SIZE HETEROGENEITY OF MONOPARTICLES FROM NUCLEAR RIBONUCLEOPROTEINS CONTAINING PREMESSENGER RNA

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1. Introduction

One of the first observations on the structure of the nuclear particles containing premessenger RNA (premRNP) was that a mild ribonuclease treatment converted the initially polydisperse material into smaller structures considered as monodisperse [1–3]. A polysomal-like structure was then suggested [1] and the premRNP were considered as a population of polyparticles made of a variable number of identical monoparticles. These were designated according to their sedimentation coefficient whose nominal value varied between 30 S and 55 S depending on the report [1–3].

However, further work showed that the structure of the premRNP was possibly more complex and contained ribonucleoproteins heterogeneous in size in addition to the monoparticles [4]. Furthermore, the monoparticles themselves were of 2 kinds (Mα and Mβ) with different protein compositions and ribonuclease sensitivities.

The present study shows an additional level of structural complexity in the premRNP. A relatively marked size heterogeneity of the monoparticle population is described. It is not related to the presence of the 2 classes of monoparticles.

2. Materials and methods

Rats were injected intracisternally with [^{3}H]uridine 4 h before sacrifice. A nuclear extract containing nuclear particles and soluble proteins was prepared by sonication from purified brain nuclei as in [5].

The extract was enriched in free monoparticles by incubation for 15 min at 20°C. The monoparticles were fractionated by sedimentation on sucrose gradient. Unless otherwise specified, the buffer was 10 mM triethanolamine–HCl, pH 7.4, 25 mM KCl, 1 mM $MgCl_2$. The conditions of centrifugation are indicated in the figure legends. In each case, calibration was done according to [6] with ribosomal subunits prepared by EDTA dissociation of brain polysomes.

Proteins were precipitated with 10% trichloracetic acid, treated with urea, dithiothreitol and sodium dodecylsulfate as in [7]. They were separated on 10% acrylamide gels and stained with Coomassie blue [7]. Profiles after scanning are presented.

For electron microscopic examination, a drop of the sucrose gradient fractions was deposited on a copper grid coated with a carbon collodion film, fixed with 0.5% glutaraldehyde for 5 min and stained with 1% uranyl acetate.

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3. Results and discussion

3.1. *Sedimentation heterogeneity of the monoparticle population*

Brain nuclear monoparticles were prepared by incubation of a nuclear extract containing the premRNP and the nucleosol [8] for 15 min at 20°C. This is a mild procedure since less than 10% premRNP was solubilized (against 50% after treatment in the presence of 0.1 μg exogeneous ribonuclease under the same conditions). The endogeneous ribonuclease converted

