Structural study of translating 70 S ribosomes from *Escherichia coli*

I. Electron microscopy

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Translating 70 S ribosomes of *Escherichia coli* either in the pre-translocation or in the post-translocation state have been prepared by using the cell-free translation system in poly(U)–S–S–Sepharose columns [Methods Enzymol. (1979) 59, 382–398]. Electron microscopy study of the preparations has demonstrated that: (1) the mutual orientation of the ribosomal subunits in the translating ribosomes is the same as proposed by Lake for routine 30 S·50 S couples [J. Mol. Biol. (1976) 105, 111–130]; (2) the L7/L12 stalk of the 50 S subunit sticks out from the 70 S particle and does not join the 30 S subunit; (3) pre-translocation and post-translocation state ribosomes do not differ in mutual orientation of the subunits and in the position of the L7/L12 stalk, within the limits of electron microscopy resolution.

Electron microscopy *Ribosome* *Ribosomal subunit arrangement* *Pre-translocation state* *Post-translocation state*

1. INTRODUCTION

During recent years, electron microscopy of isolated ribosomal subunits has led to the elucidation of a number of their important structural features [1–7]. At the same time, contradictory results have been obtained concerning the mutual orientation of the subunits in the 70 S ribosome [3,4,6] using non-translating 30 S·50 S couples isolated by routine procedures from *Escherichia coli*. It was not excluded that the subunit orientation in the non-translating 70 S particles was not very uniform, and that the different results of the various groups reflected the real variety of association modes of the uncharged ribosomal subunits.

The column technique for the translation of poly(U) covalently bound by its 3′-end with cellulose or Sepharose through split-table disulfide bridges [8,9] has opened the possibility of the isolation of the homogeneous translating 70 S monoribosomes in different functional states. In this work we used the electron microscopy method to study the ribosomal preparations obtained in this way. The aim of this work was to compare:

(1) The images of the translating ribosomes with those of non-translating ones, with special attention to the mutual orientation of the ribosomal subunits;
(2) The ribosomes in the pre-translocation and post-translocation states.

2. MATERIALS AND METHODS

Escherichia coli MRE-600 ribosomes washed 4 times with 1 M NH_4Cl containing 0.01 M $MgCl_2$ were taken as starting material for preparation of translating ribosomes by using the poly(U)–S–S–Sepharose column technique [8,9]. If only Phe-tRNA (without EF-G and GTP) or only EF-G with GTP (without aa-tRNA) were passed through the poly(U)–S–S–Sepharose columns containing translating ribosomes then pre-translocation or post-translocation state ribosomes were obtained in the columns, respectively [9]. Elution of the

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columns with dithiothreitol resulted in ribosomal preparations where all the particles were active in translation and existed as monoribosomes bound to poly(U) fragments of about 100 nucleotides long. The translating ribosomes were passed through the 5–30% glycerol gradient by centrifugation and dialyzed against the standard buffer for electron microscopy containing 10 mM Tris-acetate, 50 mM ammonium acetate and 10 mM magnesium acetate (pH 7.5).

The non-translating ribosomal particles for comparisons were obtained from 'tight' 70 S couples [10] by their dissociation and separation of the subunits by sucrose gradient centrifugation in 10 mM Tris–HCl, 100 mM NH_4Cl and 1 mM $MgCl_2$ (pH 7.5) with subsequent re-association at 6 mM magnesium acetate.

Samples for electron microscopy were prepared as in [11]. A thin carbon film was partially floated from the surface of mica firstly onto the ribosome suspension and then, after adsorption of the ribosomes on the film, onto 1% aqueous uranyl acetate. Copper grids coated by carbon micro nets were placed on the floating carbon film and picked up together with it. The excess suspension was sucked off by filter paper and the grids were allowed to dry. The samples were examined in a JEM-100C microscope equipped with a liquid nitrogen anti-contamination trap at an operating voltage of 80 kV and magnification of 80000. All the micrographs represent the ribosomal particles as viewed from the specimen side.

