# NUCLEAR RIBONUCLEOPROTEIN PARTICLES OF HIGHER PLANTS

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## 1. Introduction

Soon after the discovery of cytoplasmic messenger ribonucleoproteins (mRNP) or informosomes [1], ribonucleoproteins containing heterogenous non-ribosomal RNA (the so-called '30 S particles') were also found in nuclear extracts of animal cells [2,3]. The buoyant density of these particles in CsCl proved to be identical with that of cytoplasmic informosomes [4,5]. It was later shown that if extraction is done in the presence of a ribonuclease inhibitor, larger heterogeneous RNP particles with sedimentation coefficients of 30 S to 250 S can be isolated from animal nuclei [6]. Thus, judging from the main criteria, such as buoyant density, sedimentation heterogeneity and others, nuclear mRNP particles have proved to be similar to the cytoplasmic mRNP particles and can be considered as nuclear informosomes [7].

In previous communications we reported data on the presence in plant cytoplasmic extracts of free and polysome-bound informosomes similar in all characteristics to the analogous animal particles [8,9]. The present paper is devoted to the isolation and some characteristics of nuclear informosomes from germinating wheat embryos.

## 2. Materials and methods

The object of the study were embryos of wheat *Triticum vulgare,* sort 'Kazakhstan 126', prepared according to Johnston and Stern [10]. The embryos were germinated as described earlier [8] for 4 hr at 30°C and incubated with [$^3$H]uridine (0.5 mCi/ml) in a medium containing 0.02 M KCl and 0.01 M Tris buffer, pH 7.0, for 15–20 min at 30°C. After incubation was completed, the embryos were washed free from excess radioactive uridine and homogenized in buffer consisting of 0.25 M sucrose, 0.005 M $MgCl_2$, 0.01 M KCl, 0.005 M mercaptoethanol, 4% gum arabic, 0.01 M triethanolamine, pH 7.0. The homogenate was filtered through two layers of Miracloth. The isolation of nuclei from the homogenate was done by a somewhat modified procedure of Tautvydas [11]. The filtered homogenate was centrifuged at 3000 *g* for 15 min. The nuclear pellet was suspended in a small volume of the buffer used for homogenization without gum arabic, then Triton X-100 was added to a final concentration of 0.02%, and the suspension was layered onto a discontinuous gum arabic gradient (8 ml 12%, 10 ml 10%, 12 ml 8%), prepared in the homogenization buffer, and centrifuged at 3000 *g* for 15 min. Sometimes the purification of nuclei was done by repeated centrifugation through the gum arabic gradient. The purity of nuclei was checked microscopically. The RNP particles were extracted from the purified nuclei with a high salt concentration buffer according to Penman et al. [12]; the buffer contained 0.5 M NaCl, 0.01 M $MgCl_2$, 0.01 M triethanolamine, pH 7.0, and Triton X-100 was added to a final concentration of 0.5%. The lysate was treated with DNAase (50 pg/ml) for 20 min at 30°C and centrifuged at 15 000 *g* for 20 minutes. The supernatant fraction representing the nuclear extract was fixed with 4% neutral formaldehyde, and after 30-40 min incubation was diluted with a low salt concentration buffer (0.01 M NaCl, 0.005 M $MgCl_2$, 0.01 M triethanolamine, pH 7.4) with formaldehyde and used either for sucrose gradient sedimentation or for density analysis in the CsCl gradient. The RNA was isolated form the nuclear lysate by the method of Perry et al. [13] with the addition of unlabelled

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ribosomal RNA from wheat embryos as a carrier and reference. Other details of the procedure are given in the legends to figures.

### 3. Results and discussion

Three types of procedures were used to isolate the nuclear ribonucleoprotein particles of animal material: repeated extraction with isotonic buffer in the cold with pH increasing from 7 to 8 [4], mechanical disruption of the nuclei by ultra-sound in isotonic medium at neutral pH values [14], and lysis in a high salt concentration [12].

