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Identification by RNA-protein cross-linking of ribosomal proteins located at the interface between the small and the large subunits of mammalian ribosomes

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Protein constituents at the subunit interface of rat liver ribosomes were analysed by cross-linking with the bifunctional reagent, diepoxybutane (distance between reactive groups 4 Å). Isolated 40S and 60S subunits were labelled with ^{125}I and recombined with unlabelled complementary subunits. The two kinds of selectively labelled 80S ribosomes were treated with diepoxybutane at low concentration. Radioactive ribosomal proteins covalently attached to the rRNA of the unlabelled complementary subparticles were isolated by repeated gradient centrifugation. The RNA-bound, labelled proteins were identified by two-dimensional gel electrophoresis. The experiments showed that proteins S2, S3, S4, S6, S7, S13, and S14 in the small subunit of rat liver ribosomes are located at the ribosomal interface in close proximity to 28S rRNA. Similarly, proteins L3, L6, L7, and L8 were found at the interface of the large ribosomal subunit in the close vicinity of 18S rRNA.

Key words: cross-linking/rat liver/ribosomes/RNA-protein interaction

Introduction

The interaction between the two ribosomal subparticles is essential for a proper ribosomal function in mRNA translation. Owing to this functional cooperativity, a number of active ribosomal domains would be expected to occur in the contact region between the subparticles. In mammalian ribosomes, mRNA (Nonomura *et al.*, 1971; Stahl and Kobets, 1981; Reboud *et al.*, 1981; Takahishi and Ogata, 1981), initiation factor eIF-3 (Emanuilov *et al.*, 1978, Nygård and Westermann, 1982), and the A and P sites of the peptidyltransferase center (Metspalu *et al.*, 1978; Ulbrich *et al.*, 1980a; Todokoro *et al.*, 1981) are supposed to be associated with the contact region. By analogy with the situation in bacteria (Girshovich and Kurtskhalia, 1978), the attachment site for translocation factor EF-2 is probably also located in this region. The structural organisation of ribosomal components at the interface is therefore of great potential significance for understanding ribosome function. In bacterial ribosomes the interface includes sequences of 16S and 23S rRNA and a limited number of ribosomal proteins (Sköld, 1981; Cover *et al.*, 1981; Lambert and Traut, 1981).

A substantial part of our present knowledge of the topography of eukaryotic ribosomes and ribosome-associated translational complexes originates from experiments with chemical cross-linking (Uchiomi *et al.*, 1980, 1981; Terao *et al.*, 1980a, 1980b; Westermann *et al.*, 1979, 1980, 1981; Nygård *et al.*, 1980; Tolan and Traut, 1981; Nygård and Westermann, 1982). In some of these experiments, and in similar studies of prokaryotic ribosomes, the short bifunctional reagent, diepoxybutane (distance between reactive groups 4 Å), has been used to establish near neighbour relationships between proteins and RNA (Bäumert *et al.*, 1978; Nygård *et al.*, 1980; Westermann *et al.*, 1980, 1981; Sköld, 1981; Nygård and Westermann, 1982).

In the experiments reported here the ability of individual proteins in the small and large subparticles of rat liver ribosomes to interact across the ribosomal interface with the RNA of the opposite subparticle was studied by chemical cross-linking with diepoxybutane. The existence of such close protein-RNA interactions across the interface was established, and the participating ribosomal proteins were identified.

Results

Rat liver 40S and 60S ribosomal subunits, labelled separately with [^{125}I]iodine (Bolton and Hunter, 1973), formed ^{125}I-labelled 80S monomers when incubated at slightly elevated magnesium concentrations (Trachsel and Staehelin, 1979). By combining ^{125}I-labelled subunits with complementary unlabelled subunits, 80S ribosomes were produced which contained labelled proteins exclusively in one of the two subunits. The two kinds of differentially labelled reassociated 80S ribosomes were treated with the bifunctional cross-linking reagent, diepoxybutane. Gradient sedimentation analysis (Figure 1) showed that, under the experimental conditions used, >80% of the subunit-bound radioactivity was associated with the 80S ribosomal monomers. Very little radioactive material (<5%) was associated with cross-linked particles sedimenting faster than 80S (Figure 1). Thus, the preparations were essentially free of contaminating covalently linked ribosomal aggregates.

