FREE POLY(A) TRACTS COMPLEXED WITH PROTEIN IN THE CYTOPLASM OF DRIED WHEAT EMBRYOS

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1. Introduction

It has been shown that the early stages of seed germination are characterized by a rapid increase of the content of polyribosomes in the embryo and by an increase of the rate of protein synthesis [1–5]. The formation of polyribosomes at this developmental stage does not depend on transcription [6]. It is now evident that these phenomena represent a consequence of mobilization of the previously formed and stored mRNA. The mechanism responsible for this activation is however not yet clear. Recent studies [7–9] conducted with sea urchin embryos indicate that after fertilization cytoplasmic mRNA synthesized during oogenesis undergoes polyadenylation. These authors advance a hypothesis according to which polyadenylation is regarded as a process responsible for the activation of the stored mRNA.

In this work we examined the distribution of poly(A) sequences in the cytoplasmic extracts of dry wheat embryos. It has been shown that practically all poly(A) is present as a free homopolymer complexed with protein.

2. Materials and methods

Experiments were conducted with the embryos of *Triticum vulgare*. Embryos were separated from the grains at the waxy stage of maturation, incubated with 250 μCi/ml [^{3}H]adenosine (spec. act. 28 Ci/mmol) for 20 min at 28°C and dried at room temperature. Embryos from the dry wheat grains were prepared as described by Johnston and Stern [10].

The embryos were homogenized in a medium containing 0.02 M triethanolamine (pH 7.6), 25 mM KCl, 5 mM $MgCl_2$, 0.25 M sucrose and 1 mM mercaptoethanol. To prepare postmitochondrial and postribosomal fractions of cytoplasm the homogenate was centrifuged for 20 min at 23 000 × *g* or for 3 h at 105 000 × *g*, respectively. RNA from the postmitochondrial fraction was extracted as described by Perry et al. [11]. The sucrose gradient centrifugation conditions are given in the legends to figures. To isolate the poly(A) containing fraction $(poly(A)^+$, the RNA was stirred with poly(U)–Sepharose (Pharmacia) in a buffer containing 0.01 M Tris–HCl (pH 7.5), 0.1 M NaCl, 0.2% sodium dodecylsulphate. The suspension was poured onto the column and the unbound material ($poly(A)^-$ RNA) was eluted with the same buffer at 23°C. $Poly(A)^+$ RNA was eluted at 50°C with a buffer containing 0.01 M Tris–HCl (pH 7.5) with 0.2% sodium dodecylsulphate.

For treatment of RNA with pancreatic and T_1 ribonucleases, RNA preparations were dissolved in 2 × SSC (1 × SSC = 0.15 NaCl + 15 mM sodium citrate) and 50 μg/ml of pancreatic ribonuclease (Worthington, USA) was added

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together with 20 units/ml of T_1 ribonuclease (Sankyo, Japan). The mixture was incubated for 30 min at 37°C, chilled and the acid insoluble material precipitated with 7.5% trichloroacetic acid in the presence of a carrier (*E. coli* RNA) added to a final concentration of 40–50 μg/ml; the radioactivity of the acid insoluble material was then determined.

Aliquots equal to 5 μl each were withdrawn from the corresponding fractions of the sucrose gradients and mixed with [^{3}H]poly(dT) (1500 cpm). NaCl concentration was adjusted to 0.3 M and the hybridization was performed for 1 h at 20°C in capillary tubes. The tube contents were then diluted with a solution containing 0.03 M Na-acetate (pH 4.5), 0.03 mM $ZnSO_4$, 0.20 M NaCl, 25 μg/ml denatured DNA and the mixture incubated for 90 min with S_1 nuclease purified from Takadiastase [12]. After incubation the samples were chilled, precipitated with 7.5% trichloroacetic acid and the acid insoluble radioactivity determined.

