Direct tRNA – protein interactions in ribosomal complexes

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ABSTRACT

Nucleotide residues in E. coli tRNA^Phe^ interacting directly with proteins in pre- and posttranslocated ribosomal complexes have been identified by UV-induced cross-linking. In the tRNA^Phe^ molecule located in the A_b-site (pretranslocated complex) residues A9, G18, A26 and U59 are cross-linked with proteins S10, L27, S7 and L2, respectively. In tRNA^Phe^ located in the P_b-site (posttranslocated complex) residues C17, G44, C56 and U60 are cross-linked with proteins L2, L5, L27 and S9, respectively. The same cross-links (except for G44-L5) have been found for tRNA in the P_b-site of the pretranslocated ribosomal complex. None of the tRNA^Phe^ residues cross-linked with proteins in the complexes examined by us are involved in the stabilization of the secondary structure, but residues A9, G18, A26, G44 and C56 participate in stabilization of tRNA tertiary structure. Since translocation of tRNA^Phe^ from A_b- to P_b-site is accompanied by changes of tRNA contacts with proteins L2 and L27, we postulate that this translocation is coupled with tRNA turn around the axis joining the anticodon loop with the CCA-end of the molecule. This is in agreement with the idea about the presence of a kink in mRNA between codons located in the ribosomal A- and P-sites. In all E. coli tRNAs with known primary structure positions 18 and 56, interacting with L27 protein, when tRNA is located in the A-sites of the pretranslocated and P-sites, respectively. The same cross-links (except for G44-L5) have been found for tRNA in the P_b-site of the pretranslocated ribosomal complex. None of the tRNA^Phe^ residues cross-linked with proteins in the complexes examined by us are involved in the stabilization of the secondary structure, but residues A9, G18, A26, G44 and C56 participate in stabilization of tRNA tertiary structure. Since translocation of tRNA^Phe^ from A_b- to P_b-site is accompanied by changes of tRNA contacts with proteins L2 and L27, we postulate that this translocation is coupled with tRNA turn around the axis joining the anticodon loop with the CCA-end of the molecule. This is in agreement with the idea about the presence of a kink in mRNA between codons located in the ribosomal A- and P-sites.

INTRODUCTION

Several recent papers (1, 3) suggest that ‘…ribosomal RNA (rRNA) has a primary functional role in most if not all stages of protein synthesis. Ribosomal proteins have now been relegated to an important but more auxiliary role of facilitating the function of rRNA.’ (2). But in the meantime a large number of specific direct interactions (contacts) of ribosomal proteins with rRNAs (4, 5), mRNA (6, 7) and tRNA (8) has been demonstrated. These interactions are altered in a specific way in response to the association of ribosomal subunits (4, 5), and formation of the initiation (9) or elongation complexes. These interactions are significantly altered by changes in the functional state of the ribosomal complex and tRNA location in it (4, 5, 8).

It therefore appears that not only interactions of tRNA with each other and with other RNA types play an important role in protein biosynthesis; it is likely that interactions of ribosomal proteins with rRNAs, mRNA and tRNAs are essential in this process as well. Studies of RNA – protein contacts in different ribosomal complexes may therefore give important information about the arrangement of interacting partners in these complexes, their functional role and functional topography.

Identification of proteins cross-linked with tRNA after UV-irradiation of ribosomal complexes leads to structural characterization of ribosomal tRNA-binding sites and allows classification of these sites depending on the functional state of the elongation complex (8). Identification of tRNA residues interacting with proteins at different stages of translation opens fundamentally new perspectives, leads to a more precise structural characterization of ribosomal tRNA-binding sites and contributes to the description of tRNA translocations in the ribosome and tRNA functional topography at the single nucleotide resolution.

In this study we have identified tRNA residues directly interacting with ribosomal proteins. This was done, when tRNA was located in A_b- and P_b-sites of the pretranslocated and P_b-sites of the posttranslocated ribosomal complexes.

