SHORT SEQUENCES OF POLYADENYLIC ACID AT THE 3′-ENDS OF NUCLEAR DNA–LIKE RNA

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1. Introduction

The data obtained previously strongly suggest that mRNA is localized near the 3′-end of nuclear giant dRNA* precursor [1,2]. It was also found that the 3′-end sequences of nuclear dRNA are enriched with transcripts from reiterated DNA base sequences. They may correspond to terminators of transcription and/or of translation. In respect to these data it seems to be interesting to study the nature of 3′-end sequences in nuclear dRNA fractions.

In this paper it is reported that 20 – 25% of dRNA chains possess purine oligonucleotides at the 3′-end ($n = 8 - 9$). These sequences are ended with a short lenght of polyA ($n = 6 - 7$) in most of the chains. Similar results were obtained with heavy, intermediate and light dRNAs.

2. Material and methods

dRNA was isolated by hot phenol fractionation [3,4] from Ehrlich ascites carcinoma cells [1,2]. By ultracentrifugation in sucrose gradients in the presence of sodium-dodecyl-sulphate dRNA was separated into three fractions: heavy ($\geqslant$ 35 S), intermediate (20 – 30 S) and light (10 – 18 S).

Then all fractions were labeled with ^3H by periodate treatment and sodium [^3H] borohydride reduction [5]. The details of the technique were described previously [1,2].

For 3′-end sequence analysis the RNA fractions were digested with the pyrimidylic RNAse (pancreatic ribonuclease A) (50 μg per 1 mg RNA) or with a mixture of pyrimidylic RNAse and guanylic RNAse (*Actinomyces aureovertisillatis* Kras. ribonuclease, EC 2.7.7.26) (100 units per 1 mg RNA). The mixtures were incubated in 0.02 M Tris-HCl, pH 7.8, for 1 or 18 hr at 37°. The hydrolyzates were separated on DEAE-Sephadex A-25 (Cl^-) washed by water before use. Nucleoside derivatives were not retained on the column. The latter was washed by 7 M urea – 0.05 M Tris, pH 7.5, and eluted with a NaCl gradient (0.05 – 0.5 M) in the same solution [6]. 8 to 9 peaks were revealed and collected and the remaining material eluted by 2 M NaCl or 0.5 M KOH.

The combined material of each peak was desalted and hydrolyzed with 0.5 M KOH. After neutralization (up to pH 4 – 5) the solution was passed through Dowex-1 ($HCOO^-$) to remove nucleotides. Non-retained material (nucleosides) was collected, the radioactivity in the aliquots was counted, and the rest of it was chromatographed on Whatman 3 MM paper to identify the nucleoside derivatives labeled, according to the procedure described previously [1,2]. The material not retained on Sephadex A-25 was also chromatographed on paper to detect the nucleoside content. Additional purification of 3′-end nucleosides (Dowex-1 and paper chromatography) was necessary as some ^3H is incorporated in the other places in RNA as well as into some contaminating compounds. The radioactivity was counted in an SL-40 scintillator counter (Intertechnique).

Abbreviations:
dRNA, RNA with DNA-like base composition;
polyA, polyadenylic acid;
RNAse, ribonuclease.