The cross-linked 80S ribosomes were disrupted with lithium dodecylsulphate, and the mixtures of rRNA and covalent rRNA-protein complexes were isolated by repeated gradient centrifugation in the presence of lithium dodecylsulphate (Figure 2). From the cross-linked [^{125}I]40S·60S ribosomes, a mixture of 28S rRNA and covalent 28S rRNA-protein complexes was isolated (Figure 2A). From the cross-linked 40S·[^{125}I]60S ribosomes, 18S and 28S rRNA were isolated separately, together with the proteins covalently bound to these rRNA species (Figure 2B and C). As in earlier reports (Nygård *et al.*, 1980; Westermann *et al.*, 1980; Sköld, 1981), no ^{125}I-labelled ribosomal proteins cosedimented with the rRNAs without prior treatment with cross-linking reagent (not illustrated). As can be seen from Figure 2, the isolated 18S and 28S rRNA fractions derived from the unlabelled subunits of the two kinds of recombined 80S ribosomes contained covalently linked ^{125}I-labelled ribosomal proteins, indicating that cross-linking had occurred across the ribosomal interface (Figure 2A and C). Cross-linking had also taken place within the subunits (Figure 2B) (Westermann *et al.*, 1981).

After degradation of the rRNA by a combined treatment with ribonucleases T1 and A, followed by alkaline hydrolysis,

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the released proteins were identified by two-dimensional gel electrophoresis. To ensure unambiguous identification of the ribosomal proteins, they were analysed by use of two independent gel systems. By electrophoresis system I (basic pH/acidic pH system) (Lastick and McConkey, 1976) the 40S interface proteins cross-linked to 28S rRNA were identified as S3, S4, and S6. In addition, some radioactivity co-migrated with proteins S2, S7, S11, S13, S14, and S23 (Figure 3). In electrophoresis system II (acidic pH/SDS system) (Nika and Hultin, 1980) radioactive spots were associated with proteins S2, S3, and S4 and to a lesser extent with proteins S6, S7, S13/16, S14/15, S15a/20, and S25 (Figure 4). The 60S interface proteins covalently attached to 18S rRNA were identified in system I as L3, L6/L7, and L8 while less dense radioautographic spots were associated with proteins L4 and L13/13a/15 (Figure 5C). In system II proteins L3 and L8 were the most radioactive while proteins L6, L7, and L15 were more weakly labelled (Figure 6B). From these results, it was concluded that proteins S2, S3, S4, S6, S7, S13, and S14 from the small ribosomal subunit and proteins L3, L6, L7, and L8 from the large ribosomal subunit are wholly or partly located at the ribosomal interface (Table I). Identical results were obtained in preliminary experiments in which isolated 80S ribosomes were used for cross-linking (data not shown). The proteins of the 60S subparticle involved in intrasubunit cross-links to 28S rRNA were identified in system I as L3, L6/7, L8, L13/13a/15, L19, and L23/23a. Less radioactive spots were observed at the positions of proteins L4 and L10 (Figure 5D). In system II proteins L3, L4, L6, L7, L7a, L8, L15, and L19 and, to a lesser extent proteins L10 and L23/23a, were identified as being cross-linked to 28S rRNA

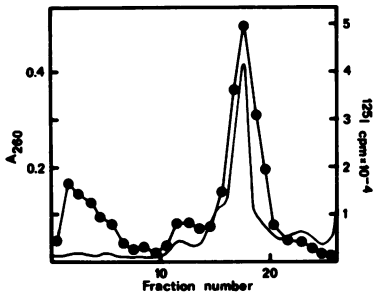

Fig. 1. Sedimentation analysis of the cross-linked 80S ribosomes. Recombined [^{125}I]40S·60S ribosomes, 3.5 mg in 1.6 ml buffer, were treated with 5 mM diepoxybutane. Small samples, 20 μl, of the reaction mixtures were analysed by centrifugation for 110 min at 260 000 g_{av} in 4 ml 10–30% (w/v) linear sucrose gradients containing 70 mM KCl, 30 mM TEA-HCl, pH 7.6, 6 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. The gradients were monitored at 260 nm and the radioactivity of consecutive 0.15 ml fractions determined in a gamma counter. —A_{260}, ● radioactivity.

