# TOPOGRAPHY OF RNA IN THE RIBOSOME: LOCALIZATION OF THE 3′-END OF THE 23 S RNA ON THE SURFACE OF THE 50 S RIBOSOMAL SUBUNIT BY IMMUNE ELECTRON MICROSCOPY

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## 1. Introduction

The macromolecular structure of the major components of ribosomes (their RNAs) is now under intensive investigation. In spite of the substantial progress in the elucidation of primary and secondary structures of *Escherichia coli* 5 S, 16 S, and 23 S RNA, our knowledge of the spatial organization of rRNA in both intact ribosomal subunits and 70 S ribosomes is still very limited (for references, see [1,2]). In [3] we proposed a general approach to the identification of external RNA regions in ribosomal subunits. It is based on the chemical modification of selected points in RNA by reagents containing a hapten phenyl-β-D-lactoside residue. This approach has been applied to the location of the 3′-end on the 16 S RNA on 30 S subunits [3]. A similar method has been used in [4] utilizing the dinitrophenyl hapten.

Here we have located the 3′-end of *Escherichia coli* 23 S RNA on the lateral surface of the 50 S subunit under its side elongated protuberance (rod-like appendage).

## 2. Materials and methods

Preparation of antibodies specific to *p*-aminophenyl-β-D-lactoside (anti-pAPL), oxidation with sodium periodate and modification of the 3′-terminal nucleoside residues of RNA by 1-*N*-[*p*-(β-D-lactosyl)-benzyl]-6-aminohexylamine (LBA) as well as estimation of the extent of RNA modification were described [3]. Reconstitution of 50 S subunits was done basically as in [5] with 23 S RNA isolated from 70 S ribosomes as recommended in [6]. 23 S RNA was separated from 16 S and 5 S RNA by 5–20% sucrose gradient centrifugation. 5 S RNA was isolated from purified 50 S subunits as in [7]. After reconstitution from a modified 23 S RNA, 5 S RNA and total 50 S subunit proteins, 50 S subunits were precipitated from the incubation mixture by addition of polyethyleneglycol (PEG) [8], dissolved in 10 mM Tris–HCl (pH 7.8), 5 mM $Mg(CH_3COO)_2$, 100 mM $NH_4Cl$, 6 mM β-mercaptoethanol and purified by centrifugation through a 10–30% sucrose gradient prepared with the same buffer (Spinco SW 27 rotor, 21 000 rev./min, 14 h). Fractions containing 50 S subunits were pooled, $Mg(CH_3COO)_2$ was brought to 10 mM and subunits were precipitated with PEG, pelleted by low speed centrifugation, dissolved in Tris–HCl (pH 7.3), 10 mM $Mg(CH_3COO)_2$, 100 mM $NH_4Cl$ and stored at −45°C. All subsequent operations were done in Tris–HCl (pH 7.3), 5 mM $Mg(CH_3COO)_2$, 100 mM $NH_4Cl$. Incubation of 50 S subunits with anti-pAPL and selection of optimal antibody : 50 S subunit ratios were as for 30 S subunits [3]. Samples were prepared for electron microscopy by negative staining with 1% uranyl acetate according to [9]. Electron micrographs were taken on a JEM-100C microscope equipped with a liquid nitrogen anti-contamination trap at a magnification of 60 000 ×. The grids were inserted in the electron microscope so that the specimen faced the electron source and the plates were printed with the emulsion away from the photographic paper.

## 3. Results

To reconstitute 50 S subunits from modified 23 S

RNA, 5 S RNA and proteins, we followed the method developed in [5] except that 23 S RNA isolated from 70 S ribosomes rather than from 50 S subunits was used to minimize the possibility of nuclease degradation of RNA during its preparation. As shown in [10], such 23 S RNA preparations yielded 50 S particles with very low activity in polyphenylalanine (poly(Phe)) synthesis (2% of control). However, in our hands reconstituted 50 S particles with modified 23 S RNA had much higher activity in poly(U)-dependent poly(Phe) synthesis (25–30% from control, not shown). One of the possible reasons for this discrepancy could be conformational changes of 23 S RNA during the modification procedure.