In our experiments numerous attempts to extract the nuclei of plant cells at a low salt concentration with pH increasing up to 8 did not result in satisfactory sedimentation patterns. Sucrose gradient centrifugation displayed the greater part of the uridine-labelled material sedimenting in the low molecular weight zone. In the cesium chloride gradient the radioactivity was distributed heterogeneously over a wide range of density of 1.35 to 1.60 g/cm$^3$ (data are not shown). Thus the method in its standard version seems to have a limited application to different materials. For some cultural animal cells it was possible to extract nuclear mRNP particles by this method in combination with increasing the temperature in the course of extraction to 20–37°C [15] and the pH up to 9 [14]. However, in our experiments the increases of temperature and pH were also found unsuccessful for extraction of plant nuclear mRNP particles.

As an alternative we used the lysis of nuclei in a high salt concentration according to Penman et al. [12]. The sucrose gradient sedimentation pattern of the wheat embryo nuclear extract obtained by such a high salt concentration technique is represent in fig.1. The figure shows that all the labelled RNA is in the structure distributed heterodispersely over a wide range of sedimentation coefficients characteristics of rapidly-labelled non-ribosomal ribonucleoprotein particles. To elucidate the density distribution of the structures studied, fractionation of the same extract was done in the CsCl gradient. As shown in fig.2, practically all the labelled RNA forms a homogeneous band with a buoyant density of 1.40 g/cm$^3$. The latter permits one to conclude that all the

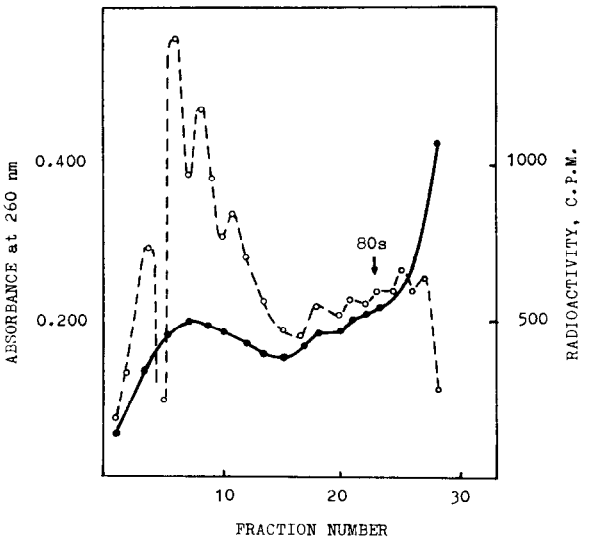


Fig.1. Sedimentation distribution in the 10–60% sucrose gradient of RNA-containing material of wheat embryo nuclear extract pre-incubated for 20 min with [$^3$H]uridine. Centrifugation at 38 000 rev/min in an SW-65 rotor for 90 min at 3°C. Solid line, UV absorption; dotted line, radioactivity.

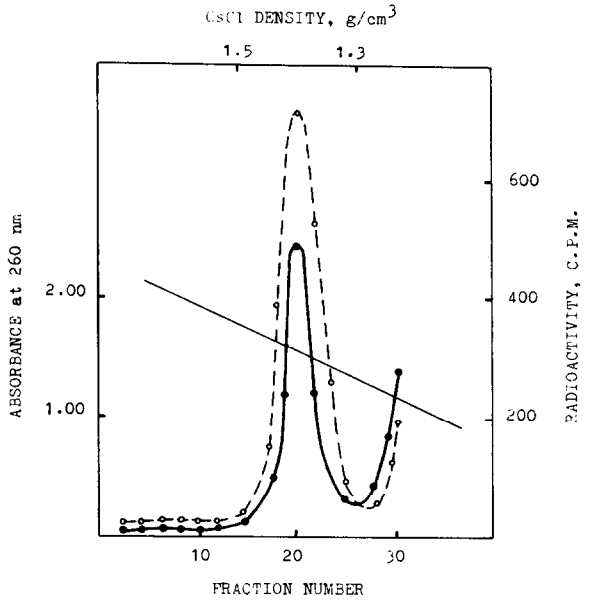


Fig.2. Density distribution of RNA-containing material of the nuclear extract in the cesium chloride gradient. Centrifugation at 40 000 rev/min in an SW-65 rotor for 20 hr at 3°C. Solid line, UV absorption; dotted line, radioactivity.

newly-synthesized RNA extracted from the plant cell nuclei after the short time of incubation with the radioactive precursor in the form of complexes with protein of the informosome type characterized by a ratio of RNA to protein of about 1:4. It can be seen in the same figure that the procedure used to purify the nuclei is quite effective, as cytoplasmic ribosomes and polyribosomes (buoyant density of 1.51–1.55 g/cm$^3$) are completely absent from the preparation.

