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REVIEW

Location of Template on the Human Ribosome as Revealed from Data on Cross-Linking with Reactive mRNA Analogs

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Abstract—In this review we summarize data on the location of template on the human ribosome that we obtained from cross-linking (affinity labeling) experiments using reactive mRNA analogs. Types of mRNA analogs, model complexes of these analogs with 80S ribosomes, and methods for analysis of the ribosomal components (proteins and rRNA nucleotides) cross-linked with the mRNA analogs are reviewed. From analysis of the cross-linking data, we suggest a scheme for the arrangement of mRNA on the human ribosome and compare the organization of the mRNA binding center on human and *Escherichia coli* ribosomes.

Key words: human ribosome, mRNA analog, mRNA binding center, affinity cross-linking, ribosomal RNA, ribosomal proteins

Protein synthesis on ribosomes, the final step in the realization of the genetic program of the cell, is based on the translation of information encoded as trinucleotide codons of messenger RNA (mRNA) into the amino acid sequence of the corresponding protein. The key moment in this process is the interaction of the ribosome with the mRNA and transfer RNAs (tRNAs) that carry amino acid residues for the nascent polypeptide chain. The organization of the decoding site, where this interaction takes place, is well studied in the case of the bacterial ribosomes (for a review, see [1]). However, there are only a few papers, with the except for the authors' works, on studying the decoding site of eukaryotic ribosomes. This can be explained not only by the lesser availability of eukaryotic (especially, human) ribosomes compared with prokaryotic ribosomes, but also by the fact that a number of approaches that have been fruitfully used for studying bacterial ribosomes are not applicable for mammalian ribosomes. First, this concerns approaches based on the reconstitution of active ribosomal subunits from the total protein and rRNA, since there is still no methodology for the reconstitution of the eukaryotic ribosomal subunits *in vitro*. Such approaches include site-directed mutagenesis (to determine the importance of particular nucleotides of rRNA for the working of the ribosomal machinery and so on) and site-directed introduction of cross-linking groups into rRNA or proteins (to identify the inter- or intra-subunit contacts). During recent years, X-ray studies of ribosomes from thermophilic bacteria have developed intensively (e.g., see [2]). However, crystals of eukaryotic ribosomal subunits suitable for X-ray analysis are still not available. Thus, the affinity cross-linking (affinity labeling) approach, which was fruitfully applied for studying bacterial ribosomes (for reviews, see [1, 3]), is especially useful for studying the structural and functional topography of eukaryotic, in particular human ribosomes. The approach is based on use of mRNA analogs bearing reactive groups that do not interfere with the formation of the specific complex of the mRNA analog with the ribosome to reveal the ribosomal components contacting mRNA or located near the mRNA binding site. After formation of the cross-links within the complex and identification of the cross-linked ribosomal components, conclusions concerning the structure of the ribosomal mRNA binding center are drawn.

In the present review, we summarize experimental data on the study of the structural and functional topography of human ribosomes with the use of reactive mRNA analogs, derivatives of oligoribonucleotides, and suggest a scheme for the arrangement of mRNA in the region of the decoding site of the human ribosome.

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REACTIVE mRNA ANALOGS USED FOR AFFINITY LABELING OF HUMAN RIBOSOMES

Studies of mRNA binding center of *Escherichia coli* ribosomes using reactive mRNA analogs showed that the cross-linking patterns depend not only on the type of complex (i.e., on the location of the mRNA analog on the ribosome and the functional state of the ribosome), but also on the nature of the cross-linking group [4, 5]. Therefore, complete and reliable information on the arrangement of mRNA on the ribosome requires accumulating and comparing data obtained using mRNA analogs with various cross-linking groups and with various sites of attachment of the groups to the oligonucleotide.

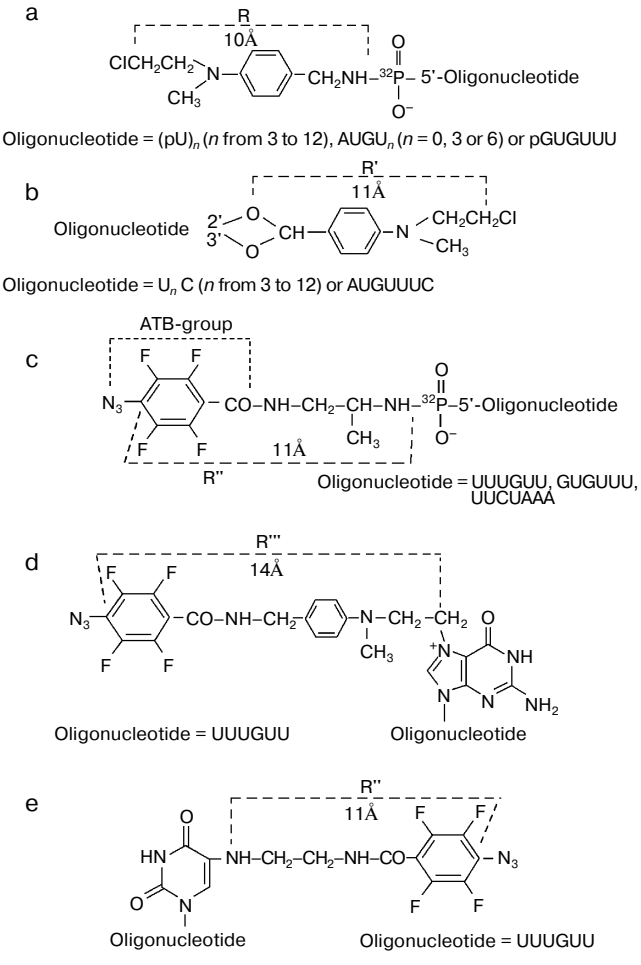

Fig. 1. Derivatives of oligoribonucleotides that have been used for studying human ribosomes: with an alkylating group at either the 5′-phosphate (a) or the 3′-terminal ribose (b) and with a photoactivatable group at the 5′-phosphate (c), atom N7 of the guanine residue (d), or atom C5 of the uridine residue (e).

Derivatives of oligoribonucleotides. In early studies of the structural and functional topography of human ribosomes, we fruitfully used oligoribonucleotides bearing from one to four coding triplets together with an aromatic N-2-chloroethylamine group (RCl or R′Cl, see Fig. 1) attached to either the 5′-terminal phosphate *via* a phosphoramide bond (Fig. 1a) or to the 2′,3′-terminal cis-diol group of the 3′-terminal ribose *via* a benzylidene bond (Fig. 1b), as mRNA analogs. Methods for synthesis of these derivatives have been described in detail [5, 6]. Earlier, use of alkylating derivatives of oligoribonucleotides resulted in substantial progress in the study of *Escherichia coli* ribosomes (for reviews, see [4, 5]). The aromatic N-2-chloroethylamine group has at least two advantages as the reactive moiety of an affinity reagent. First, the chemical selectivity of this group is relatively low, and it can alkylate guanine, adenine, cytosine, and phosphodiester groups of nucleic acids as well as carboxyl-, amino-, mercapto-, and imidazole groups of proteins [7]. The second advantage is the mechanism of the alkylation with aromatic N-2-chloroethylamines. The reaction proceeds *via* the rate-limiting step of formation of the highly reactive intermediate ethylene immonium cation, which subsequently rapidly interacts with nucleophilic centers of the biopolymer as well as with water and nucleophilic buffer components [5, 6]. Hence, when the reagent molecules are converted into the active intermediates outside the complex with the biopolymer, they react with water and low molecular weight buffer components and do not attack the biopolymer if the reagent concentration is not very high. This allows the affinity reagent to be used at relatively high concentrations that do not lead to any substantial unspecific alkylation of the biopolymer.

During recent years, photoactivatable derivatives of oligoribonucleotides bearing the *p*-azidotetrafluorobenzoyl (ATB) group (see Fig. 1, c-e) have been widely used for studying human ribosomes. This group can give a high yield of cross-links with both nucleic acids and proteins upon relatively mild ($270 < \lambda < 380$ nm) UV irradiation [8-10]. To introduce the ATB group in various positions of oligoribonucleotides, a general approach based on the initial insertion of a spacer containing an aliphatic amine group at the designed nucleotide residue is used. Then the ATB group is selectively coupled to the amino group by its acylation with N-hydroxysuccinimide ester of *p*-azidotetrafluorobenzoic acid. The approach for introduction of the spacer depends on the designed site of modification. In the case of the 5′-terminal phosphate (Fig. 1c), the oligomer is condensed with ethylene diamine or propylene diamine in the presence of triphenylphosphine and dipyridyldisulfide (see [6]). To couple the spacer to a guanine residue (Fig. 1d), a principally different approach has been applied. An oligoribonucleotide containing a single guanine residue is alkylated with 4-[(N-2-chloroethyl-N-methylamino)]benzylamine under conditions practically excluding alkylation of the other nucleotide residues [11]. Finally, an approach for

synthesis of derivatives bearing a reactive group at the selected uridine residue (an oligomer can contain other uridines that are unaffected, see Fig. 1e) is based on the use of 5-bromouridine instead of uridine in chemical synthesis of the oligomer at the step of incorporation of the respective residue in the oligonucleotide chain. After termination of the oligoribonucleotide synthesis, a bromine atom is substituted for the $-NHCH_2CH_2NH_2$ group by the reaction with ethylene diamine [12].

mRNA analogs containing 4-thiouridine (s^4U). While alkylating and photoactivatable derivatives of oligoribonucleotides can provide information on the mRNA environment on the ribosome, mRNA analogs containing s^4U residues can form "zero-length" cross-links that reflect direct contacts of mRNA with the ribosome. Synthetic mRNA analogs containing s^4U residues are obtained by *in vitro* transcription using T7 RNA polymerase and specially designed DNA templates. The templates are either DNA fragments containing the RNA polymerase promoter upstream from the sequence to be transcribed (rDNA) [13] or linearized plasmids containing the promoter and the rDNA sequence [14]. The mixture of ribonucleoside triphosphates for the transcription contains s^4UTP together with UTP, which results in the incorporation of the photoreactive s^4U residues into the synthesized mRNA analog together with the U residues [14]. The locations of the s^4U residues in the RNA chain are determined from the rDNA sequence. Three types of s^4U-containing mRNA analogs, each approximately 50 nucleotides long, have been used to study human ribosomes (see Table 1): those with a random distribution of s^4U residues [14]; those with s^4U residues located at either the 3′- or the 5′-terminal region [15], or those with the residues located at position 3′ or 5′ to the coding triplet at the central part of the RNA molecule [15]. Some of the mRNA analogs contained a Shine–Dalgarno sequence at the 3′-ends.

