THE LOCALIZATION OF POLYADENYLIC SEQUENCE AT THE 5′-END OF LIGHT NUCLEAR dRNA

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1. Introduction

It was reported previously that the 3′-end sequences of giant nuclear DNA-like RNA (dRNA) are transferred to polysomes [1, 2]. This shows the localization of mRNA sequences near the 3′-end of the precursor molecules [3]. Recently, on the other hand, relatively long poly A stretches have been discovered in cytoplasmic mRNA as well as in nuclear dRNA [4–7]. The poly A sequences consist of about 100–200 nucleotides. It was suggested that poly A is localized at the 3′-end of mRNA. However, we observed that only short poly A sequences consisting of 4–7 nucleotides are localized near the 3′-end of some nuclear dRNAs [8]. The same result was obtained by Lingrel with purified hemoglobin mRNA isolated from reticulocytes [9].

For this reason one can suggest that long poly A stretches are localized near the 5′-end of dRNA transferred into the cytoplasm. In this paper we have studied the properties of poly A isolated from the light fraction of nuclear dRNA which is enriched by sequences corresponding to cytoplasmic mRNA. It was found that poly A alkaline hydrolysates contained pAp groups and do not contain free adenosine. This shows poly A sequences to be at the 5′-ends of light dRNA chains. In other words poly A is probably localized before the message.

2. Materials and methods

Nuclear dRNA was isolated by the hot phenol fractionation technique [10, 11] from Ehrlich ascites carcinoma cells labelled *in vitro* with ^{32}P during 1.5 or 4 hr. Light dRNA (10–20 S or 10–30 S) was then isolated with the aid of sucrose gradient centrifugation in the presence of sodium dodecylsulphate (SDS) [2].

Some samples were treated with $NaIO_4$ and then with NaB^3H_4 to introduce the ^3H label in the 3′-end nucleoside of RNA. The technique used [12] was essentially the same as described previously [2].

RNA was treated by a mixture of pyrimidyl and guanyl RNases (50 μg of the former and 200 units of the latter per 1 mg RNA in 0.3 M NaCl–0.03 M Na citrate, pH 7, 1 hr at 37°). Then pronase (200 μg) was added and the sample was additionally incubated for 30 min to destroy RNase. SDS (up to 1%) and a mixture of rRNA and tRNA (used as markers) were added and the sample was passed through a column of Sephadex G-75 equilibrated with 0.1 M sodium acetate. Elution was performed with the same solution. In all fractions the acid-insoluble and total radioactivities were measured. Acid-insoluble material recovered as a sharp peak between rRNA and tRNA was collected, precipitated with ethanol, washed with 5% TCA in the cold, and hydrolyzed by 0.5 M KOH for 24 hr at 37°. The base composition of material in the aliquot was analyzed [13]. The main part of the hydrolysate was mixed with marker (incomplete RNase hydrolysate of rRNA) and separated on a DEAE-Sephadex A-25 column in 7 M urea [14]. The radioactivity was measured in an SL-40 Intertechnique counter using the Cerenkov effect [15].

The material of the third peak (containing labeled pXp) was desalted and analyzed with the aid of

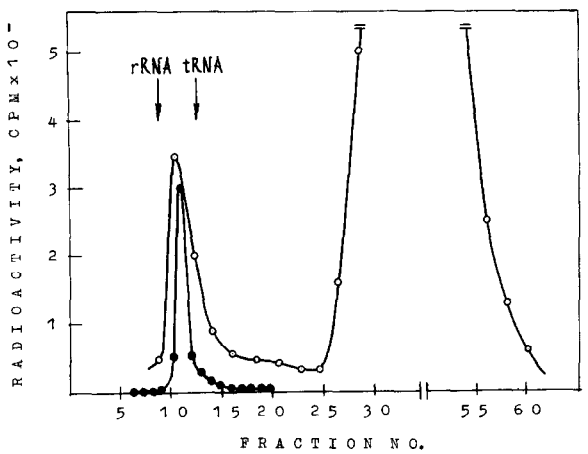

Fig. 1. The isolation of poly A from RNase hydrolysate of light dRNA. About 2.0 ml of hydrolysate (1.6 mg RNA) was passed through a Sephadex G-75 column (1 × 60 cm). (○—○—○): Total radioactivity (determined using the Cerenkov effect). (●—●—●): Acid-insoluble radioactivity (determined in aliquots on millipore filters in a toluene scintillator).

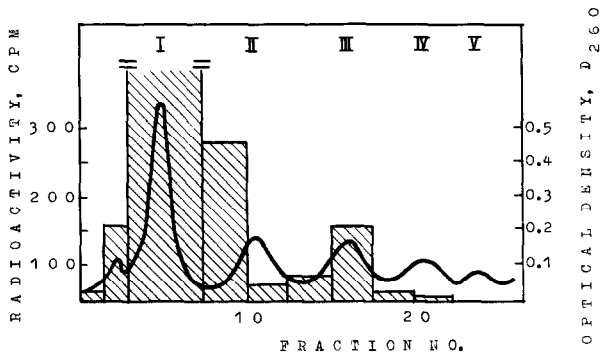

Fig. 2. Chromatography of poly A (from fig. 1) alkaline hydrolysate in the presence of non-labeled oligonucleotides. DEAE-Sephadex column (0.5 × 50 cm); elution was developed by a 0.05 M–0.5 M NaCl gradient in 7 M urea–0.05 M Tris, pH 7.5. The radioactivity was determined in combined fractions of eluate, as indicated. (Peaks I, II, III, etc., contain the mono-, di-, tri-, etc., nucleotides of non-labeled marker RNA).

