mRNA Binding Track in the Human 80S Ribosome for mRNA Analogues Randomly Substituted with 4-Thiouridine Residues†

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ABSTRACT: The interaction between mRNA and 18S rRNA in human 80S ribosomes has been studied using synthetic mRNA analogues randomly substituted with 4-thiouridine, which can be photoactivated for cross-linking. Two mRNA analogues with different sequences have been used for complex formation with ribosomes without or with the presence of a cognate tRNA. Cross-linked 18S rRNA nucleotides were identified by reverse transcription analysis. The base U630 in 18S rRNA was the main target of cross-linking for both of the mRNA analogues studied, and three minor sites of cross-linking, A1060, U1046, and U966, were also identified. Thus, in the case of human 80S ribosomes, the set of nucleotide residues cross-linked to the mRNA analogues is significantly smaller than the twelve sites seen for *Escherichia coli* with these same two mRNA analogues [Bhangu, R., & Wollenzien, P. (1992) *Biochemistry 31*, 5937–5944]. The residue U630 is within a highly conserved region corresponding to the 530 loop region of eubacterial 16S rRNA; the cross-link to this site indicates that it plays a key role in interacting with mRNA on 80S ribosomes independently of the presence of a cognate tRNA at the P site.

A substantial amount of experimental data on the contacts of different translation participants—tRNA, mRNA, and translation factors—with bacterial ribosomes has been accumulated to the present time. According to these data, rRNA plays a significant role in the working of the ribosomal machinery [see Noller (1991)]. rRNA sites contacting different parts of the tRNA, including the 3′-end (Wower et al., 1989), the aminoacyl residue (Barta et al., 1984; Steiner et al., 1988), and the 5′-anticodon base (Prince et al., 1982; Wower et al., 1989), and contacting the synthetic template poly(A) (Stiege et al., 1988) have been determined. Recently, significant progress has been achieved in studying mRNA interactions with ribosomes with the use of photoreactive derivatives of mRNAs bearing 4-thiouridine (s^4U)[1] residues in different positions [e.g., see Stade et al. (1989), Rinke-Appel et al. (1991, 1993), Dontsova et al. (1991, 1992), Wollenzien et al. (1991), Bhangu and Wollenzien (1992), and Bhangu et al. (1994)].

There is much less data on the interaction of mRNA with eukaryotic ribosomes. The first studies involved affinity labeling of human placenta ribosomes with derivatives of short oligoribonucleotide mRNA analogues bearing alkylating groups at either the 3′- or 5′-end (Karpova et al., 1992; Mundus et al., 1993). In the course of these studies 18S rRNA fragments containing sites of attachment of the mRNA analogues within the initiation complex and the pretranslocation complex were identified. For the 5′-oligouridylate derivatives the exact 18S rRNA residues involved in formation of the 5′-side of the codon–anticodon interaction area have been identified recently as A1023, C1026, A1058, and G1059 (Malygin et al., 1993). For the 3′-oligouridylate derivatives the 18S rRNA residues identified exactly so far are G1702 and G1763/G1764 (A. A. Malygin, D. M. Graifer, K. N. Bulygin, M. A. Zenkova, V. I. Yamkovy, J. Stahl, and G. G. Karpova, unpublished data).

It seems attractive to use longer mRNA analogues bearing s^4U residues as photoaffinity probes for labeling of human ribosomes due to the absence of a spacer group between the mRNA and the potential target sites and due to the possibility of examining a larger part of the mRNA track. In addition this approach offers the possibility to insert the reactive groups in the desired positions within the mRNA through reaction with the sulfhydryl group of s^4U. In a previous study mRNA analogues 51–54 nt in length were used that contained s^4U in restricted regions of the 3′, middle, or 5′ third of the RNA; these were able to cross-link to 18S rRNA at positions U630 and U1111/A1112 (Graifer et al., 1994). In the present report we have used two synthetic 56- and 57-nucleotide-long mRNA analogues bearing 16 uridine residues scattered over the whole molecule which are randomly substituted with s^4U during *in vitro* transcription. These two mRNA analogues have purine compositions of 38 and 53%. The purpose of this experiment is to find more of the sites in the 18S rRNA that can be cross-linked by this type of probe even if we do not know their order along the mRNA track. The locations of the RNA cross-linking sites were determined by primer extension experiments. This has allowed us to identify several more 18S rRNA positions that participate in the formation of the mRNA binding channel and to reveal the key role of nucleotide U630 in the interaction with mRNA.

