Localization of elongation factor Tu on the ribosome

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The EF-Tu-binding center of the *E. coli* ribosome has been localized by immunoelectron microscopy after cross-linking of the specific EF-Tu·70 S ribosomal complex with dimethylsuberimidate. EF-Tu has been found to be in contact with the 50 S subunit in the region of the L7/L12 stalk and with the 30 S subunit in the upper part of its body on the side opposite the top of the ledge (the platform). The EF-Tu position on a model of the 70 S ribosome is presented.

Elongation factor Tu *Ribosome* *Dimethylsuberimidate* *Immunoelectron microscopy*

1. INTRODUCTION

Elongation factor Tu (EF-Tu) plays a fundamental role in protein biosynthesis, promoting the codon-dependent binding of aminoacyl-tRNA to the ribosome. The importance of determining the ribosomal region interacting with EF-Tu is evident. Numerous investigations using cross-linking [1,2], singlet energy transfer technique [3], inhibition by ribosomal protein-specific antibodies [4], ribosomal protein omission tests [5–7], etc. have left this question still open.

This paper describes experiments on a direct localization of EF-Tu on the ribosome by immunoelectron microscopy.

2. MATERIALS AND METHODS

Tight 70 S ribosome couples were prepared from *E. coli* MRE 600 as described in [8]. Samples of electrophoretically homogeneous EF-Tu·GDP and EF-Ts were kindly provided by Dr O. Stengrevics (Institute of Applied Biochemistry, Olaine, USSR). $tRNA^{Phe}$ was from Boehringer Mannheim (1.5 nmol Phe/A_{260}). ^3H labeling of EF-Tu·GDP was carried out by reductive methylation [9] with 1 mM formaldehyde in the presence of NaB^3H_4 (Amersham, 8 Ci/mmol) in 0.1 M borate (pH 9.0), 5 mM $MgCl_2$, 100 mM KCl, 2 mM β-mercaptoethanol and 20 μM GDP; the specific activity of the preparation was $1–2.5 \times 10^3$ cpm per pmol. Specific rabbit antisera against EF-Tu·GDP were prepared by a standard technique. The IgG fraction was purified by $(NH_4)_2SO_4$ fractionation, chromatography on DE 52 and gel filtration on Sephadex G-200 and stored at −80°C in 50 mM Tris-HCl (pH 7.5), 150 mM NH_4Cl, 8 mM $MgCl_2$, 10 mM β-mercaptoethanol (30 mg protein/ml). An Ouchterlony test showed that IgG did not react with ribosomes and isolated protein L12.

The ternary complex [^{3}H]EF-Tu·GMPPCP·[^{12}C]Phe-$tRNA^{Phe}$ (complex I) was prepared via two steps: (i) an equimolar mixture of [^{3}H]EF-Tu·GDP and EF-Ts (18 nmol/ml each) was incubated for 10 min at 30°C in the presence of 20 mM EDTA, 20 mM Bicine (pH 8.2) and the [^{3}H]-EF-Tu·Ts complex was isolated on Sephadex G-50 in 20 mM Bicine (pH 8.2), 2 mM EDTA and 2 mM β-mercaptoethanol [12]; (ii) then an equimolar amount of [^{12}C]Phe-$tRNA^{Phe}$ was added and also GMPPCP, $MgCl_2$ and KCl to 1, 10 and 100 mM, respectively, and the mixture was incubated for 5 min at 30°C. The complex ribosome·poly(U)·$tRNA^{Phe}$ (complex II) was prepared separately: an equimolar mixture of the ribosomes and deacylated $tRNA^{Phe}$ (250 pmol each) and 1.8 A_{260} of poly(U)

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(M_r about 2×10^4) in 20 mM Bicine (pH 8.2), 10 mM $MgCl_2$, 100 mM KCl (final volume 120 μl) were incubated for 5 min at 30°C. Complexes I and II were mixed in a ratio of 1.2:1 (final volume 200 μl) and, after 10 min incubation at 30°C, cooled in ice. 1/16 vol. of a fresh 80 mM solution of dimethylsuberimidate (Pierce) in 0.5 M Bicine (pH 8.2) was added and the mixture was incubated for 10 h in ice. The excess of the reagent was inactivated with NH_4Cl (0.1 M).

