RELEASE OF mRNP-PARTICLES OF THE INFORMOSOME TYPE FROM POLYRIBOSOMES OF HIGHER PLANT EMBRYOS

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1. Introduction

In a previous paper [1] we reported the existence in wheat embryo of RNP-particles of the informosome type with a buoyant density in CsCl of about 1.40–1.44 g/cm^3 observed in the pre-ribosomal zone of the sucrose gradient of postmitochondrial extract. These particles differed from polyribosomes of buoyant density 1.52 g/cm^3. In their density characteristics the plant informosomes were similar to analogous particles isolated from animal cells [2,3].

It is shown in the present paper that EDTA treatment leads to dissociation of the wheat embryo polyribosome component and release of mRNP-particles with a buoyant density of 1.40 g/cm^3. It is concluded that in addition to free informosomes there are polysome-bound mRNP-particles of the informosome type in the cytoplasmic extract of germinating wheat embryos. Similar data have also been obtained on germinating pea seedlings.

2. Materials and methods

Embryos of *Triticum vulgare* wheat of the 'Kazakhstan 126' variety were used. They were prepared, germinated and incubated with radioactive precursors as described previously [1]. Homogenization was done in a standard buffer of the following composition: 0.02 M triethanolamine, pH 7.8, 0.005 M $MgCl_2$, 0.15 M KCl, 0.25 M sucrose, 0.001 M mercaptoethanol. The homogenate was centrifuged at 3000 rpm for 3–5 min and then at 20 000 *g* for 20 min. 20% Triton X-100 was added to the postmitochondrial extract to a final concentration of 0.5%, the extract was incubated for 20 min at 2°C and the total RNP-particle fraction was pelleted by centrifugation in an SW-65 rotor for the time indicated in the legends to figures. The RNP particle fraction was suspended in buffer containing 0.02 M triethanolamine, pH 7.6, 0.005 M $MgCl_2$, 0.025 M KCl, 0.001 M mercaptoethanol and fractionated in a 10–60% linear sucrose gradient in an SW-65 rotor of a Spinco L2-65 ultracentrifuge with 0.5 ml 70% sucrose as a cushion.

Fractionation in a caesium chloride density gradient was done as described previously [1].

In EDTA dissociation experiments the RNP-particle fraction was suspended at 2°C in a buffer containing 0.02 M triethanolamine, pH 7.6, 0.025 M KCl and 0.01 M EDTA. After 10 min the suspension was fixed with 4% formaldehyde.

Pisum sativum seedlings of the 'Komsomol' variety were also used. The seedlings were sterilized with 12% H_2O_2 and germinated in damp paper for 12 hr at 20°C. The embryos were separated from the cotyledons by hand and incubated in a medium containing [^{3}H]uridine for 30 min. The procedure of homogenization and subsequent analysis was the same as for wheat embryos, except that the KCl concentration in the buffer for homogenization was decreased to 0.025 M.

3. Results and discussion

Fig. 1 shows the sedimentation profile of the RNP-particle preparation pelleted by centrifugation

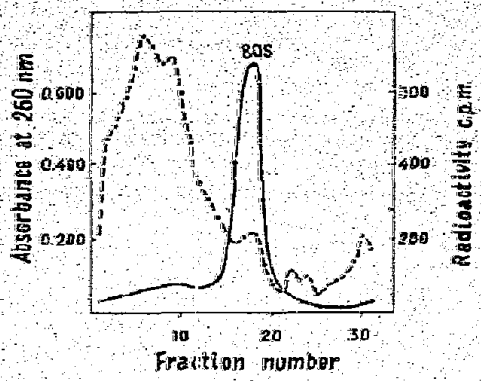

Fig. 1. Sedimentation distribution in a 10–70% sucrose gradient of RNP-particles pelleted for 4 hr at 38 000 rpm from post-mitochondrial extract of wheat embryos. After 5.5 hr germination the embryos were incubated with [^{3}H]uridine for 30 min. Sucrose gradient centrifugation was done at 38 000 rpm for 3.5 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

of germinating wheat embryo cytoplasmic extract for 4 hr. It is seen that the bulk of radioactivity ([^{3}H]uridine) sediments in the preribosomal zone; in the zones of monoribosomes, ribosomal subparticles and light RNP-particles the radioactivity is small. Analysis in a caesium chloride gradient (fig. 2) shows that the newly-synthesized RNA is distributed over a rather wide band with a maximum of radioactivity in the density region of 1.44 g/cm^3. This main peak

