RNA-BINDING PROTEIN FACTOR OF WHEAT EMBRYO EXTRACTS

M. A. AJTKHOZHIN and T. N. KIM
Institute of Botany, Academy of Sciences of the Kazakh SSR, Alma-Ata, Kazakh SSR, USSR

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1. Introduction

Non-ribosomal ribonucleoprotein particles containing messenger or 'messenger-like' RNA were first described in cytoplasmic extracts of fish embryos in 1964; the particles were called informosomes [1]. Later, informosomes (mRNP) were also found in many animal cells, both in the cytoplasm and in the nucleus. Detailed reviews have been published by Spirin [2,3]. Recently informosomes have also been found in plant cells [4,5]. Soon after discovery of the particles, it was shown that ribonucleoprotein particles of the informosome type could be obtained in vitro by adding exogenous RNA to a ribosome-free extract of animal cells [6–8]. Free informosome-forming (RNA-binding) protein was isolated from animal cytoplasmic extracts and some of its physico-chemical characteristics studied [9–14]. Its characteristic feature is a high molecular weight; the sedimentation coefficient of animal RNA-binding protein is 6–10 S. An assumption was made that RNA-binding protein could be a pool from which informosomes in vivo are formed.

This paper describes the RNA-binding protein factor from wheat embryo cytoplasmic extracts. Similarly to the animal factor, the plant factor is adsorbed on nitrocellulose filters if exogenous RNA is added and forms stoichiometric complexes with RNA of a buoyant density of 1.4 g/cm^3. The sedimentation coefficient of the main component was found to be 6.5–8 S.

2. Materials and methods

Dry embryos of wheat *Triticum vulgare*, variety 'Kazakhstan 126', prepared by the method of Johnston and Stern [15] were used. The embryos were homogenized in the following buffer: 0.15 M sucrose, 0.01 M KCl, 0.001 M $MgCl_2$, 0.001 M mercaptoethanol, 0.01 M triethanolamine, pH 7.8. The homogenate was centrifuged at 3000 rev/min for 3–5 min and then at 20 000 rev/min for 20 min. 4 ml of the extract was layered on 1 ml 10% sucrose prepared in the buffer for homogenization but not containing sucrose and centrifuged in a Spinco L SW-50 rotor at 38 000 rev/min for 5 hr after which the upper two-thirds of the supernatant were collected. This fraction was used as a ribosome-free extract.

To study the conditions of complex formation with exogenous RNA, highly-labeled RNA from *Aspergillus nidulans* with a spec. act of 23 $\times 10^6$ cpm per mg was used. The highly-labled RNA was added to the ribosome-free wheat embryo extract in different proportions, incubation was done at 4°C for 15 minutes, and then the mixture was fixed with neutral 4% formaldehyde. The preparation was dialyzed and then analyzed in a cesium chloride density gradient as described earlier [16].

The sedimentation distribution of the RNA-binding factor was studied by sucrose gradient centrifugation of the ribosome-free extract followed by analysis of the fractions by the technique of sorption on nitro-cellulose filters [9].

3. Results and discussion

Fig.1 represents the density distribution of particles resulting from interaction of exogenous *Asp. nidulans* RNA with the ribosome-free extract of wheat embryos. The addition of the RNA to the

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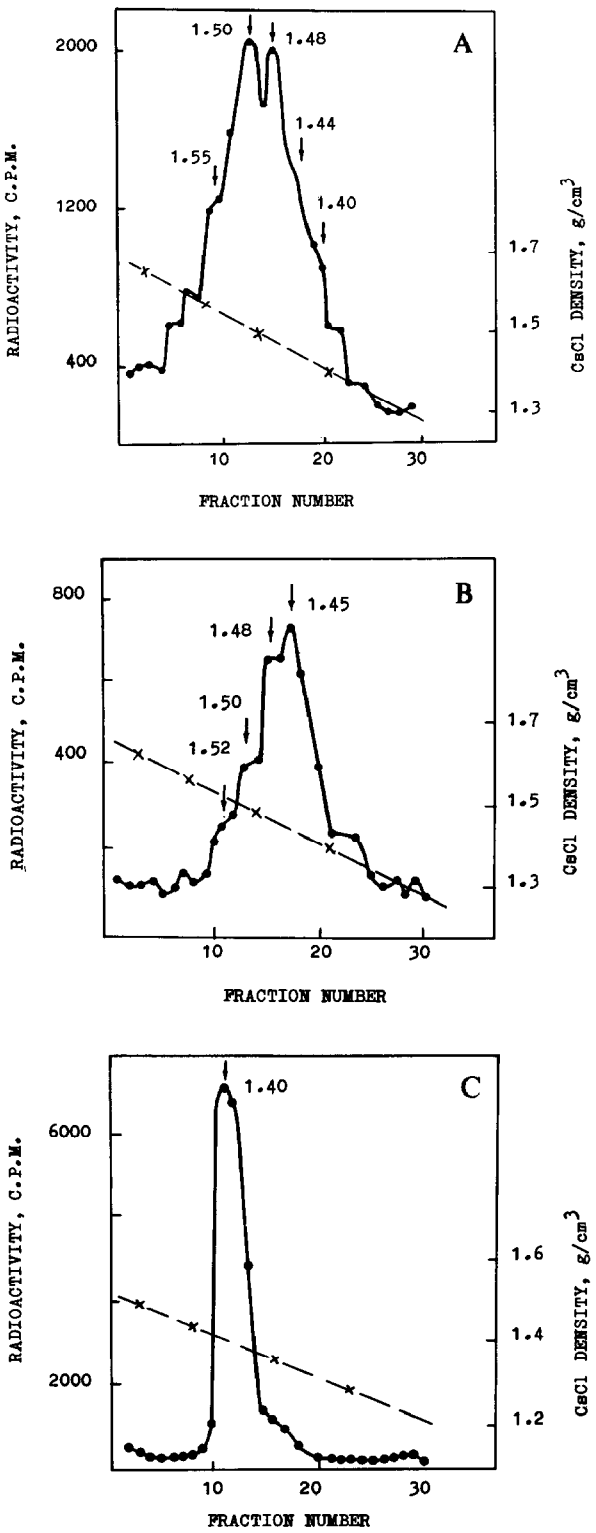