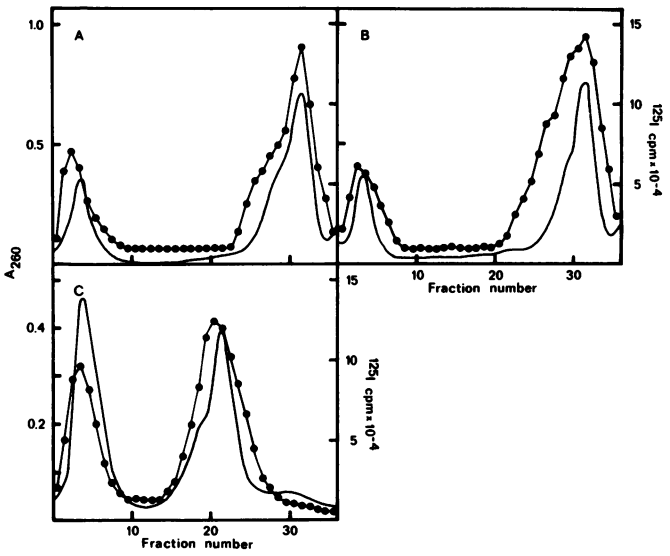

Fig. 2. Isolation of covalent protein-RNA complexes. The cross-linked 80S ribosomes were dissolved in Buffer A (50 mM LiCl, 1 mM lithium ethylenediamine tetra-acetic acid, 2% (w/v) lithium dodecylsulphate) containing 1 mM iodoacetamide, and incubated for 5 min at 56°C. The incubation mixtures were layered onto 11 ml linear 10–30% (w/v) (**A** and **B**) or 10–25% (w/v) (**C**) linear sucrose gradients in Buffer A. After centrifugation for 16 h at 200 000 g_{av}, the gradients were monitored at 260 nm (—) and the radioactivity (●) of the consecutive 0.32 ml fractions determined in a gamma counter. Isolation of: (**A**) ^{125}I-labelled 40S proteins covalently attached to 28S rRNA by inter-subunit cross-links; (**B**) intrasubunit cross-linked complexes of ^{125}I-labelled 60S proteins and 28S rRNA; and (**C**) ^{125}I-labelled 60S proteins covalently attached to 18S rRNA by inter-subunit cross-links.

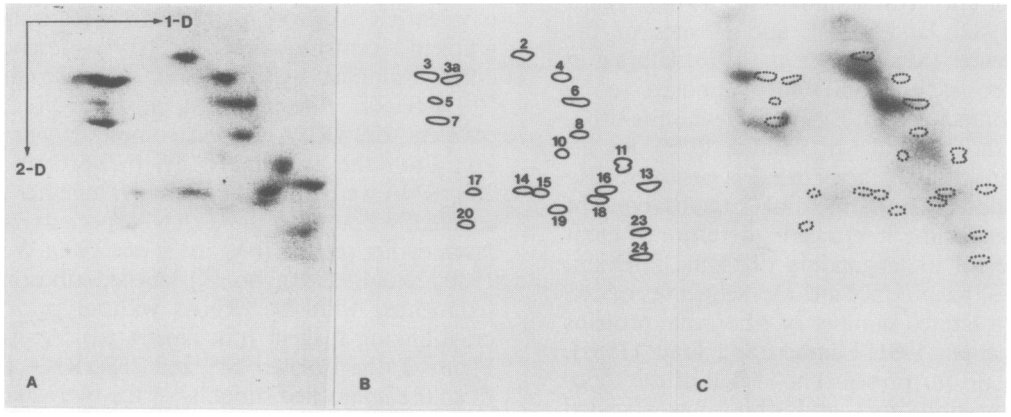

Fig. 3. Identification of 40S ribosomal proteins covalently attached to 28S rRNA by inter-subunit cross-links. The 28S rRNA in the isolated covalent complexes between ^{125}I-labelled 40S proteins and 28S rRNA were hydrolysed by treatment with RNAse A and T1 followed by 0.1 M NaOH treatment in the presence of 300 μg 40S proteins, SDS and iodoacetamide. The incubation mixtures were neutralised with H_3PO_4, dialysed against 1 mM HCl, and precipitated with acetone/HCl. The protein components were identified by two-dimensional gel electrophoresis in the basic/acidic pH system (pH 8.6/pH 4.2) described by Lastick and McConkey (1976). The first-dimension electrophoresis was in 1.8 x 55 mm gel rods and the second-dimension electrophoresis in 200 x 140 x 1 mm slab-gels. The gels contained 4% and 15% (w/v) acrylamide respectively. After electrophoresis the gels were stained with Coomassie Brilliant Blue, dried, and radio-autographed for 21 days using a Cronex Lightening Plus intensifying screen. **A** Stained gel containing 50 μg (14 000 c.p.m.) 40S proteins. **B** Designation of the stained ribosomal proteins according to McConkey *et al.* (1979). **C** Radioautograph of the stained gel.

(Figure 6C). These cross-linking data are also summarised in Table I.