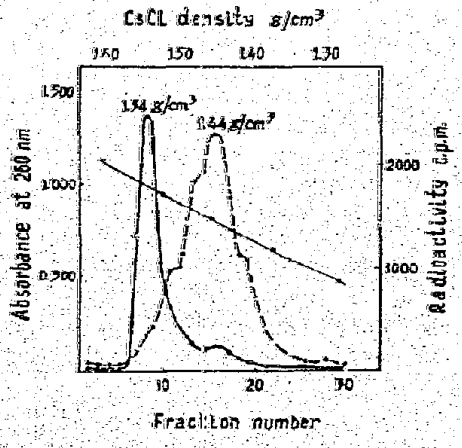

Fig. 2. Density distribution in a caesium chloride gradient of RNP-particles pelleted from the post-mitochondrial extract of wheat embryos for 4 hr at 38 000 rpm. Centrifugation was done at 40 000 rpm for 20 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

displays a left shoulder with a density in the region of 1.50–1.52 g/cm^3 which coincides with the density of polyribosomes. There is also a right shoulder with a density in the region of 1.40 g/cm^3.

It is possible to improve the resolution of the structures investigated in the post-mitochondrial extract by means of two consecutive centrifugations: firstly a 50 min sedimentation and then the supernatant was again centrifuged for 4 hr. Fig. 3 represents

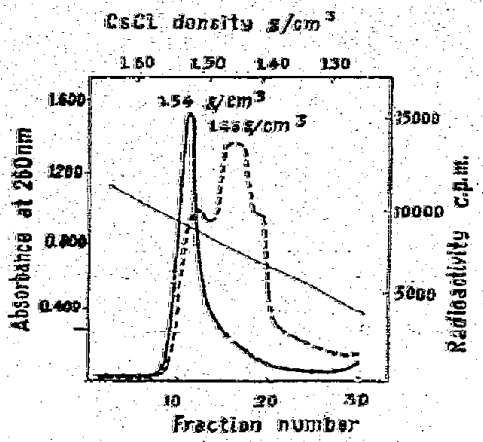

Fig. 3. Density distribution in a caesium chloride gradient of RNP-particles pelleted from the post-mitochondrial extract of wheat embryos at 38 000 rpm for 50 min. Centrifugation was done at 40 000 rpm for 20 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

the density distribution profile of the total RNP-particle preparation pelleted from the post-mitochondrial cytoplasmic extract for 50 min. Here the RNP-fraction is more distinctly displayed as three components with densities of 1.51–1.52 g/cm^3, 1.46 g/cm^3 and 1.40 g/cm^3. The preparation pelleted following centrifugation for 4 hr (fig. 4) exhibits only two components with densities of 1.51–1.52 g/cm^3 and 1.40 g/cm^3.

It was shown previously [1] that the fraction with a buoyant density of 1.51–1.52 g/cm^3 must be ascribed to polyribosomes. It should be noted that the lower buoyant density value of plant polyribosomes as compared to monoribosomes is analogous to the situation with animal materials [3].

Perry and Kelley [4], Henshaw [5] and Cartouzou et al. [6] have shown the presence of polyribosome-bound RNP-particles containing mRNA in animal

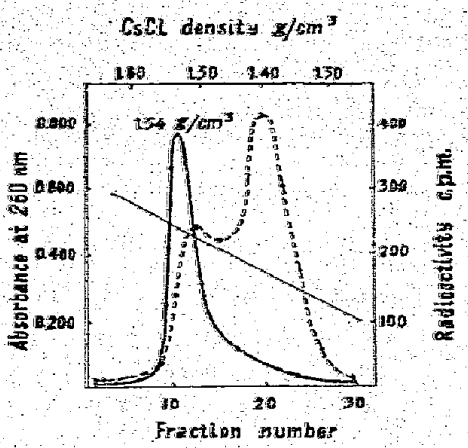

Fig. 4. Density distribution in a caesium chloride gradient of RNP-particles pelleted for 4 hr at 38 000 rpm from the supernatant fraction after 50 min preliminary centrifugation of the wheat embryo post-mitochondrial extract. Caesium chloride centrifugation was done at 40 000 rpm for 20 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