According to centrifugation in a 5–20% sucrose gradient the product of cross-linking contained about 8–10 mol [^{3}H]EF-Tu per 100 mol ribosomes. The cross-linked 70 S ribosomes or their subunits were purified by repeated centrifugation in the sucrose gradient and concentrated by PEG 6000 precipitation.

The pellets were dissolved in 10 mM Tris-HCl (pH 7.5), 100 mM NH_4Cl, 10 or 2 mM $MgCl_2$ and incubated with antibody against EF-Tu for 30 min in ice. The amount of antibody for optimal formation of the 50 S and 30 S dimers was 30 and 40 μg per pmol cross-linked EF-Tu, respectively. Analysis of the product was carried out by 5–20% sucrose gradient centrifugation in the above buffers. After sucrose removal by dialysis, the dimer fractions were negatively stained with uranyl acetate using the single-layer carbon technique [10] and examined in a JEM-100C microscope.

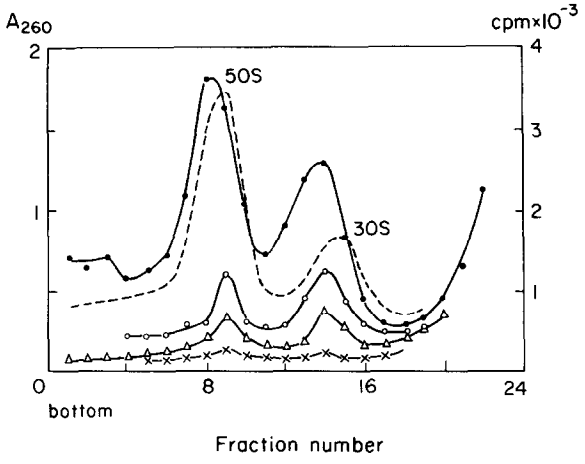


Fig.1. Specificity of [^{3}H]EF-Tu cross-linking with the ribosome by dimethylsuberimidate. (●—●) Whole mixture, (×—×) same but without the reagent, (○—○) same but without poly(U), (△—△) the mixture containing only [^{3}H]EF-Tu·Ts, ribosome and reagent, (– – –) A_{260}.

3. RESULTS

As in the case of EF-G [11], the localization of EF-Tu on the ribosome by immunoelectron microscopy requires prior fixation of the readily dissociating complex of the factor with the ribosome. To this end we used cross-linking of the complex with dimethylsuberimidate. The results are shown in fig.1. As seen, in the whole system

