The central part of the 5.8 S rRNA is differently arranged in programmed and free human ribosomes

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A sequence-specific modification of the human 5.8 S rRNA in isolated 60 S subunits, non-programmed 80 S ribosomes and ribosomes complexed with mRNA and tRNAs was studied with the use of a derivative of the nonaribonucleotide UCUGUGUUU bearing a perfluorophenylazide group on the C-5 atom of the 5'-terminal uridine. Part of the oligonucleotide moiety of the derivative was complementary to the 5.8 S rRNA sequence ACACA in positions 82–86 flanked by two guanines at the 5'-terminus. The target for the cross-linking was identified as nucleotide G89 on the 5.8 S RNA. In addition, several ribosomal proteins were modified by the oligonucleotide derivative bound to the 5.8 S rRNA and proteins L6 and L8 were among them. Application of these results to known cryo-electron microscopy images of eukaryotic 60 S subunits made it possible to suggest that the

central part of the 5.8 S rRNA containing the sequence 82–86 and proteins L6 and L8 are located at the base of the L1 stalk of the 60 S subunit. The efficacy of cross-linking in non-programmed 80 S ribosomes was much lower than in isolated 60 S subunits and in programmed 80 S ribosomes. We suggest that the difference in the accessibilities of the central part of the 5.8 S rRNA in the programmed and non-programmed 80 S ribosomes is caused by a conformational switch that seems to be required to dissociate the 80 S ribosomes into the subunits after termination of translation to allow initiation of translation of a new template.

Key words: human ribosome, oligonucleotide derivative, photo-cross-linking, 5.8 S rRNA, ribosomal protein.

INTRODUCTION

Despite remarkable progress made in studying the structural and functional topography of the ribosome, many aspects concerning relationships between structure and function remain unclear for eukaryotic ribosomes that have not yet been studied by X-ray crystallography. To date, mammalian ribosomes were studied mainly using two approaches: by site-directed cross-linking with mRNA analogues [1–3] and by cryo-EM (cryo-electron microscopy) [4,5]. The latter provides information on the overall structure and morphology of the ribosomal subunits and the ribosome. Thus several fragments of the rRNA structure and ribosomal proteins that have prokaryotic counterparts have been mapped on cryo-EM models of the ribosomes. Cross-linking makes it possible to determine nucleotides of the small subunit rRNA and ribosomal proteins that are located close to a nucleotide of the mRNA analogue bearing the cross-linker. Applying this approach, the conserved nature of the decoding site of the small subunit rRNA has been confirmed, and several differences in mRNA tracks on mammalian and prokaryotic ribosomes have been revealed [3,6]. In particular, with the use of perfluorophenylazide-modified oligoribonucleotides it has been shown that human protein S26, which has no prokaryotic homologues, surrounds a relatively long stretch of the upstream part of the mRNA [3].

For all of the mRNA analogues with perfluorophenylazide-modified nucleotides used to study the mRNA binding centre of human ribosomes, the 40 S subunit was the main target for cross-linking within the 80 S ribosomal complexes [3,6–8], and, in some cases, cross-linking to 5.8 S rRNA was detected (E. Laletina, D. Griafer and G. Karpova, unpublished work). This cross-linking was not blocked by poly(U) (polyuridylylate) and thus occurred outside of the mRNA-binding site. One of the probable reasons for the cross-linking of modified oligoribonucleotides to

5.8 S rRNA away from the mRNA-binding site might be binding of the oligomer to an accessible complementary sequence in the rRNA, making cross-linking possible. If so, photoactivatable oligoribonucleotide derivatives may be useful tools to study possible structural rearrangements in the 5.8 S rRNA during the course of translation (subunit association, binding of various ligands to the 80 S ribosome, etc.). This small rRNA specific to eukaryotes forms a complex in the ribosome with the 28 S rRNA [9–11], and has been studied for almost three decades. Direct evidence for a functional role of the 5.8 S rRNA was first provided by a study demonstrating that oligonucleotides complementary to specific exposed regions of the ribosome-associated 5.8 S rRNA inhibited protein synthesis *in vitro* [12]. Later studies *in vivo* with mutant 5.8 S rRNA carrying alterations in the universally conserved sequences GAAC showed that this rRNA participates directly in ribosome translocation [13,14], although the molecular basis for the involvement of 5.8 S rRNA in this process remains unclear. The topography of the 5.8 S rRNA in the ribosomes has been studied by chemical probing with diethyl pyrocarbonate [15]. The reactivity of nucleotides of 5.8 S rRNA towards this reagent depended on the state of the 5.8 S–28 S rRNA complex (whether isolated, in the 60 S subunit or in the ribosome). This made it possible to reveal the sites whose accessibility was changed when the 5.8 S–28 S rRNA complex was integrated into the 60 S subunit and when the subunits were within polysomes [15].

