

LOCALIZATION OF THE ELONGATION FACTOR G ON *ESCHERICHIA COLI* RIBOSOME

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1. Introduction

The translocation step of protein biosynthesis on the ribosome is promoted by the elongation factor EF-G. The ribosomal EF-G-binding center is known to be formed mainly by its 50 S subunit [1–3]. At the same time the ability of this subunit to interact with EF-G is significantly stimulated by the presence of the ribosomal 30 S subunit [4–8]. Affinity labelling has shown that EF-G in the specific complex with the ribosome is crosslinked to 50 S as well as to 30 S subunit, i.e., the factor is evidently located on the ribosomal interface [9,10].

The possibility of covalent fixation of EF-G on the ribosome permitted us to use the IEM technique for the direct localization of the ribosomal EF-G-binding center. Here we show that:

- (1) EF-G is located on the 50 S subunit surface facing the 30 S subunit at the base of the side elongated protuberance (rod-like appendage);
- (2) EF-G contacts with the 30 S subunit in the region of the groove between the head and body from the side of the ledge (platform).

2. Materials and methods

Ribosomes (70 S tight couples) and their 50 S subunits were obtained from *E. coli* MRE-600 as in [11,12]. EF-G was isolated according to [13], its ^3H -labelling was carried out by reductive methylation [14]. The preparation and properties of the photoac-

tivated azido(N)- and azido(S)-[^3H]EF-G derivatives are described in [9,15]; the conditions for the formation of their complexes with the 70 S ribosomes (or isolated 50 S subunits), and for photoaffinity cross-linking are the same as those in [9,15] except for the use of equimolar amounts of ribosomes and EF-G for the complex formation. The irradiated [^3H]EF-G derivative · ribosome complex was ultracentrifuged in a sucrose gradient (5–20%) containing the dissociating buffer (1 mM MgCl_2 , 200 mM NH_4Cl , 10 mM Tris-HCl, pH 7.5). Isolated ribosomal subunits were concentrated by precipitation with polyethylene glycol 6000 [16]. Anti-EF-G was obtained by immunization of rabbits with homogeneous EF-G and purified by affinity chromatography on Sepharose with immobilized EF-G. According to the data of double immunodiffusion and IEM analysis, the preparation of anti-EF-G obtained does not interact with the ribosomes.

The optimal conditions for preparing of the dimers (pairs of ribosomal subunits linked by anti-EF-G) are: anti-EF-G was added in ice to ~ 1 nmol modified 50 S or 30 S subunits so that its molar ratio to the cross-linked EF-G was 2:1 or 1:1, respectively, in a buffer with 10 mM Mg-acetate, 100 mM NH_4 -acetate, 10 mM Tris-acetate (pH 7.5). The sample volume was 0.4 ml. This mixture was immediately layered on the pre-cooled sucrose gradient (5–20%) in the same buffer but with 2 mM Mg-acetate. Dimers were isolated by ultracentrifugation on the Spinco L-5-50 (SW41 rotor, 22 000 rev./min, $+2^\circ\text{C}$, 8.5 or 16 h for 50 S or 30 S subunits, respectively, the fraction volume ~ 0.25 ml). The dimer fractions chosen for IEM analysis were pooled and dialyzed against the same buffer for sucrose removal.

The samples for electron microscopy analysis were prepared according to the Valentine technique [17] or to its doublecarbon-layer modification [18,19].

Abbreviations: IEM, immune electron microscopy; azido(S)-EF-G, EF-G with the arylazido-residue connected to the single exposed SH-group; azido(N)-EF-G, EF-G with the arylazido-residue connected to the exposed NH_2 -group(s); anti-EF-G, antibody directed against EF-G

3. Results

Azido(S)-EF-G was used for the localization of EF-G on the 50 S subunit as it crosslinks mainly to the 50 S subunit in the specific EF-G · ribosome complex (the yield was ~ 0.1 mol EF-G/mol 50 S, $\sim 90\%$ of this amount was in 23 S RNA [15]). Fig.1(a) shows the result of anti-EF-G interaction with the 50 S subunits containing the crosslinked [3 H]EF-G. It is seen that the addition of anti-EF-G leads to a considerable change of the radioactivity profile: up to 30–40% of the label appears in the region of the 50 S subunit dimers. The pooled dimer fractions number 9 and 10 with a specific radioactivity of ~ 0.7 – 0.8 mol EF-G crosslinked/mol 50 S subunit were used for IEM analysis.