Discussion

Because of the short distance between the reactive groups (4 Å), diepoxybutane is a powerful bifunctional reagent in the study of close neighbourhood relationships between proteins and RNA (Bäumert *et al.*, 1978; Nygård *et al.*, 1980; Westermann *et al.*, 1980, 1981; Sköld, 1981; Nygård and Westermann, 1982). In chemical cross-linking a major problem is often the cooperativity of the cross-linking events, resulting in mixtures of covalently linked aggregates. With diepoxybutane, at high reagent concentrations, cooperativity that disturbs the native structure has been reported (Bäumert *et al.*, 1978). However, a reagent concentration of 1% (116 mM) has been considered sufficiently low to avoid this complication (Bäumert *et al.*, 1978; Sköld, 1981). In the present investigation we used a concentration of 5 mM, which has proved effective in producing cross-linked products without detectable covalent aggregates (Nygård *et al.*, 1980; Westermann *et al.*, 1980, 1981; Nygård and Westermann, 1982).

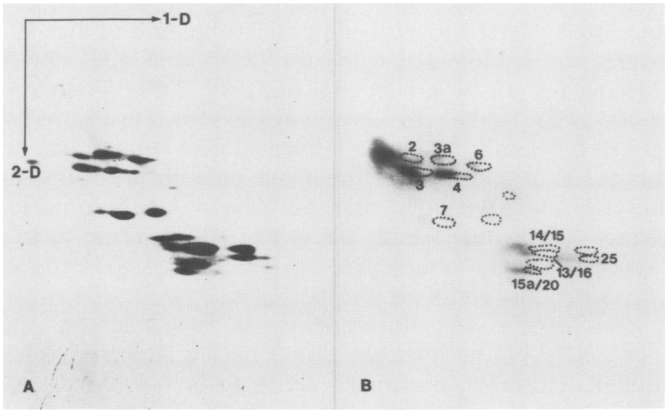

Fig. 4. Identification of 40S ribosomal interface proteins. The samples were prepared for gel electrophoresis as described in Figure 3. Proteins from the small ribosomal subunit (100 μg, 28 000 c.p.m.) were electrophoresed in the pH 4.3/SDS two dimensional system described by Nika and Hultin (1980). Gradient slab-gels were used in both dimensions. The first dimension gels were 160 mm long and contained a 10–15% (w/v) acrylamide gradient. The second dimension gels were 160 x 160 x 1.5 mm and contained a 10–20% (w/v) gradient. Staining and radioautography were as described in Figure 3. **A** Stained gel. **B** Radio-autograph (14 days exposure) with proteins designated according to McConkey *et al.* (1979) (H.Nika, unpublished data).

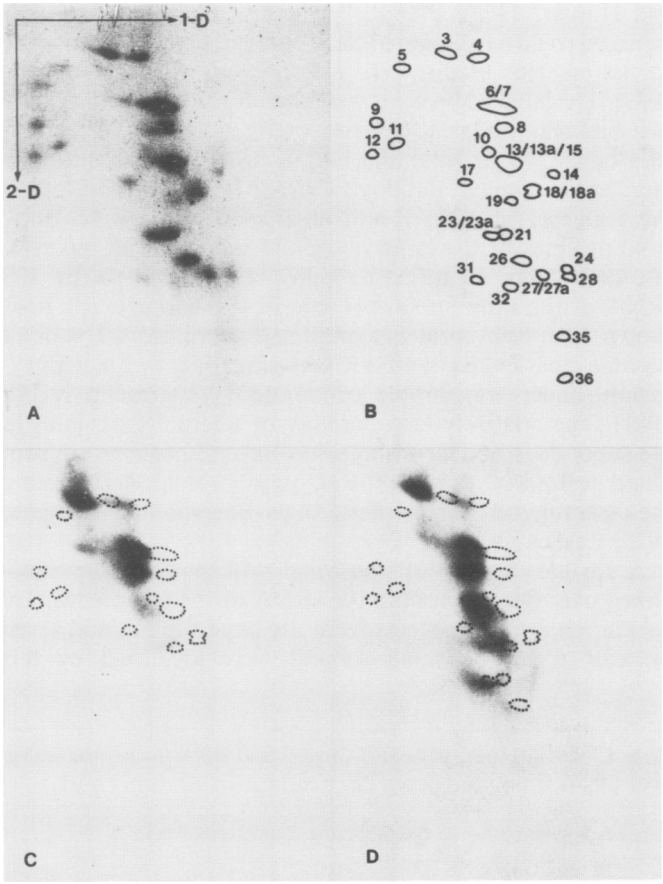

Fig. 5. Identification of 60S proteins cross-linked to 18S and 28S rRNA. The protein samples were prepared and analysed by two-dimensional electrophoresis as described in Figure 3 with the exception that the 40S carrier proteins were replaced by 300 μg 60S proteins. **A** Stained gel containing 50 μg protein. **B** Designation of the stained ribosomal protein according to McConkey *et al.* (1979). **C** Radio-autograph of 60S proteins (19 400 c.p.m.) covalently linked to 18S rRNA. Radio-autography was for 14 days using an intensifying screen. **D** Radio-autograph of 60S proteins (24 500 c.p.m.) covalently linked to 28S rRNA. Radio-autography was for 21 days as described above.

