INFORMOSOMES AS A STORED FORM OF mRNA IN WHEAT EMBRYOS

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1. Introduction

Investigations carried out in the last 10–15 years suggest the presence of a temporary inactive stored (masked) form of mRNA in unfertilized eggs of animals [1–3]. An analogous storage of mRNA was also demonstrated in higher plant seeds [4–7]. However, it is not yet known in what form the stored mRNA is present in animal eggs and higher plant seeds.

The discovery of messenger ribonucleoproteins (informosomes) in animal cells [8] permitted the formulation of the hypothesis that *informosomes* can be the stored form of mRNA in embryonic animal objects [9–11]. Recently informosomes have been also found in embryonic plant objects [12–14].

In the present study we attempted to approach this problem by investigating the distribution of newly synthesized RNA in cytoplasmic extract of wheat embryos at different stages of ripening. The advantage of plant objects for this purpose is that their embryos in many cases can be easily separated from the seed without losing their germinating power and vitality. They are able to rapidly incorporate radioactive precursors when immersed in an aqueous medium, thus permitting brief exposures with an isotope for selective labelling of mRNA. It is found that ripening of seeds is accompanied by accumulation of free informosomes (not bound to ribosomes) in the cytoplasm of embryos.

2. Materials and methods

Embryos of spring wheat of the 'Kazakhstanskaya 126' variety, at different stages of ripening, were used for the experiments. Embryos were separated from the seeds manually with a preparatory needle and placed in an incubation buffer (0.01 M Tris, 0.02 M KCl, pH 7.0) with [^{3}H]uridine (0.5 mCi/ml). Incubation with the radioactive precursor lasted for 20 min at 26–27°C. After incubation the embryos were washed with cold incubation buffer and the cytoplasmic extract was obtained as described earlier [13,15]. Total RNP particles were pelleted from the cytoplasmic extract by a 4 h sedimentation at 100 000 *g* and after fixing with 4% neutral formaldehyde were fractionated by sucrose and CsCl gradient centrifugations [13,15].

3. Results and discussion

The distribution of the newly synthesized mRNA in the cytoplasmic RNP particle fraction at different stages of ripening of wheat embryos (early milky, milky-waxy, waxy) was studied. Parallel analyses of RNP particle preparations of the cytoplasmic extracts were made in the sucrose and CsCl gradients. Here only the results of fractionation of RNP particle preparations in the CsCl gradient are given.

Fig.1 shows the results of density analysis of cytoplasmic RNP particles isolated from the embryos at different stages of ripening. At the early milky stage (fig.1a), the only type of particles containing mRNA are structures with a buoyant density of 1.50–1.52 g/cm^3 identified earlier as polyribosomes [12]. At the milky-waxy stage (fig.1b) the cytoplasmic extract has two types of particles containing the newly synthesized mRNA: polyribosomes with a buoyant density of 1.50–1.52 g/cm^3 and free informosomes with a buoyant density of 1.40–1.45 g/cm^3.

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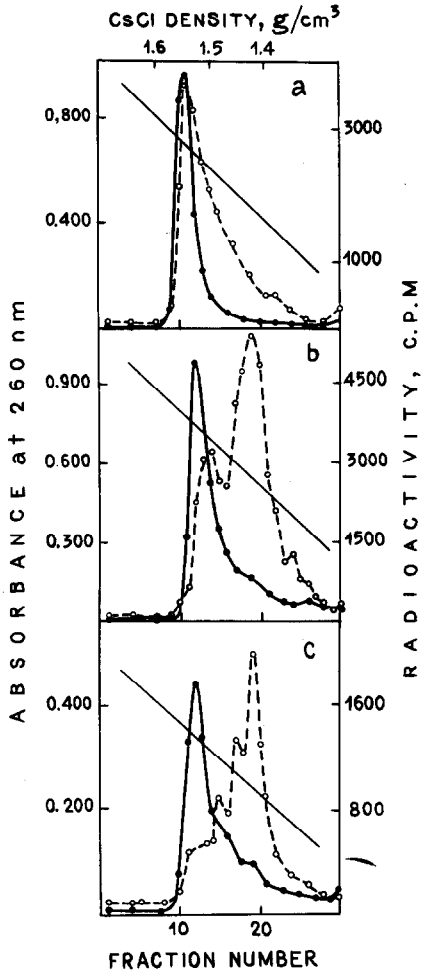