Two main and well-known [19–21] views or projections can be seen on electron micrographs: asymmetrical 'crown' views and 'kidney' ones (fig.2). The asymmetrical 'crown' view is characterized by a central protuberance and two side protuberances of unequal length. The longer of these two protuberances, or rod-like appendage, containing proteins L7/L12 [22] is an obligatory structural feature of the intact 50 S subunit. The 'kidney' view is characterized by a notch asymmetrically arranged on the irregular concave side, which is interface side of the 50 S subunit. Many 50 S dimers contain one or more easily identified antibodies. Altogether we have examined ~ 200 dimers such as presented in fig.2(b). In all cases the site of anti-EF-G attachment to the 50 S subunit, i.e., the position of the crosslinked EF-G, has been determined unambiguously: it is located on the interface side of the subunit at the base of the rod-like appendage.

It should be noted that this result has been obtained for the azido(S)-EF-G · 70 S ribosome complex formed in the presence of GTP (+ fusidic acid). The same location of EF-G was found (not shown) for the ribosomal complex formed in the presence of the non-hydrolyzable GTP analog, (β,γ -methylene)guanosine 5'-triphosphate, and for the complex of the EF-G derivative with the isolated 50 S subunit (+ GTP and fusidic acid).

For localization of EF-G on the ribosomal 30 S subunit we chose the crosslinking reaction of 70 S ribosome with azido(N)-EF-G (+ GTP and fusidic acid) which attacks preferably the 30 S subunit ($\sim 70\%$ of crosslinked EF-G with an yield of ~ 0.06 mol EF-G/mol 30 S) [9]. The result of the modified 30 S subunit interaction with anti-EF-G is shown in fig.1(b). The

pooled dimer fractions 8 and 9 with a specific radioactivity of ~ 0.3 mol crosslinked EF-G/mol 30 S subunit were examined. The typical images of the antibody-linked 30 S subunit pairs and single 30 S subunits with attached anti-EF-G are presented in fig.3. About 100 such images have been analyzed. At least 2 anti-EF-G-binding sites can be detected on the electron micrographs. In terms of the three-dimensional 30 S subunit model proposed in [23], the region of their location can be interpreted as a narrow zone on the

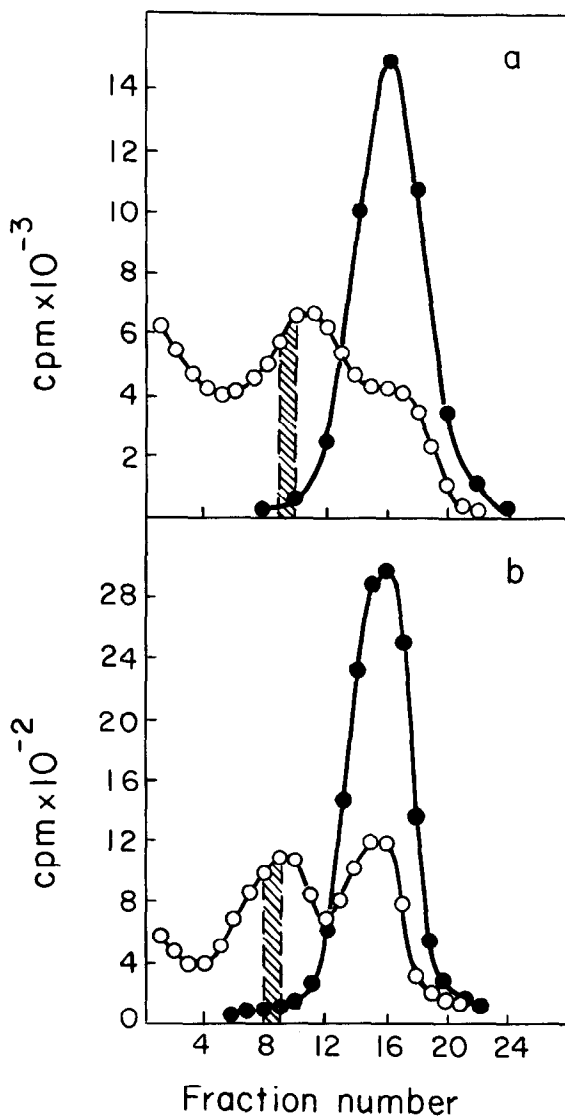


Fig.1. Effect of anti-EF-G on the sedimentation of the 50 S (a) and 30 S (b) ribosomal subunits containing the crosslinked [3 H]EF-G: (●) minus anti-EF-G; (○) plus anti-EF-G.

