-site. On the other hand only proteins of the P'-site are crosslinked to the tRNA in the ribosomal P-site. It is noteworthy that some of the proteins participating in the binding of, or close to chloramphenicol and puromycin in the ribosome belong to proteins contacting tRNA in the A-site (Table 3). Information on the proteins involved in formation of the decoding site (clustering about the anticodon loop) is much more sparse. Changes (chemical or mutational), elimination of proteins or the attachment of antibodies leading to altered translation fidelity permit assignment to the decoding site of the proteins S4, S5, S11 and S12 /2, 43/. Moreover, at least some of the proteins (S4, S5, S11, S12, S13 and S21; see /44/ and references the-

rein) clustering around or interacting with the poly- and oligonucleotide template should also be found in the decoding site. Of all these proteins only S5 and S7 directly contact tRNA in the A-site while S7 and S11 - in the P-site of 70 S ribosome.

Thus, a major part of the proteins contacting tRNA in the ribosomal complexes belong either to the peptidyl transferase center or to the decoding site. The functional role of the tRNA contacts with S10 and L7/L12 proteins, however, is still obscure. Possibly, these proteins are required for correct orientation of the tRNA in the ribosome and/or for its translocation in the course of the translation process.

As can be seen from Table 3 there are apparently significant differences between the ternary complexes containing only the 30 S subunit and those involving the whole 70 S ribosome. When in the 70 S ribosome complex, the 30 S proteins contact tRNA in much less number than when the 30 S subunit is in the Phe-tRNA^{Phe}.poly(U).30 S complex /45/ (Table 3). Thus, while in the latter case identification of proteins containing tRNA fragments was less precise than in the present work, nevertheless, almost half the radioactivity of the tRNA fragments crosslinked to proteins was detected on the electropherogram in the region of proteins S3+S4, S6, S8, S13+S14, S15+S16+S17, S18 and S19+S20 /45/. However when [³²P]-tRNA-containing 70 S complexes were irradiated the spots corresponding to the above proteins showed practically no radioactive [³²P]-tRNA fragments (Fig. 3, Table 3). The proteins S7, S10 and S9 and/or S11 on the other hand crosslink to tRNA in both Phe-tRNA^{Phe}.poly(U).30 S and Phe-tRNA^{Phe}.poly(U).70 S complexes (Table 3). This difference can be due either to the fact that in the first complex tRNA has a less compact structure so that it can contact with a significantly greater number of proteins, or that in the absence of a 50 S subunit the complex is less homogeneous. In favor of the second proposal is the fact that Phe-tRNA^{Phe}, poly(U) and 30 S subunit form several ternary complexes differing in the tRNA binding constants /46/. Apparently this is due to differences in the contacts between tRNA

and ribosomal proteins.

In conclusion it should be mentioned that by far not all the proteins contacting tRNA in the complete ribosomal complexes (Table 2) bind to immobilized tRNA /47, 48/ even under the optimal binding conditions /49/. Among the crosslinking, but non-binding proteins are S10, S11, L12 and L27 (Table 2, cf. /49/). This is a strong argument for holding that reliable data on the polynucleotide-protein contacts can be obtained only by using native complexes as objects for study.

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