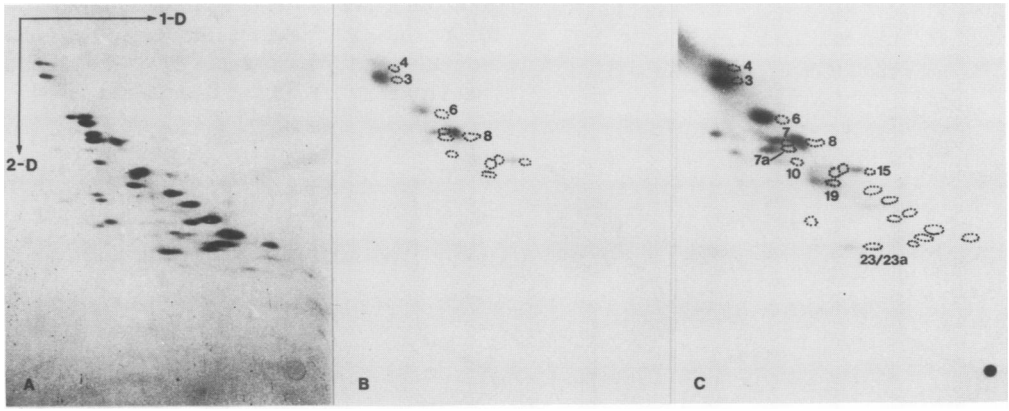

Fig. 6. Identification of 60S proteins involved in inter- and intra-subunit cross-linking to 18S and 28S rRNA. The protein samples were prepared for electrophoresis as described in Figure 5. Two-dimensional gel electrophoresis was as described in Figure 4. Radio-autography was as described in Figure 5. The protein spots were designated according to McConkey *et al.* (1979) (H.Nika, unpublished data). **A** Stained gel containing 100 μg protein. **B** Radio-autograph (7 days exposure) of 60S proteins (39 000 c.p.m.) cross-linked to 18S rRNA. **C** Radio-autography (14 days exposure) of 60S proteins (49 000 c.p.m.) cross-linked to 28S rRNA.

The RNA components of the covalent protein-RNA complexes were degraded by enzymic and alkaline hydrolysis. Because of the negative charge of the remaining, covalently-bound nucleotide, as well as of the labelling reagent (Bolton and Hunter, 1973), the labelled, cross-linked proteins were expected to differ slightly in electrophoretic mobility from the carrier proteins used as reference (Anderson and Hickman, 1979). The labelled proteins were therefore cross-identified by two basically different electrophoretic systems (Lastick and McConkey, 1976; Nika and Hultin, 1980). The results were qualitatively similar, although some difference was noticed in the degree of labelling of individual spots. At least 10 of the >40 proteins of the large ribosomal subunit (McConkey *et al.*, 1979) were identified as being cross-linked to the 28S rRNA of the same subunit (Table I). It has previously been shown that eight proteins of the small ribosomal subunit become cross-linked to 18S rRNA by treatment with diepoxybutane under comparable conditions (Westermann *et al.*, 1981). The relatively large number of internal cross-links in the subunits is not surprising, since several proteins are supposed to interact primarily with preribosomal RNA during the assembly of the particles (Auger-Buendia and Longuet, 1978; Lastick, 1980).

A smaller number of ribosomal proteins could be cross-linked over the interface to the rRNA of the complementary subparticles. Four proteins from the large subunit and seven proteins from the small subunit were identified by this criterion as belonging to the interface (Table I). The situation is comparable to that in bacterial ribosomes, where the number of interface proteins that could be cross-linked to rRNA of the opposite particle was also larger on the small (30S) subunit (Sköld, 1981).