MATERIALS AND METHODS

70S ribosomes of E. coli MRE 600 (Biolar, Olaine, USSR) were used throughout this work. Monomercurated tRNA^Phe^ was obtained according to (10) using tRNA^Phe^ of E. coli (Boehringer, Mannheim). Aminoacylation of tRNA^Phe^ with [3H]-phenylalanine (Amersham, England, specific radioactivity 137 Ci/mole) and acetylation of Phe-tRNA^Phe^ was carried out according to (11).

The following buffers were used: Buffer A: 10 mM phosphate buffer, pH 7.4; 150 mM NaCl, 3% BSA, 0.05% Tween 20; Buffer B: 10 mM phosphate buffer, pH 7.4; 150 mM NaCl, 0.1% Tween 20; Buffer C: 10 mM phosphate buffer, pH 7.4; 150 mM NaCl, 0.05% Tween 20.

Preparation of pretranslocated complexes tRNA^Phe^ 70S.poly(U).NacPhePhe-tRNA^Phe^-Hg (I) and tRNA^Phe^ Hg.70S.poly(U).NacPhePhe-tRNA^Phe^ (II) as well as the posttranslocated complex NacPhePhe-tRNA^Phe^-Hg.70S.poly(U) (III) (monomercurated tRNA in A_b-, P_b- and P_b-sites, respectively) was described earlier (8). According to composition and functional state of these complexes their homogeneity was better than 80% (cf. (8, 12).
UV-irradiation of complexes (0.5—1.0 nmol of ribosomes) was conducted under a low pressure mercury lamp (254 nm) in Petri dishes with continuous stirring, at +4°C (8). The irradiation time was 10—20 min and the absorbed dose was 10—15 quanta per nucleotide (calculated according to (13), taking into consideration optical density of the solution and the incident UV-light intensity). Under these irradiation conditions dissociation of the complex leading to tRNA release or aminocyl- and peptidyl-tRNA deacylation did not exceed 5%.

Isolation and labelling of tRNA located in definite ribosomal tRNA-binding sites

Monomercurated tRNA (free and cross-linked with proteins) was separated from other components of ribosomal complexes by adsorption to Thiopropyl-Sepharose (19). At this stage after dissociation of UV-irradiated complexes other RNA molecules and proteins, which did not participate in the cross-link formation with mercurated tRNA, were removed. The use of mercurated tRNA is critically important if the starting complex contains more than one tRNA molecule occupying different tRNA-binding sites in the ribosome.

For identification of the protein cross-linked with tRNA and determination of tRNA residue involved in the cross-link the [32P] label was introduced (after deacylation (14)) into the 3'-end of tRNA using [32P]pCp and T4 RNA ligase (15).

Preparation of nitrocellulose-immobilized complexes of individual ribosomal proteins with antibodies (individual immune complexes)

Mixture of polyclonal antibodies against ribosomal proteins (IgG fraction) was prepared at the Institute of Hematology and Blood Transfusion (Moscow) from the serum of rabbits immunized by the standard technique (with Freund’s adjuvant) with a complete mixture of proteins from E. coli 70S ribosomes.

Proteins of 70S ribosomes (400—500 μg) isolated according to (16), were separated by two-dimensional PAGE according to (17). After electrophoresis the separated proteins were transferred electrophoretically onto nitrocellulose (BA83, Schleicher and Schuell) presoaked in 1% Tween-20. The transfer solution contained 10% methanol in 0.7% acetic acid. Proteins transferred to nitrocellulose were stained for 1—2 min with Amido Black (0.5%) in methanol-acetic acid-water (40:10:50). After destaining in the same solution but without the dye protein-containing areas of the nitrocellulose sheet were cut out and placed into the wells (3 ml in volume) of the titration plate. Then 0.5 ml of Buffer A was added to each well.

After the incubation (12 hours at 20°C with continuous stirring) the wells were washed with Buffer B (3 times, 0.5 ml per well) and 0.2 ml of rabbit antibodies against the ribosomal proteins (IgG, 20 mg/ml protein, diluted 1:50 with Buffer A) was added to each well. After incubation for 2—4 hours (at 20°C, with stirring) the liquid was removed and the wells washed three times with 0.5 ml of Buffer C.