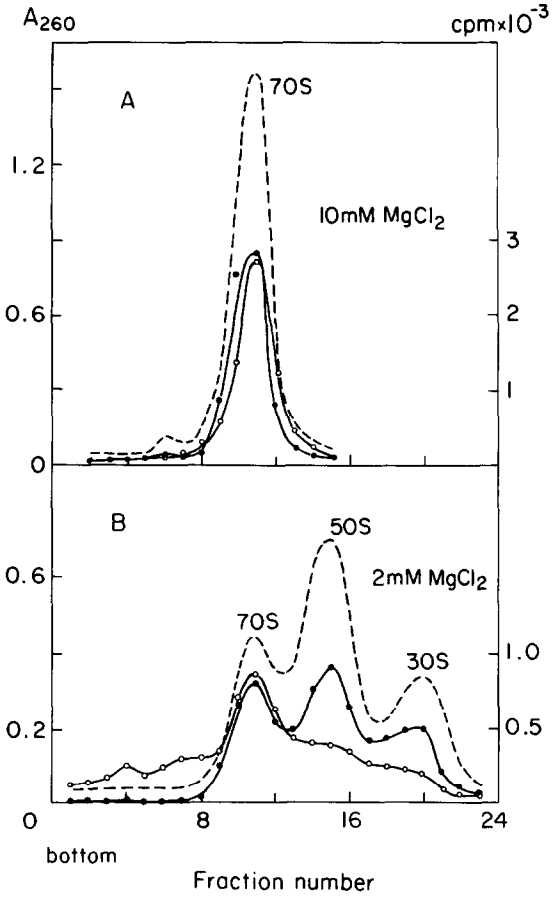


Fig.2. Accessibility to antibody action at 10 or 2 mM $MgCl_2$ of [^{3}H]EF-Tu specifically cross-linked with the ribosome. Mixtures: 130 pmol ribosomes (10.6 pmol cross-linked factor) ± 0.33–1.0 mg antibody at 10 mM $MgCl_2$ or ± 0.33 mg antibody at 2 mM $MgCl_2$. (●—●) Without antibody, (○—○) in the presence of antibody, (– – –) A_{260}.

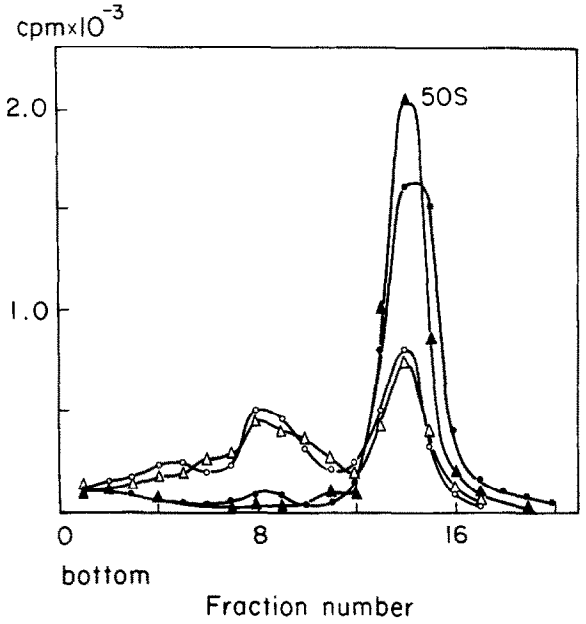


Fig.3. Interaction of the antibody with [^{3}H]EF-Tu cross-linked in the ribosome with the 50 S subunit. (●—●) Without antibody (120 pmol 50 S, 5 pmol cross-linked factor), (○—○) plus 0.15 mg antibody, (▲—▲) same as preceding data, but in the presence of native EF-Tu (60 pmol), (△—△) same as (○—○) but in the presence of purified protein L12 (2.6 nmol).

[^{3}H]EF-Tu cross-links with both the ribosomal subunits. In the absence of poly(U) cross-linking sharply decreases. Treatment of only the mixture of [^{3}H]EF-Tu · Ts and ribosomes with the reagent in the absence of the other components does not lead to efficient cross-linking. In other words, the appearance of the radioactive label, i.e. [^{3}H]EF-Tu, in fractions of the ribosomal subunits is due to the formation of the specific complex between the factor and the ribosome.

