Volume 248, number 1,2, 92–96 FEB 07141 May 1989

Structure of the rat liver ribosome 40 S subunit: freeze-drying and high-resolution shadow casting

V.D. Vasiliev, O.M. Selivanova, G. Lutsch*, P. Westermann+ and H. Bielka+

*Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR, *Department of Electron Microscopy* and *+Department of Cell Physiology, Central Institute of Molecular Biology, Academy of Sciences of the GDR, Berlin-Buch, GDR*

Received 27 March 1989

Small ribosomal subunits from rat liver have been studied by electron microscopy using freeze-drying and high-resolution shadow casting. The absolute hand of the asymmetric subunit has been determined and its three-dimensional model with a 'right' location of the side protuberance has been constructed. The results evidence that pro- and eukaryotic ribosomes have a unique and principally similar structural organization.

Eukaryotic ribosome; Subunit structure, small; Handedness determination; Electron microscopy

1. INTRODUCTION

Information about the three-dimensional structure of ribosomes is a prerequisite for localization of functional sites of these particles. In the case of the eukaryotic small ribosomal subunit the models of their three-dimensional structure derived from electron microscopic studies differ in fine structure and handedness [1–6]. The models proposed by Sabatini and co-workers [1,4] and by Kiselev et al. [3] show only few structural features, whereas those proposed by Lutsch et al. [2,5] and by Boublik et al. [6] are more detailed with respect to their profile and substructure. On the other hand, most of the models are 'right-handed', whereas that of Lutsch et al. [2,5] is of the opposite handedness. The different models have been derived from investigations of negatively stained specimens [1–3,6] as well as from a comparison of negatively stained particles with those critical-point-dried and shadowed [4].

In order to get a more uniform picture about the structure of the 40 S ribosomal subunit, specimens from rat liver have been reinvestigated by freeze-drying and high-resolution shadow casting. It has been shown that the 40 S particle possess a right-handed structure. On the other hand, the shape and surface features of the model proposed by Lutsch et al. [2,5] could be confirmed and more precisely determined by an independent method.

Correspondence address: V.D. Vasiliev, Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR

2. MATERIALS AND METHODS

Small ribosomal subunits have been prepared as described in [7]. Negative staining was performed with 1% uranyl acetate (uncorrected pH) using a single-layer carbon technique [8]. Freeze-drying and shadow casting were done as described in [9]. The specimens were washed before freeze-drying in a buffer containing 50 mM NH_4Ac, 6 mM MgAc, 1 M ethanol, pH 7.5. Electron micrographs were taken with a JEM-100C microscope at 80 kV and a magnification of 60000 × (or of 56400 × for fig.2). The micrographs represent the ribosomal particles as viewed from the specimen side.

3. RESULTS AND DISCUSSION

Large fields of homogeneously distributed well contrasted particles can be obtained by freeze-drying and high-resolution shadow casting of unfixed rat liver small subunits (fig.1a). Several dif-

Published by Elsevier Science Publishers B.V. (Biomedical Division)

