MAK COLUMN CHROMATOGRAPHY OF THE FIRST RNA SYNTHESIZED DURING GERMINATION OF *ZEA MAYS* EMBRYOS

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1. Introduction

It has been shown by autoradiography that tritiated urdine incorporated in excised *Zea mays* embryos between the 6th and 8th hr of germination labels exclusively the RNA of the chromatin in the root cells [1]. Labeling of the nucleolar and cytoplasmic RNA occurs when incorporation of uridine is performed a few hours later during the germination.

In order to characterize further these RNA species, MAK column chromatography was undertaken. The RNA synthesized between the 6th and 8th hr of germination is parently eluted on the heavy side of the ribosomal RNA peak and partly retained on the column. As a working hypothesis, this RNA species is assumed to be similar to the heterodisperse nuclear RNA of animal cells.

2. Material and methods

2.1. *Germination procedure*

Zea mays seeds (var. INRA 258) were germinated in a dark chamber at 16°. During the first six hours the seeds were soaked in tap water, if germination proceeded further, they were transferred to Petri dishes on wet filter paper.

2.2. *Isotope incorporation*

The embryos were excised from the seeds and incubated 2 hr at 16° with tritiated uridine (5-T-uridine from the Radiochemical Center, UK, specific activity 19.4 Ci/mM, 250 μCi/ml) in 1 ml of water containing 50 μg of streptomycin. After incorporation, the embryos were stored in liquid nitrogen.

2.3. *Extraction of nucleic acids*

The frozen embryos were ground at 4° with a precooled porcelain mortar and pestle containing a little sand and 2 ml of the extraction buffer (NaCl 4×10^{-1} M; EDTA 5×10^{-3} M; SDS 1%, Tris 1×10^{-2} M, pH 7.4 with HCl). After addition of 8 ml of buffer, the homogenate was mixed with 10 ml of buffer saturated phenol. The mixture was shaken vigorously for 15 min at room temperature and centrifuged at 2590 *g* for 15 min. The aqueous phase was removed and reextracted with 10 ml of fresh saturated phenol. Nucleic acids were precipitated overnight with 2 volumes of ethanol at −20°. The precipitation procedure was repeated twice.

2.4. *Fractionation of nucleic acids*

Nucleic acids were fractionated on single-layer columns of methylated albumin kieselgur (MAK) similar to that used by Mandell and Hershey [2]. Fractionation of approximately 2 mg nucleic acids was performed at room temperature with a linear salt gradient from 0.1 to 1.6 M NaCl. 3.7 ml fractions were collected in 8 min. The fractions were scanned for absorption at 260 nm and then for radioactivity in a Packard liquid scintillation spectrometer. The nucleic acids bound to the column were washed at 60° with a 2% SDS solution according to Ellem [3].

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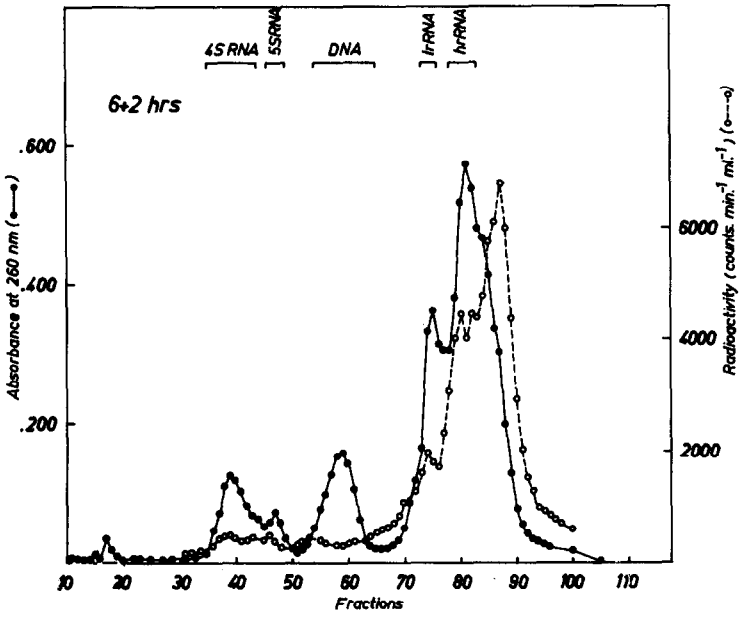

Fig. 1. MAK column chromatography of the nucleic acids extracted from *Zea mays* embryos germinated during 8 hr. Tritiated uridine was incorporated for 2 hr before extraction. (lrRNA: light ribosomal RNA; hrRNA: heavy ribosonal RNA; ●—● absorption at 260 nm; o- - -o radioactivity).