3. RESULTS AND DISCUSSION

3.1. *Non-translating 30 S·50 S couples*

Fig.1 represents a field of ribosomal particles prepared by partial re-association of the 30 S and 50 S subunits in the buffer containing 10 mM Tris-acetate, 50 mM ammonium acetate and 6 mM magnesium acetate (pH 7.5). The projections of the 30 S and 50 S subunits have been detailed in [1–7]. Two main projections of 30 S·50 S couples have been also characterized thoroughly by Lake [3] who proposed a non-contradictory three-dimensional model of the 70 S ribosome. However, this model based on the analysis of negatively stained preparations and supported by immuno-electron microscopy data was not finally proved and is not generally accepted. At present,

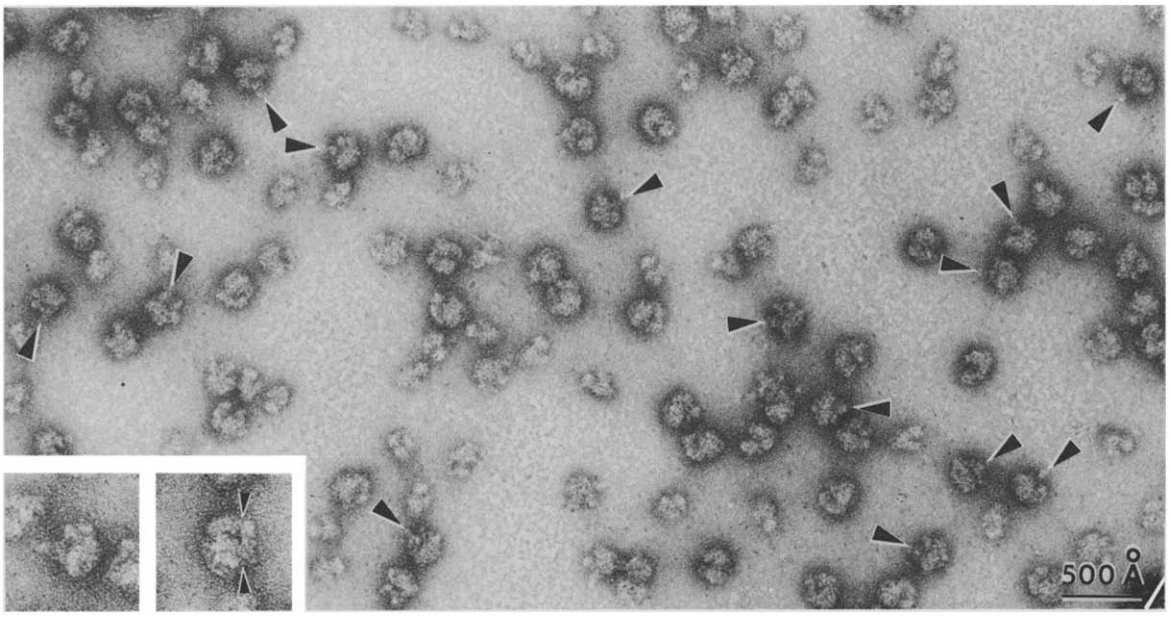

Fig.1. Electron micrograph of a field of non-translating ribosomal particles prepared in the buffer containing 6 mM magnesium acetate, 50 mM ammonium acetate and 10 mM Tris·acetate (pH 7.5). Arrows indicate the 70 S couples in the overlap projection. Two main projections of the 70 S couples are shown in the insertion at higher magnification.

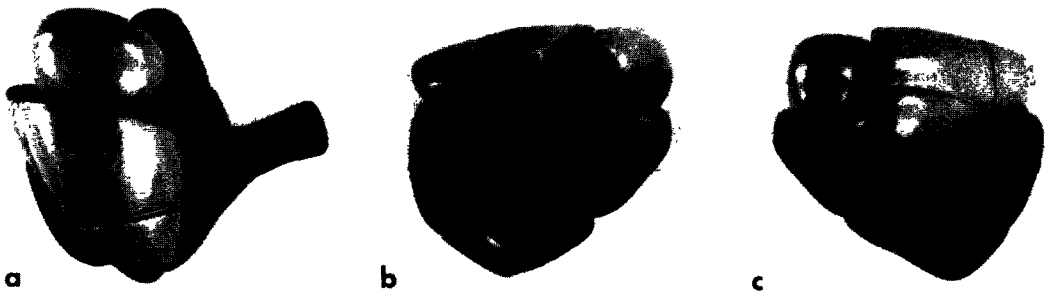

Fig.2. Three-dimensional model of the 70 S ribosome in overlap (a) and non-overlap (b,c) views.

