Intersubunit RNA-protein contacts in pre- and post-translocated *E.coli* ribosome

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Ribosomal proteins participating in intersubunit RNA-protein contacts (directly interacting with RNA of the opposite subunit) were determined by means of ultraviolet-induced cross-links in pre- and post-translocated ribosomal complexes, as well as in the free 70 S ribosome (tight couple) of *E coli* In these 3 complexes at least L1 and L9 proteins interact with 16 S RNA, while S6, S9/11 and S15 react with 23 S RNA All these proteins ('hinge-joint' proteins) are clustered on the small protuberance of the 50 S subunit and on the platform of the 30 S subunit. Reduction in the number of other (variable) intersubunit RNA-protein contacts in the course of transition from the tight couple to the pre- and, finally, to the post-translocated state, demonstrates gradual loosening of intersubunit interactions in 70 S ribosome Such a loosening ('opening') of the 70 S ribosome is determined by conformational changes in ribosomal subunits and/or in their relative arrangement, conjugated with alteration of the functional state of the ribosomal complex

E coli *ribosome* *Pre-translocated state* *Post-translocated state* *Intersubunit RNA-protein contact* *Ultraviolet-induced cross-link*

1. INTRODUCTION

One of the steps in the elongation process is translocation – synchronous displacement of mRNA and tRNA molecules along the translating ribosome. Evidently, such a displacement should be determined and/or accompanied by considerable alteration of the higher structure of the translating ribosome and, hence, of intermolecular and intersubunit interactions. According to Spirin's assumption [1] the driving force of this displacement is unlocking of ribosomal subunits by transition of the translating complex from the pre- to post-translocated state This transition has been shown to be accompanied by a minute (about 1 S) increase in the complex sedimentation constant [2]. However, transition from the pre- to post-translocated state is accompanied by significant conformational changes of both subunits (to be discussed separately). This could lead to alterations of the size and shape of the complex and, hence, to alteration of its hydrodynamic properties.

Here, the proteins directly participating in the intersubunit RNA-protein interactions inside free 70 S *E. coli* ribosome (tight couple), pre- and post-translocated ribosomal complexes have been determined by means of UV-induced cross-links.

2. MATERIALS AND METHODS

70 S ribosomes of *E. coli* MRE 600 (tight couple, I) were prepared as described in [3]. Upon binding of Phe-tRNA^{Phe} and NAcPhe-tRNA^{Phe} in the presence of poly(U) [4] activity was 85–90%. Pre-translocated complex (II) – NAcPhePhe-tRNA^{Phe}·70 S poly(U)·tRNA^{Phe} was obtained according to [5], the ratio 70 S:NAcPhePhe (cf. [6]) being about 1.0:0.8. Post-translocated complexes (IIIa and IIIb) – 70 S·poly(U)·

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NAcPhePhe-tRNA^{Phe} and 70 S poly(U)·NAcPhe-tRNA^{Phe}, respectively, were obtained by EF-G-promoted translocation of complex II [7] and by direct binding of NAcPhe-tRNA^{Phe} to the 70 S ribosome in the presence of poly(U) The ratios 70 S:NAcPhePhe or 70 S.NAcPhe were about 1.0.0 9 for complexes IIIa and IIIb, respectively.

Complexes were irradiated according to a conventional procedure [8] (254 nm, 20–30 quanta per nucleotide) Irradiated complexes were dissociated by addition of SDS and EDTA (final concentrations 1% and 20 mM, respectively) and ribosomal RNAs were separated electrophoretically in 4% polyacrylamide gel under denaturing conditions (8 M urea, 60°C). Gel pieces, containing 23 S and 16 S RNA, were iodinated by ^{125}I in the presence of chloramine T [9] and treated with a mixture of RNases A and T_1. Iodinated proteins (cross-linked to respective RNAs during irradiation of the complexes) were separated by two-dimensional electrophoresis in polyacrylamide gel according to [10] with some modifications (to be published) Iodinated proteins were identified by comparison of the radioactivity distribution with the position of authentic free ribosomal proteins, separated in the same gel and stained with Coomassie blue Two-dimensional distribution of radioactivity after electrophoresis was determined with the help of a multi-wire detector, conjugated with a computer [11] The relative efficiency of protein cross-linking was calculated as the ratio of radioactivity in the respective spot to the total radioactivity in the gel, subtracting the background and ignoring difference in iodination efficiency of different ribosomal proteins.