The reconstituted modified 50 S subunits had a coefficient of sedimentation of 50.0 ± 0.5 S (as determined in an analytical ultracentrifuge) and, as one can see from fig.1a, they were highly homogeneous.

The sedimentation pattern of modified 50 S subunits incubated with anti-pAPL is shown in fig.1b. The 50 S · anti-pAPL · 50 S complexes appear as a shoulder with a mean of ~63 S. The formation of 50 S subunit 'dimers' is specific since the incubation of modified 50 S subunits with anti-pAPL in the presence of free hapten does not produce the 'heavy' shoulder and the sedimentation pattern becomes entirely similar to that of the control subunits (fig.1c). For large scale isolation of the 50 S · IgG · 50 S complexes the concentrations of the components were raised 3–5-fold. This resulted in an increase of the relative amount of the 'dimer' fraction and its better separation from the 50 S subunit peak (fig.1d).

The 'heavy' fraction (shaded region in fig.1d) has been used for electron microscopy study. The general view of the preparation from this fraction is shown in fig.2a. One can see that the preparation contains a large quantity of the 50 S · IgG · 50 S complexes and single subunits with attached antibodies. The electron microscopic images of both single reconstituted 50 S subunits and 50 S subunits in complexes with antibodies can be subdivided into two well-known types: 'crown'-like particles and 'crescent'- or 'kindey'-like

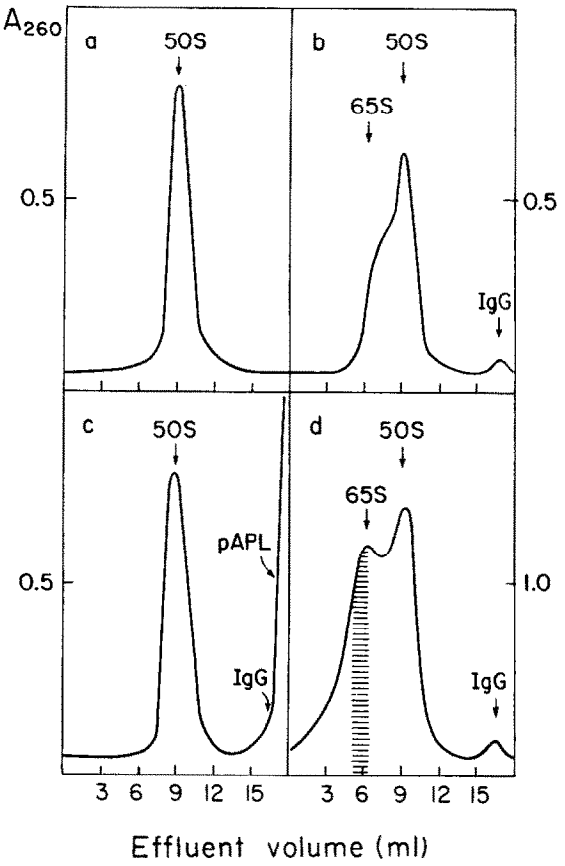


Fig.1. Sedimentation of reconstituted, LBA-modified 50 S subunits treated with anti-pAPL in a 5–20% sucrose gradient. (a) Modified 50 S subunits (2 $A_{260}$ units, 80 pmol) in the absence of antibodies; (b) +50 μg anti-pAPL, 312 pmol; (c) +50 μg anti-pAPL and pAPL to 50 mM final conc.; anti-pAPL peak is masked by the absorbance of the large excess of the free hapten; (d) large scale preparation of 'dimers': 10 $A_{260}$ units of LBA-modified 50 S subunits + 250 μg anti-pAPL; shaded region indicates fractions used in electron microscopy analysis.