In the present paper, we show that the nonaribonucleotide UCUGUGUUU bearing the perfluorophenylazide cross-linker at the C-5 atom of either the first or the third uridine is able to cross-link to nucleotide G89 in the central part of the 5.8 S rRNA. Cross-linking occurred due to the formation of a complementary complex of the oligonucleotide derivative with the 5.8 S rRNA sequence ACACA at positions 82–86 3' of G89. The level of cross-linking to this nucleotide in free 80 S ribosomes was much lower

Abbreviations used: cryo-EM, cryo-electron microscopy; eIF, eukaryotic initiation factor; poly(U), polyuridylylate.

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Figure 1 Oligoribonucleotide derivatives used for cross-linking experiments

The distance separating the modified base and the first nitrogen of the azido group is no more than 11 Å (1 Å = 0.1 nm) [6].

than in isolated 60 S subunits or in programmed 80 S ribosomes. The results indicate that the central part of the 5.8 S rRNA is arranged differently in free and programmed 80 S ribosomes. A possible mechanism of the conformational switch in 60 S subunits caused by a transition from programmed to non-programmed ribosomes is discussed. We have also identified the ribosomal proteins proximal to the central part of the 5.8 S rRNA in the 60 S subunit, and have applied these results to the known cryo-EM maps of eukaryotic large ribosomal subunits.

EXPERIMENTAL

Materials

tRNA^{Val} (1800 pmol/*A*₂₆₀ unit), [¹⁴C]Phe-tRNA^{Phe} (1800 pmol/*A*₂₆₀ unit) and tRNA^{Phe} (1300 pmol/*A*₂₆₀ unit) from *Escherichia coli* were kindly provided by Dr T. Shapkina (B. P. Konstantinov's St. Petersburg Institute of Nuclear Physics, Gatchina, Russia). A 16-mer DNA complementary to the 3'-terminal sequence 144–159 of the human 5.8 S rRNA was synthesized in-house. The dodecaribonucleotide UCUCUCGUGUUU was synthesized chemically using the solid-phase H-phosphonate method as described previously [16].

Photoreactive UCUGUGUUU derivatives

Photoreactive derivatives of the nonaribonucleotide UCUGUGUUU bearing a 4-azido-2,3,5,6-tetrafluorobenzoyl group attached to the C-5 atom of the first or the third uridine residue via an ethylene diamine spacer (reagents I and II respectively) (Figure 1) were synthesized as described in [17]. Before the cross-linking experiments, the photoreactive derivatives of the nonaribonucleotide were labelled with ³²P at the 5'-termini as described in [18].

Ribosomal 40 S and 60 S subunits

Ribosomal 40 S and 60 S subunits with intact rRNAs were isolated from unfrozen human placenta as described [19]. Before use, the subunits were re-activated by incubation in binding buffer A (120 mM KCl, 13 mM MgCl₂, 0.6 mM EDTA and 20 mM Tris/HCl, pH 7.5) at 37 °C for 10 min. 80 S ribosomes were obtained by association of the re-activated 40 S and 60 S subunits in a 40 S/60 S ratio of 1.3:1 (40 S subunits were in excess over 60 S subunits to ensure binding to all 60 S subunits in the 80 S ribosome).

Ribosomal complexes

Ribosomal complexes were obtained by incubating 8×10^{-7} M of 60 S subunits or 80 S ribosomes with reagent I or II (2×10^{-6} M if not specified) for 50 min at 20 °C in buffer A as described in [2]. In experiments in which 80 S ribosomes were complexed with mRNAs and tRNAs before oligomer binding, the ribosomes were pre-incubated at 20 °C in buffer A with (i) poly(U) (*A*₂₆₀ of 0.1) and, if specified, 4×10^{-6} M Phe-tRNA^{Phe} for 30 min, or with (ii) 4×10^{-6} M mRNA analogue UCUCUCGUGUUU and 4×10^{-6} M tRNA^{Phe} for 50 min. In experiments with both tRNA^{Phe} and tRNA^{Val}, 80 S ribosomes were first incubated for 50 min with the mRNA analogue and tRNA^{Phe}, then 7×10^{-6} M tRNA^{Val} was added, and incubation was carried out for a further 50 min.

