SPECIFIC SELFPACKING OF THE RIBOSOMAL 16 S RNA

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1. Introduction

In 1963 Spirin et al. discovered that ribosomal particles can be unfolded into ribonucleoprotein strands where most of the ribosomal proteins remain associated with covalently continuous ribosomal RNA [1,2]. From this a concept of the role of ribosomal RNA as a structural matrix for the arrangement of numerous ribosomal proteins was proposed [2]. The questions of principal importance are:

(1) At what level of its structural organization does the ribosomal RNA perform its matrix role?

(2) Is free ribosomal RNA capable of forming the specific tertiary structure anticipating the features of the compact ribosomal particle?

Earlier we studied, by electron-microscopy the ribonucleoprotein derivatives of the 30 S ribosomal subparticle containing proteins S4, S7, S8, S15, S16, S17, S18 and S19 [3], or just proteins S4, S7, S8 and S15 [4], or only protein S4 complexed with 16 S RNA [5]. In all three studies it was shown that the particles are compact and possess the main morphological features of the original 30 S ribosomal subparticle. These results suggested that the ribosomal RNA itself could be responsible for formation of the main elements of the unique ribosomal three-dimensional structure.

This report presents the results of a study by electron-microscopy of isolated ribosomal 16 S RNA, without any proteins. It is shown that under certain ionic conditions the 16 S RNA is capable of self-packing into a specific compact structure bearing morphological features of the ribosomal 30 S subparticle.

2. Materials and methods

Ribosomal 30 S subparticles were prepared from *Escherichia coli* MRE-600 ribosomes by sucrose gradient zonal centrifugation in the presence of 0.5 M NH_4Cl and 1 mM $MgCl_2$ [6]. Ribosomal 16 S RNA was obtained from 30 S subparticles by splitting off the proteins in 3 M LiCl with 4 M urea [7,8] followed by phenol deproteinization in the presence of sodium dodecyl sulfate [9]. Protein contamination in the RNA preparations was <1% both after the LiCl–urea dissociation and after the additional phenol treatment. The isolated ribosomal 16 S RNA was stored as a precipitate in ethanol–50 mM acetate buffer (pH 5.5) mixture (2:1) at −10°C up to 3 months; no degradation of the RNA was observed. Before the experiment the RNA was pelleted by centrifugation at 10 000 × g for 10 min. The RNA pellet was dissolved in the buffer containing 30 mM CH_3COONH_4, 6 mM $(CH_3COO)_2Mg$, 1 M ethanol (pH 7.5), to 1.0–1.5 A_{260}/ml and dialyzed overnight against 2 changes of the same buffer. The solution was clarified from possible aggregates by centrifugation at 20 000 × g for 15 min. The preparation was heated for 10 min at 40°C, cooled to 4°C and then used for the experiment.

The technique of fast freezing to the temperature of liquid nitrogen, freeze-drying in vacuum and high resolution shadowing with tungsten–tantalum was used for the electron microscopy [3,10,11]. This technique made it possible to fix the preparation instantly without change of the initial conditions.

3. Results and discussion

Ribosomal 16 S RNA was studied in buffer con-

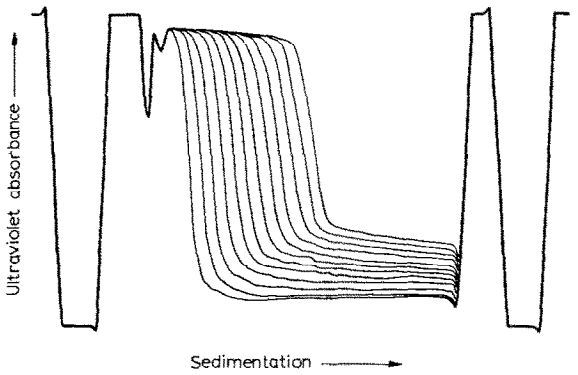

Fig.1. Sedimentation diagram of the ribosomal 16 S RNA preparation in buffer containing 30 mM CH_3COONH_4, 6 mM $(CH_3COO)_2Mg$, 1 M ethanol (pH 7.5). Centrifugation was done in a UCA-10 analytical ultracentrifuge (USSR) at 40 000 rev./min, 20°C; records were taken at 4 min intervals.

taining 30 mM CH_3COONH_4, 6 mM $(CH_3COO)_2Mg$, 1 M ethanol, pH 7.5, in which it had a sedimentation coefficient of $s^0_{20,w}$ = 21.5 ± 0.5 S. Sedimentation analysis showed homogeneity of the preparation (fig.1). Higher Mg^{2+} concentrations in the buffer did not result in further increase of the sedimentation coefficient.