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[1] Abbreviations: EDTA, (ethylenedinitrilo)tetraacetic acid; IF-3, initiation factor 3; nt, nucleotide(s); poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); s^4U, 4-thiouridine; TBE buffer, 89 mM Tris, 89 mM boric acid, and 10 mM EDTA; Tris, 2-amino-2-(hydroxymethyl)-propane-1,3-diol.

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Table 1: Sequences of RNAs Used As mRNA Analogues

mRNA 1c	5′ GGGAAAGCUCUCACGCCUCCUCU,AUG,GUC,UUC,UAC,UAGCCUCCUUGAUCCAGGGAUC 3′ fMet Val Phe Tyr
mRNA 7	5′ GGGAAAGCUCAGUGGUCGUAGUCG,AUG,UGG,UAGUUGCCGUCGUUUGAUCCAGGAUC 3′ Trp fMet Trp

MATERIALS AND METHODS

Materials. Nucleotide triphosphates and dideoxynucleotide triphosphates were purchased from Pharmacia. DNA oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer and were purified by electrophoresis on 12% polyacrylamide–urea gels before use. AMV reverse transcriptase (type 007) was purchased from Life Sciences. T4 polynucleotide kinase was from U.S. Biochemicals. $tRNA^{Trp}$ was purchased from Subriden RNA, and $tRNA^{Phe}$ and glycogen were purchased from Boehringer Mannhein. [γ-^{32}P]-ATP was from Amersham. 40S and 60S ribosomal subunits containing intact ribosomal RNA were isolated from unfrozen human placenta by the method described by Matasova et al. (1991). The subunits were reactivated by incubation at 37 °C for 10 min in binding buffer (13 mM $MgCl_2$, 0.5 mM EDTA, 120 mM KCl, and 20 mM Tris·HCl, pH 7.5) before use. 80S ribosomes were obtained by reassociation of the subunits in the binding buffer at room temperature. Activity of 80S ribosomes in poly(U)-dependent binding of [^{14}C]Phe-$tRNA^{Phe}$ was 80% [binding of 1.6 mol of tRNA/(mol of ribosomes), compared to a maximum binding of 2 mol of tRNA/(mol of ribosomes)].

Preparation of mRNA Analogues. Construction of the plasmids containing the T7 promoter adjacent to suitable synthetic DNA inserts was performed as described by Wollenzien et al. (1991). The plasmid DNAs were cut with nuclease *Bam*HI before use in *in vitro* transcription. *In vitro* transcriptions were performed with ATP, CTP, GTP, and GMP and with UTP or a mixture of UTP and s^{4}UTP as described by Wollenzien et al. (1991). The mRNA analogues were purified on Qiagen columns using conditions supplied by the manufacturer and were labeled at their 5′-termini by [γ-^{32}P]ATP and T4 polynucleotide kinase in the exchange reaction with ADP (Bhangu & Wollenzien, 1992).

Ribosome mRNA Complex Formation and Cross-Linking. Complexes of 80S ribosomes with mRNA analogues and tRNAs (when used) were obtained by incubation of the components in binding buffer at 20 °C for 1 h (Graifer et al., 1990). The reaction mixtures contained 7 pmol of 80S ribosomes, 28 pmol of mRNA, and in some experiments 40 pmol of $tRNA^{Phe}$ or $tRNA^{Trp}$ in a final volume of 30 μL. The samples were irradiated with UV light at 320–365 nm from a high-intensity mercury lamp for 10 min at 20 °C (Isaacs et al., 1977). The intensity of light in this device after filtration with a circulating $Co(NO_3)_2$ solution is about 100 mW/cm^2.