In order to determine the amount of bound antibodies, 50 μl of Buffer A containing 1 mg/ml of iodinated [125I] protein A (iodination protocol as in (18), radioactivity did not exceed 10^5 cpm/ml) was added to each well. After removal of the excess protein A by repeated washing (5×1 ml of Buffer B) the radioactivity adsorbed on nitrocellulose in each well was determined.

Serum of rabbits immunized with a mixture of all 70S ribosomal proteins contains sufficient amount of antibodies against all proteins of the small ribosomal subunit (S-proteins) and most proteins of the large ribosomal subunit (L-proteins) (Fig. 1). The immobilized immune complexes are highly specific: binding of a ribosomal protein with the homologous complex (i.e. containing antibodies against this protein) is more than one order of magnitude higher as compared with heterologous controls (see Table 1).

Table 1. Identification of ribosomal proteins, cross-linked with E. coli tRNA^{32P} in ribosomal complexes.

<table>
<thead>
<tr>
<th>Ribosomal complexes</th>
<th>tRNA-binding sites (8)</th>
<th>S5</th>
<th>S7</th>
<th>S9</th>
<th>S10</th>
<th>L2</th>
<th>L5</th>
<th>L27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretranslocated complex (I)</td>
<td>A_b</td>
<td>200</td>
<td>800</td>
<td>56</td>
<td>670</td>
<td>1200</td>
<td>48</td>
<td>1420</td>
</tr>
<tr>
<td>Pretranslocated complex (II)</td>
<td>P_b</td>
<td>180</td>
<td>50</td>
<td>1980</td>
<td>52</td>
<td>1020</td>
<td>580</td>
<td>750</td>
</tr>
<tr>
<td>Posttranslocated complex (III)</td>
<td>P_l</td>
<td>180</td>
<td>53</td>
<td>2100</td>
<td>55</td>
<td>1900</td>
<td>62</td>
<td>1050</td>
</tr>
</tbody>
</table>
Identification of proteins cross-linked to tRNA after UV-irradiation of ribosomal complexes

Aliquots (50 μl) of solution of [32P]-labelled mixture of free and protein-cross-linked mercurated tRNA were added to the wells containing individual immobilized immune complexes. After 1–2 hours of incubation (20°C, with stirring) the wells were rinsed with 0.5 ml of Buffer B 5 times and the radioactivity in each well determined (Table 1).

Isolation of individual cross-linked tRNA–protein complexes

Pieces of nitrocellulose with immune complexes containing tRNA–protein cross-links (detected by [32P] radioactivity), were placed into tubes, to which 100–200 μl of 0.2 M glycine buffer (pH 2.8) was added. After intensive stirring for 10 minutes at 20°C the solution was transferred into clean tubes, adjusted to a neutral pH with NaOH and three volumes of ethyl alcohol was added. The precipitate was collected by centrifugation at 15000 rpm for 10 min and washed with 80% ethanol (3×100 μl).

Identification of tRNA residues cross-linked with proteins

Position in tRNA of nucleoside residues cross-linked with protein was determined as described (14) in the material, isolated by adsorption to immune complexes.

After limited random hydrolysis of phosphodiester bonds the oligonucleotides containing the cross-linked protein may be removed from the mixture by phenol deproteinization. Separation

Figure 2. Radioactivity distribution on the sequencing PAAG after electrophoretic separation of oligonucleotides after statistical cleavage of the phosphodiester bonds in [32P]pCp-labeled original and protein cross-linked E. coli tRNA. A, P, and P, tRNA-protein cross-links, isolated from complexes I, II and III, respectively. tRNA-cross-linked proteins are marked over ladders, C - control (free tRNA).
of the remaining free oligonucleotides in the sequencing gel allows us to determine the distance in nucleotides from the labelled end of tRNA to the nucleotide preceding the one, which is cross-linked with protein (14).

RESULTS

Mercurred tRNA may be selectively introduced into any of ribosomal tRNA-binding sites during preparation of the ribosomal complexes. Using sorption on Thiopropyl-Sepharose we have isolated from irradiated complexes I, II and III individual tRNAs (free and cross-linked to protein), located in A₅₃, P₅₃ and P₇₃ sites, respectively. By this approach we have isolated only those cross-links in which the mercurized base of tRNA is not prevented by cross-linked protein from interacting with Thiopropyl-Sepharose. Obviously, introduction of the label into 3'-end of isolated tRNA is possible only for those molecules in which the cross-linked protein does not interfere with the ligation reaction.

