TOPOGRAPHY OF RNA IN THE RIBOSOME: LOCATION OF THE 3′-END OF 5 S RNA ON THE CENTRAL PROTUBERANCE OF THE 50 S SUBUNIT

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1. Introduction

The 5 S RNA is an integral part of the prokaryotic ribosome and plays an important role in polypeptide synthesis. However, the specific function of 5 S RNA and/or associated proteins is unknown. The removal of 5 S RNA from 50 S ribosomal subunits strongly impairs various functional activities of ribosomes [1,2]. The only exception is the EF-G-dependent GTP hydrolysis which is not influenced by the presence of 5 S RNA [2]. Therefore, the direct localization of the 5 S RNA–protein domain with respect to other ribosomal components with known functions is of great interest.

Here we report the localization of the 3′,5′-terminal stem of the *Escherichia coli* 5 S RNA on the surface of the 50 S subunit. This was done using the immune-electron microscopy approach applied to localize the 3′-ends of the 16 S [3] and 23 S RNA [4] on the 30 S and 50 S subunits, respectively. The 3′-end nucleotide residue of 5 S RNA was found to be located on the outward surface of the central protuberance of the 50 S subunit. These data together with the known secondary structure of the 3′,5′-terminal stem of the 5 S RNA allow one to conclude that its 5′-end is also located in this region of the 50 S subunit.

2. Materials and methods

Ribosomes and ribosomal subunits were isolated from *Escherichia coli* strain MRE 600 as described [3]. 5 S RNA was prepared as in [5] except that 50 S subunits rather than 70 S ribosomes were used as a source of RNA. The homogeneity of 5 S RNA preparations was checked by polyacrylamide gel electrophoresis and, if necessary, they were additionally purified by gel-filtration on Sephadex G-100. Oxidation of the 3′-terminal nucleoside residue of the 5 S RNA, modification of oxidized RNA by 1,*N*-[*p*-(β-D-lactosyl)benzyl]-6-aminohexylamine (LBA), estimation of the extent of 5 S RNA modification were done as described for 16 S RNA [3]. Antibodies specific to phenyl-β-D-lactoside hapten (anti-pAPL) were prepared as in [3]. 50 S subunits were reconstituted from modified 5 S RNA, 23 S RNA and total 50 S subunit protein (TP50) by the method developed in [6] with some modifications [4]. Purification of reconstituted 50 S subunits, incubation of subunits with anti-pAPL and the electron microscopy technique were also described [3,4]. Buffer, 10 mM Tris–HCl (pH 7.3), containing 5 mM $Mg(CH_3COO)_2$ and 100 mM NH_4CH_3COO was used in all experiments.

3. Results

As one can see from fig.1a, 50 S subunits reconstituted from 23 S RNA, TP50, and 5 S RNA modified by phenyl-β-D-lactoside hapten at its 3′-end (modification was 40–50%) give the symmetrical homogeneous peak in a sucrose gradient. After incubation of these particles with anti-pAPL, a 'dimer' fraction of 63 S av. appears (fig.1b). The formation of the 50 S · IgG · 50 S complexes is specific for the modified 5 S RNA in 50 S subunits since the incubation of the reconstituted 50 S subunits with anti-pAPL in the presence of free hapten does not give rise to the 'dimer' fraction (fig.1c). For electron microscopy the 'dimer' fraction was isolated on a large scale.

