INFORMOSOMES OF GERMINATING WHEAT EMBRYOS

M.A. AJTKHOZHIN, A.U. AKHANOV and Kh.I. DOSCHANOV
Institute of Botany, Academy of Sciences of the Kazakh SSR, Alma-Ata, USSR

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1. Introduction

There are at present numerous data on the presence of complexes of messenger or messenger-like RNA with protein in animal cells [1–9]. These complexes are characterized by a heterogeneous sedimentation distribution but they are comparatively homogeneous in buoyant densities. Their RNA:protein ratio is approx. 1:3. It was suggested that they be called "informosomes" [1].

Up to now it remains unknown whether these particles are limited to animal systems or whether they are universal for all eukaryotic organisms.

We have attempted to find these particles in plant cells. In this paper data are reported on the presence of informosomes in germinating wheat embryos.

2. Materials and methods

Embryos of spring wheat *Triticum vulgare* of the "Kazakhstanskaya" 126 variety were used for the experiments. Embryos were obtained according to a standard technique [10]. Germination was carried out according to Chen et al. [11] at 32° for 5.5 hr. At the end of germination the embryos were washed with distilled water and placed in a medium with radioactive precursors, [^{3}H]uridine (1 mCi/ml) or [^{14}C]amino acids (50 μCi/ml each). Upon completion of incubation, the embryos were washed free of the radioactive medium and homogenized in a glass homogenizer with a teflon pestle in a buffer of the following composition: 0.02 M triethanolamine-HCl, pH 7.6, 0.005 M $MgCl_2$, 0.025 M KCl, 0.25 M sucrose. In some cases the KCl concentration was increased to 0.15 M. The homogenate was centrifuged at 2,000 rpm for 20 min. Triton X-100 was added to the post-mitochondrial supernatant to a final conc. of 0.5% and then the total ribonucleoprotein (RNP) fraction was pelleted at 38,000 rpm for 4 hr. Preparations of RNP particles were fractionated by centrifugation in a sucrose gradient (10%–70%) or in a preformed cesium chloride gradient with 4% formaldehyde [3, 12]. The isolation of RNA was carried out by the detergent method [13].

3. Results and discussion

Fractionation of the RNA preparation isolated from the total RNP particles (fig. 1) shows that the

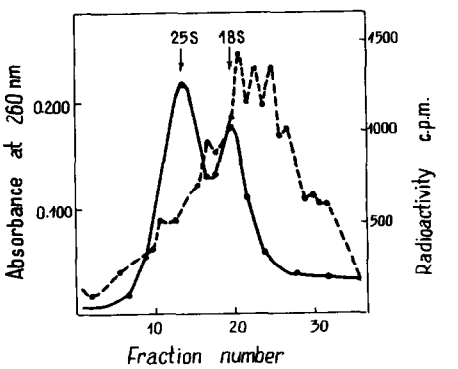

Fig. 1. Sedimentation distribution in a sucrose gradient of RNA of the total RNP fraction from wheat embryos. RNP particles were deproteinized with 2% sodium dodecyl-sulfate at 37° for 2 min and the RNA preparation was layered on a 15–30% sucrose gradient. Centrifugation was carried out in the SW-50 rotor of the Spinco L2-65 ultracentrifuge at 38,000 rpm for 5.5 hr at 16°. UV-absorption is represented by a solid line and radioactivity by the dotted one.

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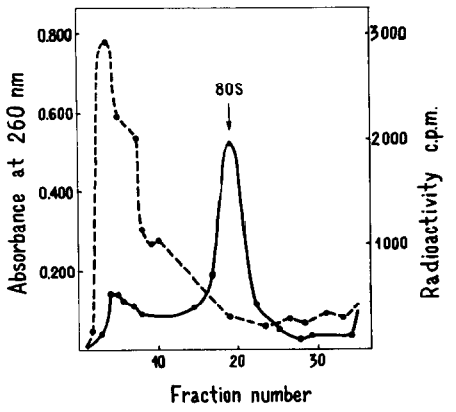

Fig. 2. Sedimentation distribution in a 10–70% sucrose gradient of the fraction of RNP particles labelled for 30 min with [^{3}H]uridine. Centrifugation was at 38,000 rpm for 3 hr at 3°. The solid line represents UV-absorption, the dotted one shows radioactivity.

main bulk of newly-synthesized RNA sediments in the 6 S–16 S zone; no essential labelling of ribosomal RNA is revealed.

To study the distribution of newly-synthesized RNA in cytoplasmic structures, a fractionation of the RNP particle preparation was carried out by sucrose gradient centrifugation (fig. 2). The greater part of the newly-formed RNA of RNP particles is revealed in the zone of fast sedimenting structures, ahead of

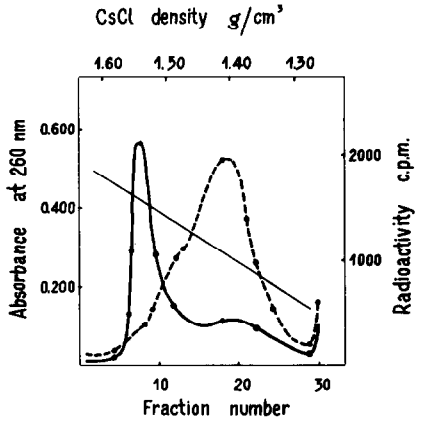

Fig. 3. Density distribution of the RNP particle fraction in a cesium chloride gradient. The embryos were incubated for 30 min with [^{3}H]uridine. Centrifugation was at 38,000 rpm for 18 hr at 3°. The solid line represents UV-absorption, the dotted one shows radioactivity.