Table 1. Results on cross-linking of synthetic mRNAs substituted for s^4U residues (designations of the mRNAs are as originally appeared in the respective given papers [14, 15]) to 18S rRNA in the 80S ribosomal complexes obtained in the presence of the cognate tRNAs. Triplets used as the codons together with uridine residues substituted for s^4U and positions of these residues on the ribosome upon binding of the codon (in the case of two codons, the first of them) at the P site are marked in the mRNA sequences

Designation	Sequence	Cross-linked nucleotide of 18S rRNA**
mRNA 8'	+20.....+26 5'GGCAGAGCGGCACAGGAGCGCAAC**GGGACC**GCACAGCCGAGAG**U**C**U**G**U**C**U**A3' **Gly Thr**	**U-630**
mRNA 9e'	−20... −16 5'GGCACAC**U**A**U**C**U**GCACAGGAGCGCAAC**GGGACC**GCACAGCCGAGAGCCAGACGA3' **Gly Thr**	**U-630**
mRNA 10	−3 −1 +4 +6 5'GGCAGAGCGGCACAGGAGCGC**U**A**U**GGG**U**G**U**GCACAGCCGAGAGCCAGACGA3' **Gly**	**U-630, U-1111/ A-1112**
mRNA 10c	−3 −1 5'GGCAGAGCGGCACAGGAGCGC**U**A**U**GGGACCGCACAGCCGAGAGCCAGACGA3' **Gly Thr**	**U-1111/ A-1112**
mRNA 10b	+4 +6 5'GGCAGAGCGGCACAGGAGCGCCAC**GGG**U**G**U**G**CACAGCCGAGAGCCAGACGA3' **Gly**	
mRNA 1c**	5'GGGAAAGC**U**C**U**CACGCC**U**CC**U**C**U**A**U**GG**U**C**UUC**U**A**C**U**A**GCC**U**CC**UU**GA**U**CCAGGGA**U**C3' **Phe**	**U-630**, A-1060, U-1046, U-966
mRNA 7*	5'GGGAAAGC**U**CAG**U**GG**U**CG**U**AG**U**CGA**U**G**UGG**U**AG**UU**GCCG**U**CG**UUU**GA**U**CCAGGA**U**C3' **Trp**	**U-630**

* U residues were substituted for s^4U incompletely, but randomly. The s^4U content was from 3.5 to 7 mol per mol of the mRNA.
** Major cross-links are given as bold.

EXPERIMENTAL ASPECTS OF AFFINITY LABELING OF HUMAN RIBOSOMES AND OF ANALYSIS OF RIBOSOMAL COMPONENTS CROSS-LINKED WITH mRNA ANALOGS

Model complexes of ribosomes with mRNA analogs. Cross-linking of mRNA analogs with ribosomes was investigated in model complexes obtained either nonenzymatically (i.e., without translation factors) or using a cell-free protein synthesizing system from rabbit reticulocytes. The nonenzymatic binding of the short (up to 12 nucleotides long) mRNA analogs with 80S ribosomes under optimal conditions (at 20°C and 13 mM Mg^{2+} concentration) practically completely depends on the presence of the cognate tRNA (i.e., is not detected without tRNA [16-20]) and, therefore, takes place only at the decoding site. The source of the tRNA seems not to be of principal importance. The qualitative and quantitative binding properties were practically the same for human and *Escherichia coli* $tRNA^{Phe}$, in the presence of either poly(U) or pUUUUUU as the mRNA analog [21]. This allowed the use of the much more available individual *Escherichia coli* tRNAs instead of the human species to obtain model complexes of 80S ribosomes with mRNA analogs and the cognate tRNAs. The location of codons of the mRNA analog on the ribosome at either A, P, or E sites has been adjusted by the cognate tRNAs (see Fig. 2). The scheme for complex formation was based on the generally accepted idea that tRNAs in all forms have higher affinity to the ribosomal P site in the absence of translation factors [21-23]. The P site binding of N-acetylaminoacyl-tRNA or peptidyl-tRNA in some cases has been confirmed by the puromycin reaction [19]. For the oligo(U) derivatives, definitely fixed location of the mRNA analog on the ribosome in the presence of cognate Phe-$tRNA^{Phe}$ could be achieved only for the derivative of pUUU consisting of the single codon, and for the derivative of pUUUUUU, which contained two UUU triplets. In the first case, codon UUU and the $tRNA^{Phe}$ molecule were bound at the P site (Fig. 3a), and in the second, the codons and the $tRNA^{Phe}$ molecules occupied simultaneously the A and the P sites of the 80S ribosome (Fig. 3b). In the latter case, the peptidyl transfer reaction occurred [17, 21], this resulting in formation of the pre-translocational complex containing deacylated tRNA at the P site and dipeptidyl-tRNA at the A site (Fig. 3b). In the cases of other oligomers bearing only Phe codons, namely, $(pU)_4$, $(Up)_3C$, $(Up)_6C$, $(pU)_{12}$, and $(Up)_{12}C$, indefinite location of the oligoribonucleotide moieties of the reagents at the decoding region was possible. This might result in formation of a heterogeneous mixture of complexes varying in the location of the reactive group on the ribosome.

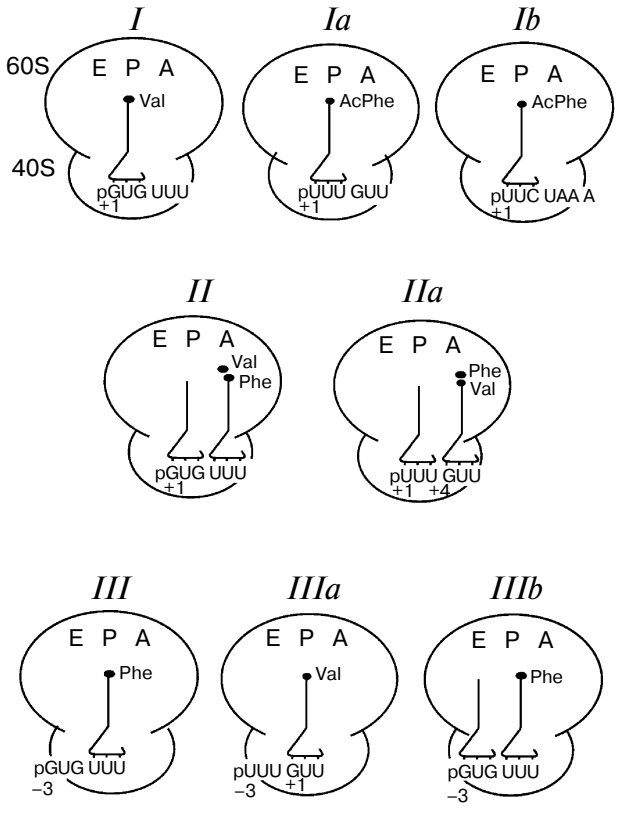

Fig. 2. Types of 80S ribosomal complexes with derivatives of oligoribonucleotides consisting of two different codons. mRNA positions in which the cross-linking groups have been introduced are marked. A, P, and E are the tRNA binding sites.

To obtain 80S ribosomal complexes of derivatives of $AUGU_n$ (n = 0, 3, or 6) bearing an alkylating group at either the 3′- or the 5′-terminus, a cell-free protein synthesizing system from rabbit reticulocytes containing all translation factors and deprived from the endogenous ribosomes and mRNAs has been used [24]. The system was supplied with all components necessary for the translation in a cell-free system. In the cases of the derivatives with n = 3 or 6, stable complexes of the ribosomes with the mRNA analog and MetPhe-$tRNA^{Phe}$ or MetPhePhe-$tRNA^{Phe}$ were formed. The peptidyl-tRNAs were bound at the P sites together with the respective last codon of the mRNA analog (see Fig. 4, a and b). With the derivative of pAUG, a complex presented in Fig. 4c, was formed. The P site binding of the peptidyl-tRNAs was confirmed by the puromycin reaction using labeled amino acids ([^{14}C]phenylalanine or [^{35}S]methionine). Upon puromycin treatment of the complexes, all label associated with the 80S ribosomes was liberated from the ribosomes as peptidyl-puromycin.

Alkylating derivatives of $AUGU_n$ (n = 0 or 3) were also used for studying 40S initiation complex formed by human initiator Met-$tRNA_i^{Met}$, initiation factor eIF-2 from rabbit liver and GTP (Fig. 4, d and e) [25]. The

complexes were obtained under conditions in which factor-independent binding of the mRNA analog was negligible.

The extent of binding of the mRNA analogs with 80S ribosomes in the presence of cognate tRNAs was typically from 0.7 to 0.9 mol/mol 80S ribosomes, with exception for complexes formed by hexaribonucleotides bearing Val codons GUG or GUU and Val-tRNA^{Val} at the P site. In the latter case, the extent of the mRNA analog binding was substantially lower, about 0.2 mol/mol 80S ribosomes. This is explained by the lower affinity of codon GUG (or GUU) in the presence of Val-tRNA^{Val} to the P site of human 80S ribosomes as compared with that of codon UUU (or UUC) in the presence of the tRNA^{Phe} molecule. The extent of binding of the derivatives of AUGU$_n$ in the initiation complex was from 0.06 to 0.2 mol/mol 40S subunits.

The position of modified nucleotides of the mRNA analogs on the ribosome was designated as generally accepted. Position +1 corresponds to the first nucleotide of the P site bound codon, and positions +4 and −3 correspond to the first nucleotides of the A- and E-site bound codons, respectively.

Affinity labeling of ribosomes and analysis of the cross-linked ribosomal subunits. To perform affinity labeling with alkylating mRNA analogs, the respective ribosomal complexes were incubated at 20-25°C for a time corresponding to 3-5 periods of half-conversion of the alkylating group to the ethylene immonium cation (28 or 5 h for the 3′-benzylidene or the 5′-phosphoramide derivatives, respectively [17]). To obtain cross-links with photoreactive mRNA analogs, the respective ribosomal complexes were irradiated with relatively mild UV light ($270 < \lambda < 360$ nm in the case of the perfluoroaryl azide derivatives [11, 19, 20, 26] and $320 < \lambda < 365$ nm in the experiments with s^{4}U-containing mRNA analogs [14, 15]). For the short mRNA analogs, distribution of the cross-linked ^{32}P-labeled reagent between the ribosomal subunits can be easily examined by centrifugation in a sucrose density gradient (10-30%) under conditions of dissociation of the 80S ribosomes into the subunits (at Mg^{2+} concentration decreased to 3 mM and KCl concentration increased to 300-500 mM [11, 16-20, 24-26]). Under these conditions, complexes of short mRNA analogs with the 80S ribosomes and the cognate tRNAs dissociate, hence, radioactive label in the fractions of the subunits indicates covalent attachment of the labeled mRNA analog. In practically all cases, mRNA analogs were cross-linked only to the 40S subunits. The efficiency of the cross-linking of both the alkylating and perfluoroaryl azide derivatives of oligoribonucleotides to the ribosomes was very high; in the majority of the experiments, up to 50-80% of a mRNA analog being bound in the complex became cross-linked to the ribosomes. The described method for analysis of the subunit labeling is not applicable for longer (more than 12-15 nucleotide long) mRNA analogs,

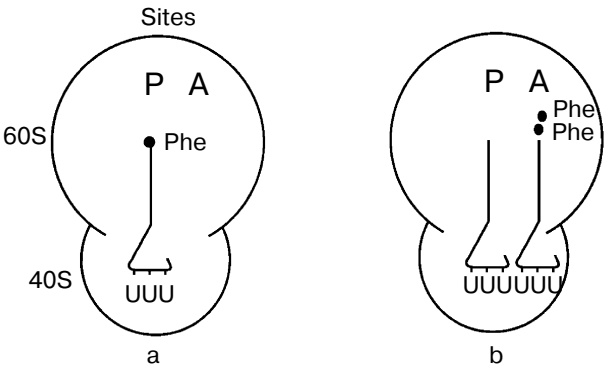

Fig. 3. Types of 80S ribosomal complexes with oligo(U) derivatives bearing either one (a) or two (b) UUU codons. P and E are as in Fig. 2.