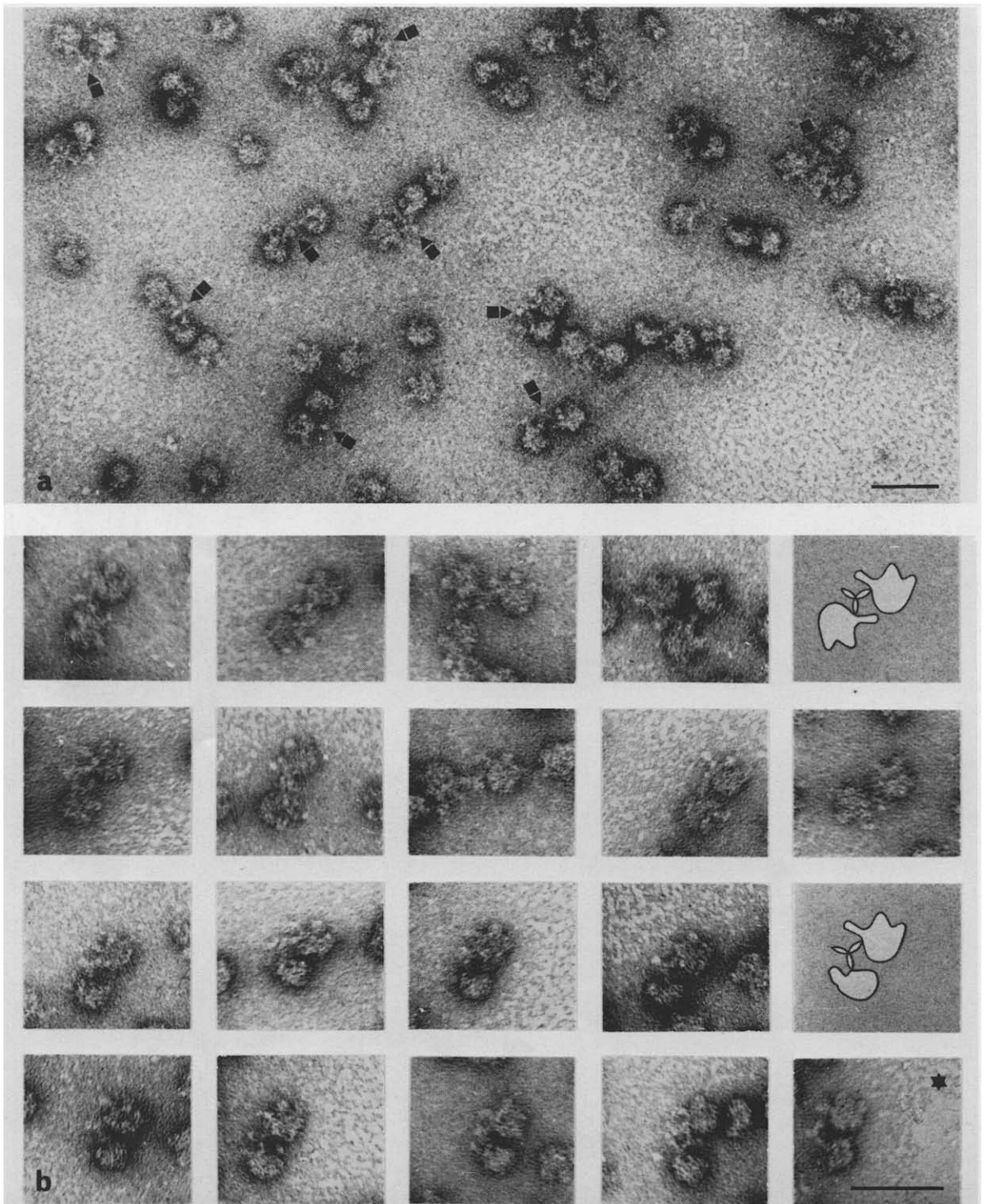


Fig.2. Electron micrographs of 50 S subunits modified by azido(S)-EF-G after reaction with anti-EF-G. Negative staining by 1% uranyl acetate. Single-carbon-layer technique. Microscope JEM-100C. Direct magnification $\times 60\,000$. Prints represent the specimen as viewed from the electron source side: (a) General view of the dimer fraction preparation (fig.1a); arrows indicate antibodies in 50 S · anti-EF-G · 50 S complexes; bar = 500 Å; (b) 50 S ribosomal subunits linked with anti-EF-G. Two upper rows represent 'crown-crown' types of images as schematically shown in the right frame; the two next rows represent 'crown-kidney' types. The last frame (marked by asterisk) shows an example of the 50 S · anti-EF-G · 30 S complex; bar = 500 Å.

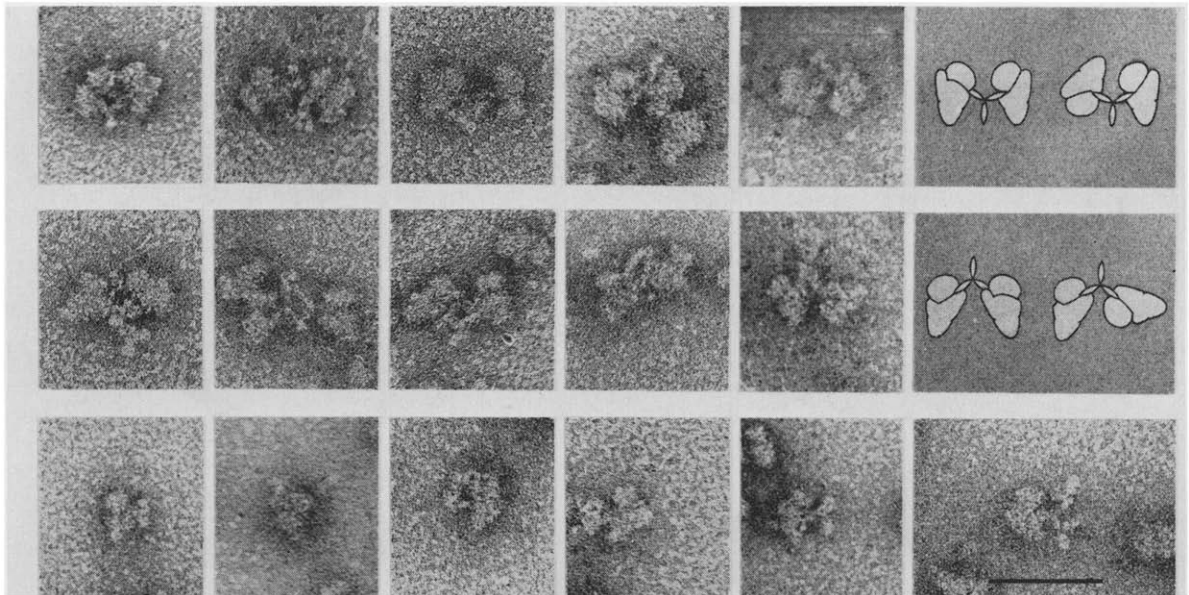


Fig.3. A gallery of electron micrographs of the 30 S subunits modified by azido(N)-EF-G after reaction with anti-EF-G. Negative staining using the double-carbon-layer technique. The two upper rows represent pairs of the 30 S ribosomal subunits linked by antibodies. The subunits in the pairs are oriented mostly in intermediate positions between the lateral (0° and 180°) and the frontal (90°) or dorsal (270°) views, when the width of the subunit is maximal [23]. Schematic drawings of the pairs of the subunits in these characteristic projections (45° and 225°) are shown in the right frames. When the subunit is oriented in a position close to the frontal or dorsal views one can see that the antibody is attached to the subunit from the side of the ledge (e.g., the frame 3 in the row 2). The last row shows single subunits with attached antibody molecules; bar = 500 Å.