Isolation of RNA from Irradiated and Control Samples. Sodium dodecyl sulfate and EDTA were added to the samples to final concentrations of 1% and 20 mM, respectively. After incubation at 37 °C for 5 min, proteins were removed by phenol extraction (using an equal volume of water-saturated phenol). RNA was precipitated from the water phases by addition of 10 vol of 2% lithium perchlorate in acetone with subsequent centrifugation at room temperature for 5 min at 14000*g*. The RNA pellets were washed with 70% ethanol, dried in vacuum, and redissolved in water at a final concentration of about 0.5 pmol of 18S rRNA/μL. Initial analysis of the mRNA–rRNA cross-links was carried out by electrophoresis in 1% agarose gels in TBE buffer. The gel contained 0.1% ethidium bromide, allowing the detection of the rRNA bands by UV light. Before electrophoresis, the samples, containing about 0.5 pmol of rRNA, were denatured by heating at 45 °C for 10 min in 40% water/60% formamide.

Reverse Transcription Analysis. Primer extension analysis of the mRNA–rRNA cross-links using reverse transcriptase was done as previously described (Wollenzien, 1988). In the current experiments each reaction mixture contained 0.5 pmol of 18S rRNA, 1 pmol of 5′-^{32}P-labeled DNA primer, and 2 units of AMV reverse transcriptase in a final volume of 20 μL. Eight primers (20-mers) were used for 18S rRNA. These primers allowed the examination of most of the 18S rRNA at sequence resolution with the exception of about 150 nucleotides from the 5′-end; in addition, 60 nucleotides from the 3′-terminus of the 18S rRNA were not examined. The complementary sites in the 18S rRNA are 18S-1A, 1812–1831; 18S-2, 1222–1241; 18S-3, 655–674; 18S-10, 1398–1417; 18S-12, 1142–1161; 18S-20, 950–969; 18S-23, 372–391; 18S-27, 1548–1567.

RESULTS

Characteristics of the Synthetic mRNAs. The mRNA analogues for these experiments were synthesized by *in vitro* transcription of linearized plasmid DNAs containing a T7 promoter and the desired DNA sequences. The sequences of the mRNA analogues are presented in Table 1. The mRNA analogue designated 7 (56 nucleotides) was exactly as described before and contains codons for fMet and Trp (Bhangu & Wollenzien, 1992). The mRNA analogue designated 1c (57 nucleotides) is like the molecule 1b described before (Bhangu & Wollenzien, 1992) except for three nucleotide substitutions that remove the similarity to a Shine–Dalgarno sequence upstream from unique codons for fMet, Val, Phe, and Tyr. Each mRNA analogue has 16 uridine residues, which were randomly substituted with s^{4}U during transcription to levels of 3.5–7 mol of s^{4}U/(mol mRNA molecule) to make the mRNA analogues photoreactive (Lemaigre-Debreuil et al., 1991; Wollenzien et al., 1991).

Preparation of Complexes of Ribosomes with mRNA Analogues. Complexes of 80S ribosomes with the mRNAs in the absence or presence of $tRNA^{Phe}$ (in the case of mRNA 1c) and $tRNA^{Trp}$ (in the case of mRNA 7) were formed under the ionic conditions described in Graifer et al. (1990) by the incubation of the components at 20 °C for 1 h and were then irradiated. Under the conditions used in the experiment—4× molar excess of mRNA analogue over 80S ribosomes at a ribosome concentration of 0.23 μM—mRNA analogues of the same length and base composition bind with stoichiometries of 0.8–0.9 per 80S ribosome (unpublished results); this is taken as evidence that for RNA molecules of this length only one is bound to the 80S ribosome. In control experiments, complexes were formed using mRNA analogues of the same sequence containing uridine residues instead of s^{4}U and were then irradiated. 80S ribosomes irradiated without mRNA and samples of the complexes made with photoreactive mRNA analogues that were not irradiated were also used as additional controls.