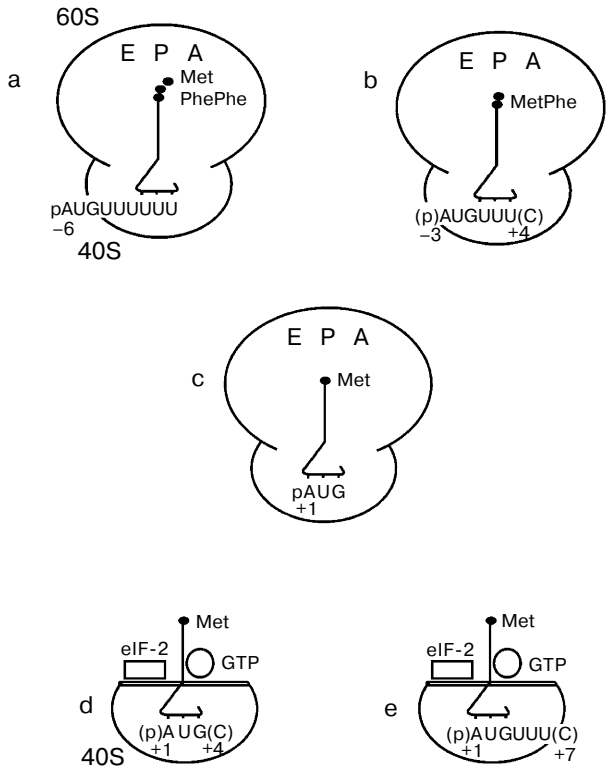

Fig. 4. Types of complexes in which cross-linking of ribosomes with AUG-containing oligoribonucleotide derivatives has been studied: 80S ribosomal complexes formed in a cell-free protein synthesizing system (a, b, c) and 40S initiation complexes (d, e). mRNA positions in which the cross-linking groups have been introduced are marked. A, P, and E are as in Fig. 2.

which remain bound to 40S subunits to a substantial extent even after dissociation of the 80S ribosomes into the subunits.

Short mRNA analogs were cross-linked to the ribosomes in a specific way (i.e., at the decoding site) since these analogs were unable either to bind or to cross-link to human 80S ribosomes without the cognate tRNAs. Longer (about 50 nucleotide long) s^4U-containing mRNA analogs were capable of tRNA-independent binding (and therefore of cross-linking) with 80S ribosomes. In the case of these derivatives, the cross-links occurred, in fact, due to the s^4U residues, since mRNA analogs of the same sequences but containing only U residues were unable to form the cross-links [14, 15].

For analysis of the distribution of the cross-links between the 18S rRNA and proteins, the labeled 40S subunits were denatured by SDS and EDTA with the subsequent separation of the rRNA and proteins by a centrifugation in a sucrose density gradient in the presence of SDS and EDTA [16-19, 24, 25].

Determination of sites of cross-linking of mRNA analogs with 18S rRNA. 18S rRNA sequences containing sites of cross-linking of ^{32}P-labeled mRNA analogs were determined using two alternative approaches. First was based on the blot-hybridization of the cross-linked 18S rRNA with restriction fragments of the corresponding rDNA [16, 24, 25]. The fragments of known length were obtained by hydrolysis of the rDNA with definite restrictases in parallel experiments, then the fragments were separated by PAGE and immobilized on a nylon membrane. Identification of the hybridization signals has been made by a comparison of the lengths of the hybridized fragments with the rDNA restriction map and the sequence of 18S rRNA. In the later reports, another approach has been applied. The cross-linked 18S rRNA was hydrolyzed with RNase H in the presence of deoxy 20-mers complementary to various rRNA sequences (cDNAs) with subsequent separation of the resulting RNA fragments by PAGE. The gels were stained and radioautographed. The staining allowed to detect both parts of 18S rRNA digested in the presence of each cDNA, and autoradiography indicated which fragment contained a site of cross-linking with the labeled mRNA analog [11, 19, 20, 26]. Comparison of the results obtained with various cDNAs allowed to restrict the cross-linking site location within rRNA sequence of several dozen nucleotides long.

The exact identification of the cross-linked 18S rRNA nucleotides was performed by primer extension using the cross-linked 18S rRNA as a template. Primers, typically deoxy-20-mers, were chosen from the results of determination of the fragments containing the cross-linking site (see above). The primer extension makes a stop or pause at the cross-linked nucleotide. The position of the stopping (or pause) site was determined by PAGE analysis of the primer extension products in a denaturing gel, with a parallel analysis of the sequencing and control reaction mixtures. In the sequencing reactions, unmodified 18S rRNA was used; in control experiments, 18S rRNA isolated from unmodified 40S subunits that passed through all the procedures as the modified ones but without mRNA analogs was applied. The cross-linking site was generally assumed to be the nucleotide 5′ to the primer extension stopping site.

Identification of ribosomal proteins cross-linked to mRNA analogs. Labeled proteins were identified using one- and two-dimensional PAGE with subsequent autoradiography of the gels [11, 17-19]. In some cases, either immunoblotting [18] or immunoprecipitation [17] was used. One-dimensional PAGE in the presence of SDS was used to evaluate the molecular masses of the proteins cross-linked to mRNA analogs, and two-dimensional PAGE was used to identify the cross-linked proteins according to their known relative locations in the electrophoregrams. In the majority of experiments, the two-dimensional PAGE was performed in a system in which separation was made in the first dimension at pH 8.3, and in the second dimension in the presence of SDS [11, 17-19]. If the separation in this system did not unequivocally identify the cross-linked proteins, an additional analysis in an alternative electrophoretic system (separation in the first dimension at pH 8.3 and in the second dimension at pH 4.0) was made [11]. Comparing autoradiograms and the stained gels, one has to take into account that the mRNA analog cross-linked to a protein changed the electrophoretic mobility of the protein. The magnitude of a shift of a cross-linked protein with respect to the unmodified protein was evaluated from experiments in which the location of the cross-linked protein in the electrophoregram was confirmed by immunoblotting [18].

RIBOSOMAL COMPONENTS CROSS-LINKED TO DERIVATIVES OF OLIGOURIDYLATES BEARING AN ALKYLATING GROUP AT EITHER THE 3′- OR THE 5′-TERMINUS

The first study on affinity labeling of human ribosomes was performed using a derivative of a hexaribonucleotide bearing UUU triplets coding for phenylalanine and an alkylating group at the 5′-terminus [16]. Using a blot hybridization approach, an 80-nucleotide-long fragment containing the cross-linking site was found in the central domain of 18S rRNA. In further studies, oligouridylates of various lengths with an alkylating group at either the 3′- or the 5′-terminus were used for investigation of the mRNA binding center of human ribosomes. The data are summarized in Table 2. In the case of the 5′-derivatives, increasing the length of the oligo(U) moiety decreased the fraction of the cross-linking to 18S rRNA from 96% (n = 3) to 4% (n = 12) and, accordingly,

increased the fraction of cross-linking to 40S proteins. Protein S26 was the only target of the cross-linking among the proteins for ClR-p(U)$_n$ (n = 6 or 12); in the case of the shorter derivatives, protein labeling was insignificant. Sites of cross-linking of the oligo(U) derivatives with the 18S rRNA also were dependent on the length of the oligo(U) moiety. The derivatives of (pU)$_3$ and p(U)$_4$ were cross-linked with several 18S rRNA nucleotides (mainly, A1023, C1026, C1057, and A1058) whereas the (pU)$_6$ derivative was cross-linked only to nucleotide C1057. The main "jump" in the change in the environment of the 5′-terminus of the mRNA analog is observed in moving from the (pU)$_4$ to the (pU)$_6$ derivative, i.e., on changing the type of complex of the mRNA analog with the 80S ribosomes (see above). These data indicate that appearance of a codon at the A site causes changes in the environment of the 5′-terminal nucleotide of the P site bound codon of the mRNA analog. For ClR-(pU)$_{12}$, three 18S rRNA fragments (593-674, 975-1172, and 1748-1869) containing the cross-linking sites have been found, possibly due to uncertain location of the mRNA analog on the ribosome. In this case, labeling of protein S26 and 18S rRNA within nucleotides 975-1172 probably occurred in the complex where the alkylating group was at position +1, as for the (pU)$_6$ derivative (see Fig. 3b and Table 2). Therefore, 18S rRNA nucleotides in the region 1020-1058 together with protein S26 may be attributed to the environment of mRNA position +1 on the ribosome.

In the case of the 3′-alkylating derivatives, (Up)$_n$C-R′Cl (n = 3, 6, or 12), the distribution of the cross-links between the proteins and the 18S rRNA was less dependent on n than in the case of the 5′-derivatives. As for the cross-linking sites in the 18S rRNA, all three mRNA analogs were cross-linked with the same nucleotides, G1702 and G1763/G1764, although the distribution of the labeling between these nucleotides depended on the length of the oligo(U) moiety of the mRNA analog. For n =

Table 2. Cross-linking of 80S ribosomes with derivatives of oligouridylates (complex types are given in Fig. 3)

mRNA analog	Position of cross-linking group on 80S ribosome	Cross-linking to 40S subunits				Reference
		18S rRNA		proteins		
		relative fraction of labeling, %	site of cross-linking*	relative fraction of labeling, %	cross-linked proteins*	
ClR-pUUU	+1	96	**A-1023, C-1026, C-1057, A-1058,** A-1020, A-1027	4	n.d.**	[17]
ClR-pUUUU	+1/–1	93	**A-1023, C-1026, C-1057, A-1058,** A-1020, A-1027	7	n.d.	[17]
ClR-(pUUU)$_2$	+1	24	C-1057	76	**S26**	[17]
ClR-(pUUU)$_4$	indefinite (from –6 to +1)	4	593-674, 975-1172, 1749-1869	96	**S26**	[17]
UUUC-R'Cl	+3/+4***	80	**G-1763/G-1764**, G-1702	20	**S26**, S3, S3a	[17], [29]
(UUU)$_2$C-R'Cl	+6/+7***	30	G-1763/G-1764, **G-1702**	70	**S3, S3a, S26**	[17], [29]
(UUU)$_4$C-R'Cl	indefinite (from +6 to +13)	35	**G-1763/G-1764**, G-1702	75	**S3, S3a**, S26	[17], [29]

* Major cross-links are given in bold.
** Not determined.
*** Indefinite position of alkylating group is caused by possibility of fixation of the mRNA analog on the ribosome by either the UUU or the UUC codon.