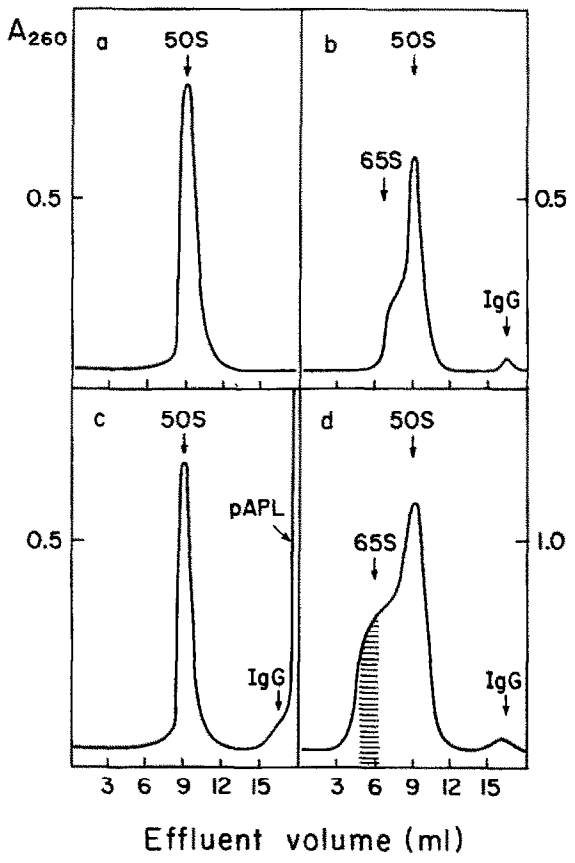

Fig.1. Sedimentation of reconstituted 50 S subunits with LBA modified 5 S RNA treated with anti-pAPL in a 5–20% sucrose gradient. (a) Modified 50 S subunits (2 A_{260} units, 80 pmol) in the absence of antibodies; (b) +50 μg anti-pAPL, 312 pmol; (c) +50 μg of anti-pAPL and pAPL to 50 mM final conc.; anti-pAPL peak is masked by the absorbance of the large excess of the free hapten; (d) large scale preparation of 'dimers': 5 A_{260} units of LBA-modified 50 S subunits + 125 μg anti-pAPL; the shaded region indicates fractions used in electron microscopical analysis.

It is interesting that increasing of the concentrations of 50 S subunits and anti-pAPL in the incubation mixture results in a better separation of the 'dimer' fraction from monomeric 50 S subunits.

The results of electron microscopical analysis are presented in fig.2. As one can see from fig.2a, ~30% of the total number of reconstituted 50 S subunits form pairs in which single subunits are linked by antibodies. The asymmetric 'crown'-like images of 50 S subunits are predominant as in the case of 50 S subunits reconstituted from the 23 S RNA modified by the same hapten [4]. Fig.2b depicts both 50 S · IgG · 50 S and single 50 S · IgG complexes in two characteristic projections. Altogether, we have examined 70 complexes and we have not observed any 50 S subunit which would be bound with more than one antibody molecule. The binding site of antibodies at the 50 S subunits can be easily and unambiguously identified: it is located on the outward (not contacting with the 30 S subunit) side of the central protuberance of the 50 S subunit 20–30 Å lower than its top (fig.3).

4. Discussion

It has to be emphasized that the mapping of the 3′-end of the 5 S RNA on the 50 S subunit spells out simultaneously the localization of its 5′-end. Indeed, it was proved by direct crosslinking experiments that the complementary terminal sequences 1–10 and 110–119 in the *Escherichia coli* 5 S RNA form the double-helical stem [7]. Thus, the central protuberance of the 50 S subunit is the site of location of the 3′,5′-terminal stem of the 5 S RNA. This morphological part of the 50 S subunit is the universal and very characteristic feature of both prokaryotic and eukaryotic ribosomes [8]. Further, it is retained after removal of a significant portion of ribosomal proteins from 50 S subunits [9]. One can suggest that the majority of the 'body' of the central protuberance consists of rRNA with a stable tertiary structure. It is also important that the 5 S RNA–protein complex can be specifically associated with different protein-deficient core-particles [2] and even with the free 23 S RNA [10]. Hence we can assume that one of the 5 S RNA binding sites formed by 23 S RNA segment in the region of the large subunit central protuberance.

Fig.2. Electron micrographs of 50 S subunits modified by LBA in the 3′-end of their 5 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 the images of subunits in characteristic projections schematically shown in the right frames. The last row gives single subunits with attached antibody molecules; bar = 500 Å.