Additional proof that the structures observed are of a true nuclear nature was obtained from analysis of the RNA of the particles. The sedimentation pattern of the RNA preparation isolated from the nuclear extract is given in fig.3. It is seen that all the newly-synthesized RNA sediments heterodispersely with an average sedimentation coefficient from 15 S to 30 S and over, characteristic of nuclear RNA.

The possibility of contamination of the nuclear mRNP particle preparation by chromatin proteins was eliminated in experiments where the non-fixed nuclear extract was centrifuged in the sucrose gradient containing 0.1 M NaCl and 4% formaldehyde. After centrifugation the radioactive material was collected from the two zones (fig.4), dialysed free from sucrose

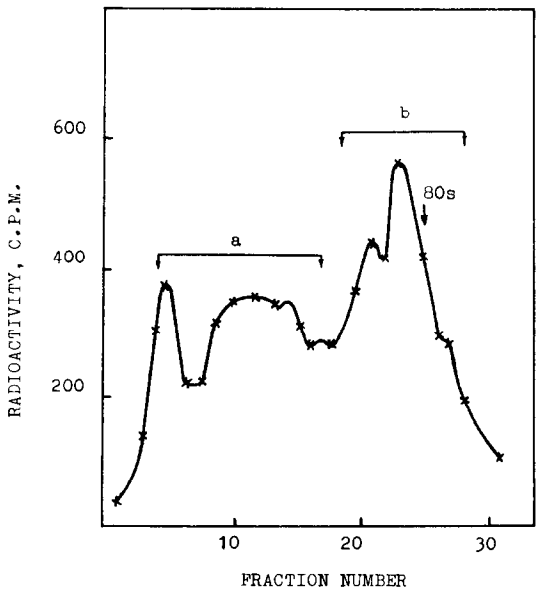


Fig.4. Sedimentation distribution of RNA-containing material of the nuclear extract in the 10–60% sucrose gradient. Centrifugation at 23 000 rev/min in an SW-25.1 rotor for 90 min at 3°C. Zones 'a' and 'b' were collected, the sucrose removed by dialysis and the material used for density analysis in the cesium chloride gradient (see fig.5).

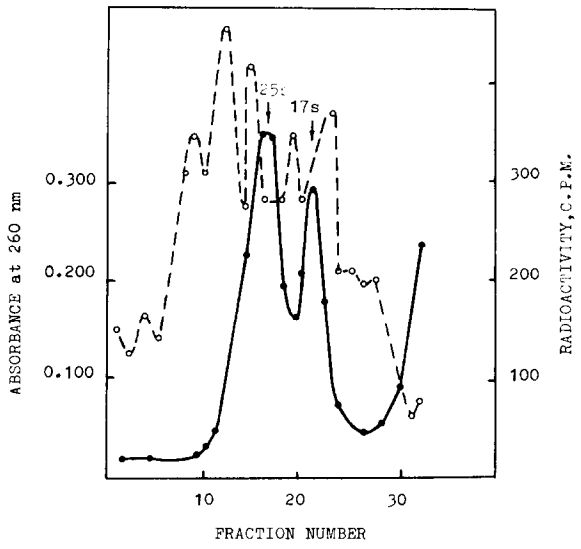


Fig.3. Sedimentation distribution in the 15–30% sucrose gradient of RNA isolated from the nuclear extract. Centrifugation at 38 000 rev/min in an SW-65 rotor for 5.5 hr at 16°C. Solid line, UV absorption of ribosomal RNA; dotted line, radioactivity.

and analyzed in the cesium chloride gradient. The nuclear mRNP particles from both zones purified in this manner had the same buoyant density in CsCl (1.40 g/cm$^3$) as the particles of the crude extract (fig.5).