the mutual orientation of the subunits in the 70 S ribosome can be determined more conclusively because all the essential structural features of both the subunits are known. The 30 S and 50 S subunits have unique asymmetric structures. It has been shown by shadow casting [1] and by tilting experiments with negatively stained preparations [12] that the 30 S subunit has a 'right' location of the side ledge or platform. High resolution shadow casting has enabled to determine independently the absolute hand of the 50 S subunit [7]. It has been established that the correct enantiomorph of the two possible mirror-related structures is that with the 'right' arrangement of the rod-like appendage, as has been proposed by Lake [3].

Two mentioned orthogonal projections of the 30 S·50 S couple are shown in the insertion. Both the overlap and the non-overlap projections [3] are presented. The images of the 30 S·50 S couples are very clear and permit the only interpretation which coincides with that of Lake [3]: the ribosomal subunits are coupled in such a manner that the head of the 30 S subunit joins the 'head' (central protuberance) of the 50 S subunit, and the side ledge (platform) of the 30 S subunit is in contact with side protuberance (L1 ridge [13]) of the 50 S subunit. The rod-like appendage of L7/L12 stalk [14] can serve as a good marker for determining the mutual orientation of the subunits. It should be noted that when the 70 S ribosome is in the non-overlap projection, the groove separating the central protuberance ('head') from the main body of the 50 S subunit [7] is very well seen. This groove faces the groove separating the head from the body of the 30 S subunit (both the grooves are indicated by arrows in the insertion, fig.1).

Another important observation is that the overlap projections of the 30 S·50 S couples with clearly visible L7/L12 stalks occur on micrographs with the same frequency as the asymmetrical crown-like projections of the original 50 S

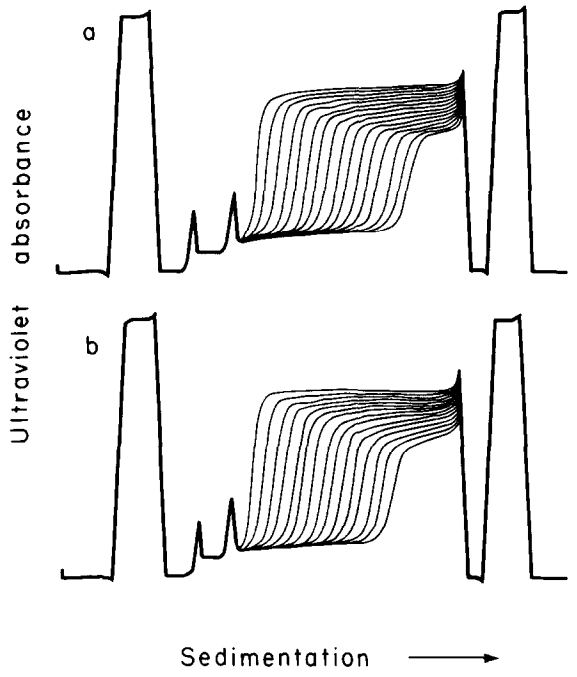

Fig.3. Sedimentation patterns of the translating ribosome preparations under investigation: (a) pre-translocation ribosomes; (b) post-translocation ribosomes. Sedimentation was done in 10 mM Tris-acetate, 50 mM ammonium acetate and 10 mM magnesium acetate, pH 7.5 at 20°C using the analytical ultracentrifuge UCA-10 (USSR) equipped with ultraviolet optics; the speed was 30000 rev./min; the scanning interval was 2 min.