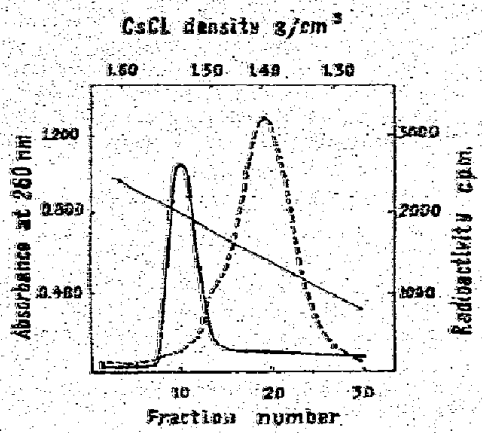

Fig. 6. Density distribution in a caesium chloride gradient of RNP-particles pelleted for 4 hr at 38 000 rpm from the wheat embryo post-mitochondrial extract after EDTA treatment. Centrifugation was done at 40 000 rpm for 20 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

cells; after dissociation of polyribosomes with EDTA these particles had a buoyant density of 1.40–1.46 g/cm³.

To check whether this was so in plants, we suspended the total RNP-particle fraction, obtained as a result of 4 hr centrifugation, in EDTA and then fixed them in formaldehyde after a 10 min incubation. Half of the suspension was analyzed in a sucrose gradient (fig. 5) and the other half in a caesium chloride gradient (fig. 6).

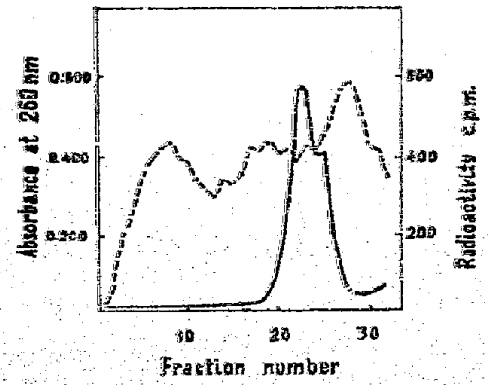

Fig. 5. Sedimentation distribution in a 10–70% sucrose gradient of RNP-particles pelleted for 4 hr at 38 000 rpm from the wheat embryo post-mitochondrial extract after EDTA treatment. Centrifugation was done at 38 000 rpm for 3.5 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

It is seen from the UV-absorption profile in fig. 5 that there is a complete dissociation of ribosomes into two subparticles. The radioactivity is distributed heterogeneously along the whole gradient, the label being observed both in the heavy and the light zones.

On fractionation of the same suspension in the caesium chloride (fig. 6) the UV-absorption profile shows that the ribosome subparticles occupy the band at 1.53–1.54 g/cm³, i.e., the same band as monoribosomes. The radioactive label is localized in the band with a density around 1.40 g/cm³. A comparison of figs. 2, 5 and 6 quite distinctly demonstrates the complete dissociation of the polyribosomal component with a density of 1.51–1.52 g/cm³ and a sharp increase of radioactivity in the 1.40 g/cm³ band. This result can be interpreted as a release of polyribosome-bound mRNA-particles with a density of 1.4 g/cm³ by EDTA.

As to the component with a density of 1.46 g/cm³, the picture here is not clear enough and the decrease of radioactivity in this band is probably connected with the partial dissociation of particles with a density of 1.46 g/cm³. It is quite possible that this structure represents a complex of informosomes with the monoribosome or the small subparticle.