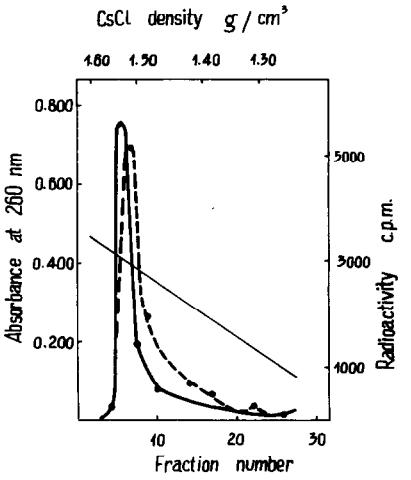

Fig. 4. Density distribution of the RNP particle fraction in the cesium chloride gradient. The embryos were incubated for 10 min in a medium with [^{14}C]amino acids (valine, leucine, lysine). Centrifugation was at 38,000 rpm for 18 hr at 3°. The solid line represents UV-absorption, the dotted one shows radioactivity.

the UV peak of monoribosomes. They can be either polyribosomes or free high molecular-weight RNP particles of non-ribosomal type (informosomes). To decide this question centrifugation in a cesium chloride density gradient was carried out (fig. 3). The main band of the UV absorbing material corresponding to monoribosomes and having a buoyant density of 1.545 g/cm^3 is seen in the densitogram as well as the second component with a buoyant density of 1.40–1.44 g/cm^3 where almost all the radioactive material is concentrated. By the usual criteria (characteristic value of buoyant density, presence of rapidly labelled RNA of non-ribosomal type, high sensitivity to ribonuclease [9]) the latter component corresponds to informosomes of animal cells.

In view of the available data on the possibility of sorption of alien proteins on polyribosomes when using non-ionic detergents in a buffer with a low ionic strength [14], the same experiments were repeated in a buffer where the KCl concentration was increased to 0.15 M. In our experiments no changes in sedimentation and density characteristics of the particles studied were observed.

To obtain better evidence that the observed component with a buoyant density of 1.40–1.44 g/cm^3

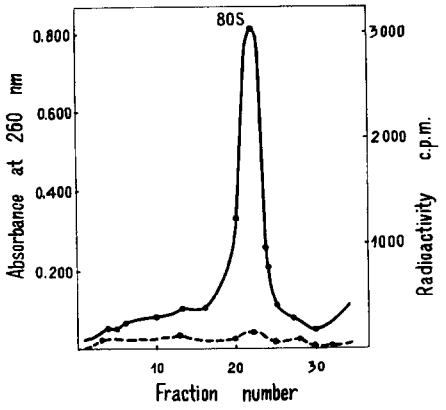

Fig. 5. Sedimentation distribution in a sucrose gradient of the RNP particle fraction labelled for 30 min with [^{3}H]uridine and treated with ribonuclease. Centrifugation was at 38,000 rpm for 3 hr at 3°. The solid line represents UV-absorption, the dotted one shows radioactivity.

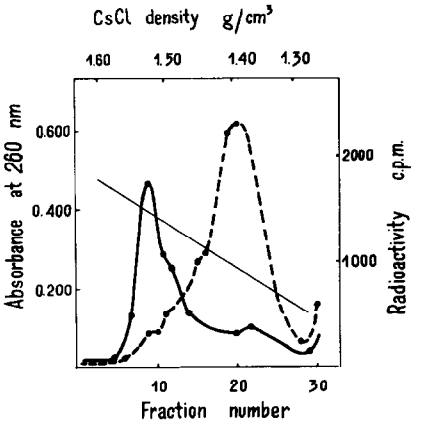

Fig. 6. Density distribution in a cesium chloride gradient of the RNP particle fraction (the same preparation as in fig. 3) treated with EDTA. Centrifugation was at 38,000 rpm for 18 hr at 3°. The solid line represents UV-absorption, the dotted one shows radioactivity.

is not a result of sorption of alien proteins on polyribosomes, a pulse label experiment with [^{14}C]amino acids was carried out followed by analysis in the cesium chloride gradient (fig. 4). As seen in the figure, the single observed radioactive component has a buoyant density of 1.52 g/cm^3 which corresponds to the buoyant density of animal polyribosomes [8].

Some properties of the RNP particles with a buoyant density of 1.40–1.44 g/cm^3 were studied. Treatment by pancreatic ribonuclease at a low concentration completely destroys them (fig. 5), while the pattern of ribosome sedimentation does not change. EDTA, 0.01 M, treatment for 10 min at 0° did not destroy the particles (fig. 6).

In connection with these data it should be noted that indirect hints on the possible presence of informosomes in dry wheat embryos were obtained recently by Marcus and Weeks [15] during testing of an RNP particle fraction in a cell-free system.

Thus, on the basis of the results obtained we have come to the conclusion that there exist high molecular-weight complexes of non-ribosomal (messenger or messenger-like) RNA with protein, i.e. informosomes, in cytoplasmic extracts of germinating wheat embryos. In density characteristics and some other properties they are similar to informosomes of animal cells. It is probable that informosomes are universal in eukaryotic cells.

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