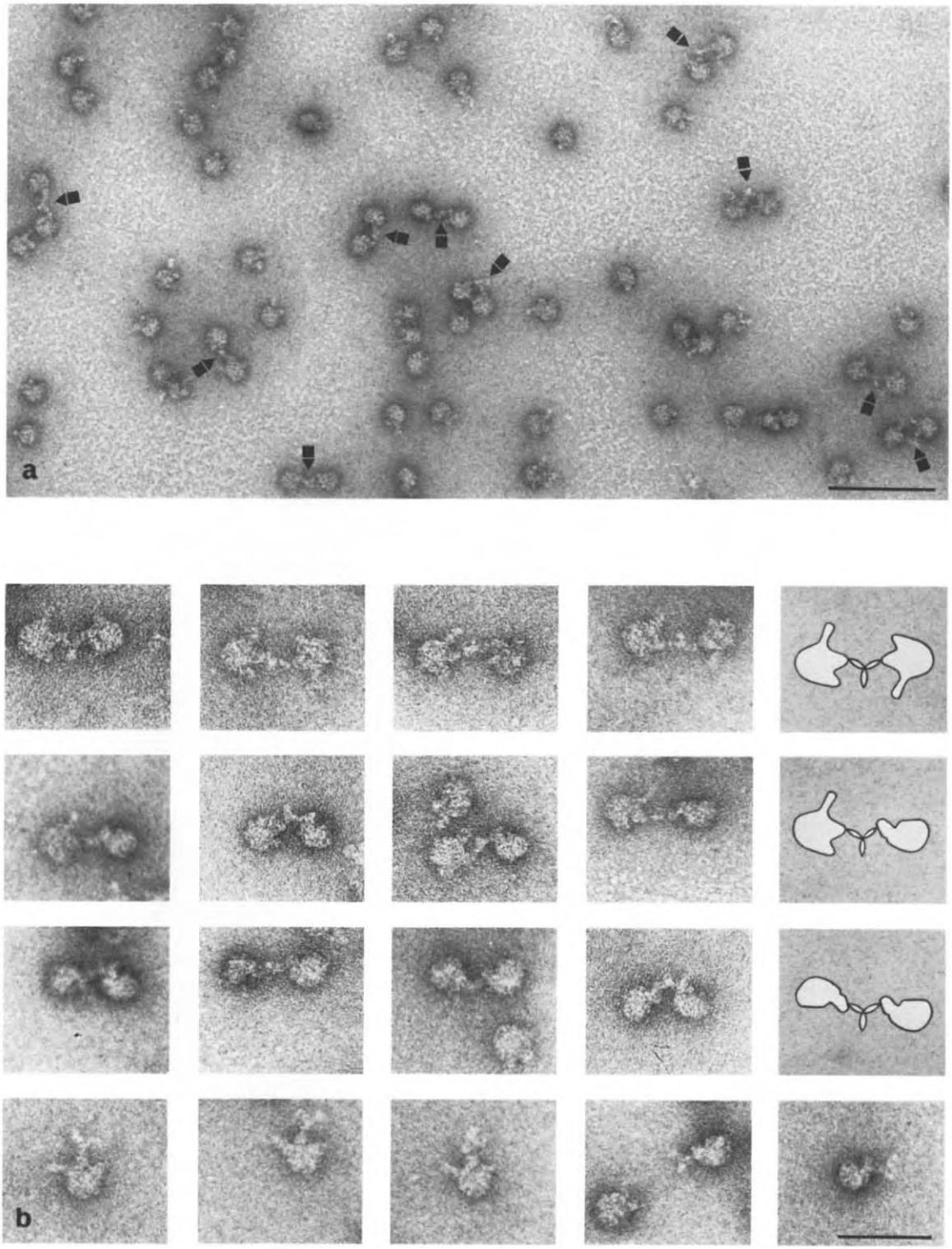

Fig.2a,b

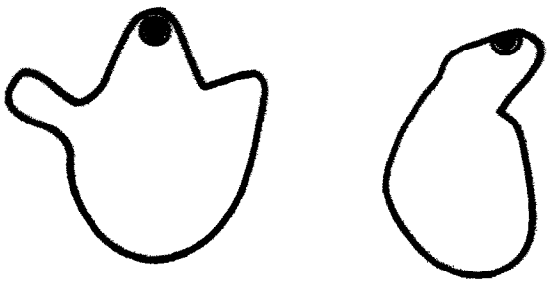

Fig.3. Localization of the 3'-end of 5 S RNA on the 50 S subunit: the 3'-end position on the two main projections of the large ribosomal subunit is denoted by solid circles.

These data allow one to map the 5 S RNA-binding protein L25 on the 50 S subunit. Since the L25-binding site occurs near the 5 S RNA 3',5'-terminal stem [11] ⩽30 Å in length, it also has to be located in the region of the central protuberance. This conclusion is in strong contradiction with the model of Stöffler et al. who placed all 5 S RNA-binding proteins (L5, L18 and L25) at the edge of the large subunit interface opposite to the central protuberance (e.g., see fig.34 in [12]). At the same time our data are in better correlation with Lake's preliminary map of large subunit proteins, on which proteins L5 and L25 are placed on the 'right' (short) protuberance [13].

It is interesting that Lake et al. have located protein L27, which is very likely the component of the peptidyl transferase (for references see [14]), near the top of the central protuberance of the 50 S subunit [15] and hence near the 3',5'-terminal stem of 5 S RNA. Since the presence of 5 S RNA is very significant for binding of aminoacyl-tRNA in the A-site of the ribosome and peptidyl transferase activity [2], and the P-site of the ribosome is mainly formed from 23 S RNA ([16], for references see [14]) one can suggest that the central protuberance of the 50 S subunit is a very important if not the primary part of the peptidyl transferase center of the ribosome.

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