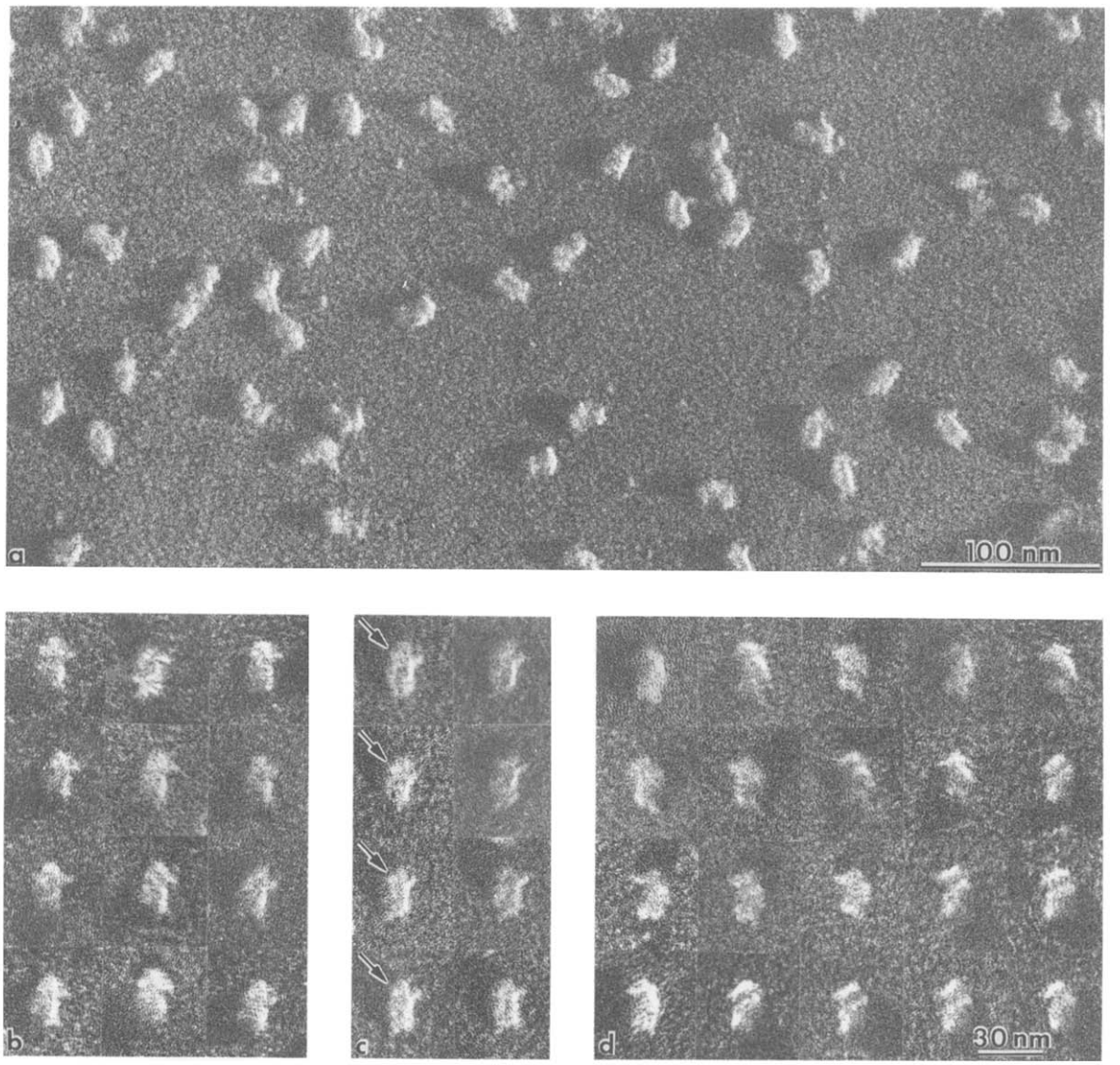

Fig.1. Electron micrographs of freeze-dried and shadowed 40 S ribosomal subunits. Shadowing with tungsten-40% rhenium. Shadowing angle is 26°. Metal cap is 10 Å thick. General view (a) and selected images (b–d). The number of 40 S subunit images in the left lateral view (b), frontal view (c) and right lateral view (d) are approximately proportional to their frequencies in the fields. Arrows indicate the eukaryotic bill on the head of the subunit.

ferent particle images with characteristic features are identifiable and can be interpreted as different views of the 40 S subunit. Examples of three characteristic types of 40 S subunit images selected from several different fields are presented in the galleries in fig.1b,c,d. The images presented are very similar with respect to their contours and fine structure to the well-known images of negatively stained 40 S subunits. The characteristic features of the 40 S subunit such as the so-called 'head' with the 'eukaryotic bill', the 'body' with the 'hump' and the 'eukaryotic lobes' [2,5,10,11] are well recognizable. The hump, in its turn, consists of two lobes and is bordered by the 'subdivision line'. The upper lobe of the hump forms the 'protuberance' on one of the lateral sides of the

subunit. It is evident that each of these features is revealed well only at certain 40 S subunit orientations relative to the direction of the shadowing, when it is not in the 'shadow'. Examples of shadow graphs with well recognizable characteristic features are presented in fig.2 (upper two rows).