Immune electronmicroscopic data indicate that the antigenic determinants of interface proteins S2, S3, S6, and S7 are located in the neck region of the 40S particle, with proteins S2, S3, and S7 near the attachment site of the 60S subunit (Lutsch *et al.*, 1979; Bommer *et al.*, 1980). Initiation factor eIF-3 has been found to attach to the 40S subunit close to the interface region (Emanuilov *et al.*, 1978) in contact with proteins S3 and S4 among others (Nygård and Westermann, 1982). Proteins S3, S3a, and S6 have been found in close contact with both artificial and physiological messengers (Terao and Ogata, 1979; Stahl and Kobets, 1981; Reboud *et al.*, 1981; Takahishi and Ogata, 1981). These proteins have been further designated as presumptive P-site proteins because of their ability to cross-link to initiator tRNA (Westermann *et al.*, 1981). Proteins S6, S13, S14, and S15 have been referred to as A-site proteins because of their affinities for immobilised elongator tRNA and 5.8S rRNA (Metspalu *et al.*, 1978; Ulbrich *et al.*, 1979, 1980a; Todokoro *et al.*, 1981). In the cross-linking model shown in Figure 7A and B, our results have been integrated with current data on the spatial arrangement of the above proteins in relation to 18S rRNA and functional domains close to the ribosomal interface (cf. Svoboda and McConkey, 1978; Terao *et al.*, 1980b; Tolan and Traut, 1981; Uchiumi *et al.*, 1981; Gross *et al.*, 1981; Kelly and Cox, 1981).

Considerably less information is available on the topography of the interface region of the large ribosomal subunit. However, protein L6 seems to be associated with both A and P sites (Ulbrich *et al.*, 1980a; Todokoro *et al.*, 1981). Proteins L6, L7, and L8 together with 5S and 5.8S rRNA belong to the peptidyltransferase centre (Metspalu *et al.*, 1978; Ulbrich *et al.*, 1980a, 1980b; Todokoro *et al.*, 1981). The 5S rRNA can be cross-linked to protein L5 (Terao *et al.*, 1980a) and both 5S and 5.8S rRNA can be bound to proteins L5, L6, L7, and L8 (Metspalu *et al.*, 1978; Ulbrich *et al.*, 1979, 1980b). Furthermore, immobilised 5.8S rRNA binds 40S subunits, indicating a direct interaction of 5.8S rRNA with the small ribosomal subparticle (Villems *et al.*, 1979). The present and previous data on rRNA and proteins at the interface of the large ribosomal subunits are summarised in the cross-linking model shown in Figure 7C (cf. Svoboda and McConkey, 1978; Nika and Hultin, 1979; Uchiumi *et al.*, 1980; Kelly and Cox, 1981).

Table I. Neighbouring ribosomal proteins and RNA in the mammalian ribosome

Type of cross-linked product	Neighbouring components A and B	
	A	B
interface cross-links	S2, S3, S4, and S6 > S7, S13, and S14	28S rRNA
	L3 and L8 > L6 and L7	18S rRNA
intra subunit cross-links	S3a, S6, S7, S11, and S16/18 > S8, S23/24, and S25[a]	18S rRNA
	L3, L4, L6, L7, L7a, L8, L15, and L19 > L10 and L23/23a	28S rRNA
	S3a[b]	3′ end of 18S rRNA
	L3[b]	3′ end of 28S rRNA
	L5[c]	5S rRNA

[a]Westermann *et al.*, 1981; [b]Svoboda and MConkey, 1978; [c]Terao *et al.*, 1980.

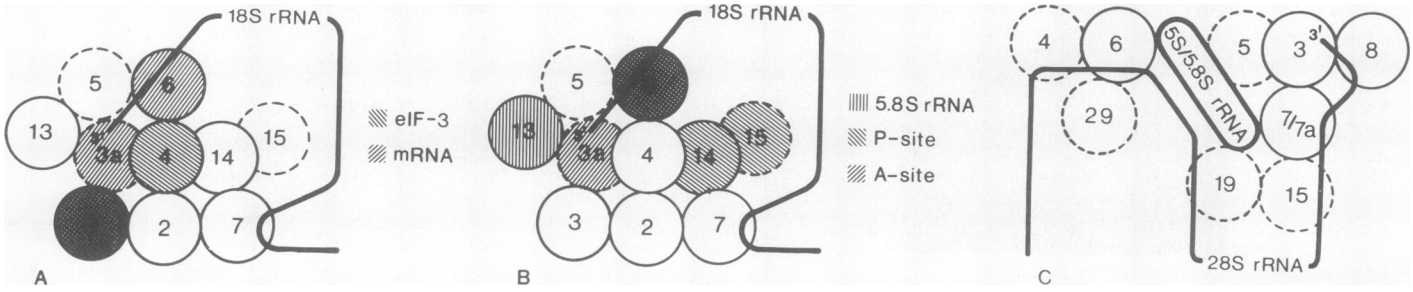

Fig. 7. Schematic representation of the spatial arrangement of proteins and rRNA in the interface region of 40S and 60S ribosomal subparticles. In **A** and **B** the 40S proteins cross-linked to 28S rRNA (continuous circles) are collated with current data on 18S rRNA neighbourhoods and functional domains at the interface. In **C** the 60S proteins covalently linked to 18S rRNA (continuous circles) are shown in the context of present knowledge about the protein interactions of 5S/5.8S rRNA and 28S rRNA.