3 or 12, G1763/G1764 were cross-linked predominantly, and G1702 was modified more effectively in the case of n = 6. These data suggest that 18S rRNA nucleotides G1702 and G1763/G1764 are in the environment of the mRNA nucleotides 3′ to the P site bound codon (see Table 2). Among the proteins, S3, S3a, and S26 were cross-linked to the 3′-alkylating oligo(U) derivatives, the distribution of the cross-linking among these proteins being strongly dependent on n. At n = 6 or 12, proteins S3 and S3a were mainly cross-linked, whereas protein S26 was labeled preferentially at n = 3. Evidently, protein S26 is located at the decoding region in such way that it is in the vicinity of mRNA nucleotides 3′ and 5′ to the P site bound codon, and proteins S3 and S3a are placed more closely to the mRNA nucleotides 3′ to the P site bound codon (see Table 2). It should be noted that the results on the protein labeling in human ribosomes with the alkylating oligo(U) derivatives correlate well with the data obtained earlier on 80S ribosomes from rat liver using similar derivatives. The mRNA analogs bearing an alkylating group at the 3′-termini were cross-linked mainly to proteins S3/S3a [27], and 5′-alkylating mRNA analogs modified only protein S26 [28].

Data obtained using the oligo(U) derivatives (see Table 2) showed that the 5′-alkylating derivative of $(pU)_{12}$ was cross-linked to 18S rRNA region 1748-1869 typical for the cross-linking with the 3′-alkylating derivatives (for the 3′-derivatives of UUUC and of $(UUU)_4C$, modification patterns were found in this region of 18S rRNA). Based on these data, one can suggest that mRNA parts 3′ and 5′ to the area of the codon–anticodon interactions are located in the vicinity of each other, i.e., mRNA parts that are not fixed on the ribosome by the codon–anticodon interactions, make a loop or turn. All subsequent data have confirmed this suggestion (see below).

CROSS-LINKS WITH DERIVATIVES OF $AUGU_n$

The use of oligouridylates as mRNA analogs in nonenzymatic systems is significantly limited because their unequivocal location can be achieved only for two types of complexes and only for the derivatives of tri- and hexauridylates (see above). Definitely fixed location of an mRNA analog on the ribosome can be achieved using derivatives that carry the initiator AUG codon (see Fig. 4). Results of the cross-linking of ribosomes with such derivatives are summarized in Table 3.

Cross-linking in the 40S initiation complex 40S·GTP·eIF-2·Met-$tRNA_i^{Met}$·mRNA analog was studied using derivatives of [5′-^{32}P]$pAUGU_n$ and $AUGU_n$[^{32}P]C (n = 0 or 3) that carried an alkylating group at the 5′- or the 3′-terminus, respectively [25]. In all cases, the mRNA analogs were cross-linked to 18S rRNA and 40S proteins to a comparable extent. Fragments of 18S rRNA containing mRNA cross-linking sites were determined by blot-hybridization. For ClR-pAUGUUU, the cross-linking site was located within fragment 976-1057, as in the case of the analogous derivative of pUUUUUU (see Tables 2 and 3). The AUG derivative was cross-linked to the 18S rRNA at least at two sites, the first located in the region mentioned above and second at the adjacent sequence 1058-1164. The latter also correlates with the data on labeling of the 80S ribosomes with ClR-pUUU and ClR-pUUUU (see Table 2). As for the 3′-alkylating derivatives, the main cross-linking site of AUGUUUC-R′Cl was within 18S rRNA positions 593-673, and AUGC-R′Cl had two main cross-linking sites in the 3′-terminal domain of the 18S rRNA, namely, within positions 1610-1747 and 1748-1869. In the latter case, the similarity of the data on labeling of 80S ribosomes with UUUC-R′Cl and that cross-linked to nucleotides G1702 and G1763/G1764 of 18S rRNA [17] is evident. This most probably reflects similarity between the 40S initiation complex formed by AUGC-R′Cl and 80S ribosomal complex containing UUUC-R′Cl and Phe-$tRNA^{Phe}$ at the P site (in both cases, the A site was free both from a codon and tRNA), i.e., mRNA position +4 in the both complexes is arranged in regard to the 18S rRNA similarly. Comparison of the data on cross-linking of the pAUGUUU and pUUUUUU derivatives to 18S rRNA shows that the mRNA position +1 in the 40S initiation complex and in 80S ribosomal complex imitating the pre-translocational state is also located similarly. On the other hand, cross-linking patterns for UUUUUUC-R′Cl (see Table 2) in the 80S ribosomal complex and for AUGUUUC-R′Cl (see Table 3) in the 40S initiation complex differ considerably. This may indicate differences in the environment of the 3′-terminal fragments of the mRNA analogs in the pre-translocational (Fig. 3b) and initiation (Fig. 4e) complexes because in the pre-translocational complex codon at the A site is fixed by the codon–anticodon interaction with cognate tRNA, in contrast to the initiation complex. Besides, in the 80S ribosomal complex with UUUUUUC-R′Cl, the A site might be occupied with the 3′-terminal codon UUC (not UUU) that resulted in the placement of the alkylating group at position +6 rather than +7, as in the case of AUGUUUC-R′Cl. This might be the reason for the differences in the cross-linking patterns for the AUGUUC and UUUUUUC derivatives.

The mRNA analogs AUGUUU[^{32}P]pC-R′Cl and ClR-[^{32}P]$pAUGU_n$ (n = 0, 3, or 6) were used for cross-linking in complexes obtained with the protein synthesizing system from rabbit reticulocytes (see Fig. 4, a, b, c) [24, 29, 30]. For ClR-$pAUGU_n$, the fraction of cross-linking to the 18S rRNA was the highest in the case of the derivative of the trinucleotide (95%); increasing the length of the mRNA analog oligonucleotide moiety strongly decreased the fraction of cross-linking to the 18S rRNA with a concomitant increase in that to the

Table 3. Cross-linking of derivatives of oligoribonucleotides $AUGU_n$ to ribosomes in the 40S initiation complexes and in the 80S ribosomal complexes obtained using a cell-free protein synthesizing system

mRNA analog*	Ribosome or 40S subunit	Position of cross-linking group on ribosome	Cross-linking to 40S subunits				Reference
			18S rRNA		proteins		
			relative fraction of labeling, %	site of cross-linking	relative fraction of labeling, %	cross-linked proteins	
ClR-p**AUG**	40S	+1	69	976-1057, 1058-1164	31	n.d.**	[25]
ClR-p**AUG**UUU	40S	+1	55	976-1057	45	n.d.	[25]
AUGC-R'Cl	40S	+4	40	1610-1747, 1748-1869	60	n.d.	[25]
AUGUUUC-R'Cl	40S	+7	44	593-673	66	n.d.	[25]
ClR-p**AUG**	80S	+1	95	G-1010, G-1029, G-1033, G-1051, G-1054, G-1059	5	n.d.	[24, 30]
ClR-pAUG**UUU**	80S	−3	30	G-1010, G-1029, G-1033, G-1051, G-1054, G-1059	70	S3, S3a	[24, 29, 30]
ClR-pAUGUUU**UUU**	80S	−6	15	593-674, 975-1172, 1748-1869	85	S3, S3a	[24, 29]
AUG**UUU**C-R'Cl	80S	+4	5	n.d.	95	S3a	[24, 29]

* P site bound codons are given in bold.
** Not determined.

40S proteins, as was detected for the analogous oligo(U) derivatives (see Table 2). The derivatives of pAUG and pAUGUUU were cross-linked to the same nucleotides (G1010, G1029, G1033, G1051, G1054, and G1059) in a fragment of 18S rRNA in which cross-linking sites of ClR-pUUU have been found. The fact that an alkylating group at mRNA position +1 (the AUG derivative) and −3 (the AUGUUU derivative) was cross-linked to the same nucleotides of 18S rRNA points out that the rRNA fragment 1010-1059 is in the vicinity of both the mRNA positions, probably closer to position +1 (since the RCl group at position +1 was cross-linked much more effectively than that at position −3, see Table 3). Small divergence in the modification patterns for the derivatives of $pAUGU_n$ ($n = 0$ or 3) and the same derivatives of pUUU and pUUUU that were cross-linked with 18S rRNA nucleotides A1020, A1023, C1026, A1027, A1058, and G1059 (see Table 2), may be explained by the principally different ways of formation of the respective complexes. A relatively large number of the cross-linking sites in both cases probably reflects high flexibility of the mRNA analog in complexes where the A site is free of both codon and tRNA. This suggestion correlates well with the fact that a derivative of $(pUUU)_2$ was cross-linked only to nucleotide C1057 in the complex stabilized by codon–anticodon interaction at both the A and the P sites compared with the multiple labeling of 18S rRNA by the respective derivatives of pUUU and pUUUU [17]. Additional evidence for this suggestion is more selective labeling of 18S rRNA in the 40S initiation complex formed by the pAUGUUU derivative compared with the similar complex formed by the pAUG derivative (see Table 3).

Fragments of the 18S rRNA containing cross-linking sites are the same for ClR-$pAUGU_6$ (alkylating group at position −6, see Table 3) and for ClR-$(pU)_{12}$ (see Table 2). This clarifies the results obtained using ClR-$(pU)_{12}$. In the latter case, labeling of 18S rRNA within nucleotides

593-674 and 1748-1869 most probably occurred in the complexes where the alkylating group was distant from the area of the codon–anticodon interactions (in particular, at position –6). Cross-linking of ClR-pAUGU$_6$ in the region 975-1172 probably occurred in the complexes, where the alkylating group was in position +1 or –3 (i.e., in the small portion of the complexes in which translocation did not occur at all or only one translocation step occurred). As for the labeling of proteins, ClR-pAUGU$_n$ (n = 3 or 6) was cross-linked to proteins S3 and S3a to comparable extent, and AUGUUUC-R′Cl modified protein S3a preferentially.

Comparing the data on cross-linking of 80S ribosomes with ClR-pAUGU$_n$ (n = 3 or 6) and (Up)$_n$C-R′Cl (n = 6 or 12) [29], one can see that both types of derivatives cross-link to the same proteins S3 and S3a. Besides, two of three fragments containing cross-linking sites of the 5′-alkylating derivative of pAUGU$_6$ (as well as of the analogous (pU)$_{12}$ derivative), namely, 593-674 and 1748-1869, are typical for the cross-linking of 3′-alkylating derivatives of AUGC and AUGUUUC in the 40S initiation complexes (see Table 3) and for 3′-alkylating oligo(U) derivatives in the 80S ribosomal complexes (see Table 2). This indicates that mRNA regions 3′ and 5′ to the area of the codon–anticodon interactions are close to the same ribosomal components, and this may also be evidence for the suggestion on the loop or turn of the mRNA in the region of the codon–anticodon interactions (see section above).