A model of the three-dimensional structure of the 40 S ribosomal subunit with a 'left' location of the protuberance has been derived from an analysis of the three types of images of negatively stained 40 S subunits such as presented in fig.2 (lower two rows) [2,5]. The negatively stained subunit images of type b in fig.2 were previously referred to as those of the convex 'rear' side of the 40 S subunit. However, such images cannot be interpreted unambiguously as they involve contributions from both sides of the object. Two mirror-related models of the asymmetric 40 S subunit can be derived from such images.

Shadow casting reveals that the surface and the shadow graphs can, therefore, be treated as reflection images. It has been shown earlier [12,13] that if the resolution is high enough the correct enantiomorph of the two mirror-related structures can be chosen from shadow graphs using one of the well-defined features of the object as a mark.

The bill is a convenient mark to show that the images of type b in fig.2 do not represent the rear, but the frontal side of the 40 S subunit, and that the structure with a right location of the protuberance is the correct one. Indeed, the bill is well recognizable on shadow graphs in fig.1c (indicated by arrows). Its apex is somewhat shifted from the middle of the subunit to the side opposite the protuberance. The bill is the only acutely protruding part of the subunit on its frontal side. Therefore, the shadow from the subunit in the frontal view must have a characteristic pointed shape. Actually, such shadows can often be observed on the micrographs (fig.3).

The shadow graphs also demonstrate the difference between the opposite lateral sides of the 40 S subunit. The left side of the subunit body (fig.1b) does not have a deep relief and is more or less uniformly contrasted by the metal. Unlike this, the right side of the subunit body (fig.1d) is characterized by the protuberance which accumulates the metal and causes the pointed shape of the shadow.

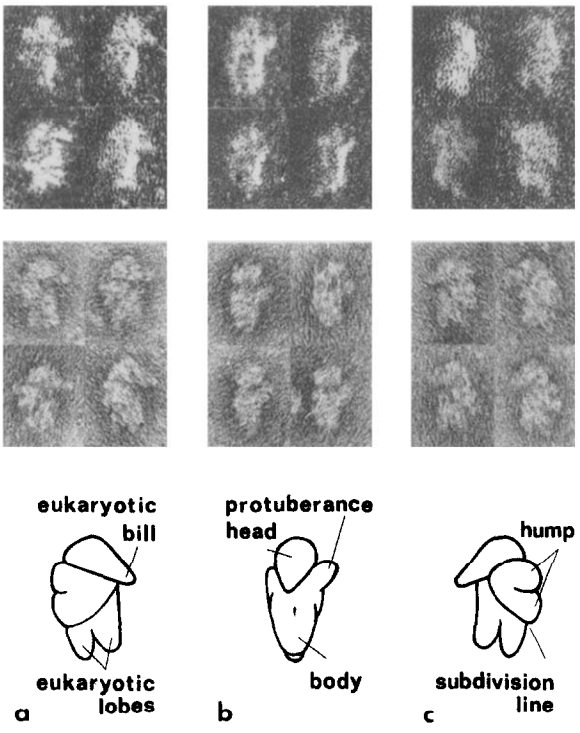

Fig.2. Electron microscopy images of the 40 S ribosomal subunits and their schematic drawings with a description of the main terms used. The resolution of the freeze-dried and shadowed subunit images (upper two rows) is close to that of the negatively stained ones (next two rows). The same structural features of the subunit are revealed in both cases. (a,c) Lateral views; (b) frontal view.

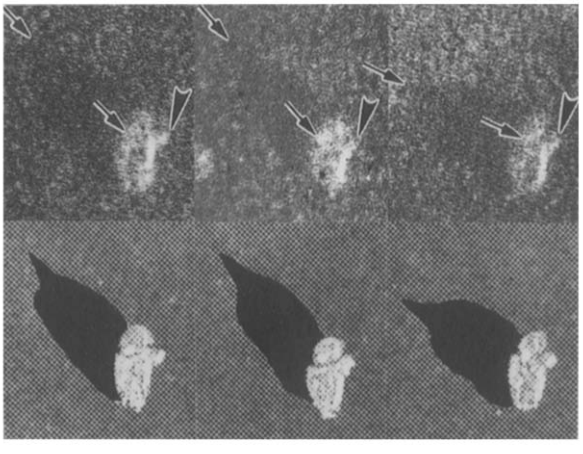

Fig.3. Shadow graphs of the 40 S ribosomal subunits in frontal view and their interpretative drawings. Arrows indicate the apexes of the eukaryotic bill and its characteristic-pointed shadow. Arrowheads indicate the protuberance.