For identification of proteins, cross-linked with tRNA molecules located in A₅₃, P₅₃ and P₇₃-sites of the ribosome (Table 1), as well as for isolation of individual (with respect to tRNA and protein) cross-links we have used immobilized individual immune complexes. Immune complexes containing polyclonal antibodies are preferred for this purpose, since some antigenic determinants of the cross-linked proteins may be modified by irradiation or shielded by the cross-linked tRNA molecule.

The majority of tRNA-protein cross-links (with proteins S₅, S₉, S₁₀, L₂, L₅ and L₂₇ in ribosomal complexes I, II and III) can be determined either by the technique of protein iodination with [¹²⁵I] (8) or after introducing [³²P] label into cross-linked tRNA (Table 1). Cross-link of tRNA with protein S₇ was determined only by the adsorption of [³²P]-labelled material at the site of protein cross-linking with tRNA. Using this approach we have isolated only those cross-links, since this approach not only allows precise identification of the cross-linked protein, but also permits isolation and further analysis of individual (with respect to protein and tRNA) cross-links.

Using monomercurred tRNA and immobilized immune complexes we were able to isolate cross-links, homogeneous in composition and specificity, i.e. containing an individual protein, cross-linked with tRNA, located at a definite site of the ribosomal complex in the defined functional state. This material may, however, be heterogeneous in terms of the position of the intermolecular covalent bond, i.e. position of the cross-linked residue in tRNA chain and/or amino acid residue in the polypeptide.

If the original material contains a mixture of cross-links differing from each other only in the position of protein cross-link within tRNA chain, then more than one set of labelled oligonucleotides will be formed during random hydrolysis. Although the radioactivity of oligonucleotides in each ladder gradually changes depending on their length, such a change is usually small and smooth. But after the electrophoretic separation of a mixture of sets the longest oligonucleotide in each set should be followed by a significant change of radioactivity. The step height (the extent of the radioactivity change between two successive bands) should be proportional to the contribution of the shorter set in the mixture. However, we find only smooth changes of radioactivity along the ladder in all studied cross-links (Fig. 2). Hence, in every individual cross-link only one nucleotide in tRNA is cross-linked (directly interacting) with the corresponding protein in the initial complex (Fig. 2, Table 2).

Thus all these cross-links appear to be homogeneous not only in terms of their composition and specificity, but also in terms of the site of protein cross-linking with tRNA. Using this

Table 2. Residues in the E. coli tRNAs directly interacting with proteins in the ribosomal complexes

<table>
<thead>
<tr>
<th>Proteins, cross-linked with tRNA</th>
<th>A₁₀⁻site</th>
<th>P₅₃⁻ and P₇₃-sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location and type of the base in E. coli tRNA²⁶, cross-linked with the protein</td>
<td>A₂₆</td>
<td>A₉</td>
</tr>
<tr>
<td>The number of residues of the respective type</td>
<td>A</td>
<td>2₄</td>
</tr>
<tr>
<td>in these positions in all E. coli tRNAs</td>
<td>G</td>
<td>1₇</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2₄</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>—</td>
</tr>
</tbody>
</table>