It is clearly seen that modification patterns for UUUC-R′Cl (cross-linking mainly to 18S rRNA nucleotides G1702 and G1763/G1764, see Table 2) and for AUGUUUC-R′Cl (cross-linking preferentially to protein S3a, see Table 3) in the complexes that seem to be similar for the position of the alkylating group on the ribosome (UUU codon at the P site, A site free) differ significantly. These differences might be due to the occupation of the P site with the UUU codon in the case of AUGUUUC-R′Cl (resulting in location of the alkylating group at position +4) and with the UUC codon in the case of UUUC-R′Cl (leading to location of the alkylating group at position +3), since the UUC codon may be more preferable than UUU codon for binding of the tRNA^{Phe} molecule in the absence of translation factors (such an assumption has already been made in the above section concerning the results on cross-linking of UUUUUUC-R′Cl to 80S ribosomes). One could not also exclude that the differences in the 80S ribosomal cross-linking patterns for UUUC-R′Cl and for AUGUUUC-R′Cl might be due to the principally different ways of forming the respective complexes. Upon formation of the complex with AUGUUUC-R′Cl, a deacylated tRNA molecule could remain at the E site as a result of the translocation that in turn affected the environment of the P site bound UUU codon.

RIBOSOMAL COMPONENTS CROSS-LINKED TO DERIVATIVES OF OLIGORIBONUCLEOTIDES THAT BEAR TWO CODONS

The use of derivatives of AUGU$_n$, that bear the initiation AUG codon, and of a cell-free protein synthesizing system provides only one type of complex, namely, a complex with the 3′-terminal codon at the P site and the A site free (Fig. 4, a and b). Using mRNA analogs carrying at least two different codons (in particular, triplet GUG or GUU coding for Val and triplet UUU or UUC coding for Phe), one can adjust the location of the mRNA analog at the ribosomal decoding site nonenzymatically by addition of the respective cognate tRNAs, thus obtaining the complexes in which a codon with a modified nucleotide is placed at the designed ribosomal site. Types of model complexes used for studying of cross-linking mRNA analogs to human 80S ribosomes are presented in Fig. 2; the cross-linking results are summarized in Table 4.

Ribosomal environment of the first nucleotide of the P site bound codon (or environment of mRNA position +1). To study cross-links of the first nucleotide of the P site bound codon, three types of complexes have been used (Fig. 2): type I (codon–anticodon interaction only at the P site, there is a codon but no tRNA at the A site); type II (model pre-translocational complex containing deacylated tRNA at the P site and dipeptidyl-tRNA at the A site); type III (codon–anticodon interaction only at the P site, the A site being free of both codon and tRNA). All used photoactivatable mRNA analogs were effectively cross-linked to the 18S rRNA in complexes I, Ia, and Ib, only rRNA nucleotide G1207 being cross-linked independently both on the point of the aryl azide group attachment at the first nucleotide of the mRNA analog (either the 5′-phosphate or the C5 atom of the 5′-terminal uridine) and on the sequence of the mRNA analog (see Table 4). Sets of proteins cross-linked in the type I complex were completely dependent on the nature of the cross-linking group: the 5′-alkylating derivative of pGUGUUU labeled protein S6 (with minor cross-linking to protein S26), whereas the 5′-photoactivable derivative of the same hexaribonucleotide modified protein S2 preferentially. Evidently, both proteins contribute to the environment of a 5′-terminal nucleotide of an mRNA codon at the P site, but the 5′-alkylating group had a more suitable (from the chemical point of view) target among the proximal amino acid residues of protein S6, while the photoreactive group had the preferential target among the residues of protein S2. The mRNA analog pUUU(N$_3$R′′′-G)UU in a type IIIa complex was cross-linked to protein S15 more effectively than with proteins S2 and S6. The differences in the sets of ribosomal proteins cross-linked to mRNA analogs with the reactive group at the 5′-phosphate and at the N7 atom of the guanine seem to be due to the different spatial arrangement of these groups. In the case of derivatives

Table 4. Cross-linking of 80S ribosomes with mRNA analogs consisting of two different codons

mRNA analog*	Type of 80S ribosomal complex (see Fig. 2)	Position of cross-linking group on 80S ribosome	Cross-linking to 40S subunits				Reference
			18S rRNA		proteins		
			relative fraction of labeling, %	site of the cross-linking	relative fraction of labeling, %	cross-linked proteins**	
ClR-p**GUG**UUU	I	+1	45	n.d.***	55	**S6**, S26	[18]
N_3R"-p**GUG**UUU	I	+1	70	G-1207	30	**S2**	[19, 20]
N_3R"-p**UUU**GUU	Ia	+1	50	G-1207	50	n.d.	[20]
N_3R"-p**UUC**UAAA	Ib	+1	45	G-1207	55	n.d.	[20]
p(N_3R"'-**U**)**UU**GUU	Ia	+1	70	G-1207	30	n.d.	[26]
pUUU(N_3R"'-**G**)**UU**	IIIa	+1	30	n.d.	70	**S15**, S2, S6	[11]
ClR-p**GUG**UUU	II (III)****	+1 (−3)****	4	n.d.	96	**S26**, S6	[18]
N_3R"-p**GUG**UUU	II (III)****	+1 (−3)****	12	G-1207	88	**S2, S6**, S26	[19]
N_3R"-p**UUU**GUU	IIa	+1	60	G-1207	40	n.d.	[26]
p(N_3R"-**U**)**UU**GUU	IIa	+1	60	G-1207	40	n.d.	[26]
ClR-pGUG**UUU**	III	−3	3	n.d.	97	**S26**, S6	[18]
N_3R"-pGUG**UUU**	III	−3	8	G-1207	92	**S2, S6, S26**	[19, 20]
N_3R"-pGUG**UUU**	IIIb	−3	6	G-1207	94	**S6, S26**, S30	[19, 20]
p(N_3R"-U)UU**GUU**	IIIa	−3	50	G-961	50	n.d.	[26]
p**UUU**(N_3R"'-G)UU	Ia	+4	70	A-1823, A-1824	30	**S15**, S2, S30	[11, 31, 32]
p**UUU**(N_3R"'-G)UU	IIa	+4	55	A-1823, A-1824	45	**S15**	[11, 31, 32]

* P site bound codons are given in bold.
** Major cross-links are given in bold.
*** Not determined.
**** In fact, complex type III was formed instead of the expected complex type II (correspondingly, the mRNA cross-linking group occurred at position −3 instead of the expected position +1).

of pUUUGUU carrying a photoreactive group at either the 5′-phosphate or atom C5 of the 5′-terminal uridine, appearance of a tRNA molecule at the A site (i.e., moving from complex type Ia to complex type IIa) had practically no effect on the 18S rRNA cross-linking patterns. This implies that the tRNA molecule at the A site does not significantly affect the disposition of the 5′-terminal nucleotide of the P site bound codon with respect to 18S rRNA. It should be noted here that a derivative of pUUUGUU with a photoreactive group at the 5′-phosphate was cross-linked to 18S rRNA nucleotide G1207 in complex II, whereas a 5′-alkylating derivative of $(pUUU)_2$ was cross-linked to another 18S rRNA nucleotide, C1057, in a complex analogous to complex II (see Fig. 2 and Table 2). This difference was probably caused by the different chemical and/or stereochemical preferences of the alkylating and the photoactivatable groups, and the results indicate that both 18S rRNA

nucleotides C1057 and G1207 are in the vicinity of each other and of the mRNA position +1 in complexes resembling the pre-translocational complex (type II).

Here it is worthwhile to point out that in the case of 5′-reactive derivatives of pGUGUUU, moving from complex I to complex II resulted in drastic changes in the ribosomal cross-linking patterns. Moreover, the cross-linking patterns for the type II complexes practically coincided with those for the type III complexes. Apparently, in the case of the GUGUUU derivatives, type III complexes (Phe-tRNA^{Phe} and UUU codon at the P site) were formed instead of the designed type II complexes. The reason for this was probably higher affinity of the UUU (UUC) codon in the presence of tRNA^{Phe} than that of the GUG (GUU) codon in the presence of tRNA^{Val} to the P site of human 80S ribosomes (see the section devoted to the model complexes). This was seen in the low binding extent of the GUGUUU derivatives to the 80S ribosomes in the presence of Val-tRNA^{Val}, and there was evidently equilibrium between tRNA and mRNA analog free and bound in the complex. Addition of Phe-tRNA^{Phe} shifted the equilibrium to formation of the more stable complex containing the UUU codon and Phe-tRNA^{Phe} at the P site, i.e., of complex III.

Environment of the first nucleotide of the A site bound codon (or environment of mRNA position +4). Labeling of 80S ribosomes by a photoreactive group located at mRNA position +4 has been studied using pUUU(N_3R′′′-G)UU in type Ia and IIa complexes (Fig. 2). In both complexes, the targets of the cross-linking were 18S rRNA nucleotides A1823 and A1824 and protein S15 (see Table 4). In the type Ia complex, the cross-linking to nucleotides A1823 and A1824 was much more effective than in the type IIa complex in which tRNA was labeled more strongly than the 40S subunit [31]; besides, in the type Ia complex, in contrast to the type IIa complex, minor labeling of proteins S2 and S30 was observed. Thus, moving from the Ia complex to the IIa complex (i.e., appearance of a tRNA molecule at the A site) resulted in a significant hindrance for contacts of the photoreactive group at mRNA position +4 with 18S rRNA and in complete disappearance of the cross-links with proteins S2 and S30; for all this, no new contacts of the mRNA analog with the ribosome were revealed. Therefore, it can be concluded that the tRNA molecule at the A site shields the 18S rRNA and the proteins from the cross-linking but does not affect noticeably the arrangement of the first nucleotide of the A site bound codon. Taking all this into account, one can attribute 18S rRNA nucleotides A1823 and A1824 together with proteins S15, S2, and S30 to the environment of the first nucleotide of the mRNA codon bound at the A site. Here, major differences should be noted between the results presented above and the data on the environment of mRNA position +4 obtained using alkylating mRNA analogs (see Tables 2 and 3). The differences in the 80S ribosomal cross-linking patterns for pUUU(N_3R′′′-G)UU and UUUC-R′Cl (that labeled nucleotides G1702 and G1763/G1764 together with proteins S26, S3, and S3a, see Table 2) might be caused by the different chemical and/or stereochemical preferences of the perfluoroaryl azide and the alkylating groups as well as by uncertainty of location of the alkylating group of UUUC-R′Cl on the ribosome (positions +3/+4). At the same time, it should be pointed out that nucleotides G1702 and A1823/A1824 are located very close to each other in the secondary structure of 18S rRNA (see Fig. 5). Differences in the cross-linking patterns for pUUU(N_3R′′′-G)UU and AUGUUUC-R′Cl (that labeled protein S3a preferentially, see Table 3) were probably due to principally different ways in which the respective complexes were formed (see above).