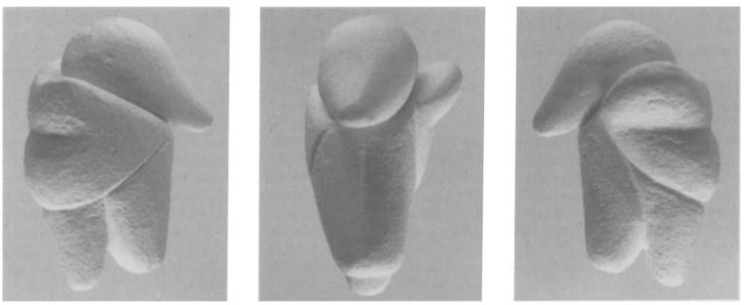

Fig.4. Three-dimensional model of the 40 S ribosomal subunit.

Thus, only the model of the three-dimensional structure of the 40 S ribosomal subunit with a right location of the protuberance results from data presented. We have constructed the model which is shown in fig.4. The model is most similar to that proposed by Lutsch et al. [2,5] but has an opposite handedness. Besides, the eukaryotic lobes and the protuberance are more expressed.

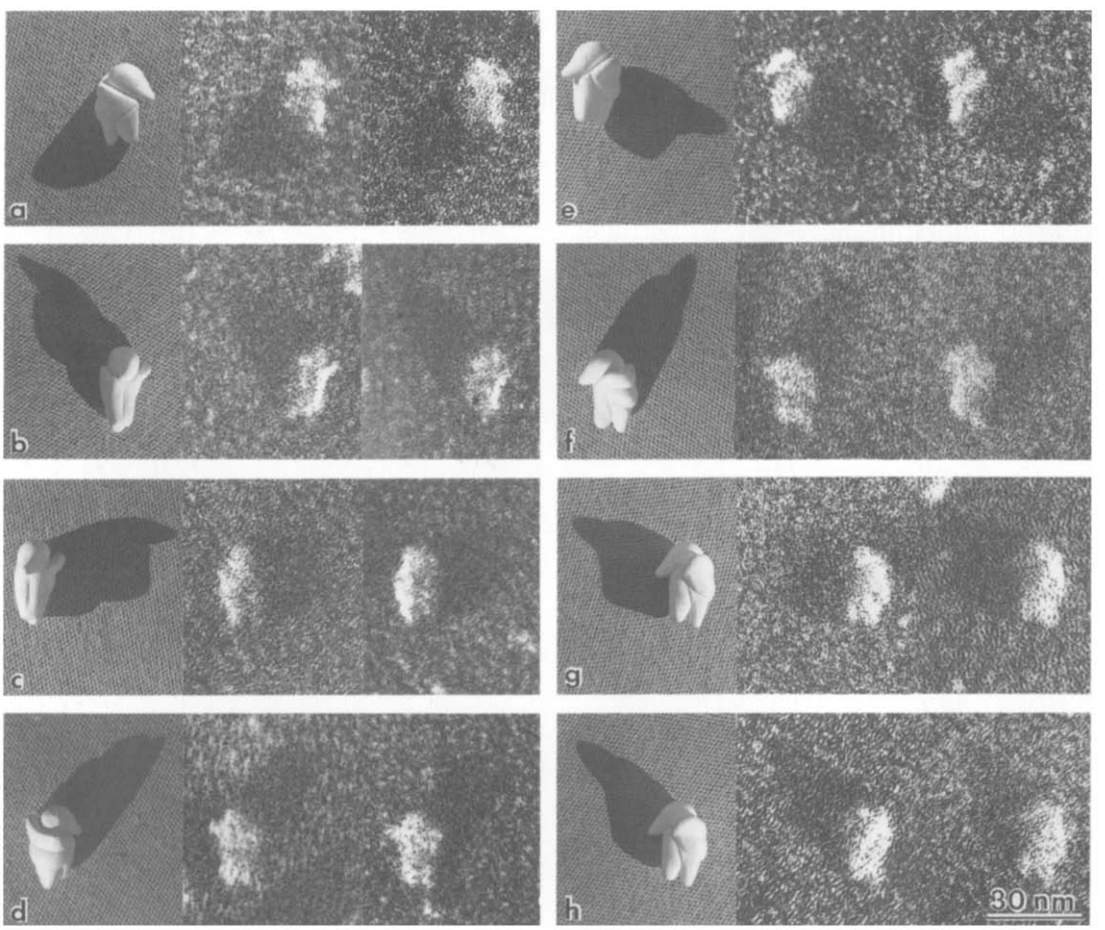

Fig.5. Electron microscopy images of the 40 S ribosomal subunits in comparison with the model illuminated at an angle of 26° (shadowing angle). (a) Left lateral view; (b,c) frontal views of the subunits each shadowed from the opposite side; (d) dorsal view; (e–h) right lateral views of the subunits at different orientations relative to the direction of shadowing.

Fig.5 represents electron micrographs of single shadowed 40 S subunits in comparison with the model. A very good accordance between the outlines, surface relief and shadows of the subunit images and those of the model is observed. The characteristic-pointed shadows from the bill on the images of the 40 S subunit in the frontal view and from the protuberance on those in the right lateral view are clearly seen. Rarely found images of the rear side of the 40 S subunit are also presented. Again, the consistency of the model with the 40 S subunit morphology is observed.

It has been well documented by freeze-drying and high-resolution shadow casting [12] and tilting experiments [14] that the prokaryotic small ribosomal subunit has a right location of the protuberance (side ledge or platform). The structural organization of the ribosome is quite highly conserved [10,15] and therefore the principal structural difference between pro- and eukaryotic ribosomes, such as the different handedness, is