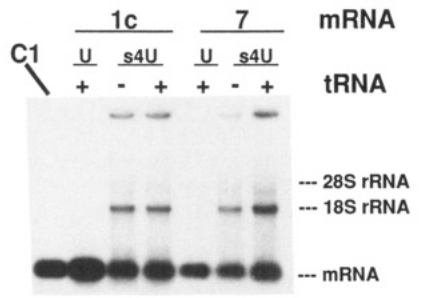

FIGURE 1: Agarose gel electrophoresis of rRNA after the cross-linking experiments (autoradiogram of the dried gel). The mRNA analogues used in each experiment, 1c and 7, and the presence of uridine (U) or 4-thiouridine (s^4U) in the message are indicated. The complexes with 80S ribosomes and the indicated mRNA analogues were formed with cognate tRNAs as indicated; for mRNA 1c, $tRNA^{Phe}$ was used, and for mRNA 7, $tRNA^{Trp}$ was used. The samples in all six lanes were irradiated with UV light. Lane C1 contains RNA from complexes containing 80S ribosomes, s^4U-containing mRNA 1c, and $tRNA^{Phe}$ which was not irradiated. The locations of 28S rRNA, 18S rRNA, and mRNA as seen by ethidium fluorescence are indicated to the right of the gel.

The level of ^{32}P-labeled mRNA cross-linked to rRNA was determined by agarose gel electrophoresis. The RNA samples were denatured prior to the analysis by heating in 60% formamide and were then applied to the gel. A fraction of the radioactive mRNA comigrated with 18S and 28S rRNAs only when thiolated mRNAs were used (Figure 1). In all cases mRNAs not containing s^4U did not comigrate with the rRNA bands at levels above background. Thus the presence of the label in the RNA bands indicates covalent cross-linking of the labeled mRNAs due to their s^4U residues. Levels of the cross-linking were evaluated by scanning of the autoradiograms and were expressed as the percentage of the rRNA being cross-linked to the mRNA analogue. The levels depended on the mRNA sequence; for the 18S rRNA these were 12% for mRNA 1c and 23% for mRNA 7. The presence of the cognate tRNA in the complexes did not reproducibly alter the frequencies of the cross-linking.

Localization of the Sites of mRNAs Cross-Linking to 18S rRNA. The sites of cross-linking in 18S rRNA were analyzed by reverse transcription elongation patterns obtained using eight different DNA oligonucleotide primers (20-mers). The primers were initially tested for their ability to direct reverse transcription on unmodified 18S rRNA. Comparison of the sequences obtained with the primers with the known 18S rRNA sequence reported by Maden et al. (1987) showed that all of the primers formed duplexes at their intended 18S rRNA target sequences.

The basis of the assay is that the reverse transcriptase does not transcribe through modified nucleotides and hence generates stronger than normal stops at sites of cross-linking compared to the control samples. The locations of these modified nucleotides can then be identified by gel electrophoresis of the reverse transcripts in parallel with lanes containing sequencing and control reactions. Experimental stops noticeably stronger than those in the corresponding control lanes reflect the presence of a cross-linking site. In the current experiments all cross-linking sites were observed in two or three independent experiments. It should be mentioned that most of the nucleotide positions show some degree of nonspecific reverse transcription stopping due to natural stops of reverse transcriptase or to some damage of the rRNA preparation. No detectable UV-induced changes in 18S rRNA isolated from 80S ribosomes irradiated without mRNAs were observed (data not shown).

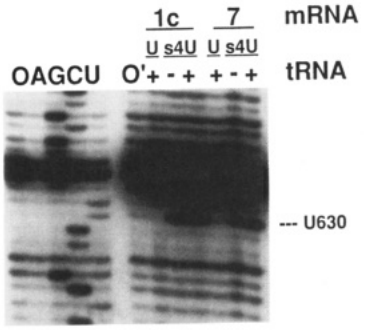

FIGURE 2: Reverse transcription analysis of mRNA cross-linking to 18S rRNA in the region 638–621. This region was examined using ^{32}P-labeled primer 18S-3 complementary to nucleotides 655–674 in the 18S rRNA. Samples from cross-linking experiments prepared with mRNA 1c and 7 containing uridine (U) or s^4U are indicated. The complexes that included cognate tRNA ($tRNA^{Phe}$ and $tRNA^{Trp}$ for mRNA 1c and 7, respectively) are indicated by a +. The control lane, marked O′, was prepared from a sample of 80S ribosomes irradiated without mRNA and tRNA. All samples on the right part of the gel were irradiated before purification of the RNA. The control and sequencing lanes on the left part of the gel were made with RNA that had not been irradiated. The band indicating the mRNA cross-link at U630 is indicated to the right of the gel.