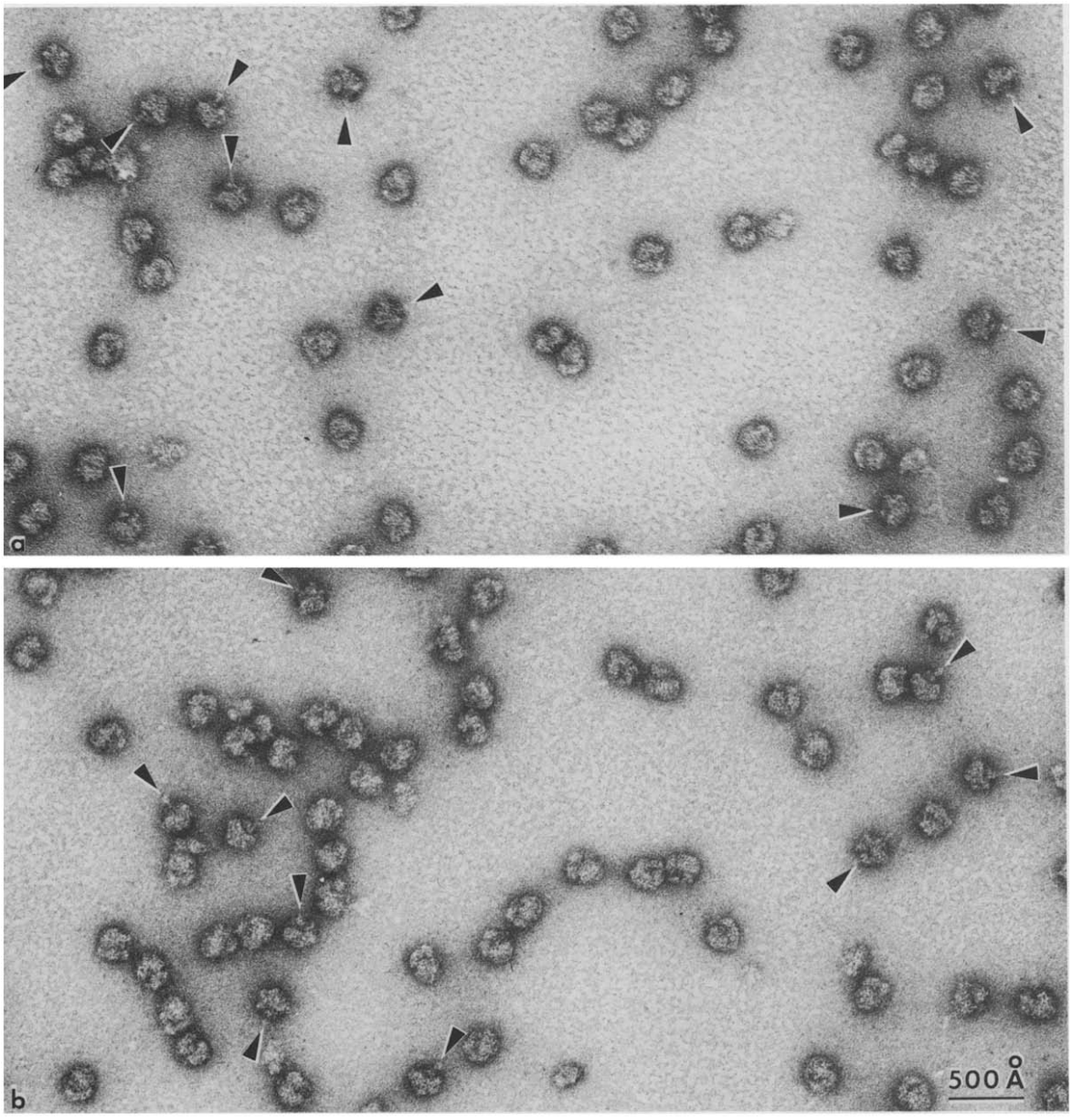

Fig.4. Electron micrographs of fields of the translating 70 S ribosomes in the pre-translocation (a) and post-translocation (b) states.

subunits. This means that intact ribosomal subunits do not change their morphology upon association with each other. Thus, our observations do not support Lake's hypothesis that the L7/L12 stalk bends to the 30 S subunit upon the association of the subunits. No contact of the L7/L12 stalk with the 30 S subunit is observed.