3. RESULTS

The total amount of proteins cross-linked to ribosomal RNAs by ultraviolet irradiation in all the complexes under study is proportional to the absorbed dose, at least up to 30 quanta per nucleotide (not shown) Hence, cross-linked proteins directly interact with the respective RNAs inside the starting complexes The electrophoretic system used here separates all the proteins of the *E. coli* 70 S ribosome, except 3 groups – S9 + S11, S13 + S14 + L27 and S20/L26 + S21 (fig.1). The presence of cross-linked oligonucleotide(s) results

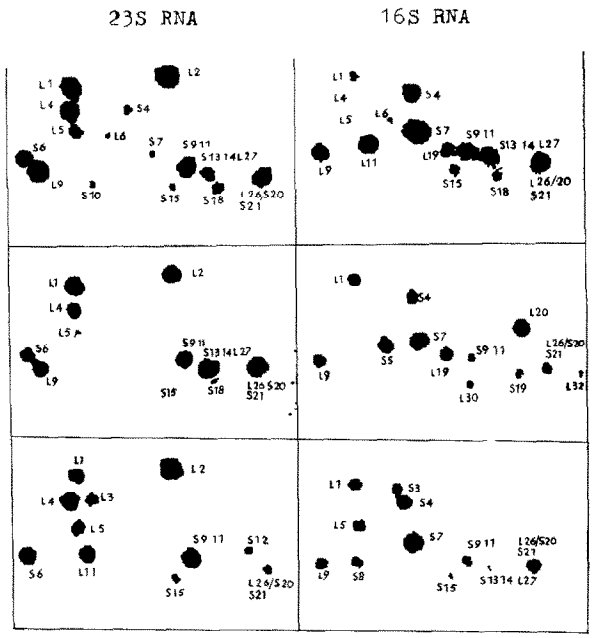

Fig 1 Distribution of radioactivity over the gel slab after two-dimensional electrophoretic separation of the proteins cross-linked to 23 S RNA and 16 S RNA Taken from the telemonitor screen of the multi-wire radioactivity detector Upper panels, free 70 S ribosome (tight couple), middle and lower panels, pre- and post-translocated complexes, respectively

in a small, but definite shift of the protein position in the polyacrylamide gel [8]. Therefore, separation of proteins, cross-linked to 16 S and 23 S RNAs, allows their exact identification (excluding proteins of the above-mentioned groups) and, hence, determination of most of the intra- and intersubunit interactions in the respective complexes. Only intersubunit RNA-protein interactions with participation of exactly identified proteins are considered below S9 and S11 proteins are also considered in this respect – although they are not resolved by electrophoresis, both belong to the 30 S subunit. Thus, the appearance of the corresponding spot after separation of proteins, cross-linked to 23 S RNA, reflects the participation of S9 and/or S11 in intersubunit interactions.

Several proteins (S6, S9/11, S15, L1 and L9) are cross-linked to RNA of the opposite subunit in all complexes studied (fig 1). Cross-linking of other proteins depends on the type of complex (table 1). It is noteworthy that the same proteins were found

Table 1

Variable intersubunit RNA-protein interactions in 70 S ribosomal complexes

Complexes	Proteins													
	S4	S7	S10	S12	S18	L4	L5	L6	L11	L16	L19	L30	L32	L33
Tight couple	+	+	+		+	+	+	+	+	+	+			
Pre-translocated					+						+	+	+	+
Post-translocated				+			+							

cross-linked to RNAs in complexes IIIa and IIIb, prepared by translocation of complex II or by binding of NAcPhe-tRNA^{Phe} to the 70 S tight couple in the presence of poly(U).

4. DISCUSSION

The position of the main part of proteins in the *E coli* ribosome has been established over the last few years. We used the 'consensus model' [12], based on the data of Stoffler et al [13] and Lake et al. [14], taking into account also the latest data from [15] (fig.2) Unfortunately, not all the proteins participating in the intersubunit RNA-protein interactions have yet been localized. Nevertheless, the different distribution of proteins, participating in the intersubunit interactions in the tight couple, pre- and post-translocated complexes, is evident.

The proteins involved in intersubunit RNA-protein interactions in all the complexes (I, II, IIIa and IIIb) are clustered on the platform of the 30 S subunit and the small protuberance of the 50 S subunit According to the consensus model [12] these clusters are situated just opposite each other in the assembled 70 S ribosome, forming a hinge-joint (fig.2) Other (variable) intersubunit RNA-protein contacts in the tight couple are distributed over all the interfaces of both subunits (fig.2). Significant reduction in variable contacts by transition from the tight couple to pre-translocated state (table 1) is most probably due to the presence of mRNA and two tRNA molecules inside the 70 S ribosome (cf. [16]) Since the location of the L20, L30, L32 and L33 proteins remains unknown, it is impossible to locate the region of new interactions appearing on transition from the tight couple to pre-translocated state. After transition from the pre- to post-translocated state only two variable