Fractions of the sucrose gradients (containing 8–16 S material) were pooled and fixed with formaldehyde added to a final concentration of 4%. They were dialyzed to remove the sucrose, and the buoyant properties of the dialyzed material were examined in a preformed CsCl density gradient [13].

3. Results

Using a sensitive method involving hybridization of [^{3}H]poly(dT) with poly(A) sequences present in the cellular RNA and its fractionations on poly(U)–Sepharose columns, we examined the distribution of poly(A) sequences in the cytoplasmic extracts of artificially dried wheat embryos. RNA isolated from postmitochondrial extracts of the homogenates of dried wheat embryos labeled with [^{3}H]adenosine was separated into poly(A)$^+$ and poly(A)$^-$ fractions (see Materials and methods). Sucrose gradient analysis (fig.1) shows that the poly(A)$^+$ material has a rather low molecular weight, having sedimentation coefficients of about 5 S.

Experiments with ribonuclease treatment of this fraction showed that RNA not bound to poly(U)–Sepharose was completely hydrolyzed by pancreatic and T_1 ribonucleases, while the 5 S (poly(A)$^+$) component was resistant to such treatment (table 1). These data permit one to conclude that isolated poly(A)$^+$ fraction contained little or no heteropolymeric sequences hydrolysible by the ribonucleases.

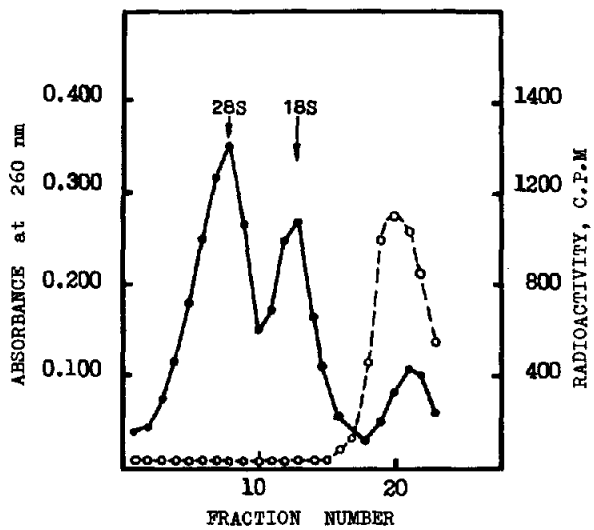

Fig.1. Sucrose gradient sedimentation of the material bound to poly(U)–Sepharose. Samples were dissolved in 0.05 M triethanolamine (pH 7.6), 0.1 M KCl, 5 M $MgCl_2$ and centrifuged in a 5–20% sucrose gradient prepared in the same buffer. Centrifugation in a SW-65 rotor for 5 h at 38 000 rev/min and +16°C. Continuous line, ultraviolet absorption of the ribosomal RNA marker. Dashed line, radioactivity of the poly(U)-bound material.

In connection with the finding of free poly(A) tracts in artificially dehydrated wheat embryos it was interesting to inquire, whether such tracts are present in the cells of embryos of normal dry seeds. We therefore isolated RNA from the postmitochondrial supernatant of the homogenate of embryos from normal dry seeds and fractionated it by sucrose

Table 1
Treatment of [^{3}H]adenosine-labeled RNA preparations with pancreatic and T_1 ribonucleases

RNA fractions	Untreated (cpm)	Treated with ribonucleases, (cpm)
Poly(A)$^-$ RNA	1300	25
Poly(A)$^+$ RNA	516	485