Complex irradiation

Irradiation of the complexes was carried out in ice-cooled cells for 0.5 min with UV light from a SpotCure (UVP) lamp via optical fibres. Shortwave UV light (wavelength < 280 nm) was cut off by a thin glass filter. The irradiated complexes were completed with 1/30 (v/v) of 5% 2-mercaptoethanol, and precipitated by 0.7 vol. of ethanol at 0 °C. If specified, the irradiated ribosomes were separated into subunits as described in [20].

RNA

RNA was isolated from the irradiated 80 S ribosomes or 60 S subunits by phenol deproteination with subsequent ethanol precipitation [21]; determination of the cross-linked 5.8 S rRNA nucleotides was carried out by primer extension as described in [22], using the deoxyoligomer complementary to the 3'-terminal sequence 144–159 of the 5.8 S rRNA as primer.

Analysis of 60 S proteins cross-linked to 5'-end-labelled reagent I

Analysis of cross-linked 60 S proteins was carried out by PAGE in the presence of SDS according to the method described in [20]. If specified, cross-linked 60 S subunits were treated with RNase A according to the method described in [22]. To identify cross-linked proteins, the autoradiograms were superimposed on the respective stained gels, and the positions of the radioactive bands with respect to those of unmodified proteins were determined.

RESULTS

Identification of the target for cross-linking in the 5.8 S rRNA

Analysis of the RNA isolated from irradiated complexes of 80 S ribosomes with reagents I or II (Figure 1) revealed a single radioactive band whose electrophoretic mobility corresponds to the 5.8 S rRNA cross-linked to a nonamer (the radioactive band was shifted to the origin with respect to the unmodified rRNA due to the cross-linked nonaribonucleotide; Figure 2). A small portion of the radioactivity remaining in the wells of the gel that was almost the same in all four lanes and did not depend on the reagent type and presence of poly(U) evidently corresponded to undissolved material rather than to the cross-linked 18 S and/or 28 S rRNAs. This conclusion is also based on our previous results that showed that reagents I and II in complexes with 80 S ribosomes do not cross-link to the 28 S rRNA, and cross-link to the 18 S rRNA only when they act as mRNA analogues in the presence of a cognate tRNA, the latter cross-linking being completely blocked by poly(U) [3]. It can be seen from Figure 2 that reagent I cross-linked to the 5.8 S rRNA much more effectively than reagent II. Poly(U) did not block, but even enhanced, cross-linking

Figure 2 Autoradiographic analysis of cross-linking of 5'-³²P-labelled reagents I (lanes 1 and 2) and II (lanes 3 and 4) to 5.8 S rRNA by electrophoresis in a 5% polyacrylamide gel

RNA was isolated from irradiated 80 S ribosomal complexes obtained without (lanes 1 and 3) or with (lanes 2 and 4) poly(U). The positions of RNA size markers are indicated as seen in the gel stained with 'Stains All'.

of both derivatives to the 5.8 S rRNA, indicating that the reaction occurred outside the mRNA-binding site. Cross-linking was specific for reagents I and II, since none of the other perfluorophenylazide-modified oligoribonucleotides (more than ten) that are used as mRNA analogues [1–3,8] cross-linked to 5.8 S rRNA. Such sequence-specific cross-linking may be realized only if the 5.8 S rRNA has a sequence complementary to nonamers I and II that allows cross-linking in a complementary complex. Computer analyses of the primary structure of the 5.8 S rRNA (<http://www.rna.icmb.utexas.edu>; free registration required) revealed only the relatively long sequence ACACA in positions 82–86 that is fully complementary to the sequence UGUGU in the nonamers. When cross-linking occurs in the complementary complex, the cross-linked nucleotides of the target RNA are generally located close to the binding site of the complementary probe [23]. To identify the nucleotides of the 5.8 S rRNA cross-linked to reagent I, a primer-extension approach was applied. With reagent II, identification was not done due to the low extent of cross-linking to the 5.8 S rRNA. Primer extension leads to a stop or pause at the cross-linked rRNA nucleotide, and the cross-linking site is generally assumed to be the nucleotide 5' of the primer extension stop site. The only stop was found at A90 of the 5.8 S rRNA (Figure 3), therefore the cross-linked nucleotide was G89, which is located close to the 3' side of the ACACA sequence, confirming the suggestion that cross-linking to the 5.8 S rRNA occurred in the complementary complex formed by the rRNA sequence ACACA and the reagent. The stop at A90 was intense in the experiments with isolated 60 S subunits (Figure 3, lane 1) and with 80 S ribosomes complexed with the mRNA analogue and tRNA (Figure 3, lane 3); in contrast, with free 80 S ribosomes, the stop was much weaker (Figure 3, lane 2).