Materials and methods

Materials

Puromycin and GTP were from Sigma Chemical Co. (St. Louis, MO). Sephadex G-15 was from Pharmacia (Uppsala, Sweden). N-succinimidyl-3-(4-hydroxy-5-[^{125}I]iodophenylpropionate) was from Amersham International Ltd. (Amersham, UK), Cronex Lightening Plus intensifying screens were from DuPont de Nemours Co. (Wilmington, DE).

Preparation of ribosomal subunits

Liver polysomes were isolated from starved rats according to Schreier and Staehelin (1973). A suspension of polysomes (80 A_{260} units/ml) in 0.5 M KCl, 20 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 2 mM puromycin, 2 mM GTP, and 11 mM 2-mercaptoethanol (Sundkvist and Howard, 1971) was incubated for 30 min at 37°C, and 1.2 ml portions were layered onto 33 ml 10–40% (w/v) linear sucrose gradients containing 0.35 M KCl, 20 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$ and 10 mM 2-mercaptoethanol. After centrifugation for 70 min at 50 000 r.p.m. in a Sorvall TV 850 vertical rotor, the gradients were monitored at 260 nm (Lake *et al.*, 1982) and the 40S and 60S ribosomal subunits collected separately. The isolated subunits were pelleted by centrifugation for 15 h at 107 000 g_{av}. The pellets were suspended in 0.25 M sucrose, 70 mM KCl, 30 mM triethanolamine hydrochloride (TEA-HCl), pH 7.6, 2 mM $MgCl_2$, and 5 mM 2-mercaptoethanol at a concentration of 80–100 A_{260} units/ml. The subunits were quickly frozen and stored at −70°C until used.

Radioactive labelling of ribosomal subunits and formation of 80S recombined ribosomes

Isolated 40S subunits (1 mg) and 60S subunits (2.5 mg) were incubated for 30 min at 0°C with N-succinimidyl-3-(4-hydroxy-5-[^{125}I]iodophenylpropionate) (Bolton and Hunter, 1973). The reaction was stopped by the addition of Tris-HCl, pH 7.6, to a final concentration of 10 mM, and the excess of non-covalently bound ^{125}I-labelled reagent was removed by gel filtration on 3 ml Sephadex G-15 columns equilibrated with 70 mM KCl, 30 mM TEA-HCl, pH 7.6, 2 mM $MgCl_2$, and 5 mM 2-mercaptoethanol.

For the identification of ribosomal interface proteins the labelled subunits were specifically recombined with the complementary unlabelled subunits to 80S ribosomes. For this purpose 1 mg of ^{125}I-labelled 40S subunits were incubated for 10 min at 37°C with 2.5 mg unlabelled 60S subunits in 70 mM KCl, 30 mM TEA-HCl, pH 7.6, 6 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. Likewise, 2.5 mg [^{125}I]60S subunits were incubated with 1 mg unlabelled 40S subunits as described above.

Cross-linking of 80S ribosomes and isolation of covalent RNA-protein complexes

The 80S recombined ribosomes were cross-linked by incubation for 30 min at 37°C in the presence of 5 mM diepoxybutane (Nygård *et al.*, 1980). The reaction was terminated by the addition of Tris-HCl, pH 7.6, to a final concentration of 10 mM.

Small samples, 20 μl, of the reaction mixtures were placed on 4 ml 10–30% (w/v) linear sucrose gradients containing 70 mM KCl, 30 mM TEA-HCl, pH 7.6, 6 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. After centrifugation for 110 min at 260 000 g_{av}, the gradients were monitored at 260 nm and the radioactivity of consecutive fractions was determined in a gamma counter (Figure 1).

The main part of the cross-linking reaction mixture was precipitated with 2 volumes of cold ethanol. The precipitates were collected by centrifugation for 20 min at 12 000 g_{av}, dissolved in Buffer A (50 mM LiCl, 1 mM lithium ethylenediamine tetraacetic acid, 2% (w/v) lithium dodecylsulphate) containing 1 mM iodoacetamide and incubated for 5 min at 56°C. The covalent ^{125}I-labelled ribosomal protein-rRNA complexes were isolated by centrifugation for 16 h at 200 000 g_{av} in 10–30% (w/v) linear sucrose gradients in Buffer A. The gradients were monitored at 260 nm. Material co-sedimenting with the 18S and 28S rRNA absorbance peaks was collected separately and precipitated with 2 volumes of cold ethanol. The precipitates were collected by centrifugation and the pellets dissolved in Buffer A containing 1 mM iodoacetamide. After incubation for 5 min at 56°C the collected 28S rRNA fractions were applied to 10–30% (w/v) sucrose gradients in Buffer A. Similarly, the collected 18S rRNA fractions were applied to 10–25% (w/v) sucrose gradients in Buffer A. Recentrifugation was for 16 h at 200 000 g_{av}. The gradients were monitored at 260 nm and the radioactivity in consecutive 0.32 ml fractions was determined in a gamma counter. Fractions containing 18S and 28S rRNA were pooled separately and the mixture of rRNA and covalent ^{125}I-labelled protein-rRNA complexes was precipitated with ethanol. The last centrifugation step was repeated once.