Environment of the first nucleotide of the E-site bound codon (or ribosomal environment of mRNA position −3). The ribosomal environment of mRNA position −3 has been studied in type III and IIIa complexes using derivatives of hexaribonucleotides with a photoactivatable group at the 5′-terminal nucleotide. Appearance of a tRNA molecule at the E site (in the case of N_3R′′-pGUGUUU, this corresponds to moving from complex III to complex IIIb) causes a noticeable effect on the protein environment of the first nucleotide of the E site bound codon (see Table 4). The lack of cross-linking to protein S2 in the IIIb complex can be reasonably attributed to the shielding effect of tRNA^{Val}, whereas the appearance of the minor labeling of protein S30 in this complex can be attributed to some change in the mRNA arrangement on the ribosome caused by the tRNA molecule at the E site. The cross-link to proteins S6 and S26 was not dependent on the presence of tRNA at the E site and was observed both for the 5′-alkylating and 5′-photoreactive derivatives. Thus, the protein environment of the first nucleotide of the E site bound codon includes proteins S2, S6, S26, and, if the E site is occupied with tRNA, protein S30. Cross-links between mRNA position −3 and 18S rRNA nucleotides G961 and G1207 imply that these rRNA nucleotides also belong to the environment of the first nucleotide of the E site bound codon. Nevertheless, the cross-link with G1207 was much less effective than those with G961 (compare relative fractions of the cross-linking to 18S rRNA in the type IIIa and IIIb complexes, Table 4) and with G1207 in the case of the modifying group attached to mRNA position +1 (compare relative fractions of the cross-linking to 18S rRNA in the type III and IIIb complexes and in the I, Ia, and Ib complexes, Table 4). Therefore, it can be reasonably suggested that the first nucleotide of the E site bound codon is located in the proximity of 18S nucleotide G961, whereas nucleotide G1207 is placed between mRNA positions −3 and +1, probably closer to position +1 rather than to −3.

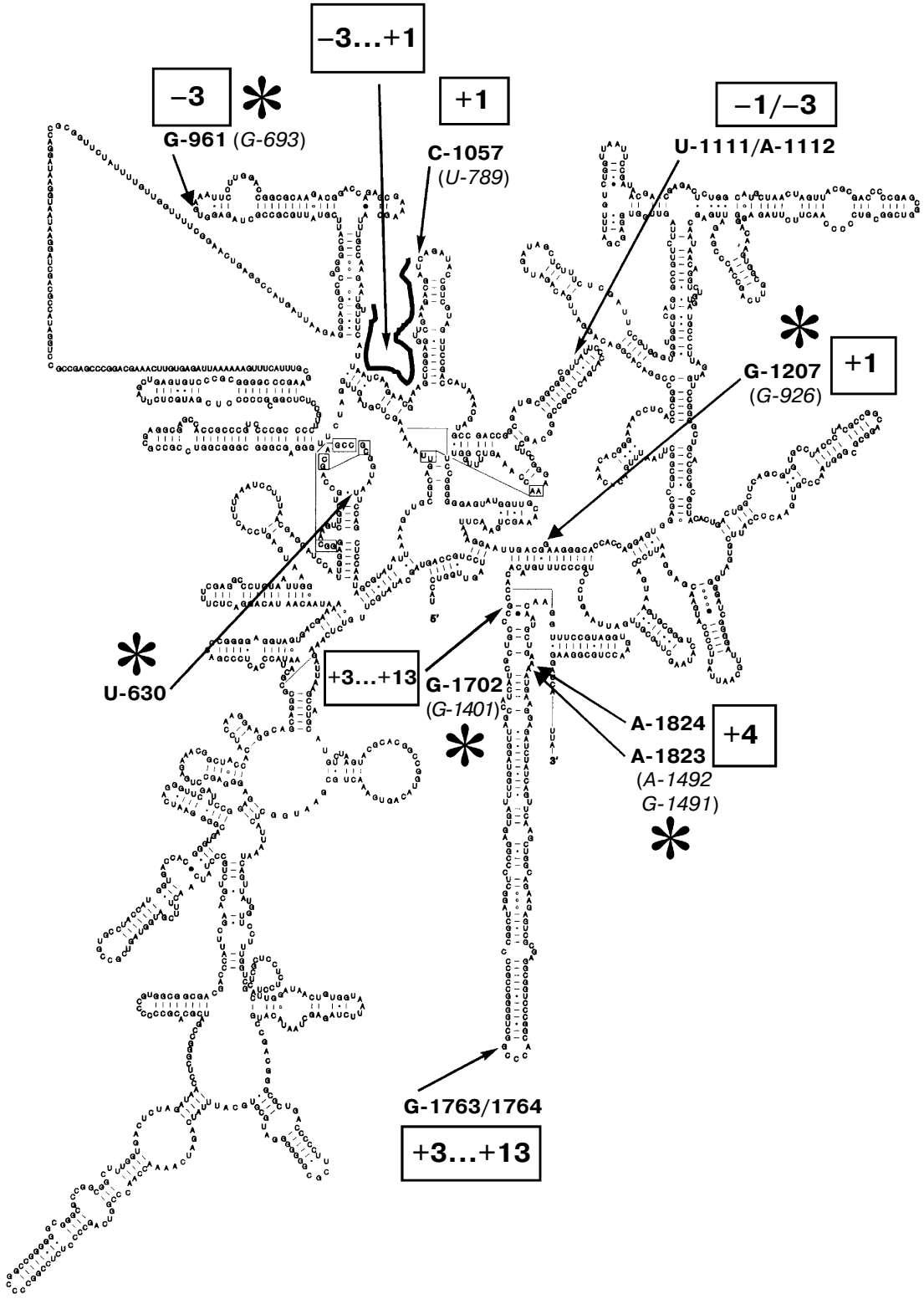

Fig. 5. Secondary structure of human 18S rRNA according to [33]. The cross-linked nucleotides are marked, and the cross-linked mRNA positions are given in boxes. The corresponding nucleotides of *Escherichia coli* 16S rRNA (if there is exact correspondence between the secondary structures of 16S and 18S rRNAs at the site under consideration) are given in brackets. The bold curve shows region 1010-1058 containing the cross-linking site for 5′-alkylating derivatives of pUUU(U) and of pAUGU$_n$ (see Tables 2 and 3). The rRNA nucleotides that have been assigned to the universal elements forming the mRNA binding center of pro- and eukaryotic ribosomes are marked with asterisks.

NUCLEOTIDES OF 18S rRNA CROSS-LINKED TO SYNTHETIC mRNA ANALOGS SUBSTITUTED FOR 4-THIOURIDINES

The data on cross-linking of synthetic mRNA analogs substituted for 4-thiouridines to 18S rRNA in complexes with 80S ribosomes and cognate tRNAs are presented in Table 1. It is seen that mRNA analogs 1c and 7 (having similar 3′- and the 5′-terminal sequences but different sequences in the region of the coding triplets) with s^4U residues distributed practically over the whole RNA sequence were cross-linked mainly to nucleotide U630 of 18S rRNA. In the case of mRNA 1c, also minor cross-linking to 18S rRNA nucleotides A1060, U1046, and U966 was observed. Also, nucleotide U630 was the main target for the cross-linking with s^4U residues placed at the distance of 16 or more nucleotides (either at the 3′- or the 5′-side) from the coding triplets (mRNAs 8′ and 9e′). Somewhat unexpected were results obtained with mRNAs 10, 10c, and 10b (see Table 1). One could expect that if mRNA 10c (s^4U residues at the positions −1 and −3) was cross-linked to 18S rRNA nucleotides U1111/A1112 and mRNA 10 (s^4U residues at the positions −1, −3, +4, and +6) cross-linked to the same nucleotides and additionally to U630, mRNA 10b (s^4U residues at the positions +4 and +6) should cross-link to U630. But the latter mRNA analog did not cross-linked with either U1111/A1112 or U630. The reason for this phenomenon could be that in the case of mRNA 10b, cross-linking to U1111/A1112 occurred initially, and this cross-linking altered the mRNA arrangement at the decoding region facilitating cross-linking to U630. Nucleotides of 18S rRNA cross-linked to the mRNAs containing s^4U residues are within the rRNA fragments where cross-linking sites of the oligoribonucleotide derivatives have been found. Namely, nucleotide U630 is within region 593-674 typical for the cross-linking of 80S ribosomes with derivatives bearing the longest oligoribonucleotide moieties, ClR-$(pU)_{12}$ (see Table 2) and ClR-$pAUGU_6$ (see Table 3). Nucleotides U1111/A1112 cross-linked to s^4U residues at the mRNA positions −1 and −3 are located within the same domain of 18S rRNA as C1057 and G1207 (see Fig. 5). Minor cross-linking sites for mRNA 1c, A1060 and U1046, are very close to the cross-linking sites for ClR-$pAUGU_n$ (see Table 3) and ClR-$(pU)_n$ (see Table 2). It is worth mentioning here that using the s^4U containing mRNA analogs no cross-linking sites were found in the 3′-terminal part of 18S rRNA. In particular, cross-links in this region might be expected for mRNA 10b carrying s^4U residues at positions +4 and +6. Nevertheless, no 18S rRNA cross-linking sites were found for this mRNA analog, although the cross-linking frequency was the same as for the other s^4U containing mRNA analogs. Probably, in the case of mRNA 10b, a cross-link in the 3′-terminal 18S rRNA sequence 1812-1869 took place, but the cross-linking sites could not be determined with the set of primers used [15]. It should be noted that all the mRNA–18S rRNA cross-links were tRNA independent.

The cross-linking data on the s^4U containing mRNA analogs, together with the results on labeling of human ribosomes with the oligoribonucleotide derivatives, indicate that the mRNA track forms a large loop across the ribosome surface. Nucleotide U630 of 18S rRNA seems to be located in a central position on the 40S subunit, as if closing to itself mRNA parts distant from each other.