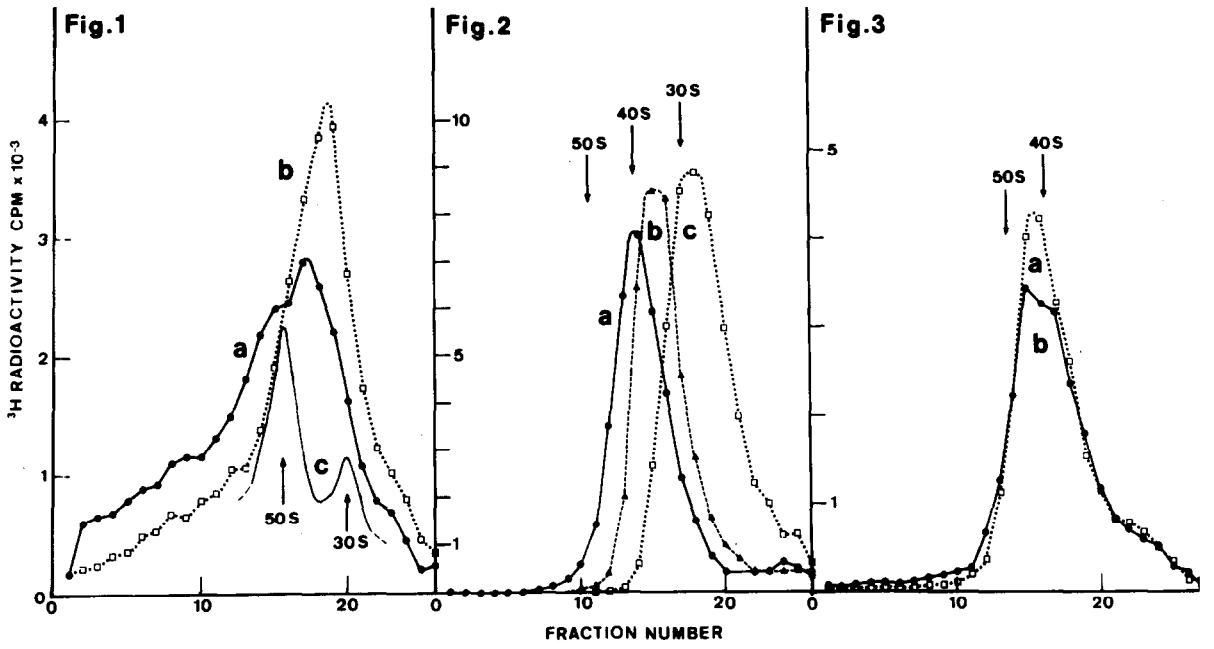

Fig.1. Preparation of monoparticles by endogeneous ribonuclease. A nuclear extract was sedimented on a sucrose gradient before (curve a) and after incubation for 15 min at 20°C (curve b). Gradient was 10–25% sucrose. Centrifugation was in an SW25-2 rotor for 6 h at 25 000 rev./min (75 000 × *g*). Brain ribosomal subunits were sedimented in another tube in the same rotor. 0.2 mM EDTA replaced $MgCl_2$ in the gradient buffer (curve c). Fig.2. Resedimentation of monoparticles. Fractions from a preparative gradient as shown in fig.1, curve b were centrifuged on a 15–30% sucrose gradient for 13 h at 25 000 rev./min (82 500 × *g*) in an SW-27 rotor. Initial sedimentation coefficients: 47 S (a), 37 S (b) and 27 S (c). Fig.3. Incubation of monoparticles after isolation. Aliquots, 2 × 0.8 ml, were taken from a 45 S fraction from a preparative sucrose gradient as shown in fig.1, curve a. 2 ml unlabelled nuclear extract was added to one of them (b) and 2 ml extraction buffer in the other (a). Both samples were incubated for 15 min at 20°C and immediately recentrifuged on a 15–30% sucrose gradient for 14 h at 23 000 rev./min (63 900 × *g*) in an SW25-2 rotor.

a fraction of the large non-incubated particles (fig.1a) into a broad peak sedimenting in the upper half of a sucrose gradient (fig.1b). This is the monoparticle peak as defined [1,2]. As shown by comparison with ribosomal subunits (fig.1c), the monoparticles sedimented from approx. 30–50 S. The separation of the ribosomal subunits was satisfactory indicating that the width of the peak was the result not of poor experimental conditions but of the presence of heterogeneous material. The method of preparation of particles (by ultrasonication of the nuclei) was not responsible of the heterogeneity, since similar results were obtained when particles were diffused out of the nuclei by the method in [1].

This heterogeneity was confirmed by resedimenting fractions of 27 S, 37 S and 47 S of the monoparticle peak, separately on new sucrose gradients (fig.2). The fractions resedimented as relatively narrow peaks at 26 S, 35 S and 40 S, respectively. This indicated that the initial heterogeneity was not primarily due to reversible interactions between monoparticles. A decrease of sedimentation coefficient was observed reproducibly for the largest particles only and was of 5 S on the average.

Endogeneous ribonucleases could be responsible for the heterogeneity of the monoparticles by transforming an initially homogeneous structure (50 S for instance) into smaller material during incubation. This was unlikely since monoparticles were found up to 30 S even in native premRNP prepared in the presence of cytoplasmic ribonuclease inhibitor ([4] and unpublished observations).

