POLYADENYLIC SEQUENCES IN PLANT RNA

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Received 15 May 1973

1. Introduction

Polynucleotide sequences rich in adenylic acid and ranging in size from 50 to 200 nucleotides have been found to be covalently bound to messenger RNA (mRNA) molecules [1—5] and to heterodisperse nuclear RNA (HnRNA) [3, 6] of animal cells. They have also been found in mRNA of the cellular slime mould, *Dictyostelium discoideum* [7], in the RNA molecules of RNA viruses [8—10] and in virus specific RNA synthesized by animal cells infected with DNA viruses [11, 12]. The poly (A) sequences have been shown to occur at the 3′ end of the RNA molecules [4, 10]. In the case of poly (A) linked to Adenovirus-specific nuclear RNA, the poly (A) segment appears to be added after transcription because the Adenovirus DNA does not contain the sequences that hybridize with poly (A) [12].

The precise role of the poly (A) region in the metabolism of RNA is still unknown. Its presence in both mRNA and HnRNA favours the hypothesis that a precursor relationship exists between these two species of RNA, the poly (A) being perhaps involved in the processing of HnRNA to mRNA [2, 6, 13, 14]. The presence of poly (A), however, is not absolutely required because the functional histone mRNA does not contain a poly (A) segment [15].

In this report, evidence is presented for the occurrence in corn RNA of polynucleotide sequences rich in adenylic acid. These sequences are most probably part of large RNA molecules, the nature of which has not been presently determined.

* Chercheur qualifié, Fonds National Belge de la Recherche Scientifique.

2. Materials and methods

Nucleic acid was extracted from 38 *Zea mays* roots (var. INRA 258) after 3 days of germination and 4 hr incubation in [^{32}P]phosphate (2 mCi in 2 ml of water containing 100 μg streptomycin). The roots were homogenized in a mortar with 1 ml of extraction buffer (0.4 M NaCl, 5 mM EDTA; 1% Sarkozyl; 0.1 M Tris, pH 9). Extraction was performed with 9 ml of buffer and 10 ml of a 1:1 mixture of phenol and chloroform containing 4% of isoamyl alcohol [21]. A second extraction of the aqueous phase was performed with 10 ml of chloroform—isoamyl alcohol [24:1]. The nucleic acid was fractionated by electrophoresis in 2.2% acrylamide gel according to Loening [22]. The gels were scanned at 265 nm, frozen and cut in 1 mm slices. Radioactivity was counted with a Geiger—Müller detector.

For measurement of acid insoluble radioactivity, an aliquot (10 μl) of nucleic acid solution was added to 1 ml of water; 100 μl of 0.1% serum albumin solution and 100 μl of 60% trichloroacetic acid solution were added later. After incubation for 30 min at 0°C, the precipitate was collected on Millipore filter (HA 0.45 μm). After drying, the filter was counted in toluene—omnifluor mixture with a liquid scintillation spectrometer.

Binding to Millipore filter was performed according to Lee [2]: an aliquot of total nucleic acid preparation was diluted in 10 vol of ice-cold buffer (0.5 M KCl, 1 mM $MgCl_2$, 10 mM Tris, pH 7.6). After 10 min in the cold, the solution was filtered through a Millipore filter (HA 0.45 μm) previously soaked in the same buffer. After drying, the filter was counted as before.