SIMILARITIES AND DIFFERENCES IN ORGANIZATION OF THE mRNA BINDING CENTER IN HUMAN AND *Escherichia coli* RIBOSOMES

Universally conserved elements of small subunit rRNA that form the mRNA binding center of both pro- and eukaryotic ribosomes. The majority of 18S rRNA nucleotides cross-linked to mRNA analogs are located in the most strongly conserved regions of the small subunit rRNA secondary structure (see Table 5 and Fig. 5). Four of these nucleotides correspond to 16S rRNA nucleotides A1492, G1401, G926, and G693 that had been found at the 30S decoding site; nucleotide U630 almost exactly corresponds to 16S rRNA nucleotide A532 that similarly contacts with mRNA positions distant (either at the 3′ or the 5′-side) from the area of the codon–anticodon interactions. Therefore, one can suggest that the mRNA forms a loop on the 30S and 40S subunit surfaces. The first suggestion of such a loop was made in report [44]; later, the possibility of mRNA looping on the 30S subunit surface was discussed in [13]. Nucleotides G1051, G1054, C1057, A1058, and G1059 of 18S rRNA that were cross-linked to 5′-alkylating derivatives of oligoribonucleotides are placed in the rRNA region which corresponds to the "790 stem-loop" fragment of *Escherichia coli* 16S rRNA. Although no mRNA–16S rRNA cross-links have been found in this region, it is located not far from the decoding site and, according to indirect data, is important for the decoding process (see Table 5). Thus, one can see that in each ribosomal site, A, P, or E site, there are universally conserved nucleotides of the small subunit rRNA that surround mRNA codons in both pro- and eukaryotic ribosomes.

Differences in organization of mRNA binding center in pro- and eukaryotic ribosomes. The main difference observed by comparison of the data on cross-linking of mRNA analogs to pro- and eukaryotic ribosomes is that the cross-linking is more selective in eukaryotic ribosomes as compared to that in the similar model complexes obtained with prokaryotic ribosomes. In eukaryotic ribosomes, cross-linking occurs to a substantially lesser number of ribosomal components. For instance, 12 main sites of cross-linking of 16S rRNA with s^4U residues dis-

Table 5. Comparison of the results on cross-linking of mRNA analogs to 18S rRNA in human ribosomes with the data on the decoding site of *Escherichia coli* ribosomes

mRNA position	Cross-linking site in 18S rRNA	Reference	Corresponding nucleotide of *E. coli* 16S rRNA		
			position	degree of conservation*	relation to decoding site
+4	A-1823	[31, 32]	G-1491	Conserved	
	A-1824		A-1492	Invariant	Probably, forms a hydrogen bond with mRNA position +5 [34]
+3/+4, +6/+7, +12/+13	G-1702	[17]	G-1401	Invariant	Keystone nucleotide at decoding site [35]; G-1402 was cross-linked to mRNA position+4 [36]
	G-1763 G-1764		1450, 1451	Variable	Not found
+1	G-1207	[20, 32]	G-926	Invariant	Was cross-linked to mRNA position +2 [37]
+1	C-1057	[17]	U-789	Conserved	Is located within 25 Å from G-926 and from G-693 [38]
+1, –3	1051-1059	[24, 30]	783-791, ("790 stem-loop")	Conserved	The region was protected against chemical modification by the P site bound tRNA [39] and antibiotics [40]; substitution of G1059 for A leads to inhibition of initiation of translation [41]
	1010-1033		742-764	Conserved	Not found
–1/–3	U-1111/A-1112	[15]	838/839	Variable	Not found
–3	G-961	[26, 32]	G-693	Invariant	Was cross-linked to mRNA positions –1/–3 [13]
–16...–20; +20...+26	U-630	[15]	A-534	Conserved	A-532 was cross-linked to mRNA positions +11...+13 [37, 42], –12...–18, –16...–20, and +20...+26 [13]

* According to [43].

tributed randomly over the whole mRNA sequence were found [45], whereas the same mRNA analogs in similar complexes with human 80S ribosomes were cross-linked mainly to nucleotide U630 of 18S rRNA [14]. Analogously, mRNAs carrying s^4U residues at the defined positions were cross-linked to one or two nucleotides of 18S rRNA in the complexes with 80S ribosomes (see Tables 1 and 5) but to 3-6 nucleotides of 16S rRNA in the 70S ribosomal complexes [13]. Similarly, from 3 to 7 proteins of both 30S and 50S subunits (depending on the complex type) were labeled with 5′-alkylating derivative of $(pU)_6$ [4], whereas in the analogous complexes with human or rat liver 80S ribosomes, the same oligo(U) derivatives were cross-linked only to protein S26 (see [28] and Table 2). All these data indicate that mRNA interacts with mammalian ribosomes by means of a smaller number of molecular contacts than in the case of bacterial ribosomes. Also, cross-linking patterns for *Escherichia coli* ribosomes were very sensitive to relatively small changes in the lengths of the oligo(U) moieties of the reagent or the cross-linking conditions, in contrast to those for eukaryotic ribosomes. For instance, sets of pro-

teins labeled with alkylating derivatives of $(pU)_4$, $(pU)_6$, and $(pU)_7$ in the 70S ribosomal complexes differed significantly from each other [4], whereas in similar experiments on rat [27, 28] or human (Table 2) ribosomes the length of the oligo(U) moiety affected only the extent of labeling of the same protein. These results together with the data on the lower number of molecular contacts of mRNA with the 80S ribosome suggest that the mammalian ribosome is less conformationally flexible than prokaryotic ribosome.

An additional difference in the organization of the mRNA binding center of pro- and eukaryotic ribosomes is concerned with the appearance of 11 additional proteins in the eukaryotic small ribosomal subunit (compared with the prokaryotic case) during evolution. Among proteins labeled with derivatives of oligoribonucleotides in the 70S ribosomal complexes, proteins S3, S4, S5, S7, and S9 were the most frequently found to be cross-linked; these proteins have been assigned to the 70S ribosomal decoding site [4]. The same proteins (with the exception of S9) were cross-linked to synthetic (about 50 nucleotide long) mRNAs [46-48]. Comparison of bacterial and mammalian (in particular, *Escherichia coli* and human) ribosomal proteins can be made to a certain extent conventionally, since the degree of homology between these proteins is rather low, from 20 to 30% in the majority of cases [49]. Under such comparison, *Escherichia coli* ribosomal proteins S3, S4, S5, S7, and S9 may be considered as homologs of the mammalian proteins S3, S9, S2, S5, and S16, respectively. From the latter set of proteins, only S2 and S3 belong to the set of proteins found in the region of the 80S ribosomal decoding site (S2, S3, S3a, S6, S15, and S26) by means of the affinity labeling approach (see Tables 2-4), whereas S3a, S6, and S26 have no homologs among *Escherichia coli* ribosomal proteins at all [49].

Finally, 18S rRNA nucleotides U1111/A1112 that were cross-linked with mRNA positions −1/−3 (see Table 1) might be assigned to the differences in the mRNA arrangement on the human and *Escherichia coli* ribosome. These nucleotides correspond to nucleotides 838/839 in 16S rRNA that are not related to the 70S ribosomal decoding site. However, a cross-link to U1111/A1112 was tRNA independent and, therefore, it possibly did not reflect real differences in arrangements of the mRNA codon at the E site of human and *Escherichia coli* ribosomes.

LOCATION OF TEMPLATE ON THE HUMAN RIBOSOME AS REVEALED FROM DATA ON CROSS-LINKING OF mRNA ANALOGS

Since the structures of mammalian ribosomes are not known at resolution that locates the ribosomal proteins and specific helices of the rRNA, data on cross-linking of mRNA analogs (see Table 5) can be interpreted in terms of the partial atomic structures of the bacterial 30S ribosomal subunit. Thus, the approximate positions of 16S rRNA nucleotides G693, the tip of helix 23 (corresponds to G961 of 18S rRNA); U789, helix 24 (C1057); G926, helix 28 (G1207); and finally, G1401 (G1702), positions 1450-1451 (G1763-G1764) and G1491-A1492 (A1823-A1824) in helix 44 can all be discerned on the crystallographic maps of the *Thermus thermophilus* 30S subunit at a 5.5 Å resolution [50]. Nucleotides G926, G1401, and G1491-A1492 correspond to G1207, G1702, and A1823-A1824, respectively, which were cross-linked preferentially to mRNA positions from +1 to +6/+7, are nicely clustered on the segment of 30S subunit known as the decoding site [50]. Nucleotide G693 (G961) is located relatively far from this cluster, while U789 (C1057) is placed between the cluster and G693 (G961). It should be noted that in a recently published paper [51] where crystallographic maps of 30S subunit at 3 Å resolution are presented, nucleotides G693 and U789 of 16S rRNA are located in this cluster. Nucleotides 1450/1451 (G1763/G1764) lie at the tip of the long penultimate helix 44 very far from the decoding site. Helix 44 is a very well-conserved feature of small subunit rRNAs [33, 43], and it is quite unlikely that it differs substantially in eukaryotes and bacteria. Thus, cross-linking of mRNA positions +3/+4 to G1763/G1764 should be considered as an artifact. The source of the error might be the prolonged incubations at room temperature required for the

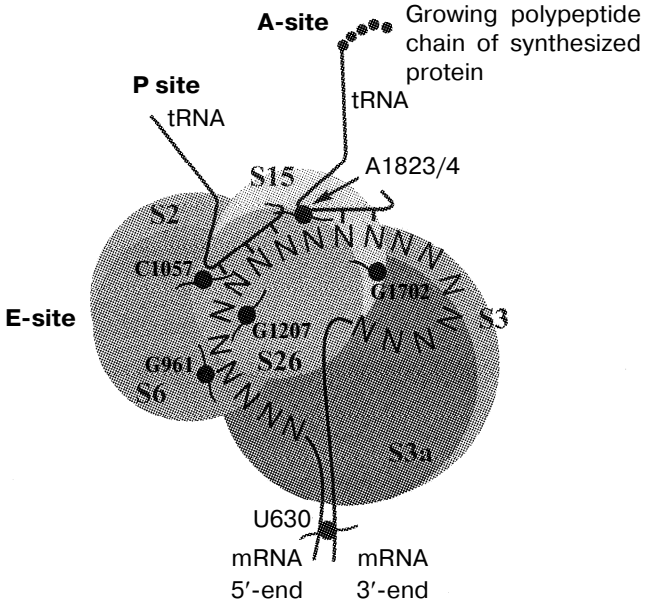

Fig. 6. Scheme for the mutual location of template and structural elements of the 40S subunit in the region of the mRNA binding center of human ribosomes. Large circles, ribosomal proteins; small circles, nucleotides of 18S rRNA.

labeling of 80S ribosomes with the 3′-alkylating mRNA analogs (see [17]), which could lead to partial degradation of the ribosomes and, subsequently, to artifactual cross-linking in the fraction of the degraded ribosomes.

Taken together, the data on cross-linking of human ribosomes with mRNA analogs of various types give an idea on the mRNA arrangement on the human ribosome. According to the scheme presented in Fig. 6, 18S rRNA nucleotide G961 is in the vicinity of mRNA position −3, nucleotide G1207 is closer to mRNA position +1 rather than to −3, and nucleotide C1057 neighbors position +1. Nucleotides A1823 and A1824 are in the proximity of position +4, and nucleotide G1702 is close to positions +6/+7, being also not far from positions +3/+4 and +12/+13. Proteins S3 and S3a are close to mRNA positions from −3 to −6 and from +3 to +13; protein S26 neighbors positions from +1 to +3 and is probably close to several mRNA nucleotides upstream from position +1. Proteins S2 and S6 are in the vicinity of positions from −3 to +1, and protein S15 neighbors mRNA positions from +1 to +4. Finally, nucleotide U630 of 18S rRNA lies remote from the area of the codon–anticodon interactions near the neck of the loop that is made by mRNA across the subunit surface.