Effect of the functional state of 60 S subunits on the efficacy of cross-linking

To examine the effects of association of 60 S and 40 S subunits to produce the 80 S ribosomes, and of binding of the 80 S ribosomes with mRNAs and tRNAs upon cross-linking of reagent I to the

Figure 3 Identification of 5.8 S rRNA cross-link sites to reagent I by reverse transcription

Extension of 5'-³²P-labelled primer complementary to 5.8 S rRNA positions 144–159 on the rRNA isolated from the irradiated complexes with 60 S subunits (lane 1), 80 S ribosomes either free (lane 2) or complexed with tRNA^{Phe} and UCUCUCGUGUUU (lane 3). Lane K is a control 5.8 S rRNA sample isolated from 80 S ribosomes irradiated without reagent I and treated as the other samples. Lanes U, G, C and A show sequencing of the 5.8 S rRNA isolated from placenta tissue. The scheme of the primer extension is given on the right; expected lengths of the sequencing tracks are indicated by arrows on the right of the sequencing panel. The position of the reverse transcription stop caused by cross-linking is indicated to the left of the Figure.

Figure 4 Autoradiographic analysis of cross-linking of 5'-³²P-labelled reagent I to 5.8 S rRNA

Cross-linking of 5'-³²P-labelled reagent I to 5.8 S rRNA in 60 S subunits (lane 1), or in 80 S ribosomes either free (lane 2), or complexed with poly(U) (lane 3), with poly(U) and Phe-tRNA^{Phe} (lane 4), with UCUCUCGUGUUU and tRNA^{Phe} on the P site (lane 5), or with UCUCUCGUGUUU, tRNA^{Phe} on the P site and tRNA^{Val} on the E site (lane 6) was analysed by electrophoresis in a 5% polyacrylamide gel followed by autoradiography.

5.8 S rRNA, RNA isolated from the irradiated mixtures was analysed by denaturing gel electrophoresis (Figure 4). Cross-linking seemed to occur with similar efficiency in the isolated 60 S subunits (Figure 4, lane 1) and in all types of 80 S ribosomal complexes (Figure 4, lanes 3–6), but, with free 80 S ribosomes (Figure 4, lane 2), cross-linking was much weaker. In control experiments, 80 S ribosomes obtained by re-association of 40 S and 60 S subunits were examined by sucrose-density-gradient centrifugation under binding conditions. The analysis showed that less than 10 % of 60 S subunits remained free (results not shown). The cross-linking results presented in Figure 4 are consistent with the data of primer extension presented in Figure 3. The difference between cross-linking efficiency with isolated 60 S subunits and free 80 S ribosomes remained significant over a wide

Figure 5 Analysis of cross-linking of 5'-³²P-labelled reagent I to 5.8 S rRNA in isolated 60 S subunits and non-programmed 80 S ribosomes at various reagent concentrations

range of reagent I concentrations (Figure 5). Quantification of the electrophoretic data from Figures 2, 4 and 5 indicates that cross-linking to isolated 60 S subunits was approx. three times stronger than to free 80 S ribosomes.