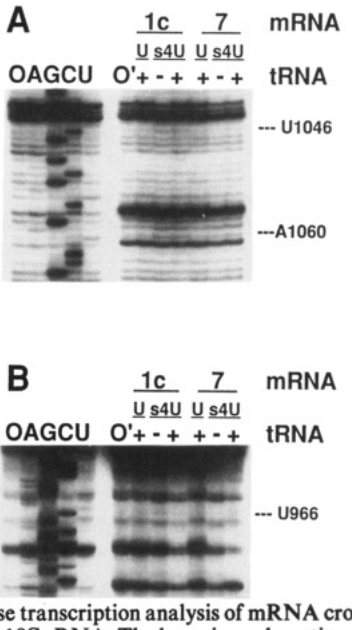

FIGURE 3: Reverse transcription analysis of mRNA cross-linking in two regions of the 18S rRNA. The lanes in each section of the figure are identified as described in the legend to Figure 2. In panel A, reverse transcription in the 18S rRNA from 1066 to 1041 is shown. In panel B, reverse transcription in the 18S rRNA from 992 to 950 is shown. ^{32}P-labeled primer 18S-12A complementary to the 18S rRNA sequence interval 1142–1160 was used for the analysis of both of these regions. The three sites indicated to the right of the gels for mRNA 1c were identified on the basis of differences in band intensity between the experimental and control lanes; all three of these sites have been seen in at least three independent experiments.

Autoradiograms showing the patterns of the reverse transcription assays are shown in Figures 2 and 3. The actual cross-linking point on the rRNA must be the nucleoside on the 5′-side of the reverse transcription stopping site (Wollenzien et al., 1991). For both mRNA analogues, U630 occurs as the single prominent cross-linking site. 18S rRNA positions A1060, U1046, and U966 occur as minor cross-linking sites for mRNA analogue 1c but not for mRNA analogue 7. All of these cross-links occur independently of the presence of the cognate tRNAs. The rest of the 18S rRNA has been inspected by reverse transcription analysis except for the last 60 nt in the 3′-terminal region, and no additional cross-links were

Table 2: Intensity of Reverse Transcription Stops at Cross-Linking Sites[a]

18S rRNA site	mRNA 1c		mRNA 7	
	without $tRNA^{Phe}$	with $tRNA^{Phe}$	without $tRNA^{Trp}$	with $tRNA^{Trp}$
U630	16 ± 5	18 ± 10	12 ± 5	21 ± 10
A1060	2.1 ± 0.5	4 ± 2	1.1 ± 0.1	0.9 ± 0.2
U1046	2.1 ± 0.5	1.8 ± 0.5	1.0 ± 0.2	1.1 ± 0.2
U966	1.5 ± 0.4	1.6 ± 0.4	1.0 ± 0.3	1.0 ± 0.1

[a] Values indicate the ratio of the intensity (absorbance times length) of each cross-link band to that of the control band at the same position. The intensities of bands in different lanes were normalized by comparison with the intensity of background bands to remove differences arising from the loading in each lane. Values are averages of two independent experiments; the ranges in values are indicated. Underlined entries were selected as positions of cross-linking by visual inspection before the densitometry was performed.

found. In particular, the regions of 18S rRNA corresponding to those where cross-links are seen in *Escherichia coli* 16S rRNA have been inspected at least three times in independent cross-linking and reverse transcription experiments without finding additional cross-links.

Table 2 indicates the intensities of the sites determined by densitometry that were identified first by visual inspection of the autoradiograms. The site U630 appears at an intensity 10–20 times that of the corresponding band in the control lanes, and the minor cross-linking sites appear at intensities 1.5–4 times those of corresponding control bands. These measurements are meant to indicate the relative strengths of the stops and may not actually reflect cross-linking frequency since the reverse transcriptase may not stop equally well at all types of s^4U-induced cross-links.