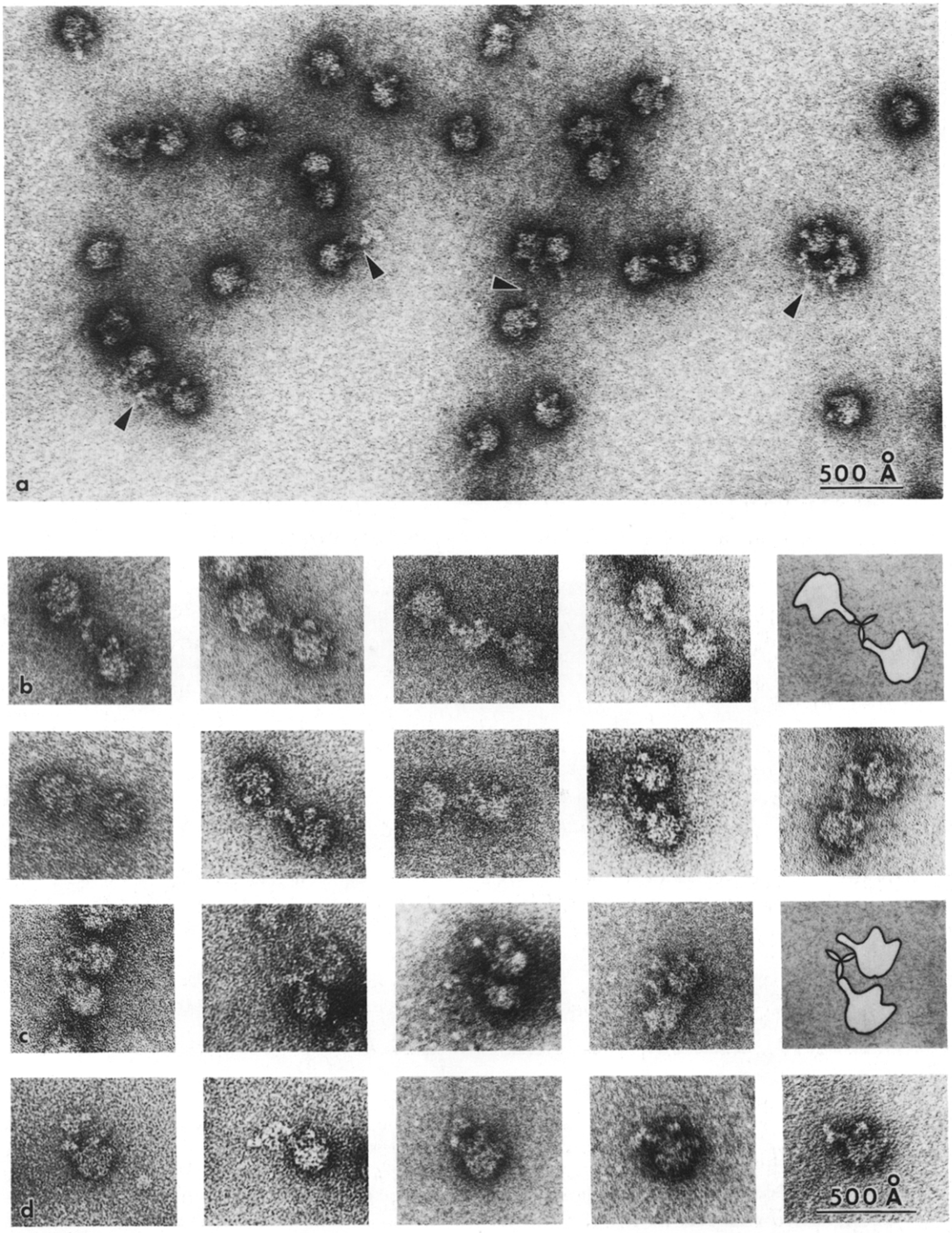
Fig.2 shows the accessibility of cross-linked EF-Tu to antibodies at 10 and 2 mM $MgCl_2$, i.e. under conditions of association and dissociation of the ribosome, respectively. The result is that the interaction of the antibody with the cross-linked factor requires the previous dissociation of the 70 S ribosome. Therefore, electron microscopy was performed after treatment of the isolated ribosomal subunits with the antibody.

Fig.3 shows the specificity of the antibody interaction with [^{3}H]EF-Tu cross-linked in the 70 S ribosomal complex to the 50 S subunits. On incubation with an optimal amount of antibody, about 50% of the radioactive label shifts to the region of the 50 S dimers. Treatment of the mixture containing an excess of added native EF-Tu with the antibody does not lead to dimer formation. At the same time the presence of an excess of protein L12 (or 50 S subunits, not shown) does not affect the yield of dimers. This means that dimer formation reflects the specific interaction of the antibody with the cross-linked [^{3}H]EF-Tu.