Preparation of cross-linked proteins for gel electrophoresis

The precipitated gradient fractions containing (a) 18S rRNA and proteins covalently bound to 18S rRNA, and (b) 28S rRNA and covalent 28S rRNA-protein complexes were precipitated with 2 volumes of ethanol. The precipitates were collected by centrifugation and the pellets dissolved in 100 μl 0.1 M Tris-HCl buffer, pH 7.6, containing 1 mM iodoacetamide. The samples were incubated for 30 min at 37°C with 20 μg RNase A and 20 units of RNase T1. After incubation, 300 μg protein from 60S subparticles was added to the 18S rRNA preparations and 300 μg protein from the 40S subunits to the 28S rRNA preparations.

For the identification of proteins covalently bound to 28S rRNA by intra-subunit cross-linking, 300 μg 60S proteins were added to the isolated covalent complexes of ^{125}I-labelled 60S protein and 28S rRNA. The incubation was continued for 2 h at 37°C in the presence of 1% (w/v) SDS, 0.2 M NaOH, and 1 mM iodoacetamide. The incubations were neutralised with 1 M H_3PO_4, dialysed overnight against two changes of 1 mM HCl and precipitated with 5 volumes of cold acetone containing 0.1 M HCl. In order to remove traces of SDS, the precipitates were dissolved in 0.1 M Tris-HCl, pH 7.6, and the material reprecipitated with acetone/HCl. The precipitates were carefully washed with acetone and dried.

Gel electrophoresis

Two-dimensional gel electrophoresis in the basic/acidic pH system (pH 8.6/pH 4.2) (system I) was carried out according to Lastick and McConkey (1976) with minor modifications. Micro-gels (1.8 x 55 mm), containing 4% (w/v) polyacrylamide, were used in the first dimension. Electrophoresis was for 4 h at 300 V (4°C). Electrophoresis in the second dimension was performed in 200 x 140 x 1 mm slab-gels, containing 15% (w/v) polyacrylamide. The gels were broad enough to allow for the simultaneous analysis of three first-dimension gels. Electrophoresis was for 13 h at 200 V (4°C). Gels were fixed and stained in 50% (v/v) methanol, 7% (v/v) acetic acid, and 0.25% (w/v) Coomassie Brilliant Blue, and destained in 50% (v/v) methanol solution containing 7% (v/v) acetic acid.

Two-dimensional electrophoresis in the acidic pH/SDS system (system II) was performed using gradient gels in both dimensions (Nika and Hultin, 1980). The linear 10–15% (w/v) polyacrylamide gradient slab-gels (140 x 160 x 1 mm) and the spacer gels used in the first dimension were based on stock solutions (pH 4.3) described by Reisfeldt *et al.* (1962), but containing 6 M urea. The 15% polyacrylamide solution contained 30% (v/v) glycerol. The concentration gels (140 x 55 x 1 mm) were provided with 25 sample slots (3 x 30 mm). Protein samples were dissolved in 8 M urea solution containing 5% (v/v) 2-mercaptoethanol and electrophoresed in the first dimension for 13 h at 300 V (4°C). The protein-containing lanes were made visible by rapid staining with Coomassie Brilliant Blue and excised from the gels. Electrophoresis in the second dimension was in 10–20% (w/v) polyacrylamide gradient slab-gels containing SDS (Laemmli, 1970). The superficially stained first-dimensional gel strips were equilibrated for 10 min at 35°C with sample buffer (Laemmli, 1970) and placed on the second-dimension gels (160 x 160 x 1.5 mm) without annealing. Electrophoresis was performed at 100 V (20°C) until the dye had reached the bottom of the gels (usually 24 h). Staining and destaining were as described above.

Radio-autography

After staining and destaining the two-dimensional gels were dehydrated in methanol for 1 h, soaked in 10% (v/v) glycerol solution for 15 min and dried. Radio-autography was performed at −80°C using Cronex Lightening Plus intensifying screens and Cronex-4 film. The ribosomal proteins were designated according to McConkey *et al.* (1979).

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