Identification of 60 S proteins cross-linked to the nonaribonucleotide derivative

Perfluorophenylazide derivatives of oligoribonucleotides were able to cross-link to ribosomal proteins as well as to rRNA when applied as mRNA analogues [3,18,20,24]. The ability of such derivatives to cross-link to proteins has been used to study the protein environment of specific sequences of 18 S rRNA in the 40 S subunits applying oligomers complementary to these sequences [22]. Thus reagent I could be used to examine ribosomal proteins that are in the vicinity of sequence 82–86 of the 5.8 S rRNA in the 60 S subunits. It should be noted that proteins of the 40 S subunit cross-linked to reagent I only when it was bound at the mRNA-binding centre, but not in a complementary complex as demonstrated by experiments in which poly(U) completely blocked cross-linking to 40 S subunits [3]. The results of SDS/PAGE separation of ribosomal proteins cross-linked to reagent I in 60 S subunits are presented in Figure 6; several groups of proteins cross-linked to different extents (Figure 6, lane 1). This cross-linking was not observed for perfluorophenylazide derivatives of other oligomers (hexa-, nona- and dodeca-mers; results not shown), and is therefore sequence-specific for reagent I. To identify the labelled proteins, the cross-linked oligoribonucleotide was hydrolysed with RNase A to decrease its effect on the electrophoretic mobilities of the proteins (Figure 6, lanes 2–5). Efficiency of cross-linking to each protein group increased similarly with increasing reagent concentration (Figure 6, lanes 2–5). The SDS/polyacrylamide electrophoretogram of the mammalian 60 S proteins is well resolved in the upper part in which bands of the individual proteins are well separated from each other (Figure 6, lane Tp60). This made it possible to unambiguously assign part of the cross-linked proteins by comparison of the positions of the radioactive bands (Figure 6, lanes 2–5) with the bands of unmodified proteins in the stained gel (Figure 6, lane Tp60). We have taken into account the fact that the radioactive spots may be shifted slightly towards the origin as compared with the corresponding unmodified proteins. This shift is caused by mono- or di-nucleotide fragments that remain cross-linked to the protein after RNase A hydrolysis. Thus we conclude that proteins L6 and L8 are among the proteins cross-linked to reagent I in the 60 S subunits. Protein bands in the lower part of the electrophoretogram are not sufficiently well resolved to identify individual cross-linked proteins; the identification of these proteins is complicated even when using two-dimensional PAGE [25] and requires a separate extensive study.

Figure 6 SDS/PAGE analysis of proteins cross-linked to 5'-³²P-labelled reagent I in the isolated 60 S subunits at various reagent concentrations

Proteins treated (lanes 2–5) and untreated (lane 1) with RNase A. Lane 6 (Tp60), stained gel. Positions of several 60 S proteins are marked according to designations given in [32].

DISCUSSION

Sequence-specific cross-linking of oligoribonucleotide derivatives to 5.8 S rRNA

Two alternative secondary structures of mammalian 5.8 S rRNA in the 5.8 S–28 S rRNA complex have been reported (Figure 7). Sequence 82–86 is in a single-stranded region in structure A (Figure 7A) and in a double-stranded region in structure B (Figure 7B). The duplex formed between 5.8 S rRNA and the oligonucleotide moiety of derivatives I and II is flanked by two terminal non-canonical G·U pairs (Figure 7). Similar to canonical A·U pairs, which exhibit comparable stability to G·U pairs, the G·U pairs should increase the stability of the duplex [26]. Clearly, the 5.8 S rRNA folded as in Figure 7(A), rather than in Figure 7(B), is preferable for binding of the reagents.

Reagents I and II contain the same oligonucleotide sequence, and only the position of the modified uridine residue differs. Nevertheless, reagent II cross-linked much less efficiently to the 5.8 S rRNA than did reagent I. It seems very unlikely that the stability of the duplex between 5.8 S rRNA and reagent II could be much lower than with reagent I, since the only difference between the duplexes is that, in the case of reagent II, one of the uridines involved in duplex formation is modified at the C-5 atom. Such a modification does not interfere with the ability of the RNA to form complementary duplexes if the respective modified uridine is in the 5'-terminal position. For example, the coding property of mRNA analogues containing a modified uridine in the first position of the UUU codon is the same as that with the unmodified codon [7]. Thus less efficient cross-linking of reagent II to the 5.8 S rRNA as compared with reagent I is due to the less suitable target for cross-linking in the 5.8 S rRNA rather than to the formation of a less stable duplex. There are no data

Figure 7 Secondary structure of the central part of human 5.8 S rRNA in the 5.8 S–28 S rRNA complex according to <http://www.rna.icmb.utexas.edu> (A) and to [33] (B)

