PRESENCE OF HETERODISPERSE NUCLEAR RNA IN A PLANT: *ZEA MAYS*

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1. Introduction

Heterodisperse nuclear RNA (hnRNA) is a rapidly labelled RNA species discovered in the cell nucleus of HeLa cells [1–3] and duck erythrocytes [4,5]. It consists of very large molecules ranging in size from 20 to 80 S [2,3]. On the basis of its low guanine + cytosine content [3–5], it was shown to be different from the rRNA precursor, the other rapidly labelled RNA species present in the nucleus. Despite the fact that most of the hnRNA is degraded within the nucleus [2,5], the hypothesis of a precursor – product relationship between this RNA and cytoplasmic mRNA was soon proposed [2–4]. Evidence for such a precursor rôle came from the presence of viral mRNA sequence in hnRNA of mouse cells transformed by SV40 virus [6]. Recently, the use of an anti-messenger DNA prepared from purified globin mRNA of duck erythrocytes allowed to demonstrate the presence of the same nucleotide sequence in hnRNA and mRNA [7]. This suggests that the cleavage of a large hnRNA molecule occurs during a maturation process of the mRNA as it happens for the rRNA. However, as pointed out by Imaizumi et al., no proof for a direct physical precursor-product relationship has been given and the presence of intermediate stages in the transfer of information cannot be definitively excluded [7].

Apart from its presence in mammalian and bird cells, hnRNA was shown to be present in an insect [8], an echinoderm [9] and an amphibia [10]. The presence of a rapidly labelled, non-ribosomal, heterodisperse RNA species has also been shown in two unicellular eukaryotes *Tetrahymena pyriformis* [11] and the cellular slime mold *Dictyostelium discoïdeum* [12]; but the heterodisperse RNA molecules present in these organisms are much shorter than those found in metazoan cells; their sedimentation coefficient is below 20 S. In higher plants, the presence of hnRNA has not been demonstrated conclusively.

In an autoradiographic study of the onset of nucleic acid during the germination of the maize embryo, we showed that incorporation of tritiated uridine was restricted to the chromatin of the root cells between 6 and 8 hr of germination [13]. The nucleolus was not labelled at this stage and exhibited some [^{3}H] uridine incorporation only after 10 hr of germination. It was heavily labelled after one day of germination. We thought that the exclusive labelling of the chromatin at the very beginning of germination offered a good opportunity for a study of the non-ribosomal nuclear RNA in a plant material. The results described here show that the RNA synthesized between 6 and 8 hr of germination is heterodisperse in size and that its base composition is characterized by a low guanine + cytosine content and a guanine/adenine ratio less than 1. These properties, together with the exclusive labelling of the chromatin, established the presence of heterodisperse nuclear RNA in this material.

2. Materials and methods

Maize seeds (*Zea mays*, cultivar CIV 2) were soaked in tap water during 6 hr at 16°C in the dark. Entire embryos were excised from the seeds and incubated 2 hr at 16°C in a solution of the radioactive percursor ([G-^{3}H] adenosine, 11.8 Ci/mM, 25 μCi in 125 μl of water containing 50 μg/ml of streptomycin for 15 embryos). During the last 15 min of incubation, the coleorhiza and the root cap were carefully discarded and

the main embryonic root was excised. The temperature of the room was maintained close to 16°C during these manipulations. At the end of the 2-hr period of incubation, the 15 roots of a batch were transferred to liquid nitrogen.

It is known that contaminating bacteria can contribute to the labelling pattern of seedling nucleic acids after incubation in [^{32}P] phosphate even in the presence of antibiotics [14]. The absence of bacteria and fungi in the roots prepared for nucleic acid extraction was checked by plating on glycerol–peptone–agar or on malt–agar, 100 µl of the radioactive solution, collected at the end of the incubation period. Less than 50 colonies of bacteria and only one mold colony were counted after 3 days of incubation. These low values, together with the fact that the primary root remains enclosed in the coleorhiza until the last minutes of incubation, established the absence of bacterial contamination.

Extraction of nucleic acids was performed as already described [15]. Nucleic acids were fractionated by electrophoresis in 2.2% acrylamide gel according to Loening [16]. The gels were scanned at 260 nm, frozen to their exact length and cut in 2 mm slices with a home-made slicer. Each slice was dissolved in 0.5 ml of acetic acid and perhydrol (1:1) at 70°C overnight; 10 ml of toluene–triton–omnifluor scintillation liquid were added to each vial and counting was performed in a Packard scintillation counter.