a—Ala 1₆₆₀, 1₆₆₁, 1₆₆₂; Gly 1₆₆₀; Ile 1₆₆₀, 1₆₆₁; Leu 1₆₆₁, 1₆₆₂; Asn 1₆₆₀; Arg 1₆₆₂, 1₆₆₄; Ser 1₆₆₁, 1₆₆₂, 1₆₆₃; Thr 1₆₆₀; Met/Init 1₆₆₀, 1₆₆₁.
b—Gly 1₆₆₀; His 1₆₆₀.
c—Leu 1₆₆₀, 1₆₆₁, 1₆₆₂; Ser 1₆₆₀, 1₆₆₁, 1₆₆₂, 1₆₆₃, 1₆₆₄; Met/Init 1₆₆₀, 1₆₆₁.
d—Glu 1₆₆₀, 1₆₆₁, 1₆₆₂; Gly 1₆₆₂; Gln 1₆₆₀, 1₆₆₁.
e—Tyr 1₆₆₀.
f—Asp 1₆₆₀; Gly 1₆₆₁; Ile 1₆₆₀, 1₆₆₁, 1₆₆₂; Leu 1₆₆₀, 1₆₆₁, 1₆₆₂; Asn 1₆₆₀; Val 1₆₆₀, 1₆₆₁; Trp 1₆₆₀.
g—Cys 1₆₆₀.
h—Ala 1₆₆₀, 1₆₆₁, 1₆₆₂; Phe 1₆₆₀; Gly 1₆₆₀, 1₆₆₂; Val 1₆₆₂; Arg 1₆₆₂.
i—2'-methylguanosine in Ile 1₆₆₂; Leu 1₆₆₀, 1₆₆₁, 1₆₆₂; Met 1₆₆₀; Gln 1₆₆₀, 1₆₆₁; Ser 1₆₆₀, 1₆₆₂, 1₆₆₃, 1₆₆₄; Tyr 1₆₆₀, 1₆₆₁.
j—Ala 1₆₆₀, 1₆₆₁, 1₆₆₂; Val 1₆₆₂.
k—Asp 1₆₆₀; Phe 1₆₆₀; Leu 1₆₆₀; Asn 1₆₆₀; Met/Init 1₆₆₀, 1₆₆₁.
l—U-Ile 1₆₆₁; Arg 1₆₆₃; in 1₇ other tRNAs—D.
m—only in P₇₃ site.
approach we have determined location of all protein cross-links, listed in Table 1 (except for S5, the amount of which was too low for this experiment) (Fig. 2, Table 2).

DISCUSSION

Structural characteristics of ribosomal tRNA-binding sites

Efficiency and accuracy of translation are due to correct positioning of tRNA molecules in the ribosome at every stage of elongation. Earlier it was proposed that the location of tRNA in the ribosome (ribosomal tRNA-binding sites) might be adequately described only in terms of ribosomal location of the codon interacting with tRNA. It has been proposed that ribosomes contain three tRNA-binding sites — A, P (21) and E (22). Furthermore, in addition to codon-anticodon interactions, the existence of specific non-covalent interactions (contacts) of tRNA with ribosomal proteins and rRNA in ribosomal complexes has been shown (12, 23). These contacts reflect mutual orientation of tRNA and ribosomal components and sets of such contacts are actually direct structural characteristics of ribosomal tRNA-binding sites. For tRNA molecules interacting with codons in A and P-sites the set of tRNA—protein contacts depends on the functional state of the complex (30S initiator complex, 70S pre- and posttranslocated complexes etc.) (13). Indeed a change of the functional state of the complex is coupled with changes of conformation of the 30S and 50S subunits (14) and their mutual arrangement in 70S complexes (5). The use of a set of contacts as a fingerprint opens the way to a more detailed classification of tRNA-binding sites, considering the functional state of the complex in addition to codon-anticodon interactions (8). Data about changes of the accessibility of certain rRNA residues after changes of the functional state of the ribosomal complex agree with these ideas (24). But these data most probably reflect not only changes in the rRNA—tRNA interactions but also conformational changes of the ribosomal complex depending on its functional state (cf. (4, 5)). Far more detailed structural characterization of tRNA-binding ribosomal sites is possible when in addition to identification of proteins interacting with tRNA, we have identified tRNA residues involved in these interactions. Thus, although proteins L2 and L27 interact with tRNAs located in both A and P-sites, these points are identical only for Pb- and Pr-sites (L2.C17 and L27.C56, respectively). When tRNA is in the Ap-site these proteins interact with other tRNA residues: U59 and G18, respectively (Table 2). Thus, for tRNA located in the A site the following direct tRNA—protein interactions were detected: A9.S10; G18.L27; A26.S7 and U59.L2; for the Pb-site: C17.L2; G44.L5; C56.L27 and U60.S9; and for Pr-site the set is the same as for Pb-, but without G44.L5.