A field from an electron-micrograph of the 16 S RNA preparation and images of individual RNA molecules are given in fig.2. As seen in fig.2a, the preparation consists mainly of compact particles of elongated shape 240 ± 20 Å long. Most of the particles are V-shaped and asymmetrical relative to their long axis as one of the shoulders is longer and thicker than the other. In some cases the gap between the two shoulders is difficult to distinguish and the particles have triangular outlines. The angle between the shoulders varies, but is never $\gtrsim 45°$. This may be an indication of a certain rigidity of the structure, and the angle variations are most likely connected with different orientations relative to the supporting film but not with the deformation of the RNA molecule. In addition to the V-shaped particles, Y-shaped ones can also be observed. Both the types of particles are schematically represented in the top left of fig.2b. The presence of Y-shaped particles means that the ribosomal 16 S RNA molecule is not a flat V-shaped structure. At the same time both types of electron-micrographic images as well as the triangular shaped particles can be readily represented as different projections of the same three-dimensional structure. If it is assumed that the small shoulder of the V-shaped particle is slightly bent in the perpendicular plane (the small shoulder must be bent up from the plane of the figure if it is to the left of the large shoulder) then the rotation around the longitudinal axis of the particle will give V-shaped, triangular and Y-shaped images of different width. Such a three-dimensional V-shaped structure with a bent small shoulder is in good accord with the asymmetrical model of the ribosomal 30 S subparticle proposed [10] and also with a similar, but slightly modified (flattened) model proposed [12,13].