For base composition analysis, the ethanol precipitate of nucleic acids, labelled with [^{32}P] phosphate (0.5 mCi in 200 µl of Streptomycin solution), was first dissolved in the electrophoresis buffer and an aliquot was submitted to electrophoresis to provide a control of the preparation. It was then filtered trough a Sephadex G-100 column (0.5 × 144 cm in NaCl 0.1M, sodium lauryl sulphate 10 g/l, Tris–Cl buffer 0.01M, pH 7) in order to separate the high molecular weight RNA from the soluble RNA and nucleotides. The fractions excluded were made 0.4M NaCl and mixed with 2 vol of ethanol to precipitate the nucleic acids. After 2 hr at–25°C, the precipitate was recovered by centrifugation, dissolved in electrophoresis buffer and an aliquot submitted to electrophoresis in 2.2% or 7% acrylamide gel to check the absence of soluble RNA. The remaining nucleic acids were submitted to alkaline hydrolysis (0.3M KOH, 18 hr, 37°C) [17]. The hydrolysate was neutralized with perchloric acid, kept in the cold for two hr and fractionated on a column of Dowex Ag-1 (X2) in chloride form (0.6 × 80 cm) with a linear HCl gradient (2 × 250 ml, from 0 to 0.17 M) [18]. The fractions corresponding to the four nucleotides were pooled and lyophylized, the residue was recovered with 1 ml of water, mixed with the scintillation liquid and counted

3. Results

We have previously reported that the chromatin was exclusively labelled when incubation of maize embryos in the presence of radioactive precursor was performed between 6 and 8 hr of germination [13]. Similar result was obtained with the cultivar CIV 2 used in the present study (unpublished result). In this cultivar, it was however required to adjust the temperature of germination to 16°C in order to maintain a period of exclusive chromatin labelling untill the 8th hr of germination. It was checked that this reduction of temperature did not affect the percentage of germination and that even at 12°C more than 95% of the seeds still germinated.

We established that the absence of labelling in the nucleolus was a general feature in the entire primary root during the investigated period of germination. This observation allowed us to use entire roots for the biochemical studies. It appeared also that the onset of RNA synthesis was more precocious in the coleorhiza and in the root cap than in the root itself. These tissues were consequently discarded in all further expiriments.

Gel electrophoresis of the total nucleic acid preparation, extracted from the roots after incubation in tritiated adenosine between 6 and 8 hr of germination, showed a population of radioactive molecules ranging in size from about 0.2×10^6 to roughly 3×10^6 daltons and culminating in the region of rRNA. There was no peak of radioactivity corresponding neither to the absorption peaks of rRNA nor to the region of the 2.3×10^6 daltons rRNA precursor [19]. After ^{32}P-labelling, the total nucleic acid preparation was submitted to Sephadex G-100 filtration in order to eliminate the highly labelled nucleotides and soluble RNA. The results of the base composition analysis of the purified high molecular weight fraction are shown in table 1. ^{32}P-labelled rRNA from maize root tips, extracted after 102 hr of germination at 16°C, was also analyzed to provide