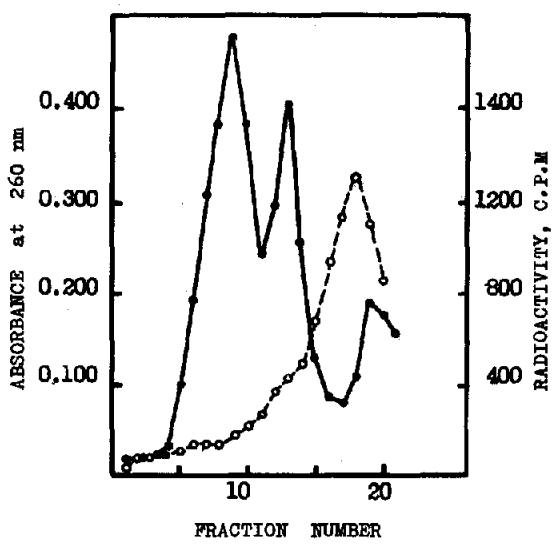

Fig.2. Hybridization of [^{3}H]poly(dT) with sucrose gradient fractions of postmitochondrial RNA from dry wheat embryos. Conditions of centrifugation were the same as in the legend to fig.1. Continuous line, ultraviolet absorption, dashed line, hybridization with [^{3}H]poly(dT).

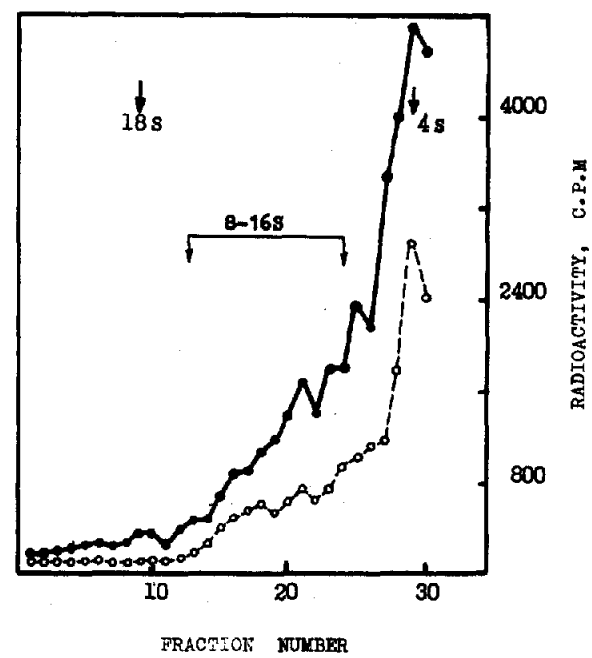

Fig.3. Fractionation in a 10–30% sucrose gradient of the postribosomal cellular fraction from dried wheat embryos preincubated with [^{3}H]adenosine. The sucrose gradient was prepared in 0.02 M triethanolamine (pH 7.6), 25 M KCl and 5 mM $MgCl_2$. Continuous line, sedimentation profile of the postribosomal cellular fraction. Dashed line, profile of the cellular fraction treated with ribonuleases. Centrifugation in a Spinco SW-41 rotor for 17 h at 36 000 rev/min and +3°C.

gradient sedimentation. Each fraction of the gradient was then hybridized with [^{3}H]poly(dT). The results shown in fig.2 demonstrate that the maximum of the poly(A) distribution in the preparation from normal dry embryos corresponds to about 5 S just as it was with artificially dried embryos.

The next question was related to the status of poly(A) tracts in non-deproteinized extracts. Sucrose gradient analysis (fig.3) of the non-deproteinized postribosomal supernatant of wheat embryos incubated with [^{3}H]adenosine and then dried showed in addition to the 5 S component, a heterogeneous material sedimenting between 8 S and 16 S. Treatment with pancreatic and T_1 ribonucleases showed that the character of distribution of poly(A) containing material is not affected by the ribonucleases treatment, confirming that the cytoplasm of dry wheat embryos does not contain large heteropolymeric RNA species bound to poly(A).

Even after ribonuclease treatment a part of the poly(A) containing material sediments at 8–16 S, as in the extract untreated with ribonucleases. Since in the deproteinized extracts the bulk of the poly(A) has a sedimentation coefficient equal to 5 S, it is suggested that the material sedimenting at 8–16 S (fig.3) represents poly(A) containing ribonucleoprotein particles.