A model of the 70 S ribosome also demonstrating new structural features of the 50 S subunit [7] is shown in fig.2.

3.2. *Translating 70 S ribosomes*

Analysis of sedimentation distribution profiles of the translating ribosome samples taken for electron microscopy showed that $\geqslant 90\%$ of the particles were in the form of 70 S monoribosomes

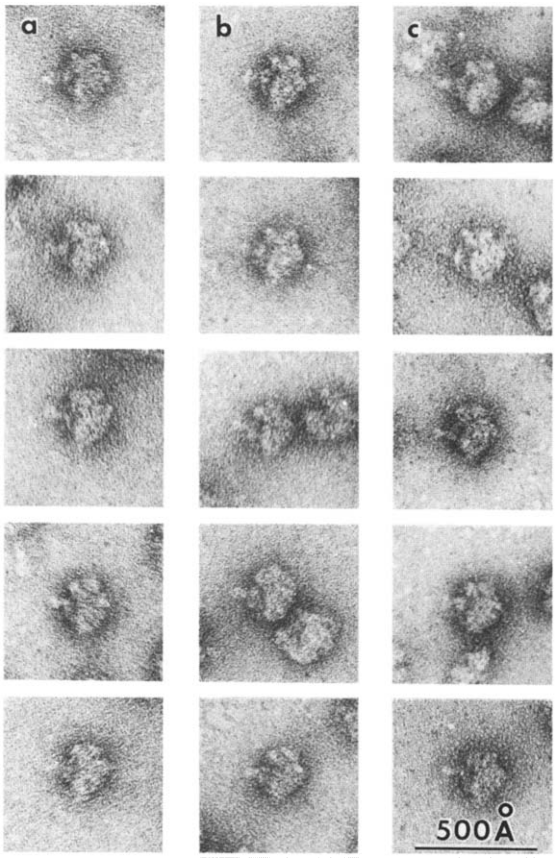

Fig.5. A gallery of electron microscopy images of the pre-translocation state ribosomes (a), post-translocation state ribosomes (b) and non-translating 30 S·50 S couples (c) in the overlap projection.

(fig.3). Using the puromycin reactivity test for translating ribosomes [9] it was found that ⩾75% of them were in the pre-translocation state and 80% in the post-translocation state, respectively.

Micrographs of the 70 S ribosomes in the pre-translocation (a) and post-translocation (b) states are shown in fig.4. Analysis of the images of about 1000 particles in each case has shown that the translating 70 S ribosomes in the two functional states do not differ from the non-translating 30 S·50 S couples and from each other, both in respect to the mutual subunit arrangement and in the frequencies of the main projections. The most informative is the overlap projection where the main structural features of the two subunits, such as the L7/L12 stalk of the 50 S subunit and the head and the side ledge of the 30 S subunit can be identified easily. A gallery of electron micrographs of the 70 S ribosomes in the pre-translocation (a) and post-translocation states (b), as well as of the non-translating 30 S·50 S couples (c) in the overlap projection is given in fig.5. No difference between these 3 types of particles is detected within the resolution limits of ~20 Å.

Thus, we conclude that:

(1) Intact ribosomal subunits do not change their morphology upon association with each other, irrespective of the presence or absence of the substrates and products of translation such as aminoacyl-tRNA, peptidyl-tRNA and deacylated tRNA;

(2) Transition from the pre-translocation state to the post-translocation one results in neither a visible re-orientation of the L7/L12 stalk nor a significant displacement of the subunits relative to each other in the plane of the subunit interface, as can be judged from electron microscopy.

A reservation should be made that the translating ribosomes in both the states under investigation did not contain elongation factors attached; hence it is not yet excluded that the transient presence of the elongation factors can affect the position of the L7/L12 stalk and the mutual orientation of the subunits.

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