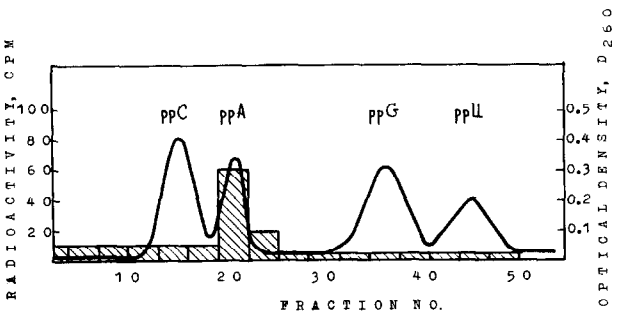

Fig. 3. Identification of labeled pAp in peak III of poly A hydrolysate (see fig. 2): Material of peak III mixed with four nucleoside-diphosphates was chromatographed on 0.5 × 40 cm Dowex-1 (Cl^-). Elution was performed by a gradient containing 0.015–0.025 M HCl and 0–0.15 M NaCl. The radioactivity was determined in combined fractions of eluate as indicated.

chromatography on Dowex-1 (Cl^-) in the presence of four nucleoside-diphosphates [16].

The acid-soluble material from the Sephadex G-75 column was also hydrolyzed by 0.5 M KOH and analyzed in the same way to determine the amount of individual pXp residues [16].

In some experiments the samples of total ^{3}H-labeled dRNA or RNase-stable material obtained from it were hydrolyzed by 0.5 M KOH, nucleotides were removed by column chromatography and the individual nucleoside derivatives were isolated by means of paper chromatography [2]. The ^{3}H-content was determined with toluene scintillator in the SL-40 counter.

3. Results

Fig. 1 demonstrates that all RNase-stable material in the RNase hydrolysate is recovered as a sharp peak in the Sephadex G-75 chromatography. It is localized between the peaks of rRNA and tRNA. Therefore its molecular weight is probably higher than that of tRNA. The following base composition was obtained: A, 92%; G, 3%; C, 3%; U, 2%. Thus the technique used allows one to isolate long poly A sequences from dRNA.

The poly A content depends on the size of the dRNA studied. It was found to be equal to 1.8% for 10–20 S dRNA; 1.1% for 20–30 S dRNA and ~ 0.2% for ⩾ 35 S dRNA.

In DEAE-Sephadex chromatography of the alkaline hydrolysate of poly A (fig. 2) about 1–2% of the radioactivity is recovered in peak III, presumably containing labeled pAp. The latter was identified by means of chromatography in another system (Dowex-1) (fig. 3). In this system radioactive material was localized in the peak with mobility of pAp

Table 1
The distribution of ^{32}P between fractions of RNase hydrolysate of dRNA.

Time of cell labelling (hr)	Sedimentation coefficient of RNA used	Fraction analyzed	Xp (Ap) (cpm)	pAp (pXp) (cpm)	pAp content in poly A (mole% P)	The ratio of pAp isolated from poly A to total pXp of RNA (%)
4*	10–20 S	Poly A	7.5×10^3	150	2	34%
	10–20 S	Other sequences	420×10^3	290 (610)	–	–
1.5**	10–30 S	Poly A	9.44×10^3	97	1	–
	10–30 S	Other sequences	900×10^3	–	–	–

* The peak of radioactivity was in the 18 S zone. Light dRNA was isolated from the 65° fraction.
** The peak of radioactivity was in the 30 S zone. The 10–30 S fraction was isolated from total nuclear dRNA.

(that is the same as of ppA). Thus 1–2% of P in poly A hydrolysate belongs to pAp.

As follows from table 1, pAp originating from poly A comprises about 35% of the total amount of pAp. The latter comprises ~ 60% of all pXp residues in the RNA hydrolysate. Thus about 20% of the light dRNA chains have long poly A-sequences at their 5′-ends.

Finally, it was found that the main part of 3′-end nucleosides (labeled with ^3H) does not belong to poly A. Only traces (less than 1%) of 3′-end ^3H-adenosine is recovered in the alkaline hydrolysate of poly A. Thus, 3′-ends of nuclear dRNA are not represented by long poly A sequences.

4. Discussion

The results of this paper show that in poly A originating from nuclear light dRNA one pAp group per 100 Ap groups may be found. As poly A blocks consist of about 100–200 nucleotides [5, 6] one can conclude that practically all poly A sequences are localized at the 5′-end of the dRNA chains.

In the light dRNA fraction the significant part of 5′-ends are represented by poly A sequences. On the other hand the experiments with 3′-end-labeled RNA demonstrate the absence of poly A at the 3′-end.

It is interesting that the pAp content in poly A isolated from heavier dRNA is twice as low as in poly A from light dRNA (table 1). It seems likely that the length of the poly A sequence in both RNAs is the same. Therefore one can assume that in heavier dRNAs about half of the poly A sequences are localized in the internal parts of molecules.

It has been suggested that each mRNA contains one poly A stretch [5–7]. Poly A content in mRNA was reported to be equal to 5%. This is three times higher than in light nuclear dRNA, which seems to contain a precursor of cytoplasmic mRNA. This difference may be explained by the assumption that only a part of light nuclear dRNA chains possess poly A. The results obtained confirm this idea showing that only about 20% of chains contain poly A at the 5′-end. Namely these chains of nuclear dRNA probably correspond to true mRNAs formed during the processing of giant dRNA. We suggest that poly A serves as a signal to protect sequences corresponding to true mRNA during this processing.

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