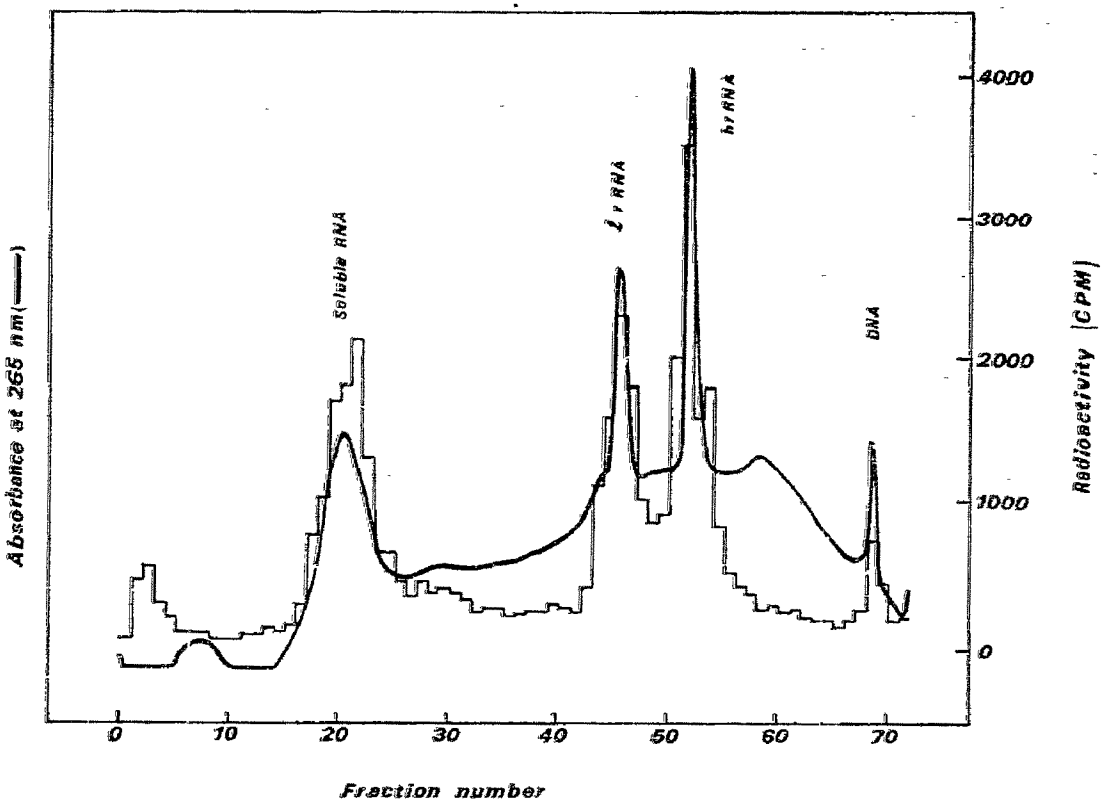

Fig. 1. Acrylamide gel fractionation of the total preparation of nucleic acid extracted from *Zea mays* roots after 3 days of germination and 4 hr of incubation in [^{32}P]phosphate. (lrRNA: light ribosomal RNA, hrRNA: heavy ribosomal RNA).

RNAase digestion (30 min, 37°C) at high ionic strength was performed in 0.3 M NaCl, 10 mM Tris pH 7.6, with pancreatic RNAase (150 μg/ml) and RNAase T_1 (770 U/ml). Digestion at low ionic strength was performed in 10 mM Tris, pH 7.6 buffer. For radioactivity measurement of the RNAase resistant material, the hydrolysate was filtered through a Millipore filter in 0.5 M KCl buffer as described for the binding of total nucleic acid.

Table 1
Binding of RNA to Millipore filter.

	cpm	
Total nucleic acid	221 850	
RNA bound to filter	18 514 (8.3% of total)	
RNA bound to filter after RNAase digestion at high salinity	2 816	difference 398 (2.1% of bound RNA)
RNA bound to filter after RNAase digestion at low salinity	2 418	

For gel electrophoresis of poly (A) sequences, total nucleic acid in 0.3 M NaCl, 10 mM Tris, pH 7.6 was digested for 30 min at 37°C with pancreatic RNAase (50 μg/ml) and RNAase T_1 (500 U/ml). Incubation proceeded 30 min more with pronase (300 μg/ml). After filtration through a Millipore filter, the bound material was eluted with 1 ml of 10 mM EDTA, 0.5% SLS, 0.1 M Tris, pH 8.8, at room temp. overnight [12]. The RNAase resistant material and 300 μg of carrier RNA were precipitated with ethanol and analysed by electrophoresis.

To determine the base composition, the RNAase resistant RNA was mixed with 1 mg of carrier RNA and hydrolysed with 0.3 M KOH overnight at 37°C. The hydrolysate was neutralized with perchloric acid and fractionated on a Dowex column (AG 1X 2, Cl^-) with a linear HCl gradient [20].