Comparison of the data on mRNA arrangement on human ribosomes with the results obtained with *Escherichia coli* ribosomes reveal for the first time both similarities in organization of the mRNA binding center of bacterial and mammalian ribosomes and peculiarities of functioning of the mammalian (in particular, human) ribosomes. The similarity is that the mRNA codons at the decoding site are surrounded by analogous (i.e., located at the same positions of the evolutionary conserved parts of the secondary structure) nucleotides of the small subunit rRNAs. A loop that mRNA makes on the human ribosome seems to exist also in the case of bacterial ribosomes. The foot of this loop is located near the highly conserved fragment "530 stem loop" of the small subunit rRNA. The peculiarities of mammalian ribosomes are related to their lower conformational flexibility compared to the bacterial ribosomes that results in the interaction with the mRNA through a smaller number of molecular contacts in the case of mammalian ribosomes.

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REFERENCES

1. Sergiev, P. V., Lavrik, I. N., Dokudovskaya, S. S., Dontsova, O. A., and Bogdanov, A. A. (1998) *Biochemistry (Moscow)*, **63**, 963-976.
2. Gabashvili, I. S., Agrawal, R. K., Spahn, C. M. T., Grassucci, R. A., Svergun, D. I., Frank, J., and Penczek, P. (2000) *Cell*, **100**, 537-549.
3. Baranov, P. V., Kubarenko, A. V., Gurvich, O. L., Shamolina, T. A., and Brimacombe, R. (1999) *Nucleic Acids Res.*, **27**, 184-185.
4. Vladimirov, S. N., Babkina, G. T., Venijaminova, A. G., Gimautdinova, O. I., Zenkova, M. A., and Karpova, G. G. (1990) *Biochim. Biophys. Acta*, **1048**, 245-256.
5. Karpova, G. G., and Knorre, D. G. (1991) *Uspekhi Biol. Khim.*, **32**, 3-49.
6. Karpova, G. G. (1987) *Izv. SO AN SSSR*, *Ser. Khim. Nauk*, **12**, 82-86.
7. Ross, W. (1964) *Biologically Active Compounds* [in Russian], Meditsina, Moscow, pp. 30, 98, 100.
8. Levina, A. S., Tabatadze, D. R., Dobrikov, M. I., Shishkin, G. V., Khalimskaya, L. M., and Zarytova, V. F. (1996) *Antisense Nucleic Acid Drug Dev.*, **6**, 119-126.
9. Repkova, M. N., Venyaminova, A. G., and Zarytova, V. F. (1997) *Nucleosides & Nucleotides*, **16**(7-9), 1797-1798.
10. Chernolovskaya, E. L., Cherepanov, P. P., Gorozhankin, A. V., Dobrikov, M. I., Vlassov, V. V., and Kobetz, N. D. (1993) *Bioorg. Khim.*, **19**, 889-896.
11. Graifer, D. M., Lyakhovich, A. V., Demeshkina, N. A., Ivanov, A. V., Repkova, M. N., Ven'yaminova, A. G., and Karpova, G. G. (1999) *Mol. Biol. (Moscow)*, **33**, 174-182.
12. Repkova, M. N., Ivanova, T. M., Komarova, N. I., Meschaninova, M. I., Kuznetsova, M. A., and Ven'yaminova, A. G. (1999) *Bioorg. Khim.*, **25**, 690-701.
13. Bhangu, R., Juzumiene, D., and Wollenzien, P. (1994) *Biochemistry*, **33**, 3063-3070.
14. Graifer, D. M., Juzumiene, D. I., Karpova, G. G., and Wollenzien, P. (1994) *Biochemistry*, **33**, 6201-6206.
15. Graifer, D. M., Juzumiene, D. I., Wollenzien, P., and Karpova, G. G. (1994) *Biochemistry*, **33**, 3878-3884.
16. Graifer, D. M., Zenkova, M. A., Malygin, A. A., Mamaev, S. V., Mundus, D. A., and Karpova, G. G. (1990) *J. Mol. Biol.*, **214**, 121-128.
17. Malygin, A. A., Graifer, D. M., Bulygin, K. N., Zenkova, M. A., Yamkovoy, V. I., Stahl, J., and Karpova, G. G. (1994) *Eur. J. Biochem.*, **226**, 715-723.
18. Smolenskaya, I. A., Graifer, D. M., Ivanov, A. V., Vladimirov, S. N., Ven'yaminova, A. G., Repkova, M. N., Stahl, J., and Karpova, G. G. (1997) *Mol. Biol. (Moscow)*, **31**, 144-151.
19. Smolenskaya, I. A., Bulygin, K. N., Graifer, D. M., Ivanov, A. V., Ven'yaminova, A. G., Repkova, M. N., and Karpova, G. G. (1998) *Mol. Biol. (Moscow)*, **32**, 233-241.
20. Bulygin, K. N., Graifer, D. M., Repkova, M. N., Smolenskaya, I. A., Veniyaminova, A. G., and Karpova, G. G. (1997) *RNA*, **3**, 1480-1485.
21. Graifer, D. M., Nekhai, S. Yu., Mundus, D. A., Fedorova, O. S., and Karpova, G. G. (1992) *Biochim. Biophys. Acta*, **1171**, 56-64.
22. Watanabe, S. (1972) *J. Mol. Biol.*, **67**, 443-457.
23. Lill, R., Robertson, J. M., and Wintermeyer, W. (1986) *Biochemistry*, **25**, 3245-3254.
24. Matasova, N. B., Vladimirov, S. N., Bulygin, K. N., Veniyaminova, A. G., Vratskikh, L. V., Mundus, D. A., Repkova, M. N., Yamkovoy, V. I., and Karpova, G. G. (1994) *Mol. Biol. (Moscow)*, **28**, 918-925.

25. Mundus, D. A., Bulygin, K. N., Yamkovoy, V. I., Malygin, A. A., Repkova, M. N., Vratskikh, L. V., Venijaminova, A. G., Vladimirov, S. N., and Karpova, G. G. (1993) *Biochim. Biophys. Acta*, **1173**, 273-282.
26. Graifer, D. M., Demeshkina, N. A., Bulygin, K. N., Repkova, M. N., Ven'yaminova, A. G., and Karpova, G. G. (2000) *Mol. Biol. (Moscow)*, **34**, 270-276.
27. Stahl, J., and Kobetz, N. D. (1981) *FEBS Lett.*, 1**23**, 269-272.
28. Stahl, J., and Karpova, G. G. (1985) *Biomed. Biochim. Acta*, **44**, 1057-1064.
29. Bulygin, K. N., Matasova, N. B., Graifer, D. M., Veniyaminova, A. G., Yamkovoy, V. I., Stahl, J., and Karpova, G. G. (1997) *Biochim. Biophys. Acta*, **1351**, 325-332.
30. Matassova, N. B., Venjaminova, A. G., and Karpova, G. G. (1998) *Biochim. Biophys. Acta*, 1**397**, 231-239.
31. Demeshkina, N. A., Repkova, M. N., Ven'yaminova, A. G., Graifer, D. M., and Karpova, G. G. (2000) *Mol. Biol. (Moscow)*, **34**, 894-898.
32. Demeshkina, N., Repkova, M., Ven'yaminova, A., Graifer, D., and Karpova, G. (2000) *RNA*, **6**, 1727-1736.
33. Gutell, R. (1993) *Nucleic Acids Res.*, **21**, 3051-3054.
34. Yoshizawa, S., Fourmy, D., and Puglisi, J. D. (1999) *Science*, **285**, 1722-1725.
35. Cunningham, P. R., Nurse, K., Weitzmann, C. J., Negre, D., and Ofengand, J. (1992) *Biochemistry*, **31**, 7629-7637.
36. Rinke-Appel, J., Junke, N., Brimacombe, R., Lavrik, I., Dokudovskaya, S., Dontsova, O., and Bogdanov, A. (1994) *Nucleic Acids Res.*, **22**, 3018-3025.
37. Sergiev, P. V., Lavrik, I. N., Wlasoff, V. A., Dokudovskaya, S. S., Dontsova, O. A., Bogdanov, A. A., and Brimacombe, R. (1997) *RNA*, **3**, 464-475.
38. Mundus, D., and Wollenzien, P. (1998) *RNA*, **4**, 1373-1385.
39. Moazed, D., and Noller, H. F. (1990) *J. Mol. Biol.*, **211**, 135-145.
40. Woodcock, J., Moazed, D., Cannon, M., Davies, J., and Noller, H. F. (1991) *EMBO J.*, **10**, 3099-3103.
41. Tapprich, W. E., Goss, D. J., and Dahlberg, A. E. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 4927-4931.
42. Juzumiene, D. I., Shapkina, T. G., and Wollenzien, P. (1995) *J. Biol. Chem.*, **270**, 12794-12800.
43. Neefs, J.-M., van de Peer, Y., Hendriks, L., and DeWachter, R. (1990) *Nucleic Acids Res.*, **18** (Suppl.), 2237-2242.
44. Evstafieva, A. G., Shatsky, I. N., Bogdanov, A. A., Semenkov, Yu. P., and Vasiliev, V. D. (1983) *EMBO J.*, **2**, 799-804.
45. Bhangu, R., and Wollenzien, P. (1992) *Biochemistry*, **31**, 5937-5944.
46. Rinke-Appel, J., Junke, N., Stade, K., and Brimacombe, R. (1991) *EMBO J.*, **10**, 2195-2202.
47. Dontsova, O., Kopylov, A., and Brimacombe, R. (1991) *EMBO J.*, **10**, 2613-2620.
48. Dontsova, O. A., Rosen, K. V., Bogdanova, S. L., Skripkin, E. A., Kopylov, A. M., and Bogdanov, A. A. (1992) *Biochimie*, **74**, 363-371.
49. Wool, I. G., Chan, Y.-L., and Glueck, A. (1996) *Mammalian Ribosomes: the Structure and the Evolution of the Proteins*, in *Translational Control* (Hershey, J. W. B., Matthews, M. B., and Sonenberg, N., eds.) Cold Spring Harbor Laboratory Press, pp. 685-732.
50. Clemons, W. M., May, J. L. C., Wimberly, B. T., McCutcheon, J. P., Capel, M. S., and Ramakrishnan, V. (1999) *Nature*, **400**, 833-840.
51. Wimberly, B. T., Brodersen, D. E., Clemons, W. M., Jr., Morgan-Warren, R. J., Carter, A. P., Vonrhein, C., Hartsch, T., and Ramakrishnan, V. (2000) *Nature*, **407**, 327-339.