This suggestion was confirmed by the following experiments. It is seen from the table 2 that the 8–16 S material in the extracts of dried embryos prelabeled with [^{3}H]adenosine quantitatively binds to membrane filters at low ionic strength. Pronase pretreatment of this material abolishes its ability to bind to the filters. The ability to bind to membrane filters at high ionic strength, characteristic of free poly(A) [14], however remains unaffected. This observation suggests that poly(A)-containing cytoplasmic components are organized into the ribonucleoproteins.

Centrifugation of the 8–16 S fractions in CsCl density gradients revealed a rather homogeneous distribution of the particles around a mean density

Table 2
Binding of 8–16 S poly(A) containing ribonucleoproteins to membrane filters

Ionic conditions	Unincubated (cpm)	Incubated[a]	
		Without pronase (cpm)	With 80 μg/ml pronase (cpm)
20 mM KCl	248	202	21
500 mM KCl	468	453	464

[a]The samples from sucrose gradient were incubated 2 h at 36°C.

of 1.36 g/cm^3, corresponding to an RNA/protein ratio about 1:3 (fig.4).

4. Discussion

The results obtained indicate that in cytoplasmic extracts of dry wheat embryos practically all the poly(A) is present as a free homopolymer complexed with protein. Such free poly(A) tracts are not found in the cells of germinating embryos (data not shown). The finding of poly(A)–protein complexes in dry embryos leads to a question about the state of mRNA in the cells during the transition of seeds to dormancy and during subsequent seed germination. Ribosomes are known not to be programmed with mRNA in the cells of embryos from the dry seeds. The formation of polyribosomes occurs immediately after seed wetting. This process is not inhibited either by α-amanitin or by cordicepin [6]. It is inferred that the formation of these polysomes involves mRNA pre-existing in the embryo. It was shown in experiments with cordycepin and α-amanitin that mRNA present in polyribosomes during the germination contains polyadenylated species. This observation implies that polyadenylated mRNA are already present in the population of stored mRNA molecules. Presence of free poly(A) tracts complexed to protein in the cytoplasm of dry wheat embryos and the absence of such tracts in the cells of germinating embryos raised the possibility that the cytoplasm of dry embryos contains not only the stored RNA species in the form of informosomes [15] but also a store of free poly(A) tracts. These latter might be utilized during the earlier periods of germination for the polyadenylation of mRNA either by direct joining of a tract to the 3′-OH terminus of RNA or via the AMP pool.

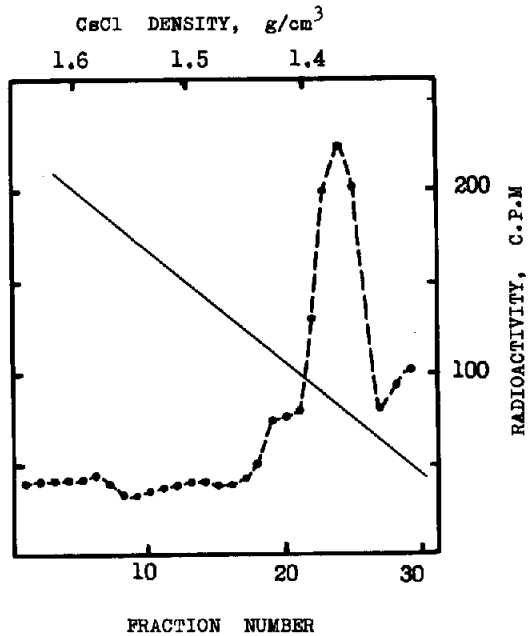

Fig.4. Density gradient analysis of ribonucleoproteins in neutral CsCl. Sucrose gradient fractions containing poly(A)$^+$ 8–16 S material (fig.3) were fixed with 4% formaldehyde, dialysed in order to remove sucrose and analyzed in CsCl. Gradients were centrifuged in a Spinco SW-65 rotor for 18 h at 40 000 rev/min and +3°C.

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