Plant nuclear mRNP particles, just as the analogous animal structures, were shown to be sensitive to low RNAase concentrations. A short treatment with the enzyme converted the greater part of the radioactivity into the acid-soluble form, and the remaining part was found in the low molecular weight zone of the sucrose gradient (fig.6). The removal of magnesium from the nuclear extract by EDTA (0.01 M) treatment did not change the density characteristics of the nuclear mRNP particles (data not shown).

Thus, the results obtained permit us to conclude that in higher plant nuclear extracts rapidly-labelled RNA exists in the form of complexes with protein of a buoyant density of 1.4 g/cm$^3$. According to all the characteristics studied, such as the non-ribosomal nature of the rapidly-labelled RNA component, the sedimentation heterogeneity, the unique buoyant

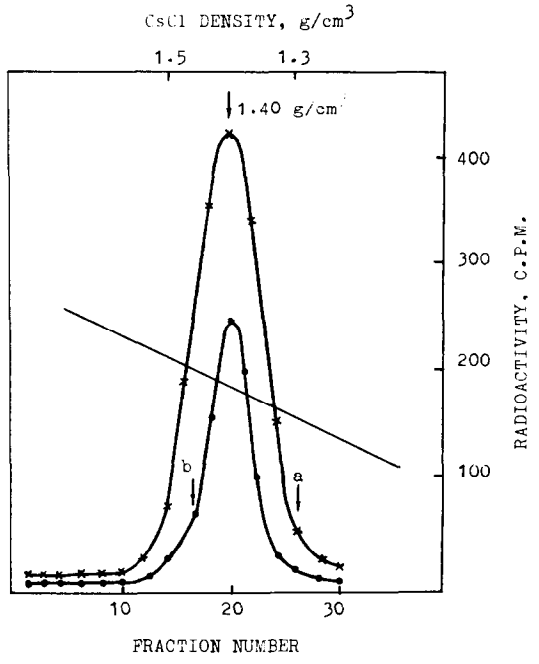


Fig.5. Density distribution in the cesium chloride gradient of nuclear mRNP particles taken from different sucrose gradient zones (see fig.4). Centrifugation in the cesium chloride gradient at 40 000 rev/min in an SW-65 rotor for 20 hr at 3°C.

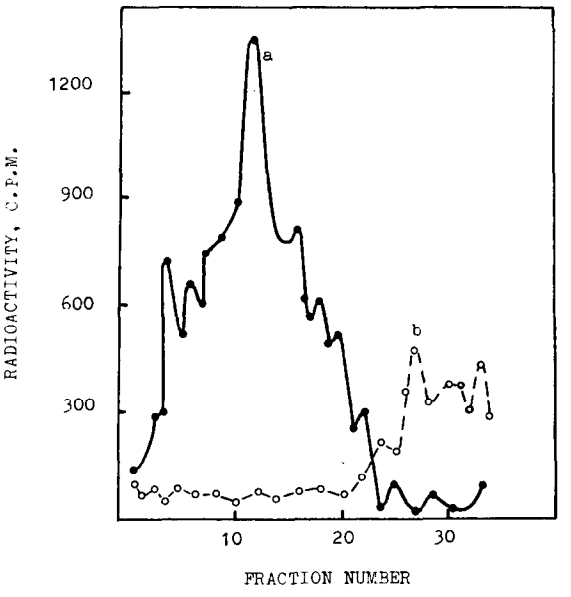


Fig.6. Effect of pancreatic RNAase on nuclear mRNP particles. The nuclear extract was incubated with pancreatic RNAase (1 pg/ml, 37°C for 5 min). Centrifugation at 38 000 rev/min in an SW-65 rotor for 90 min at 3°C. Solid line, control mRNP; dotted line, the same after the RNAase treatment.

density, sensitivity to low RNAase concentrations and resistance to EDTA, the plant nuclear mRNP particles are similar to the informosomes of animal and plant cells.

On the basis of these results we have come to the general conclusion that in addition to the earlier revealed free cytoplasmic informosomes and polysome-bound mRNP particles, nuclear informosomes are also present in plant cells. The discovery of all the three types of localization of rapidly labelled non-ribosomal ribonucleoproteins in higher plants poses the question of some common functional role of these structures in eukaryotic organisms.

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