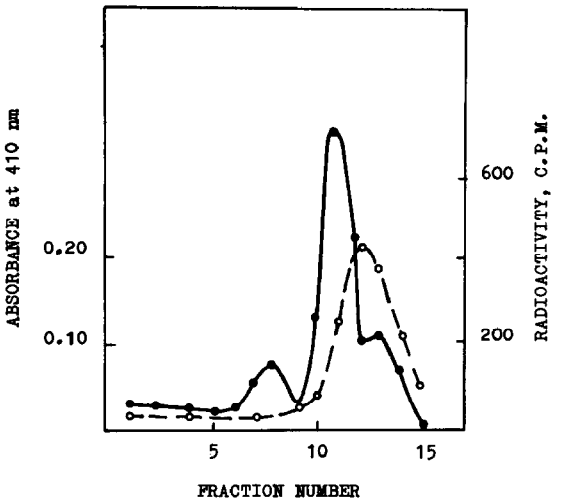

Fig.2. Sedimentation distribution of the RNA-binding factor in a 10–20% sucrose gradient. Centrifugation of the ribosome-free extract was done at 38 000 rev/min for 20 hr at 3°C. (——) Radioactivity of bound exogenous RNA (RNA-binding activity) (– – –) Reference hemoglobin absorption at 410 nm.

extract in a ratio of A_{260} extract: A_{260} RNA of 100 : 1 leads to the formation of complexes with buoyant densities in CsCl of from 1.55 to 1.45 g/cm^3, the buoyant densities of the main components being 1.50 and 1.48 g/cm^3 (fig.1a). With an increase of this ratio to 400 : 1 heterogeneity is still preserved, but at the same time a decrease of buoyant density of the particles formed to values of 1.50–1.45 g/cm^3 is observed (fig.1b). An increase of the ratio A_{260} extract: A_{260} RNA to 800 : 1 leads to the appearance of homogeneous particles with a density of 1.4 g/cm^3 (fig.1c). A further increase of this ratio to 1000 : 1 and higher did not lead to a change in the density of the particles obtained.

These results provide evidence that in the ribosome-free cytoplasmic extract of wheat embryos there exists a factor capable of interacting with exogenous RNA to form complexes similar in density to infor-

Fig.1. Density distribution in a cesium chloride gradient of particles formed at different ratios of A_{260} extract: A_{260} RNA. a) 100:1; b) 400:1; c) 800:1. Centrifugation was done at 40 000 rev/min for 20 hr at 3°C.

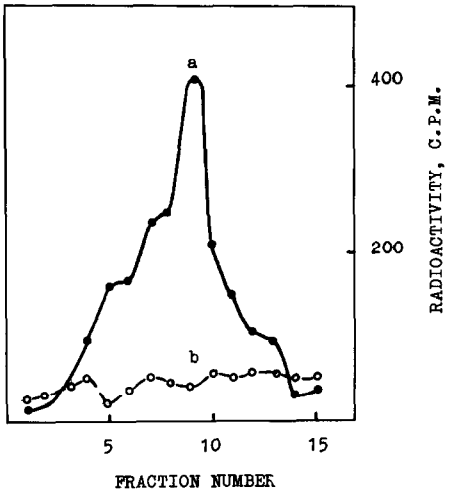

Fig.3. Effect of pronase on the RNA-binding activity of the extract: a) the extract incubated for 30 min at 35°C without addition of pronase (control); b) the extract incubated with pronase (2.5 mg/ml) for 30 min at 35°C. Centrifugation of the ribosome-free extract was done in a 10–20% sucrose gradient at 38 000 rev/min for 20 hr at 3°C.

mosomes of both animal and plant cells. Similarly to the RNA-binding protein factor of animal cells, the plant factor is capable of forming stoichiometric complexes with added RNA.

To study the character and distribution of this factor in the extract, fractionation of the ribosome-free extract by centrifugation in a sucrose gradient was done followed by the testing of different fractions of the gradient for their ability to bind RNA. The results of the sedimentation analysis are represented in fig.2. As seen from the figure, the RNA-binding factor is distributed in a rather narrow zone of the sucrose gradient. There is one major peak and few minor ones. The sedimentation coefficient of the main component, determined relative to that of hemoglobin, is 6.5–8 S.

On the basis of these data, and also by analogy with animal cells, it can be presumed that the plant RNA-binding factor is protein in nature. Indeed, pronase treatment of the extract led to a complete loss of the RNA-binding activity (fig.3).

The results provide evidence that in addition to free informosomes [4] and polysome-bound mRNP particles [5], RNA-binding proteins are present in plant cell cytoplasmic extract which are capable of giving stoichiometric complexes with exogenous RNA and thus forming informosome-like particles. In its sedimentation behaviour the plant protein factor is similar to the factor from animal cells. A further study of the plant and animal RNA-binding proteins, especially in a comparative manner, could throw light on biological functions of informosomes as universal structures of eukaryotic cells.

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