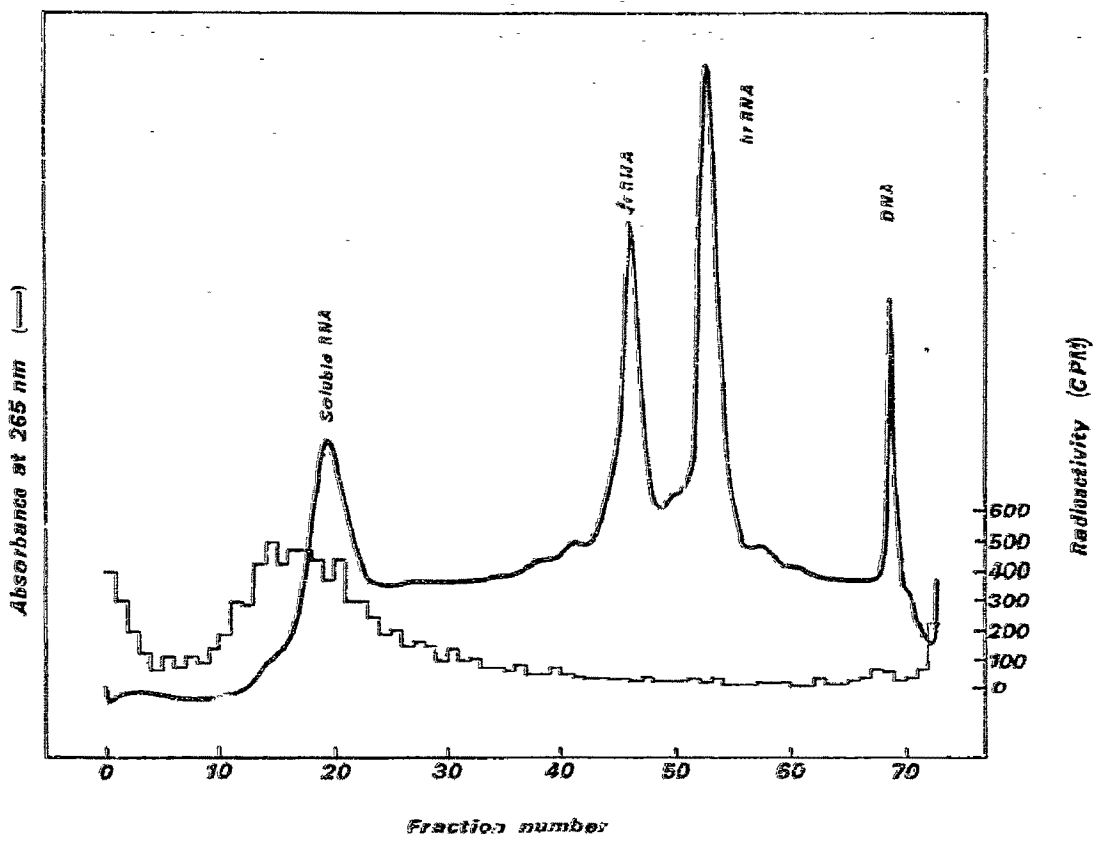

Fig. 2. Gel electrophoresis of poly (A) sequences after RNAase digestion of the total nucleic acid extracted from *Zea mays* roots. (lr RNA: light ribosomal RNA, hr RNA: heavy ribosomal RNA).

3. Results

In order to detect the presence of poly (A) sequences in our preparations of RNA, we made use of:

i) the resistance of the poly (A) to hydrolysis by pancreatic RNAase in the presence of 0.33 M KCl [16].

ii) the binding of the poly (A) or RNA containing poly (A) to Millipore filter in the presence of 0.5 M KCl [2].

RNA synthesized during 4 hr of incubation in [^{32}P]phosphate consisted mainly of soluble and ribosomal RNA (fig. 1). When filtered through a Millipore filter, a fraction of the total radioactivity bound to the filter (table 1): this was probably due to the presence of poly (A) sequences. After RNAase digestion of the total RNA preparation at high and low salinity, a significant difference was obtained between the amounts of radioactive material still retained on the Millipore filter. This showed that plant RNA contained RNAase resistant material. Similar results were obtained when [^{3}H]adenosine was used as a precursor of RNA (adenosine H3-G, 5 Ci/mM, 500 μCi/ml). Repetition of experiments showed variability in the results. The radioactivity bound to the filter ranged from 3 to 9% of the total and the RNAase resistant RNA ranged from 1.9 to 6% of the bound RNA. These discrepancies could be due to degradation of the newly synthesized RNA during extraction.

To establish if the RNAase resistant material consisted of polyadenylic acid, it was subjected to alkaline hydrolysis and its base composition was determined. The following values were obtained: 11.0% CMP, 77.6% AMP, 6.8% GMP and 4.6% UMP. 77.6% is a low value when compared with most of the data obtained from animal cells and viruses. In these cases, they ranged from 85 to 97% [7–9, 13, 17]. In rabbit hemoglobin mRNA, however, only 70.3% AMP was observed [1].

To determine the size range of the poly (A) sequences, RNAase resistant RNA was co-electrophorezed with unlabelled *Zea mays* RNA as a marker (fig. 2). The poly (A) sequences were observed as a broad peak migrating slightly faster than soluble RNA.

4. Discussion

These results demonstrate the presence of poly (A) sequences in RNA isolated from *Zea mays* seedlings. It is suggested that these sequences are most probably attached to large RNA molecules because the radioactive molecules retained on the Millipore filter after RNAase digestion represent only a small fraction of the material retained in the absence of RNAase treatment. These RNA molecules containing poly (A) are presumably mRNA and HnRNA because the ribosomal RNA and its precursor do not seem to contain poly (A) [2, 6] and because the transfer RNA and 5 S ribosomal RNA, in cases where their sequence is known, do not contain the poly (A) [18, 19].

Acknowledgements

The author is indebted to Drs. M. Lunt and M.V.S. Raju for help in translating the manuscript into English. Manuscript criticism by Drs. G. Marbaix, G. Huez and A. Burny is also acknowledged.

Sarkozyl was a generous gift from CIBA- Geigy Corporation.

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