Electron micrographs of the preparation from the 50 S dimer fraction are presented in fig.4. We have analyzed a total of 150 and 40 images of the dimers and monomers, respectively, as shown in fig.4b–d. The predominant view of the 50 S subunits is a crown-like one. In this view, antibody molecules attach to the stalk of protein L7/L12 or its base. In the infrequently observed kidney view, the antibodies attach to the base of the stalk always from the interface side of the 50 S subunit. Other antibody-binding sites occur very rarely ($< 5\%$ of the total).

For the 30 S subunits (fig.5) we have examined 160 dimers and 30 monomers. In the intermediate views (fig.5b), the antibodies attach to the upper frontal part of the 30 S body and in the frontal view (fig.5c) to the left (non-interface) side of the subunit. Hence, the antibody-binding site is located

Fig.4. Electron micrographs of the 50 S subunits with cross-linked EF-Tu after reaction with the antibodies. (a) Field of large subunits. Arrows indicate antibodies in the 50 S · IgG · 50 S complexes. (b) Gallery of the 50 S subunits linked with antibody in a characteristic crown-like view [12], as shown schematically to the right. The antibodies attach to the different parts of the L7/L12 stalk. (c) Antibodies attach to the upper subunit of each pair in the region of the base of the stalk. The two first frames represent 'crown-crown' types of images as shown schematically to the right. In the kidney view, antibodies attach to the concave (interface) side of the subunit (lower subunit in the third frame). This was observed in all 17 images of the subunit in the kidney view, which were found and examined. The fourth frame shows the 50 S · IgG · 30 S complex with typical antibody-binding sites on both large and small ribosomal subunits. (d) Single subunits with antibodies attached.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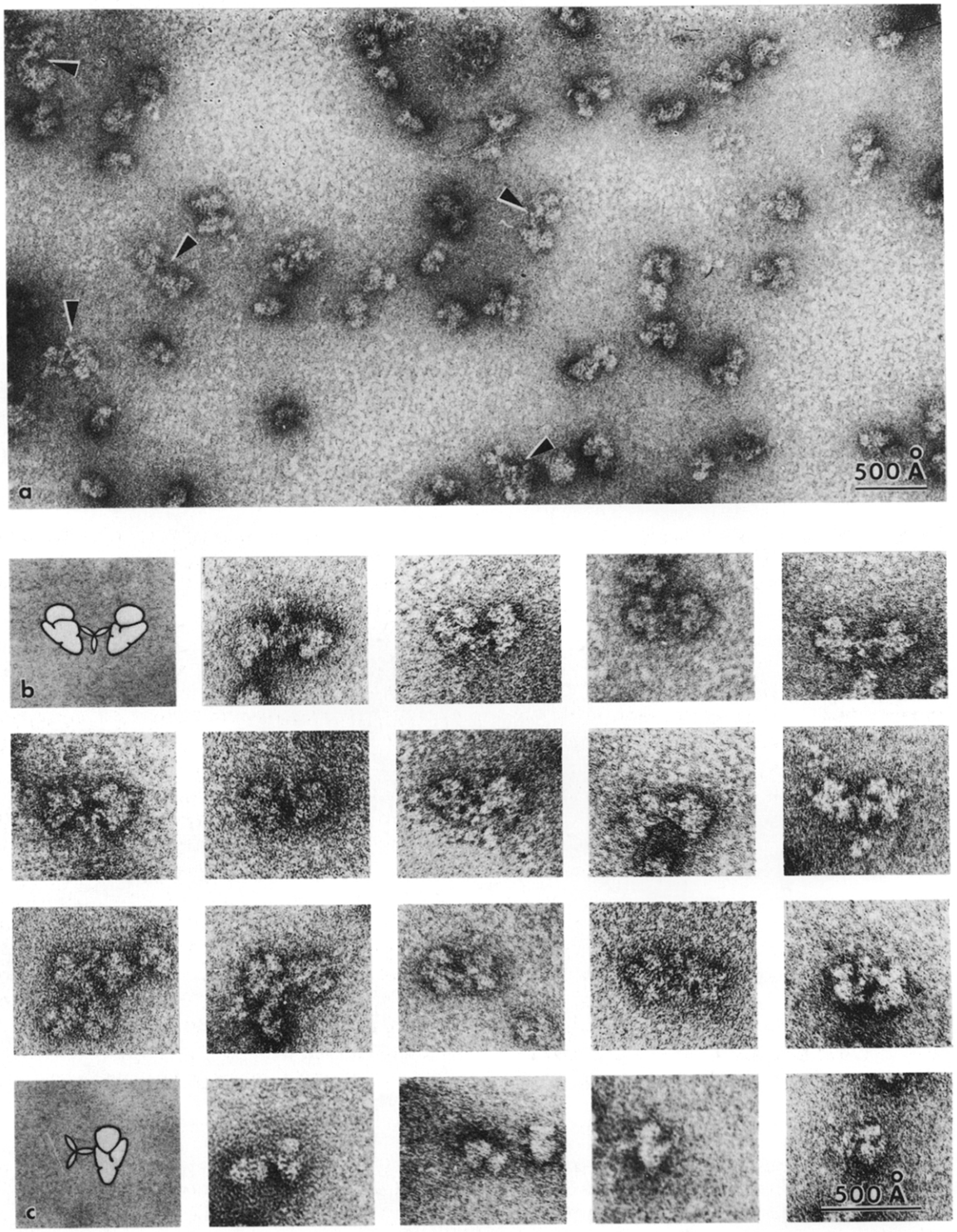
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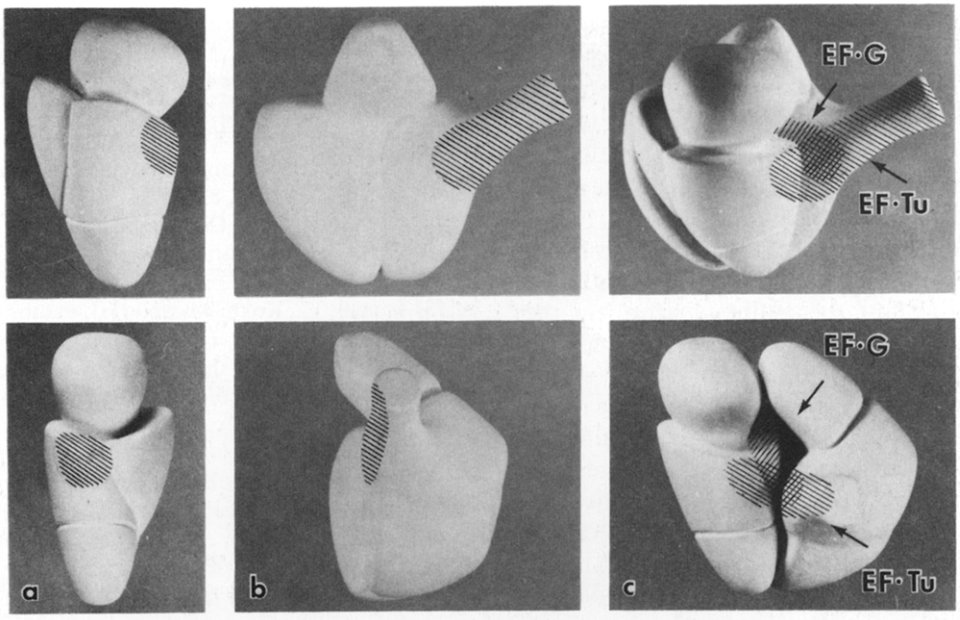