Scheme for the base pairing of the reagents with sequence 80–86 of the 5.8 S rRNA and cross-linking to G89 are shown on structure (A); on structure (B), the sequence 80–86 is marked.

indicating which RNA nucleotides are preferable for cross-linking with photoactivated perfluorophenylazide-modified oligoribonucleotides in the complementary complexes. However, when these derivatives were used as mRNA analogues, uridines were never cross-linked [2,3,6]. In the case of the duplex with reagent II, two uridine residues are adjacent to the nucleotide base-paired with the modified nucleotide of the reagent (Figure 7). Most probably, these uridines are poor targets for cross-linking, and nucleotide G89, which was easily reachable by the photoactivated group at the flexible unpaired 5'-terminal dinucleotide of reagent I, could hardly cross-link to the modified uridine fixed by base pairing.

Location of 5.8 S rRNA region 80–86 and the proximal proteins in the 60 S subunit

The large ribosomal subunit of the eukaryotic ribosome remains unavailable for X-ray crystallographic analysis, and many aspects of its structure remain obscure. No data exist on the proteins surrounding or interacting with the 5.8 S rRNA in the mammalian ribosome. Only a few early reports concern proteins that interact with the 5.8 S rRNA, identified by either affinity chromatography of the ribosomal proteins on a column with immobilized 5.8 S rRNA [27] or by nitrocellulose-filtration analysis of binding of the rRNA to proteins [28]. Both studies report only three of the approx. 50 60 S proteins that interact with 5.8 S rRNA, namely L6, L8 and L19. Two of them, L6 and L8, are major targets for cross-linking with reagent I (Figure 6), in excellent agreement with the data from these earlier reports. One of these proteins, L8,

has been mapped at the base of the L1 stalk of the 60 S subunit (from yeast), as viewed from the solvent side at the bottom of the L1 side [4]. L6 lacks a prokaryotic counterpart, and its location on the 60 S subunit is unknown as well as the location of the central part of the 5.8 S rRNA. The results of the present study indicate that protein L6 is close to the central part of the 5.8 S rRNA as well as L8. In the model of the yeast 60 S subunit presented in [4], the expansion segment of the 28 S–5.8 S rRNA complex designated ES4 that contains the 3'-extremity of the 5.8 S rRNA has been mapped at the base of the L1 stalk very close to the site of L8 location. We suggest that the central part of the 5.8 S rRNA containing sequence 82–86 and proteins L6 and L8 are located close to each other near the base of the L1 stalk of the human 60 S subunit.

Arrangement of the central part of the 5.8 S rRNA on the ribosome

Several anti-(5.8 S rRNA) oligodeoxyribonucleotides have been shown to substantially inhibit protein synthesis [12]. In particular, a pronounced effect was observed for the oligomer complementary to the sequence ACACAUU in positions 82–88, indicating involvement of this region in translation. However, more detailed studies [13,14] concerned only the universally conserved sequences GAAC in positions 42–46 and 104–107 of the 5.8 S rRNA, and showed that these sequences play an important role in ribosome translocation.

The role of the 5.8 S rRNA in translation may be understood better if each segment of the rRNA is monitored at various steps of translation. Early chemical footprinting studies with the use of diethyl pyrocarbonate indicated that the reactivity of the majority of the 5.8 S rRNA adenines in the whole ribosome was much lower than in the isolated 60 S subunit [15]. However, remarkably, three adenines, A82, A84 and A86, remained exposed in whole ribosomes, and their reactivity towards diethyl pyrocarbonate was even higher than in the isolated subunits. Further studies in which dimethyl sulphate was applied to modify the cytosines revealed that C83 and C85 of the 5.8 S rRNA were only slightly accessible in free 60 S subunits and totally inaccessible in the whole ribosomes [29].

It should be noted here that in the reports cited above, 'whole ribosomes' were in fact polysomes containing endogenous mRNA and tRNA, in contrast with the present study in which 80 S ribosomes were reassociated from 60 S and 40 S subunits. More recently, chemical footprinting (using dimethyl sulphate and a water-soluble carbodi-imide) was applied to compare the accessibility of the 5.8 S rRNA nucleotides in native polysomes, salt-washed polysomes (lacking translational factors) and salt-washed run-off ribosomes (lacking mRNA, tRNA and factors) [30]. However, no differences were detected in the various 5.8 S rRNA footprinting patterns. Therefore the footprinting data did not lead to definite conclusions concerning the arrangement of the central region of the 5.8 S rRNA in the 60 S subunits at various steps of translation.