Fig.2a

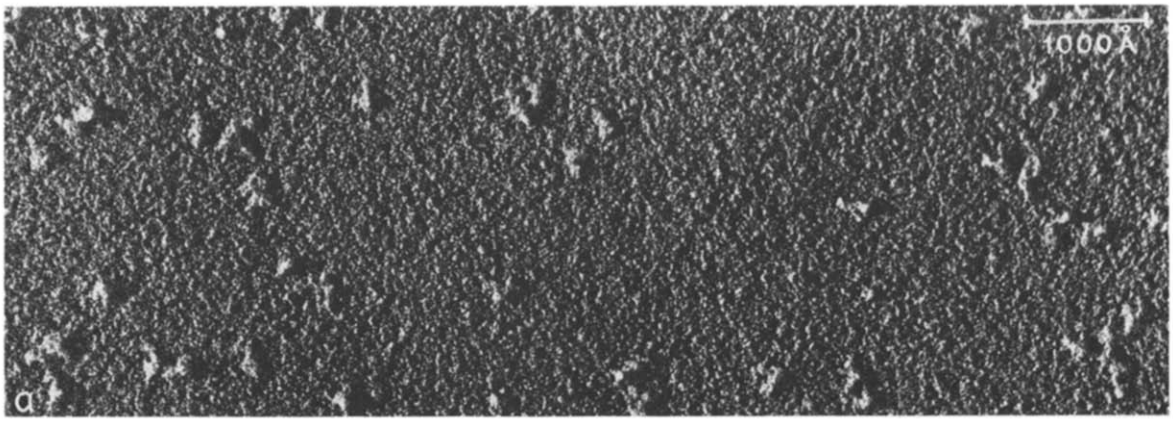

Fig.2b

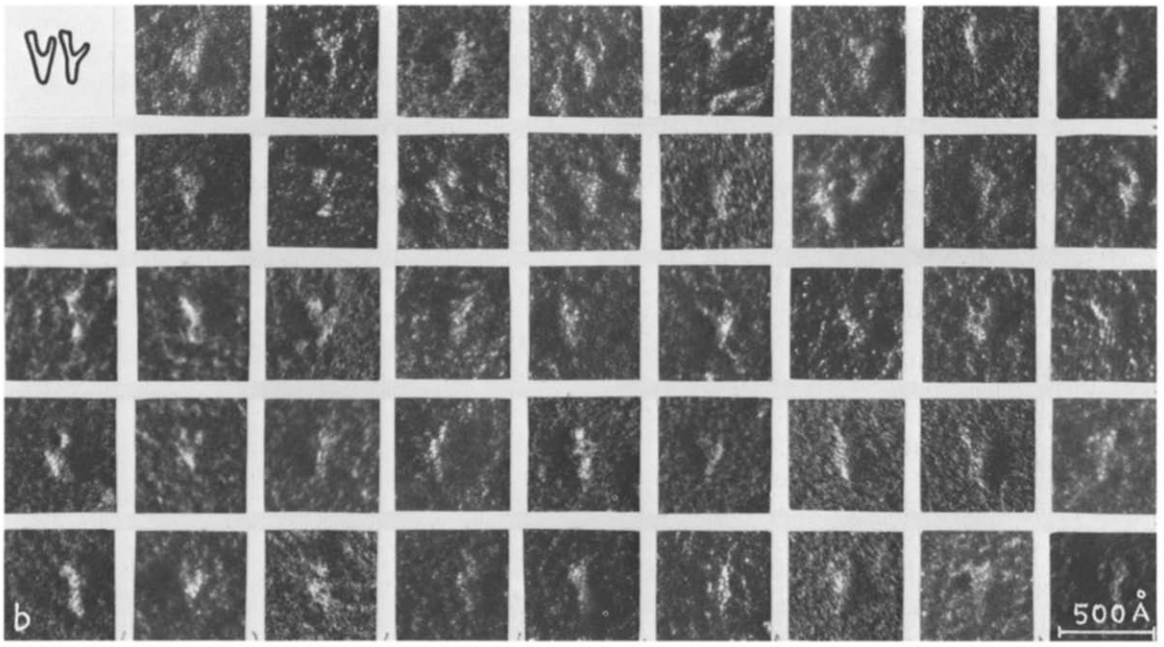

Fig.2. Electron micrographs of the ribosomal 16 S RNA preparation freeze-dried in vacuum. Shadowing with tungsten-tantalum. The shadow length: object height ratio was ~2:1. Metal layer thickness was 15 Å. Microscope JEM-100C. (a) General view of the ribosomal 16 S RNA preparation. (b) Electron-micrographic images of individual ribosomal 16 S RNA molecules. The main types of images are shown schematically at the top left. Enantiomorphic forms of these main types of images can be seen also in the micrographs.

Figure 3 shows different projections of the model of the 30 S subparticle as compared with the corresponding projections of the 16 S RNA structure (cross-hatched). It is seen that the observed types of the electron-micrographic images of the 16 S RNA molecules are well inscribed into the contours of the 30 S subparticle model. The images of the V-shaped RNA molecules must correspond to the frontal projections of the subparticle model (90° and 270°). It is likely that the large shoulder is a central elongated core of the subparticle 'body', the end of this shoulder forming its 'head'; the small shoulder forms the side ledge ('platform') of the subparticle. From this interpretation it also follows that ribosomal proteins must be grouped mainly in the 'head' and in the upper part of the subparticle 'body'.

Thus, under certain ionic conditions the ribosomal 16 S RNA molecule, without ribosomal proteins, can fold in a specific manner, thus forming a unique

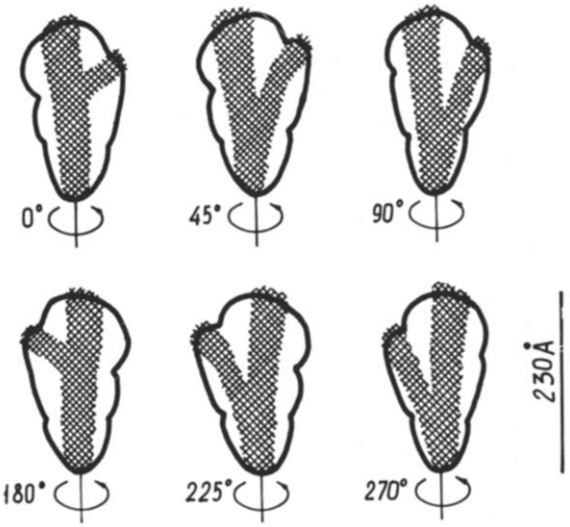

Fig.3. Contours of the ribosomal 30 S subparticle model in different projections [10]. The corresponding projections of the compact 16 S RNA molecule are shown by cross-hatching.

structure. This specific structure bears a number of the main features of the ribosomal subparticle morphology. Just as the 30 S subparticle, it can be characterized as an asymmetric polar elongated structure with an axial ratio of about 2:1. It can be thought that the 16 S RNA structure revealed reflects directly its unique structure within the complete ribosomal 30 S subparticle. It can serve as a ready three-dimensional matrix or framework for the arrangement of ribosomal proteins. Of course, this does not exclude the possibility of local rearrangements of the RNA as a result of its interactions with proteins.

The results obtained corroborate well the idea suggested earlier by Spirin that it is the RNA that plays the key role in the formation of the unique ribosomal structure [2,14]. It was pointed out that:

(1) RNA acquires the specific macromolecular organization as a result of its own intramolecular interactions.
(2) Molecules of ribosomal protein are coupled with RNA in such a way that they do not disturb seriously the RNA conformation.
(3) Thus, the principal features of the RNA conformation within the ribosome are essentially analogous to those of free RNA in its compact form (see [14], p. 340).

Acknowledgements

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