ones [11–15]. These images correspond to two main projections of the three-dimensional structure of the 50 S subunit. It should be emphasized that most of the crown forms are asymmetric, i.e., retain the side elongated rod-like appendage. This appendage com-

Fig.2. Electron micrographs of 50 S subunits modified by LBA in the 3'-end of their 23 S RNA after reaction with anti-pAPL. (a) General view of the preparation from the 'dimer' fraction (fig.1d); arrows indicate antibodies in 50 S · IgG · 50 S and 50 S · IgG complexes; bar = 1000 Å; (b) Large ribosomal subunits linked with anti-pAPL. Three upper rows represent 'crown–crown'-, 'crown–kidney'- and 'kidney–kidney'-types of images as schematically shown in the right frames. The last row gives single subunits with attached antibody molecules; bar = 500 Å.

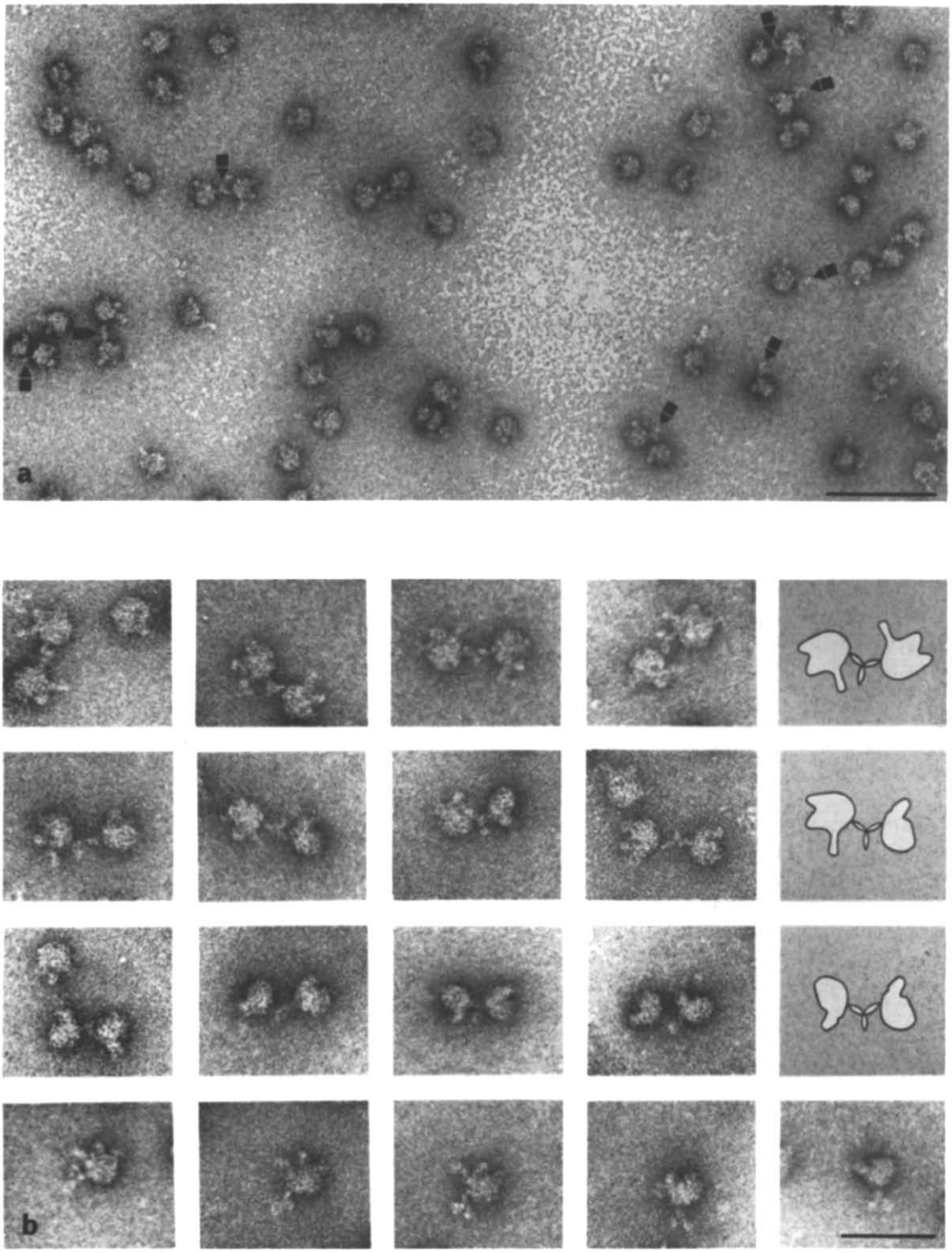


Fig.2a,b

prises proteins L7/L12 [16], and is a very characteristic structural feature of intact 50 S subunits which is the most sensitive to various kinds of treatment [15,16]. The asymmetric crown-like images in our preparations account for 60% of the total number of kidney-like and crown-like ones. This value coincides with the percentage of different forms of the images of intact 50 S subunits observed in [15].

The electron micrographs of 50 S · IgG · 50 S and single 50 S · IgG complexes representing both types of the images of the 50 S subunit are shown in fig.2b. Altogether we have examined 50 'dimers' and 50 single complexes. The ratio between the frequencies of the images of 'crown–crown' and 'kidney–kidney' types was ~10:1. Analysis of electron microscopic images shows that:

(1) Every modified 50 S subunit binds only one molecule of antibody; and
(2) The attachment site of antibodies on the 50 S subunits is always located in the same region of their surface.