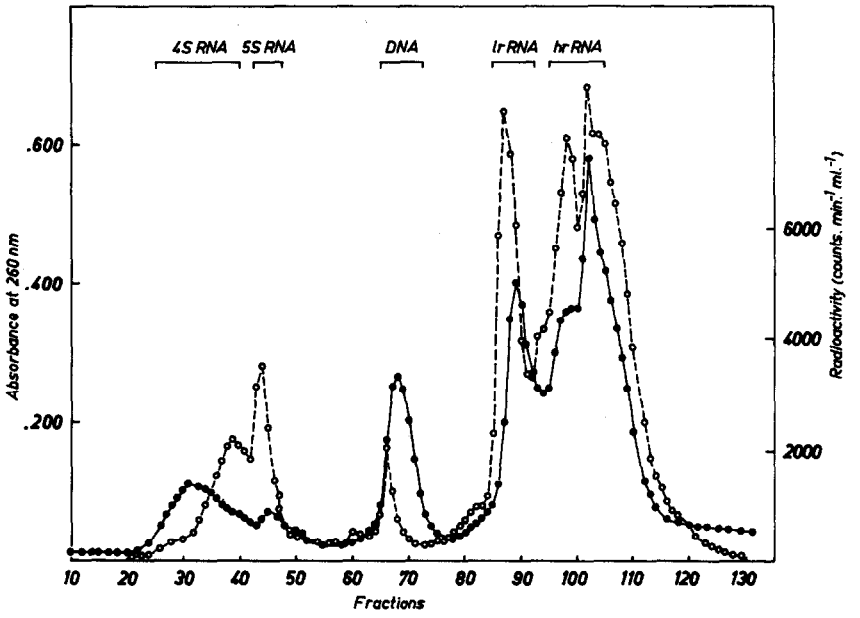

Fig. 2. MAK column chromatography of the nucleic acids extracted from *Zea mays* embryos germinated during 7 days. Tritiated uridine was incorporated for 2 hr before extraction. (lrRNA: light ribosomal RNA; hrRNA: heavy ribosomal RNA; ●—● absorption at 260 nm; o- - -o radioactivity).

3. Results

(1) The absorption profile of nucleic acids of *Zea mays* embryos, extracted after 8 hr of germination (fig. 1) followed the pattern observed in other plant tissues: pea shoot [4], peanut cotyledon [5], soybean hypocotyl [6]. The radioactivity profile showed one main peak of material with a high specific activity corresponding to the RNA eluted on the heavy side of the ribosomal RNA. Two small peaks of low specific activity material appeared in the light ribosomal and heavy ribosomal RNA regions. The radioactivity was very low in the 4S, 5S and DNA region.

After elution with the salt gradient, a large portion of the radioactivity remained in the column and could be eluted at 60° with a 2% SDS solution (12,000 cpm in 5 fractions of 3.7 ml).

(2) For comparison with the situation after 8 hr of germination, RNA was extracted from *Zea mays* embryos germinated for 7 days and incubated 2 hr in tritiated uridine (fig. 2). The absorption profile of MAK column chromatography followed the general pattern, a better separation being obtained in the ribosomal RNA region with the 7 days sample. Although radioactivity and absorption profiles were not exactly coincident, all RNA species were labeled. Radioactivity in the DNA region was attributed to DNA-RNA hybrids according to Cherry [5]. A large portion of radioactivity was retained on the column and could be recovered after deproteinization of the column (43,300 cpm in 8 fractions of 3.7 ml).

4. Discussion

(1) The MAK radioactivity profile of RNA extracted from *Zea mays* embryos after 8 hr of germination consists mainly of one peak eluting after the ribosomal RNA. This fraction corresponds to the labeled RNA of the chromatin observed in autoradiographs from control embryos incubated in the same experiment. The small radioactivity associated with the ribosomal RNA peaks can be attributed to a newly synthesized ribosomal RNA originating from the portion of the scutellum which remains attached to the scutellar node of the embryo after dissection. Autoradiography shows that in the cells of the scutellum, all nucleoli are labeled after 8 hr of germination and it is generally accepted that the nucleolar region is the site of ribosomal RNA precursor production [7].

(2) The RNA eluting on the heavy side of ribosomal RNA seems to be the first RNA synthesized during the germination process. We do not have biochemical observations made before the 8th hr of germination but no other RNA species is highly labeled at the 8th hr and autoradiographic studies in the same material showed that the labeling over the chromatin was the only one to appear as soon as the 6th hr of germination [8].

(3) RNA eluting on the heavy side of ribosomal RNA have already been described as ribosomal precursor [9], or aggregate of ribosomal RNA [10]. They have also been described in plant material as messenger RNA on the basis of heterodispersion [11], similarity of base ratio with DNA [4, 11] and stimulation of amino acids incorporation *in vitro* [12]. However, it has been shown in animal cells that the majority of the nuclear RNA (heterodisperse nuclear RNA) with characteristics of messenger RNA is apparently degraded within the nucleus [13, 14]. Its function is unknown and it was suggested that part of it undergoes maturation before migrating to the cytoplasm [15]. Preliminary studies using sucrose gradient centrifugation showed that *Zea mays* RNA synthesized between the 6th and 8th hr of germination is heterodisperse. As a working hypothesis, this RNA species may be assumed to be similar to the nuclear heterodisperse RNA of animal cells. Furhter work is in progress to characterize this RNA.

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