DISCUSSION

Arrangement of mRNA Cross-Linking Sites in Human 18S rRNA. The system of reverse transcription analysis on 18S rRNA isolated from human placenta ribosomes seems to be appropriate for the identification of cross-linking sites in this rRNA. The extent of the rRNA intactness in the purified ribosomal subunits and its relative stability in the course of the cross-linking and analysis procedures allowed us to identify the cross-linked residues in the 18S rRNA with the same precision as was obtained earlier on the system from *E. coli* (Wollenzien et al., 1991; Bhangu & Wollenzien, 1992). For human 18S rRNA, as for *E. coli* 16S rRNA, there are many positions that are partial termination sites for the reverse transcriptase, and this results in heavy banding at these sites, making it difficult to assess cross-linking. Therefore it is possible that there are some cross-linking sites that we do not detect by the present procedure alone.

One major cross-linking site (U630) and three minor cross-linking sites (A1060, U1046, and U966) in 18S rRNA have been detected in these experiments. These sites are shown on the 18S rRNA secondary structure in Figure 4. The mRNA analogues used have different sequence environments around the coding triplets but have similar sequences in their 3′ and 5′ parts. Therefore, if the patterns of contacts were sequence dependent, there should be differences in the corresponding cross-linking patterns. The results obtained indicate that in our case the sequences of the mRNA analogues do not affect their contacts with the 18S rRNA at position U630. On the other hand, the minor cross-linking sites at A1060, U1046, and U966 occur with mRNA analogue 1c but are not detectable with mRNA analogue 7. Cross-linking to position U630 was also detected in a previous study using a set of mRNA analgoues with s^4U in restricted regions of the RNA molecules, but there was no indication of cross-linking to the minor sites we detect here (Graifer et al., 1994). In addition, cross-linking did occur to another position (U1111/A1112) with mRNA analogues containing s^4U in the interval −1 to −3 with respect to the first nucleotide in the codon. s^4U should be present within this interval in the mRNA analogues 1c and 7; we do not know the reason for our failure to see this cross-link in the present experiments.

It is worthwhile to compare this result with the data obtained earlier with the same mRNA analogues on *E. coli* ribosomes (Wollenzien et al., 1991; Bhangu & Wollenzien, 1992). Some similarities may be observed. First, with *E. coli* ribosomes the majority of the cross-links do not depend on the presence of a cognate tRNA, as is the case here for human ribosomes. Second, the cross-linking occurs to a much greater extent to the small-subunit rRNA than to the large-subunit rRNA. The differences in the results obtained with the same mRNA analogues on *E. coli* and human ribosomes may be assigned mostly to the lower frequency of cross-linking of mRNA to rRNA in human ribosomes and the smaller number of rRNA cross-linking sites. In contrast to the results of the present study, in the case of *E. coli* ribosomes, 12 mRNA–16S rRNA cross-links have been determined (Bhangu & Wollenzien, 1992).

To compare the patterns of cross-linking sites in human and *E. coli* ribosomes, one has to find nucleotide residues in 16S rRNA corresponding to the cross-linking sites in 18S rRNA found here. Given the alignment of the primary and secondary structures, U630 (the main site of cross-linking in 18S rRNA) and minor sites A1060, U1046, and U966 correspond to 16S rRNA residues A534, A792, G778, and G697, respectively (Gutell, 1993). Previously, residues A532 and G693 were found as cross-linking sites for the majority of the mRNA analogues in *E. coli* (Bhangu & Wollenzien, 1992), and it is likely that the cross-linking sites U630 and U966 represent the same interactions in human ribosomes even though the positions do not match exactly (compare positions A532 *vs* A534 and G693 *vs* G697, all in the *E. coli* numbering system). It is surprising that mRNA 7 was the only one of six mRNA analogues studied that was unable to form a cross-link with A532 in *E. coli* ribosomes (Bhangu & Wollenzien, 1992); the same behavior is not seen in human ribosomes.

The cross-links made by s^4U-containing mRNA analogues at A1060 and U1046 in human 18S rRNA do not have any direct correspondence to cross-linking sites made with the same reagents in *E. coli* ribosomes. In *E. coli* these sites are in the stem–loop found in the interval 769–810 [helix 24 in the numbering system of Brimacombe et al. (1988)]. However, for *E. coli* there is other evidence linking this region to the mRNA track. Residues A794 and C795 are protected from chemical modification by tRNA in the P site (Moazed & Noller, 1986, 1990), and site-directed mutation of G791 to A altered the interaction of IF3 with the subunit and altered the association of the 30S and 50S ribosomal subunits (Tapprich et al., 1989; Dahlberg, 1989). Thus this region is very important in mRNA functions in *E. coli* 16S rRNA even though mRNA cross-links have not yet been found in it.