Our results clearly indicate that the sequence 82–86 of the 5.8 S rRNA in the vacant 80 S ribosomes obtained by re-association of the subunits is less accessible for the base pairing with a complementary probe than in free 60 S subunits, and in 80 S ribosomes complexed with mRNAs and tRNAs (Figure 4). It is clear that the difference in the accessibility of the central part of the 5.8 S rRNA in the programmed and non-programmed 80 S ribosomes is caused by a conformational switch that may accompany transition of programmed ribosomes to the free state during protein synthesis. It is known that, after termination of translation, all ligands leave the ribosome to allow it to translate another template. Initiation of translation requires free 40 S

subunits that appear as a result of dissociation of the run-off 80 S ribosomes by the eukaryotic initiation factors eIF-3 and eIF-6 (for a review, see [31]). We suggest that dissociation of all ligands from the ribosome led to a conformational switch, facilitating binding of the ribosome-dissociating factors, and this switch renders the central part of the 5.8 S rRNA less exposed. There are two possible alternative ways for the conformational switch to occur. First, the central part of the 5.8 S rRNA may become less accessible owing to alterations in its environment increasing the shielding effect of proteins and/or other rRNA regions on this part of the 5.8 S rRNA. The second may be concerned with a change of the secondary structure of the central part of the 5.8 S rRNA after which sequence 82–86 falls into a double-stranded region that is inaccessible for binding of complementary oligonucleotides. In principle, both ways are possible, but we assume that the latter is more attractive, since two alternative ways of folding of the central part of 5.8 S rRNA in the 5.8 S–28 S rRNA complex have been already reported (as discussed above). As shown in Figure 7, in structure A, the sequence 80–86 is in the single-stranded region of the 5.8 S rRNA and therefore should be accessible for the base pairing with the complementary oligomers. In contrast, in structure B, the sequence is almost completely in a double-stranded region that should impair binding of the complementary oligomers. We suggest that in free 60 S subunits and in programmed 80 S ribosomes, the 5.8 S rRNA is folded as in structure A, while in the free 80 S ribosomes it is folded as in B, promoting binding with ribosome-dissociating factors and leading to the formation of the ribosomal subunits that are able to start translation of a new template.

Relationship between functional state of the ribosome and its structure

Data showing that the functional state of the ribosome may be affected by switches in the rRNA secondary structure have been reported previously for prokaryotes [34]. Cryo-EM studies have demonstrated that alterations in the secondary structure of 16 S rRNA, namely the switch of three base pairs from positions 912–910/885–887 to 912–910/888–890 results in major rearrangements in the 70 S ribosomal three-dimensional structure [34]. This, in turn, significantly affects topography of the A site tRNA-binding region and thereby changes tRNA affinity for the ribosome and fidelity of mRNA decoding. It is thought that a similar switch occurs in eukaryotic ribosomes as well. Conformational rearrangements in the spatial structure of ribosomes also take place at other steps of translation. So, according to cryo-EM data, translocation of deacylated tRNA from the P to the E site is accompanied by a switch involving a movement of the 30 S subunit relative to the 50 S subunit and a conformational change of the L1 stalk [35]. It is suggested that these ribosomal motions could extensively disrupt the inter-subunit bridges that may be the structural basis for ribosomal splitting into subunits by RRF (ribosome recycling factor) and EF-G (elongation factor G) [35]. Similar changes in the L1 region were detected in the *Saccharomyces cerevisiae* ribosomes [36]. However, molecular mechanisms providing these conformational changes of the L1 stalk remain unknown. According to our data, the base of the L1 stalk of the human large ribosomal subunit is close to the central part of the 5.8 S rRNA whose structure depends on the state of the ribosome. It is worthwhile to notice that structural model B of the 5.8 S rRNA is compatible with the structure of prokaryotic 5 S rRNA (see <http://www.rna.icmb.utexas.edu>), which is located on the top of the central protuberance [37]. Nevertheless, to our knowledge, there are no data on any rearrangements in the secondary structure of prokaryotic 5 S

rRNA that are related to the functional state of the ribosome. Summarizing, we conclude that the conformational switch in the central part of the 5.8 S rRNA found in the present study is specific for eukaryotic ribosomes.

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