Fig.6. Localization of EF-Tu on the ribosome. The position of the EF-Tu is denoted by hatching on: (a) the exterior and frontal sides of the 30 S subunit model [13,15], (b) the interface and lateral sides of the 50 S subunit model [12], and (c) the 70 S ribosome model [14,15] approximately in overlap and non-overlap views. The position of EF-G [11] is shown also for comparison.

on the upper part of the 30 S body approximately opposite the top of its ledge. This site is observed in more than 90% of the images analyzed. In rare cases (< 10%) the antibody appears to be connected to the ledge (5%), head (3%) or other regions (1%). It is likely that these minor sites are unspecific and have no relation to the real location of EF-Tu.

4. DISCUSSION

The results of immunoelectron microscopic analysis of the ribosomal subunits containing cross-linked EF-Tu can be formulated as follows (fig.6): (i) The zone of interactions of EF-Tu with the 50 S subunit covers the L7/L12 stalk and its base; (ii) EF-Tu contacts with the 30 S subunit in the upper part of its body on the side opposite the top of the ledge.

From a comparison of these data with a model of the 70 S ribosome [14,15], it follows that both the zones described above are found in the same region of the 70 S ribosome. This permits us to outline correctly the region of the likely contacts of the EF-Tu molecule with the ribosomal surface (fig.6c). For comparison, the position of the EF-G-binding site localized by us earlier [11] is also shown. Both sites seem to be close to each other or perhaps even overlap, but do not coincide. EF-G is located deeper in the ribosomal interface, contacting with the 30 S subunit in the groove between its head and body and with the 50 S subunit at the base of the L7/L12 stalk but without visible contact with the stalk itself. EF-Tu is located nearer to

Fig.5. Electron micrographs of the 30 S subunits with cross-linked EF-Tu after reaction with the antibodies. (a) Field of the preparation. Arrows indicate antibodies in the 30 S · IgG · 30 S complexes. (b) Gallery of pairs of small subunits linked by antibody. The subunits in pairs are in characteristic intermediate (45 and 225°) views [13], as shown schematically in the first frame. The antibodies attach to the upper frontal part of the subunit body. (c) Vertically oriented subunits in the pair and in the monomeric complexes are in a frontal view. The antibodies attach to the left side of the subunit which is its interface side [14,15]. An interpretative drawing is shown in the first frame.

the ribosomal periphery, contacting with the 30 S subunit distinctly below EF-G and interacting by a substantial area of its surface with the L7/L12 stalk.

Numerous investigations of the interaction of EF-Tu with aminoacyl-tRNA show that these molecules have a fairly large contact surface [16–19] (the consequence of such a large contact surface can be significant protection of the factor antigenic determinants against the antibody action in the functional EF-Tu · 70 S ribosomal complex; see fig. 2A). In the proposed model of the EF-Tu · tRNA complex, the tRNA lies parallel to the factor molecule with the acceptor end located on domain 1 of the factor [20] and with the anticodon end protruding from its domain 3 [19]. As the anticodon and acceptor ends of the tRNA are located on the 30 S and 50 S subunits, respectively, then, in accordance with this model, the EF-Tu molecule must be placed in such a way that its domains 1 and 2 form contacts mainly with the L7/L12 stalk and/or its base on the 50 S subunit while domain 3 contacts with the upper part of the 30 S subunit body.

In conclusion, the location of EF-Tu on the ribosome shown above is the most direct experimental evidence in favour of a model of the aminoacyl-tRNA binding site being near the base of the L7/L12 stalk [21]. In fig.6c the aminoacyl-tRNA will lie above the EF-Tu molecule (perhaps adjoining the EF-G-binding site) so that its anticodon is in the groove between the head and the body of the 30 S subunit and the acceptor end is directed towards the base of the 50 S central protuberance.

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