The results of the present study may be compared also with the data obtained recently on affinity labeling of human ribosomes with other mRNA analogues—short oligoribonucleotides bearing alkylating groups [((4-(*N*-(2-chloroethyl)-*N*-methylamino)benzyl)methyl)phosphamide derivative or 2′,3′-*O*-(4-(*N*-(2-chloroethyl)-N-methylamino)benzyl-

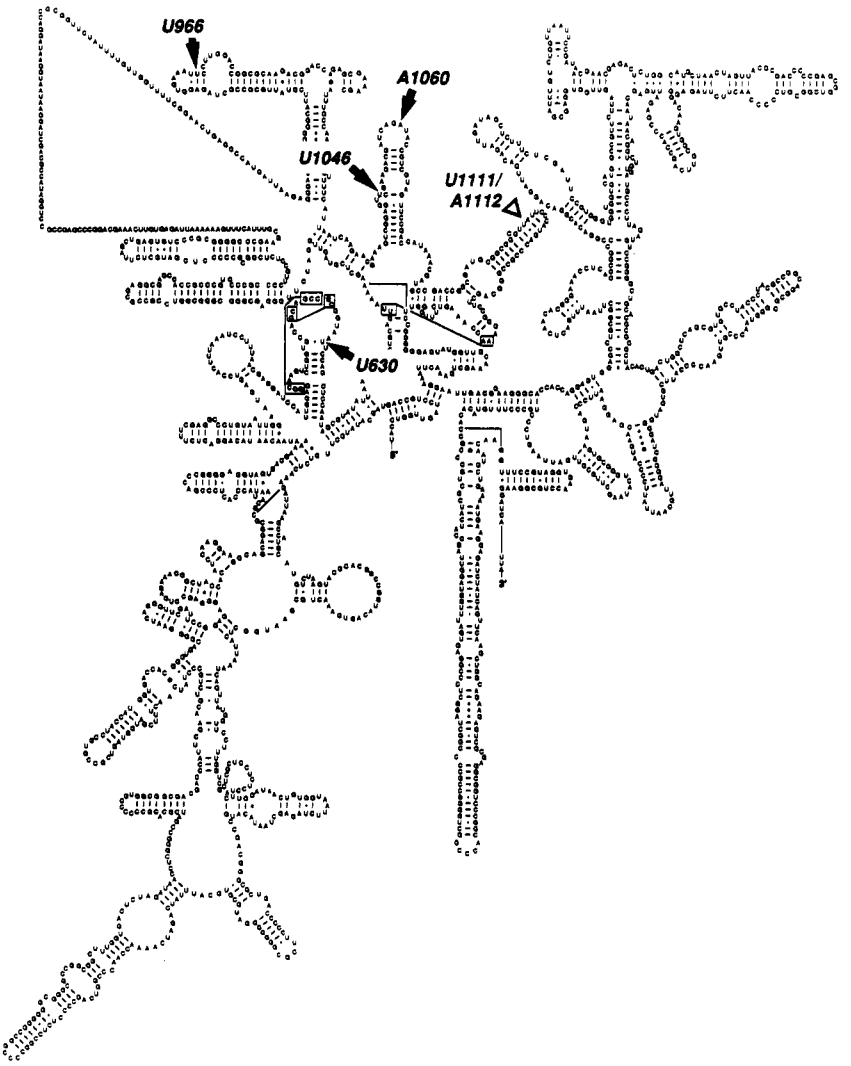

FIGURE 4: Schematic representation of cross-linking sites in human 18S rRNA. The sites identified in the present study are indicated by arrows and nucleotide numbers. The site U630 and an additional site, U1111/A1112 (indicated by the open triangle), were detected in a previous study with s^4U-containing mRNA analogues with s^4U in restricted regions of the RNA. The 18S rRNA secondary structure is that proposed by Gutell (1993).