Nevertheless, the following control was performed: a 45 S fraction was prepared from a labelled nuclear extract without incubation. Unlabelled nuclear extract was added and the mixture was incubated under the conditions used for the preparation of monoparticles (15 min, 20°C). The change of sedimentation coefficient as compared to a control incubated without any addition was insignificant (fig.3). Therefore, we conclude that the smallest monoparticles do not arise from the largest ones by endogeneous hydrolysis during preparation.

The size heterogeneity of the monoparticles might be accompanied by a variation of the size of the constitutive RNA. This size varied from 3–7 S in all 3 fractions but, on the average, decreased slightly with the sedimentation coefficient of the monoparticles (results not shown). In fact, such results are difficult to interpret. It is likely that the endogeneous ribonuclease not only nicks the RNAs at the precise sites allowing the release of monoparticles, but also starts their hydrolysis and nicks other RNA sequences belonging to the monoparticles. This would not greatly influence the sedimentation characteristics of the monoparticles since protein–protein interactions play an important role in ther stability [4,9], but would decrease the size of their RNA in a way which might be different for monoparticles of different sizes.

3.2. *Heterogeneity as visualized by electron microscopy.*

A possible cause of the heterogeneity of the sedimentation coefficient would be the heterogeneity of the actual dimensions of the particles. A nuclear extract was incubated as above and fractions sedimenting at 30 S, 40 S and 50 S were examined by electron microscopy after negative staining (fig.4). Two kinds of structures were observed: monomeric units with somewhat variable shapes and oligomeric structures apparently made up of 2 or 3 units. The proportion of oligomeric structures was similar in all 3 fractions (20–25%). The 2 main axes of the units were measured and their product was considered as an index of size. The histograms showed that there was an increase in the size of both monomeric and oligomeric structures with sedimentation coefficient. The increase was approximately linear as shown by comparing this with 2 other experiments.

The size of the units and the proportion of oligomeric structures were similar to those found in the 30–50 S region of a nonincubated nuclear extract (not shown). This indicated that the characteristics of the material were not due to the action of endogeneous ribonucleases which only allowed a better recovery of monoparticles under our experimental conditions.

On the average, the oligomeric structures were larger than the isolated units in a same fraction. This is probably to be related to differences of sedimentation coefficient brought about by the differences in shape.

Native polyparticles resembling perichromatin fibrils, made of units of different sizes, were tentatively classified as small, middle-size, large and very large [10,11]. In this work, most of the isolated units were in the middle-size and large classes, whereas oligomeric structures were rich in small units. However, a direct assimilation of units from polyparticles and from isolated units might not be correct since only 2 dimensions were measured and since a flattening may occur and be more marked in one instance than in the other.

The monoparticle peak characterized by biochemical methods contained 2 different structures upon electron microscopic visualization. They may correspond to 2 classes of monoparticles or to different configurations or arrangements of monoparticles. Whatever the interpretation, the conclusion of the heterogeneity of the monoparticle population remains, since the change of dimensions has been observed for both monomeric and oligomeric structures.

3.3. *Relative homogeneity of protein composition*

The size heterogeneity may be the consequence of the presence of classes of different monoparticles each

Fig.4. Electron microscopic visualization of the monoparticles. Right Panel: 30 S, 40 S and 50 S fractions were examined after negative staining (108 000 ×). Bars (|—|) 46 nm. Most of the structures appear as monomeric. Arrows point to oligomers. Left Panel: the main axes were measured on micrographs at a magnification of 81 000 with the help of a lens with a 0.1 mm graduation. 150 units from 3 different meshes of 2 grids were studied in each case. The number of units (ordinates) is shown as a function of the product of the 2 axes (in mm). Shaded areas correspond to oligomeric structures.