The presence of at least three types of RNP-particles containing rapidly-labeled RNA of the mRNA type has also been shown in another

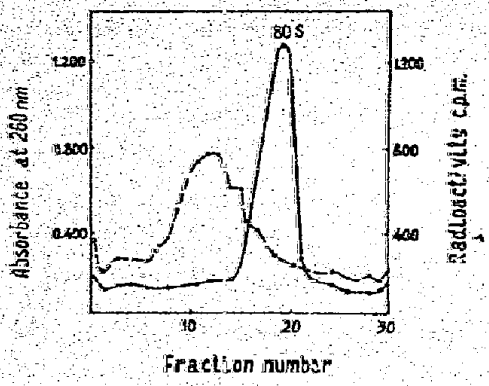

Fig. 7. Sedimentation distribution in a 10–70% sucrose gradient of RNP-particles pelleted for 4 hr at 38 000 rpm from the post-mitochondrial extract of germinating pea seedlings. Centrifugation was done at 38 000 rpm for 3 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

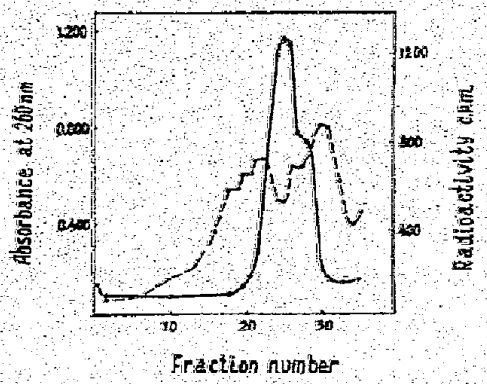

Fig. 9. Sedimentation distribution in a 10–70% sucrose gradient of pea RNP-particles after EDTA treatment. Centrifugation was done at 38 000 rpm for 3 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

embryonic plant tissue. Fig. 7 shows the sedimentation distribution in a sucrose gradient of the RNP-particle preparation isolated from germinating pea seedlings. Fig. 8 shows the density distribution profile of the same preparation. As can be seen from fig. 8, labeled RNP-particles are distributed into three classes with buoyant densities of 1.51–1.52 g/cm^3, 1.46–1.47 g/cm^3 and 1.40 g/cm^3. Figs. 9 and 10 illustrate experiments on the dissociation of pea RNP-particles by EDTA. From fig. 10 it is distinctly seen that, as a result of dissociation, a single labeled component is revealed with a density of 1.40 g/cm^3 corresponding to the RNP-particles of the informosome type.

In comparing our results with the available literature data it can be concluded that there is a similarity in the different types of rapidly-labeled RNP (both free informosomes and polyribosome-

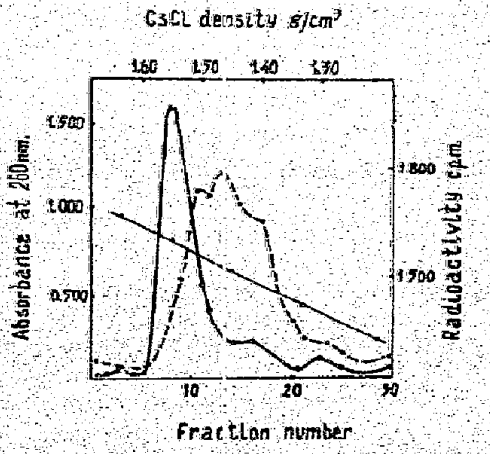

Fig. 8. Density distribution in a caesium chloride gradient of pea RNP-particles sedimented from the post-mitochondrial extract for 4 hr at 38 000 rpm. Centrifugation was done at 40 000 rpm for 20 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

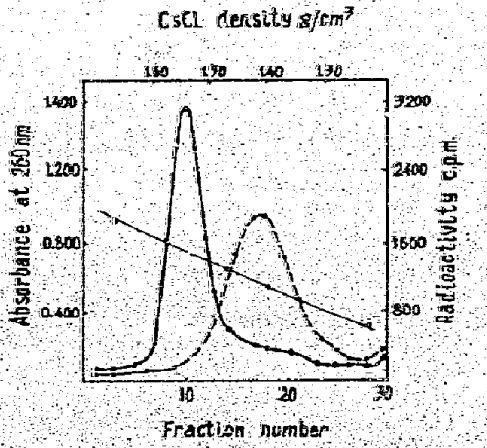

Fig. 10. Density distribution in a caesium chloride gradient of pea RNP-particle after EDTA treatment. Centrifugation was done at 40 000 rpm for 20 hr at 3°C. Solid line – UV-absorption, dotted line – radioactivity.

bound mRNP-particles) observed in cytoplasmic extracts of embryonic plant as well as animal tissues. These data support the assumption of the universality of the observed structures in eukaryotic cells and can also serve as an additional indication of an important significance of the mRNA-protein complexes.

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