idene) derivative, with spacer lengths of ~12 Å] at either the 5′- or 3′-end (Karpova et al., 1992). Because of the short length of these reagents, labeling must occur in the vicinity of the decoding area. The derivatives of tri- and tetrauridylates with alkylating groups at their 5′-ends were cross-linked to 18S nucleotides A1023, C1026, A1058, and G1059 in the presence of Phe-tRNA^{Phe}, whereas the derivative of hexauridylate fixed by the codon–anticodon interaction at both A and P sites modified only nucleotide A1058 (Malygin et al., 1993). This indicates the effect of tRNA binding at the A site on the arrangement of the template in the region of the codon–anticodon interaction. Two of the minor cross-linking sites found in the present study—A1060 and U1046—are very close to the mentioned region. In the case of the derivatives of oligouridylates with the alkylating group at the 3′-end, the cross-linking sites were found within 18S rRNA fragment 1610–1869 (the 3′-end of the rRNA molecule). Recently the exact identification of these sites has been made: the nucleotides G1702 and G1763/G1764 were identified as being cross-linked in this case (A. A. Malygin, D. M. Graifer, K. N. Bulygin, M. A. Zenkova, V. I. Yamkovy, J. Stahl, and G. G. Karpova, unpublished data).

Finally, the labeling of 18S rRNA within the highly conserved region 593–673 was observed in studies of affinity labeling of human ribosomes within the 40S initiation complex by a derivative of AUGUUUC with the alkylating group at the 3′-end. The same chemical derivative of the oligoribonucleotide AUGC modified only the region 1610–1869 (Mundus et al., 1993). These results allow us to suggest that 18S rRNA nucleotide U630 interacts with mRNA somewhat away from mRNA coding triplets at a distance greater than 12 Å from nucleotide +4 and less than 12 Å from nucleotide +7, where the nucleotides are counted from the first position in the codon used in the P site. This identification is consistent with the localization of the 16S rRNA residue A532 at positions 10–13 (Dontsova et al., 1992; Rinke-Appel et al., 1993). However, the relationship of the U630 site to the mRNA track may be somewhat more complicated than a single interaction site since the region 563–673 is also cross-linked by an alkylating reagent attached to nucleotide –6 with respect to the codon in the P site (Matasova et al., 1994). In addition, U630 is cross-linked by s^4U-containing mRNA analogues with s^4U in the interval 20–26 and in the interval from –16 to –20 (Graifer et al., 1994). These data suggest that there may be several sites at which the U630 site and the mRNA track are close.

In bacterial ribosomes the region represented by nucleotide A532 is one of a number of mRNA–rRNA contacts, but in human ribosomes the contact in this region is the main one. Hence, the contact of mRNA with residue A532 and, in the

case of human ribosomes, with the corresponding base U630 located in a highly conserved part of the small subunit rRNA secondary structure seems to be a universal element of the mRNA binding channel in the ribosome. Thus several points of evidence suggest that this interaction must play a key role in mRNA binding. The mentioned region of 16S rRNA was proposed as part of the proofreading site (Moazed & Noller, 1986) and part of an mRNA frame-monitoring mechanism together with the C1400 region (Trifonov, 1987, 1992).

The fact of higher selectivity of affinity labeling of mammalian ribosomes as compared to *E. coli* ribosomes using the same reactive mRNA analogues was observed in earlier experiments in which the proteins labeled in affinity experiments were determined. Alkylating derivatives of oligouridylates bearing the reactive group at either the 3′- or 5′-end modified a single protein in each case on rat liver ribosomes (Stahl & Kobets, 1981, 1984; Stahl & Karpova, 1985). Under the same conditions, sets of 6–10 proteins in *E. coli* ribosomes were labeled (Vladimirov et al., 1990). Hence, the mammalian ribosome seems to have a general feature of accommodating mRNA analogues in its binding center with a smaller number of identifiable contacts as compared with eubacterial ribosomes. The most straightforward explanation of this difference comes from the consideration of the properties of the cross-linking agent. Extensive differences in the nature of the mRNA–ribosome interaction are not expected; however, if the mammalian ribosomes are less flexible than the eubacterial ones, this could account for the decreased reactivity of s^4U-containing mRNA analogues on the mammalian ribosome. This could occur either if the mRNA track were much more strict in mammalian ribosomes than in eubacterial ones or if the internal flexibility of the mammalian ribosome were less than that of the eubacterial ribosomes.

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