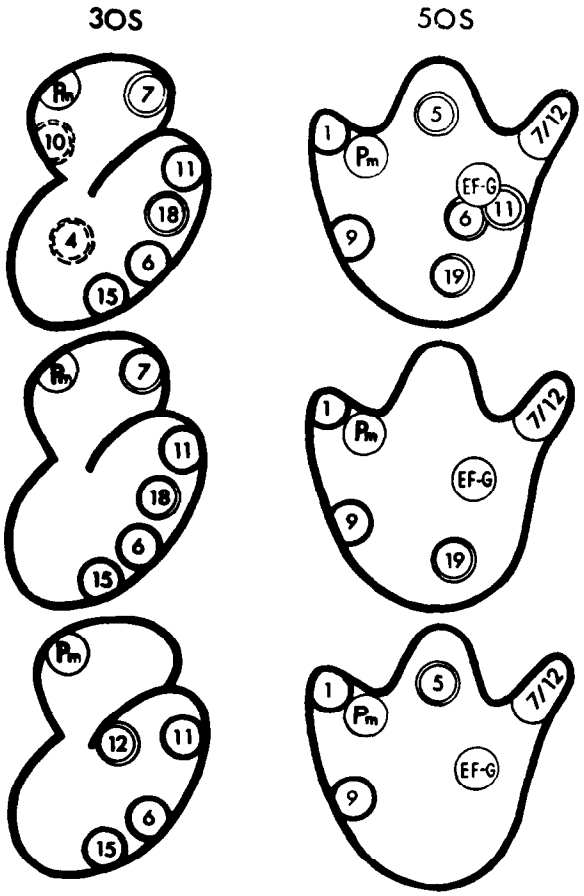

Fig.2 Location on intersubunit interfaces of ribosomal proteins, cross-linked to RNA of the opposite subunit Thick circles, hinge proteins, dashed circles, proteins whose antigenic determinants were not found on the interfaces, Pm and EF-G, proposed location of puromycin and elongation factor G, respectively Upper panels, free 70 S ribosome (tight couple), middle and lower panels, pre- and post-translocated complexes, respectively

contacts remain, involving proteins S12 and L5 (table 1), which are located near to the cleft of the 30 S subunit and on the large protuberance of the 50 S subunit, respectively. In other words, translocation is conjugated with loosening of intersubunit interactions, a process which can be thought of as opening of the 70 S ribosome.

Association of elongation factor EF-G with pre-translocated complex II leads to translocation even without hydrolysis of GTP [17]. EF-G binds to complex II somewhere below the L7/L12 stalk [18]. It should promote opening of the 70 S ribosome. Such an opened state was found for post-translocated complexes IIIa and IIIb. In the opened state after release of EF-G, the region below the L7/L12 stalk becomes available for codon-dependent binding of ternary complex aminoacyl-tRNA·Tu GTP with the R-site of the ribosome [19]. Transition of aminoacyl-tRNA into the A-site allows transpeptidation, while release of EF-Tu allows expansion of intersubunit interactions. After binding of the non-cognate ternary complex with post-translocated ribosome, hydrolysis of GTP and release of EF-Tu·GDP take place, but non-cognate aminoacyl-tRNA is also released [20]. Since these events do not prevent reassociation of the ternary complex with this ribosomal complex, they most probably do not result in transition to the pre-translocated state. Hence, transition from the pre- to post-translocated state is induced by binding of cognate aminoacyl-tRNA to the A-site, and/or by transpeptidation.

REFERENCES

[1] Spirin, A.S (1968) Dokl Akad Nauk SSSR 179, 1467–1470.

[2] Baranov, V I , Ryabova, L A. and Spirin, A.S. (1984) Mol. Biol. (Moscow) 18, 350–357

[3] Hapke, B and Noll, H (1976) J Mol Biol 105, 97–109

[4] Kirillov, S V., Katunin, V I and Semenkov, Yu P (1981) FEBS Lett 125, 15–19

[5] Semenkov, Yu P , Makarov, E M., Makhno, V I. and Kirillov, S V (1982) FEBS Lett. 144, 125–129

[6] Yokosawa, H , Inoue-Yokosawa, N , Arai, K., Kawakita, M and Kaziro, Y (1973) J Biol Chem 248, 375–377

[7] Johnson, A E and Cantor, C R. (1980) J. Mol Biol. 138, 273–297

[8] Abdurashidova, G G , Turchinsky, M F and Budowsky, E I. (1981) FEBS Lett 129, 59–61

[9] Hunter, W.H and Greenwood, F C (1962) Nature 194, 495–496

[10] Mets, L J and Bogorad, L (1974) Anal Biochem 57, 200–210

[11] Zanevsky, Yu V , Ivanov, A.B , Kaminir, L B , Kreindlin, E.Ya , Movchan, S A., Peshekhonov, V D , Chan Diik Than, Chernenko, S P and Chernyi, A A (1983) Preprint JINR, 18-83-534, Dubna

[12] Prince, J B , Gutell, R R and Garrett, R A (1983) Trends Biochem Sci 8, 359–363

[13] Kastner, B , Noah, M , Stoffler-Meilicke, M and Stoffler, G (1983) Proc. 10th Int Congr Electron Microsc 3, 99–106

[14] Winkelmann, D.A , Kahan, L and Lake, J.A (1982) Proc Natl Acad Sci USA 79, 5184–5188

[15] Breitenreuter, G., Lotti, M , Stoffler-Meilicke, M. and Stoffler, G (1984) Mol Gen Genet 197, 189–195

[16] Spirin, A S (1983) FEBS Lett 156, 217–221

[17] Girbes, T , Vazquez, D and Modolell, J (1976) Eur J Biochem 67, 257–265

[18] Girshovich, A S , Kurtskhalia, T V , Ovchinnikov, Yu A and Vasiliev, V D (1981) FEBS Lett 130, 54–59

[19] Lake, J A (1977) Proc Natl Acad Sci USA 74, 1903–1907

[20] Thompson, R C and Stone, P J (1977) Biochemistry 1, 198–202