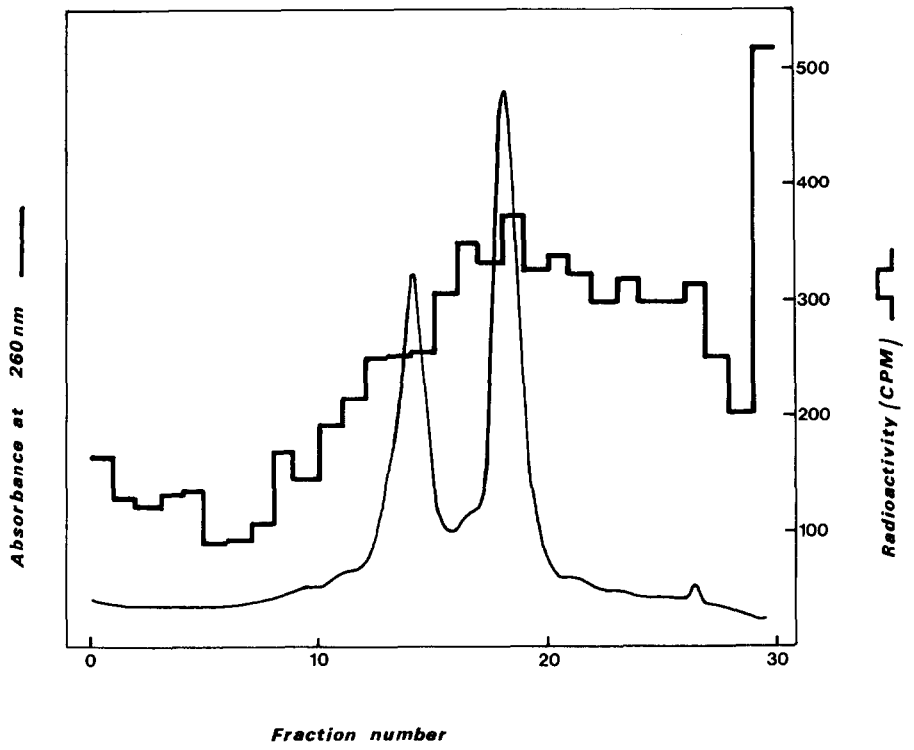

Fig. 1. Acrylamide gel fractionation of the total nucleic acid preparation extracted from *Zea mays* roots after 6 hr of germination and 2 hr of incubation in tritiated adenosine. Electrophoresis in 2.2% gel (8 X 0.7 cm) 10 mA per gel, 2 hr at room temperature. No absorbance units.

comparative data. The high molecular weight nuclear RNA is characterized by a guanine + cytosine content lower than that of the rRNA and by a value of the guanine/adenine ratio below one. This is close to the values obtained by Ingle et al. with DNA-like RNA from soybean hypocotyls [20] and by Rogers et al. with the 'heterogeneous' RNA from pea roots and artichoke tubers [19].

Table 1
Base composition of [^{32}P] RNA from maize embryonic roots

Bases	Nuclear RNA[a]	Heavy rRNA[b]
	Mol/100 mol	Mol/100 mol
Cytosine	21.8 ± 1.1	23.9 ; 23.3
Adenine	30.6 ± 2.2	25.8 ; 26.5
Guanine	27.8 ± 1.6	33.3 ; 32.2
Uracil	19.7 ± 2.5	16.9 ; 17.8
Guanine/Adenine	0.9	1.28 ; 1.21
Guanine + Cytosine	49.6	57.2 ; 55.6

[a] 5 determinations, the figures represent mean ± standard deviation.
[b] 2 determinations.

4. Discussion

Synthesis and properties of nuclear RNA have not been studied in detail in plant cells. The existence of RNA molecules with some of the required properties to fit the definition of hnRNA was known in higher plants since a long time, but in most of the cases the problem of their localization in the cell was not investigated. These molecules, rich in adenine, rapidly labelled and different from the rRNA or the rRNA precursor, were termed DNA-like RNA [20–22], heterogeneous RNA [19], or tightly-bound RNA

[22–24]. None of these RNA species were shown to be localized in the nucleoplasm and only a fraction of the DNA-like RNA was found in association with polyribosomes [25]. Leaver and Key [26] observed in carrot root discs, that after short pulses, ^{32}P-labelled rRNA precursors appear in the nuclear fraction over a background of polydisperse RNA. However, they did not conclude that hnRNA was present, probably due to the low radioactivity recovered. The same difficulty was pointed out by Verma and Marcus [27].

In embryonic maize root, heterodisperse RNA molecules are synthesized in the nucleoplasm at a stage of germination where the nucleolus is still unlabelled. These molecules have a base composition different from the ribosomal RNA as shown by their guanine/adenine ratio below one and their low guanine + cytosine content. We consider therefore this RNA as analogous to the hnRNA described in animal cells.

The absence of rRNA synthesis in our material is of great interest because it allows the study of nuclear RNA in the absence of any drug like actinomycin, used in other experimental systems to inhibit the synthesis of rRNA. Similar situations were described in plant material. In *Avena* coleoptiles, RNA that is considered to be polydisperse or messenger RNA, is synthesized in the absence of rRNA synthesis in 4 to 7 day old coleoptiles [28]. In a plant cell culture, and adenine-rich RNA species is synthesized, also in the absence of rRNA synthesis, during the lag period between the dilution of the culture and the beginning of cell divisions; it was concluded that the newly synthesized RNA was messenger RNA [27]. In the growing pollen tube of *Tradescantia*, the ribosomes necessary for the growth are already present and a polydisperse RNA species, rich in adenine, is synthesized [29]. In animal cells, hnRNA was also studied in systems where rRNA synthesis is greatly reduced as in duck erythrocytes [4,5] or absent as in the anucleolate embryos of Xenopus [10] and the sea urchin embryo [9].