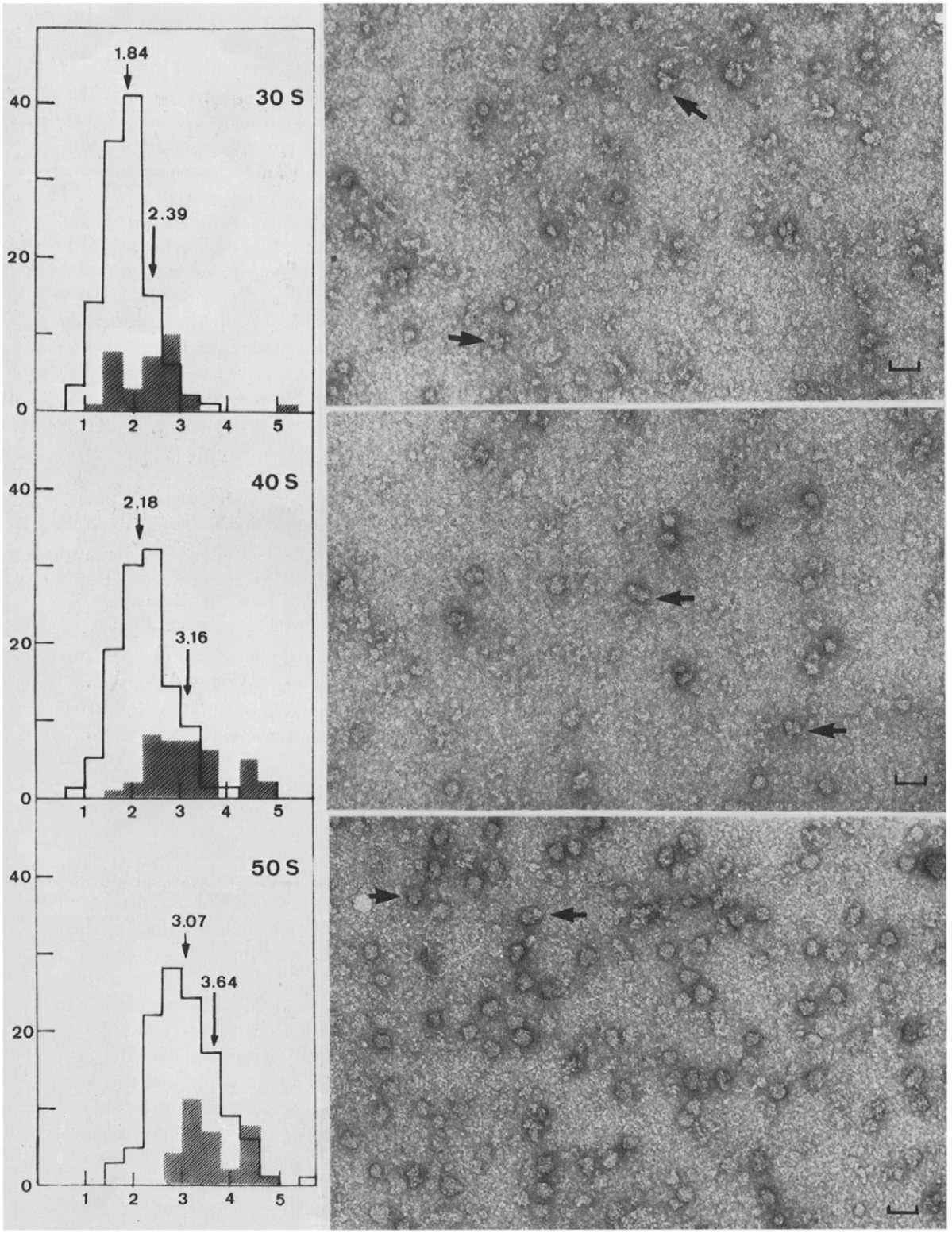

Fig.4

with a discrete size. The 2 classes of monoparticles described (Mα and Mβ) have distinct protein compositions [4] and may therefore be easily recognized. From the examination of the electrophoretic profiles of the proteins from the 30 S, 40 S and 50 S fractions of the monoparticle peak, it is clear that a separation of classes of monoparticles of different protein compositions was not achieved (fig.5). The major proteins