very unlikely. This study presents experimental evidence for the same handedness of both kinds of ribosomes and confirms, on the other hand, the existence of structural features unique for 40 S ribosomal subunits such as the eukaryotic bill and the eukaryotic lobes described until now only for negatively stained specimens.

Acknowledgement: We thank Dr S.N. Ryazantsev for fruitful discussions.

REFERENCES

[1] Nomura, Y., Blobel, G. and Sabatini, D.D. (1971) J. Mol. Biol. 60, 303–323.
[2] Lutsch, G., Bielka, H., Wahn, K. and Stahl, J. (1972) Acta Biol. Med. Germ. 29, 851–876.
[3] Kiselev, N.A., Stel'mashchuk, V.Ya., Lerman, M.I. and Abakumova, O.Yu. (1974) J. Mol. Biol. 86, 577–586.
[4] Emanuilov, I. and Sabatini, D.D. (1981) J. Ultrastruct. Res. 76, 263–276.
[5] Lutsch, G., Bielka, H., Enzmann, G. and Noll, F. (1983) Biomed. Biochim. Acta 42, 705–723.
[6] Boublik, M., Oostergetel, G.T., Wall, J.S., Hainfeld, J.F., Radermacher, M., Wagenknecht, T., Verschoor, A. and Frank, J. (1986) in: Structure, Function, and Genetics of Ribosomes (Hardesty, B. and Kramer, G. eds) pp.68–86, Springer, New York.
[7] Theise, H., Noll, F. and Bielka, H. (1978) Acta Biol. Med. Germ. 37, 1353–1362.
[8] Valentine, R.C., Shapiro, B.M. and Stadtman, E.R. (1968) Biochemistry 7, 2143–2152.
[9] Vasiliev, V.D. and Koteliansky, V.E. (1979) Methods Enzymol. 612–629.
[10] Kiselev, N.A., Stel'mashchuk, V.Ya., Lutsch, G. and Noll, F. (1977) J. Mol. Biol. 126, 109–115.
[11] Lake, J.A., Henderson, E., Oakes, M. and Clark, M.W. (1984) Proc. Natl. Acad. Sci. USA 81, 3786–3790.
[12] Vasiliev, V.D. (1974) Acta Biol. Med. Germ. 33, 779–793.
[13] Vasiliev, V.D., Selivanova, O.M. and Ryazantsev, S.N. (1983) J. Mol. Biol. 171, 561–569.
[14] Leonard, K.R. and Lake, J.A. (1979) J. Mol. Biol. 129, 155–163.
[15] Boublik, M., Wydro, R.M., Hellmann, W. and Jenkins, F. (1979) J. Supramol. Struct. 10, 397–404.