It is worth noticing that the majority of the heterodisperse RNA molecules of maize migrate in the region of the rRNA and that only 40% of them migrate in the high molecular weight region of the gel. By contrast, most of the hnRNA molecules of metazoan cells have molecular weight greater than 1.7×10^6. If the situation described here is a general feature of higher plants, plant hnRNA would represent an intermediate state, regarding the molecular weight, between similar molecules of metazoan cells and unicellular eukaryotes.

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References

[1] Scherrer, K., Latham, H. and Darnell, J. E. (1963) Proc. Natl. Acad. Sci. U.S.A. 49, 240–248.
[2] Warner, J. R., Soeiro, R., Birnboim, H. C., Girard, M. and Darnell, J. E. (1966) J. Mol. Biol. 19, 349–361.
[3] Soeiro, R., Birnboim, H. C. and Darnell, J. E. (1966) J. Mol. Biol. 19, 363–372.
[4] Scherrer, K., Marcaud, L., Zajdela, F., London, I. M. and Gros, F. (1966) Proc. Natl. Acad. Sci. U.S.A. 56, 1571–1578.
[5] Attardi, G., Parnas, H., Hwang, M. H. and Attardi, B. (1966) J. Mol. Biol. 20, 145–182.
[6] Lindberg, U. and Darnell, J. E. (1970) Proc. Natl. Acad. Sci. U.S.A. 65, 1089–1096.
[7] Imaizumi, T., Diggelmann, H. and Scherrer, K. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 1122–1126.
[8] Edström, J. E. and Daneholt, B. (1967) J. Mol. Biol. 28, 331–343.
[9] Aronson, A. and Wilt, F. H. (1969) Proc. Natl. Acad. Sci. U.S.A. 62, 186–193.
[10] Brown, D. D. and Gurdon, J. B. (1966) J. Mol. Biol. 19, 399–422.
[11] Prescott, D. M., Bostock, C., Gamow, E. and Lauth, M. (1971) Exp. Cell Res. 67, 124–128.
[12] Firtel, R. A. and Lodish, H. F. (1973) J. Mol. Biol. 79, 295–314.
[13] Van de Walle, C. and Bernier, G. (1969) Exp. Cell Res. 55, 378–384.
[14] Lonberg-Holm, K. K. (1967) Nature (Lond.) 213, 454–457.
[15] Van de Walle, C. (1973) FEBS Lett. 34, 31–34.
[16] Loening, U. E. (1967) Biochem. J. 102, 251–257.
[17] Bock, R. M. (1967) Methods Enzymol. 12, 224–228.
[18] Cohn, W. E. (1957) Methods Enzymol. 3, 724–743.
[19] Rogers, M. E., Loening, U. E. and Fraser, R. S. S. (1970) J. Mol. Biol. 49, 681–692.

[20] Ingle, J., Key, J. L. and Holm, R. E. (1965) J. Mol. Biol. 11, 730–746.
[21] Van Huystee, R. B. and Cherry, J. H. (1966) Biochem. Biophys Res. Commun. 23, 835–841.
[22] Key, J. L., Leaver, C. J., Cowles, J. R. and Anderson, J. M. (1972) Plant Physiol. 49, 783–788.
[23] Tester, C. F. and Dure III, L. S. (1967) Biochemistry, 6, 2532–2538.
[24] Johri, M. M. and Varner, J. E. (1970) Plant Physiol. 45, 348–358.
[25] Lin, C. Y., Key, J. L. and Bracker, C. E. (1966) Plant Physiol. 41, 976–982.
[26] Leaver, C. J. and Key, J. L. (1970) J. Mol. Biol. 49, 671–680.
[27] Verma, D. P. and Marcus, A. (1973) Develop. Biol. 30, 104–114.
[28] Cline, M. G. and Rehm, M. M. (1973) Abstr. Commun. 8th Int.Conf. Plant Growth Substances, Tokyo No. 23.
[29] Mascarenhas, J. P. and Bell, E. (1970) Develop. Biol. 21, 475–490.