Rotation of tRNA molecule during transition from A into P site of the ribosome

The translocation of tRNA from A into P-site is coupled with changes in tRNA contacts with L2 and L27 proteins; moreover after the translocation these proteins are involved in the interaction with different tRNA loops (Fig. 3A). Assuming that the location of proteins in 50S subunit is constant, we conclude that the translocation of tRNA from A into P site is associated with the rotation of tRNA around the axis connecting the anticodon with the aminoacyl end of molecule, relatively to the 50S ribosomal subunit. Such rotation was predicted earlier on the basis of the data about tRNA three-dimensional structure (25). This prediction makes use of the finding that the accommodation of two tRNA molecules with CCA ends brought

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Figure 3. Location on the secondary (A) and tertiary (B) structures of E. coli tRNA^Phe^ (tertiary structure is depicted by analogy with that for yeast tRNA^Glu^ (25)) of nucleoside residues which interact directly with proteins in pre- and posttranslocated ribosomal complexes. Squares refer to proteins interacting with tRNA in Ap-ribosomal site, circles — in P sites. The dotted circle shows the protein interacting with tRNA only in Pr-site. Thin lines in A connect residues involved in stabilization of tertiary structure.
that the identification of tRNA residues interacting directly with ribosomal proteins in the elongating ribosome allows us to obtain some insight into the functional topography of elongating E. coli tRNAs.

a. Location of contacts with proteins on the secondary and tertiary structure of E. coli tRNA

At least eight residues interact directly with proteins of both subunits in tRNA, located in A₀, P₀ and Pₜ-sites of ribosome (Table 2). None of these residues is involved in stabilization of secondary tRNA structure: they are located either in C (C17 and G18), T and C (C56, U59 and U60) or variable (G44) loops; another location is between the double-stranded regions (A9 and A26, Fig. 3A). All tRNAs, in spite of differences in their primary structures, have similar secondary and tertiary structures, and probably the tertiary structure of yeast tRNAₚₑ (33-35) is similar to that of E. coli tRNAₚₑ. Five out of eight nucleoside residues of E. coli tRNAₚₑ, for which direct interaction with ribosomal proteins in the elongating complexes was demonstrated, are involved in the formation of tertiary structure (Fig. 3B). By analogy with yeast tRNAₚₑ, three out of four residues interacting with proteins in E. coli tRNAₚₑ located in A₀-site participate in the formation of tertiary structure. Among the residues of E. coli tRNA interacting with proteins in P-sites, residues G44 (only in Pₑ-sites see above) and C56 (both in P₀- and Pₜ-sites) are involved in the formation of tertiary structure. The involvement of residues, interacting with proteins, in tRNA tertiary structure is an argument for hypothesis postulating changes of tRNA tertiary structure during its binding with a ribosome. Such changes, most probably, the straightening of tRNA molecule, may facilitate rotation of tRNA during its transition from A- into P-site of the ribosome.

b. Variation of E. coli tRNA residues contacting with ribosomal proteins during elongation

It is likely that tRNA-protein interactions in each site have been optimized during evolution in order to provide the necessary rate and specificity of translation. In other words, for every translation system (prokaryotic, eukaryotic, mitochondrial) the specificity and strength of these interactions (individual and/or in combination) must be similar for all elongating tRNAs of the system. Keeping this in mind, we can expect that variation of nucleoside residues, interacting with ribosomal proteins, for all tRNA molecules of a given translation system, is allowed only within the limits of some consensus, i.e. such variation should not lead to drastic changes of the overall result of tRNA-protein interactions in any of tRNA-binding sites of the elongating ribosome.

We would like to analyze from this point of view variability of bases in those positions of E. coli tRNA molecules (36) which, according to our data with tRNAₚₑ, interact with proteins in P₁, P₀ and A₀ ribosomal sites.