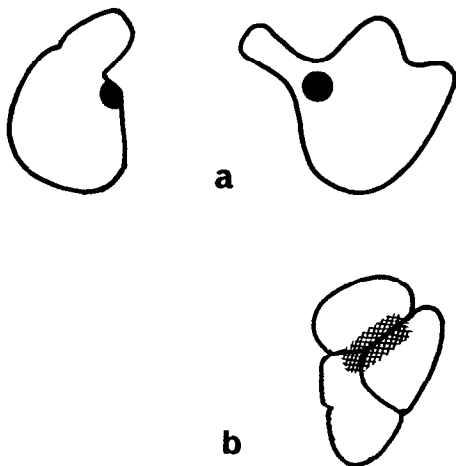


Fig.4. Localization of the elongation factor EF-G on the ribosome: (a) (●) position of the EF-G binding center on the two main projections of the 50 S ribosomal subunit; (b) (■) region of the 30 S subunit contacting with EF-G in the characteristic intermediate (45°) projection.

subunit along the groove between the head and body from the side of the ledge. One of these extreme binding sites is located at the lowest point of the groove and another near the ledge top, i.e., near the location of the 3'-end of 16 S RNA [24].

The location of EF-G on the characteristic projections of the 50 S and 30 S subunits are shown in fig.4.

4. Discussion

The possibility of EF-G localization on the ribosome by IEM analysis is essentially limited by ready dissociation of the EF-G · ribosome complex as result of anti-EF-G treatment (unpublished). It is evident that this limitation can be overcome by a prior covalent fixation of the factor on the ribosome. This approach has been used here. Effective and specific crosslinking of EF-G with the ribosome can be achieved by photoaffinity labelling. Two EF-G derivatives, azido(N)-EF-G [9] and azido(S)-EF-G [15], carrying a photoactivated arylazide residue on the

NH₂-group(s) and on the single exposed SH-group, respectively, were obtained. Both the derivatives retain the EF-G ability for specific interaction with the ribosome and photoaffinity-crosslink with the latter under mild conditions.

The results of IEM analysis of the ribosomal subunits containing the covalently bound EF-G can be formulated as follows:

- (1) The EF-G-binding center of the ribosomal 50 S subunit is located at the base of the rod-like appendage on the subunit interface side;
- (2) EF-G, being in its binding center, contacts with the 30 S subunit in the zone along the groove between the head and body from the side of the ledge.

A consequence of the results obtained is the assumption that at association of the ribosomal subunits the regions of each subunit identified by us should overlap. This assumption is logical, as the ribosome has a single EF-G-binding center in which the EF-G molecule contacts with both the subunits [9,10]. The mutual arrangement of the subunits which can be obtained at such overlapping contradicts the ribosome model proposed in [25] but not the model in [19].

It is not clear at present why the EF-G contact with 30 S subunit is observed as extended. The 30 S subunit in contrast to the 50 S subunit does not interact in an isolated state with the EF-G. Therefore the latter must evidently have some mobility around the covalent bond linking it on the subunit surface. However, the 30 S subunit in the ribosome can possess a mobility relative to the 50 S subunit owing to the association-dissociation equilibrium. Such a mobility can apparently be a specific one and can lead to the cross-linking of the EF-G derivative, bound on the 50 S subunit, with different points of the 30 S subunit surface limited to the corresponding region of its groove. The question is additionally complicated by the differences between EF-G derivatives used for cross-linking to the 50 S or the 30 S subunit (azido(S)-EF-G or azido(N)-EF-G, respectively) and by the absence of information on EF-G molecule dimensions. In any case, it should be underlined that the EF-G contact with the 30 S subunit is specific and limited to the identified narrow zone along the groove between the head and body of the subunit from the side of its ledge (fig.4).

In the EF-G · ribosome complex the factor is closely located to the protein S12 so that they can form a disulfide bond at mild oxidation [26]. These data per-

mit to suggest that the protein S12 should be located in the zone of the above 30 S subunit. Such a location correlates with the preliminary data in [27].

It is worthwhile to compare the positions of the EF-G and the proteins L7/L12 on the 50 S subunit. Proteins L7/L12 are located on the rod-like appendage [22]. Hence it follows that these proteins are neighbours of the EF-G-binding center (fig.4) but evidently cannot be regarded as the settling site itself for EF-G (cf. [6,28,29]).

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