Fig.1. Density distribution in the CsCl gradient of cytoplasmic RNP particles of wheat embryos labelled for 20 min with [^{3}H]uridine at the following stages. (a) Early milky, (b) Milky-waxy. (c) Waxy. Centrifugation was done in an SW-65 rotor at 40 000 rev/min for 18 h at 3°C. (——) u.v. absorption; (- - -) radioactivity.

At the waxy stage (fig.1c), the main type of particles containing the newly synthesized mRNA are free informosomes with a buoyant density of 1.40–1.45 g/cm^3.

From these results it can be presumed that the gradual disappearance of polyribosomes and accumulation of free informosomes at the late stages of ripening reflects a decrease in the rate of protein synthesis and an accumulation of stored mRNA in the form of informosomes during transition of the seeds into dormancy.

A question arises whether this mRNA remains in the form of informosomes in dormant dry embryos. To solve this question the following experiments were carried out. Embryos were separated from seeds at the later waxy stage and incubated with [^{3}H] uridine for 20 min, then washed free of the excess of the radioactive label and dried at room temperature (25°C). Cytoplasmic RNP particles were isolated from the dried embryos and analysed in the CsCl density gradient. The distribution of radioactive material obtained was identical to that shown in fig.1c. Consequently, the free informosomes accumulated at the late stages of ripening of wheat embryos survive embryo drying.

For a further proof that mRNA in the form of informosomes in conserved in embryos during transition into the dormant state, we incubated intact seeds with [^{3}H]uridine for 1 h at the later waxy stage. After the seeds were washed free of excess radioactive precursor and dried at room temperature (25°C), the embryos were separated and an analysis was done of the distribution of the radioactive material in the CsCl density gradient.

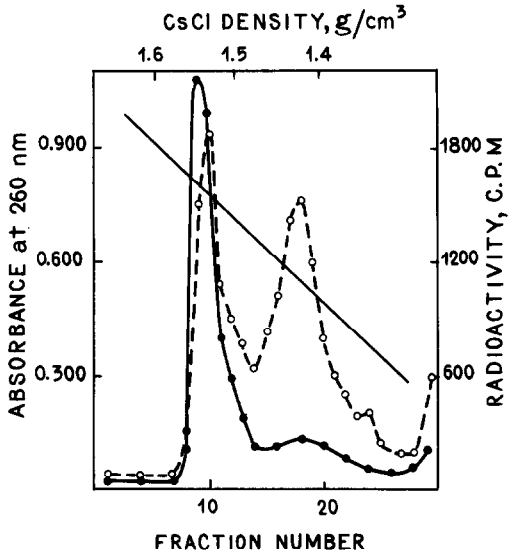

Fig.2. Density distribution in the CsCl gradient of cytoplasmic RNP particles of wheat embryos isolated from intact later waxy ripe seeds labelled for 1 h with [^{3}H]uridine and then dried. Centrifugation was carried out in an SW-65 rotor at 40 000 rev/min for 18 h at 3°C. (——) u.v. absorption; (- - -) radioactivity.

As seen in fig.2, in this case as well the predominant type of particles containing the newly synthesized RNA are informosomes with a buoyant density of 1.40–1.45 g/cm^3. The appearance of radioactivity in the band of ribosomes is apparently due to the synthesis of ribosomes because of a slower dehydration of intact seeds as compared with the isolated embryos.

It should be noted that both the embryos separated from seeds and then dried, and the embryos separated after drying of intact seeds were able to germinate and grow normally on agar in Petri dishes.

The results obtained are considered by us as a direct experimental evidence that mRNA is conserved during transition of seeds into the dormant state and that it is present there as the complexes with protein in the form of informosomes.

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