In P₁- and P₀-sites (peptidyl- and deacylated tRNA in post- and pre-translocated ribosome, respectively) proteins S9, L2 and L27 interact with bases 60, 17 and 56, respectively (Table 2). For all E. coli tRNAs position 56 is invariant, position 60 is strongly conserved and position 17 (if exists) always contains pyrimidines, preferably dihydrouridine. Only position 44 (the beginning of the variable loop) interacting with protein L5 (deacylated tRNAₚₑ in Pₑ-site of the pretranslocated ribosome), may contain any of non-modified nucleic bases (Table 2). Thus, contacts of ribosomal proteins with tRNA in P-sites involve nucleic bases in the invariant or strongly conservative positions.

The reliable data about mutual location of proteins in the ribosome are indispensable for model building (26, 27) and elucidation of the functional role of ribosomal proteins in translation. The data available now were obtained in experiments on isolated subunits (28-30). We should remember that association of subunits and functional changes of the ribosomal complexes are accompanied by conformational changes of subunits and their mutual orientation (4, 5). Therefore any information about mutual location of ribosomal proteins obtained by independent methods and for different functional states of ribosomes is quite useful.

Such information can be derived from identification of tRNA nucleotides directly interacting with proteins in the ribosomal complexes. Thus, close location of contacts with S9 and L27 proteins on tRNA primary structure as well as the identity of these contacts for tRNA in P₀- and Pₜ-sites suggests that these proteins are located closely together in both pre- and post-translocated ribosomal complexes. tRNA contacts in A₀-site with S7 and S10 proteins are close on the secondary structure (Table 2; Fig. 3A), implying that they are close to each other in 30S subunit as well. Contacts of tRNA, located in A and P sites, with L2 and L27 proteins are far from each other on tRNA sequence and located in different loops of the molecule (Table 2; Fig. 3A). Still, tRNA contacts with these proteins on the tertiary structure are very close to each other (Fig. 3B) implying close location of these proteins in 50S subunit of both pre- and posttranslocated complexes. tRNA contacts in A₀-site with S7 and S10 proteins are close on the secondary structure: they are located either in D (C17 and G18), T and C (C56, U59 and U60) or variable (G44) loops; another location is between the double-stranded regions (A9 and A26, Fig. 3A). All tRNAs, in spite of differences in their primary structures, have similar secondary and tertiary structures, and probably the tertiary structure of yeast tRNAₚₑ (33-35) is similar to that of E. coli tRNAₚₑ.

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of E. coli tRNA molecules (except for position 44, typical only of tRNA in P$_{5}$-site).

Protein L27 in case of both P-sites interacts with the invariant residue 56. When tRNA is located in the A$_{5}$-site protein L27 is in contact with residue 18 which is invariant with respect to the base.

When tRNA is located in the A$_{5}$-site (pretranslocated ribosome), residues in positions 9, 18, 26 and 59 are involved in contacts of the peptidyl-tRNA with proteins S10, L27, S7 and L2 respectively (Table 2). Although in positions 9, 26 and 59 of E. coli tRNAs purines are preferred residues (about 85%), variation in these positions is significant. But if the presence of A in all these positions reflects the 'normal' situation, then substitutions are strongly coupled. Thus, only one third of differences from A in any of these three positions is singular (i.e. not associated with non-A in any other position). Replacement of A with G, C or U in position 9 is strongly associated with a modification (2'-O-methylation) of the ribose residue in the base-invariant position 18 (11 out of 13). Thus, single differences from the 'standard' nucleoside residue in position 9, 26 and 59 are found in only about 20%. Thus, although the type of the residue contacting with ribosomal proteins in the A site varies significantly in E. coli tRNA molecules this variation follows a distinct pattern. It is necessary to emphasize that as follows from tRNA tertiary structure, bases in positions 9, 26 and 59 do not interact with each other. On the basis of all this we conclude that the associated or coupled deviations from A in these positions do not result from compensatory (from the point of view of the tertiary structure) mutations. This is in line with the idea of a universal functional topography of E. coli elongating tRNAs.

It is clear that the present discussion is far from being exhaustive in relation to the potential opened up by the experimental data and methodological principles described in this paper. More detailed discussion will be published in other papers of the series. But we want to stress that these results open new opportunities in the investigation of structure and functional features of tRNA and ribosomes by means of site-directed changes in tRNA and/or ribosomal proteins.

REFERENCES