Localization of the 3′-end of 23 S RNA in these two orthogonal projections is sufficient to map its location in three dimensions. The 3′-end of 23 S RNA is localized on the lateral convex surface of the 50 S subunit under its side-elongated protuberance as shown in fig.3.

## 4. Discussion

The importance of determining the location of the 3′-end of the 23 S RNA on the 50 S subunit surface is that this information can be used to solve the problem of the relative orientation of the 3′-terminal regions of the 16 S and 23 S RNA in the 70 S ribosome. As suggested in [17], the 3′-terminal segments of 16 S and 23 S RNA form in 70 S ribosomes a base-paired complex owing to their complementarity. It was also assumed that binding of IF-3 to 70 S ribosomes leads to dissociation of this complex and subsequent dissociation of 70 S ribosomes into subunits. The data obtained here are in strong contradiction with this assumption. Although there is no common standpoint on the relative arrangement of the 30 S and 50 S subunits in the 70 S ribosomes [12–15,18], one can see that the 3′-end of the 23 S RNA is remote from the 30 S–50 S subunit interface.

At the same time our data support the suggestion that the 3′-terminal segment of 23 S RNA can be in close proximity with its 5′-end and even form a double-stranded complex with it [19,20]. Indeed, the 3′-end of the 23 S RNA is not far from the proteins L7/L12 which at least partially form the rod-like appendage [16]. On the other hand, the complex of proteins L10 and L7/L12 was found to associate with the 5′-terminal third of the 23 S RNA molecule [21].

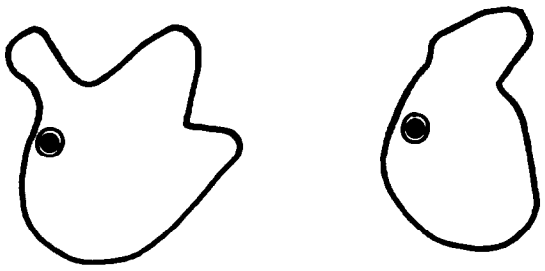

Fig.3. Localization of the 3′-end of 23 S RNA on the 50 S subunit: the 3′-end position on the two orthogonal projections of the large ribosomal subunit is shown by circles.

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