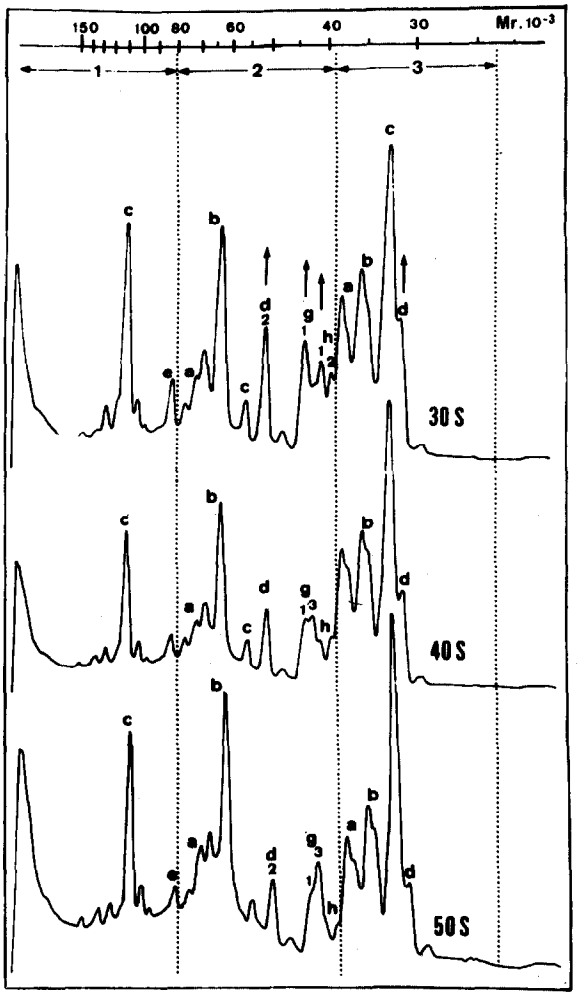

Fig.5. Protein composition of monoparticles. Fractions of 30–35 S (a), 40–45 S (b), 50–55 S (c) from a preparative sucrose gradient as shown in fig.1, curve b, were pooled and analyzed for proteins. Arrows indicate proteins whose amount increases with decreasing sedimentation coefficient, 2g3 being the only protein whose amount decreases. Nomenclature is that established in [14].

3a, 3b, 3c which are the components of Mα were present in similar proportions in the 3 fractions. This was also the case of the other major proteins. A quantitative increase of a few proteins (belonging to Mβ) with decreasing sedimentation coefficient was observed reproducibly (arrows in fig.5) but was not marked enough to account for a class fractionation. The experiments rather suggest that the size heterogeneity exists for each class of monoparticles. In addition, they show that the 2 classes of monoparticle proteins are present at a relatively constant ratio suggesting a close physical or physiological relationship.

3.4. *Implications of structural complexity*

PremRNP may be considered to be the site of the processing of premessenger RNA. The structure of the premRNP might be directly related to that of its constitutive nucleic acid, like in ribosomes, or not, like in nucleosomes. Some current concepts about the structure of premRNP assume that they are composed of single identical monoparticles [1,12,13] sometimes compared to nucleosomes. However, 2 main lines of evidence show that the structure is more complex:

1. premRNP contain a relatively high amount of heterogeneous ribonucleoprotein complexes different from the monoparticles [4];

2. Monoparticles are heterogeneous in size (present work).

With the early monotonous model, it must be assumed that there is a low degree of specificity of the premessenger RNA–protein interactions and that a given sequence of the RNA (messenger or non-messenger) may overlap 2 monoparticles. In contrast, with the heterogeneous model, it becomes possible to speculate that specific RNA sequences (messenger RNA or messenger RNA fragments on one side, non-messenger sequences or inserts on the other) are localized in different units, implying that proteins and RNA specifically interact. Interesting prospects on the mechanism of premessenger RNA processing might be open by such a model. It remains to be demonstrated that the different classes of RNA sequences are truly localized in the different classes of units.

Acknowledgements

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