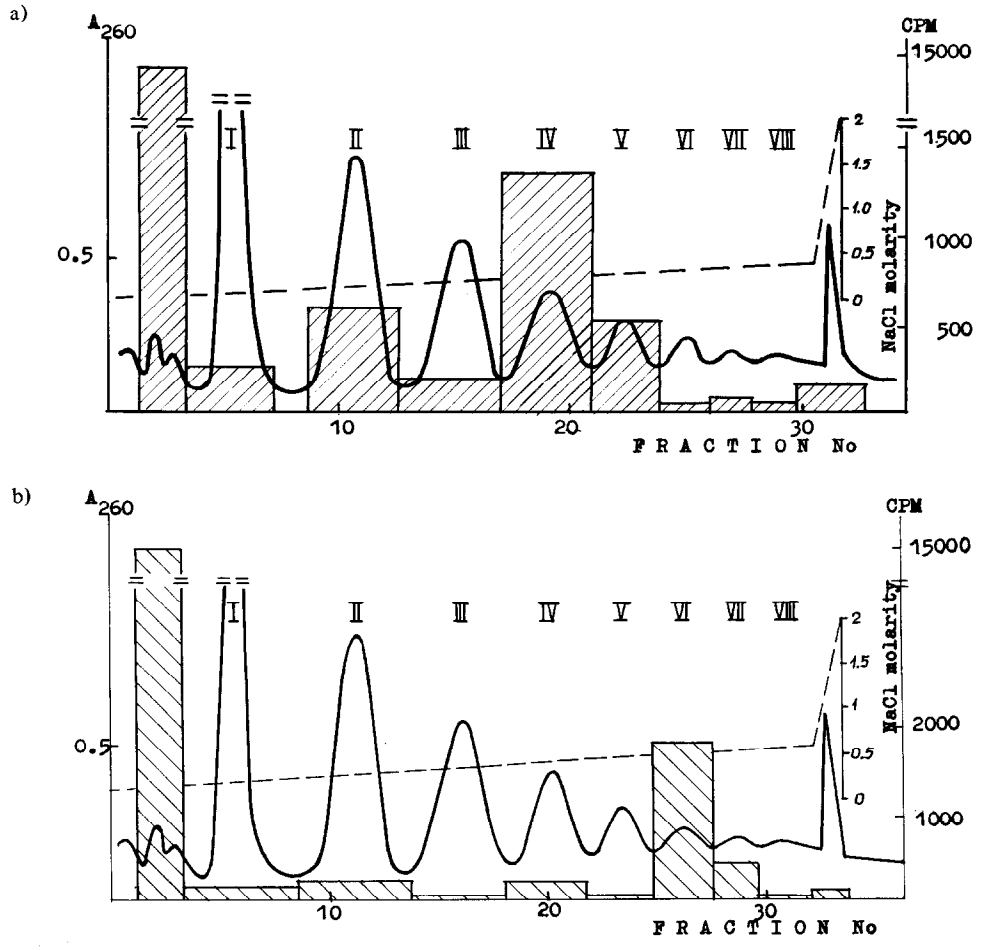

Fig.1. The typical distribution of 3′-end radioactivity among the fractions of RNAse hydrolysate of nuclear dRNA. a) dRNA treated with the mixture of two RNAses, b) dRNA treated with pyrimidylic RNAse. For conditions see the text. (———): A_{260}; hatched columns: radioactivity.

3. Results

DEAE chromatography of the RNAse hydrolyzates performed in the presence of 7 M urea separates oligonucleotides according to their negative charge. The 3′-end oligonucleotide does not contain a phosphate group at the 3′-end and it has two charge units less than the corresponding oligonucleotide from an internal part of RNA. For this reason peak I contains the 3′-end trinucleotide, peak II the 3′-end tetranucleotide, etc.

About 75 – 80% of all 3′-end nucleotides in RNAse hydrolyzates of dRNA was found in the fraction not retained by DEAE-Sephadex, i.e. with the nucleoside derivatives. 20 – 25% of the 3′-ends was recovered in the material of the I – IX UV-absorbing peaks. Only 1% of the nucleoside label is recovered in 0.5 M KOH eluate (table 1).

When the hydrolyzate is obtained from two RNAses the oligonucleotides consist of only adenylic acid residues, while after pyrimidylic RNAse digestion they may also contain guanylic acid.

After hydrolysis with the two RNAses only polyA sequences survive. One can see (table 1, fig.1a) that the main part of 3′-end oligonucleotides obtained after this hydrolysis is localized in peak II (A_3N, where

Table 1
Distribution of 3'-end label in RNAse hydrolysates of nuclear dRNA fractions on DEAE-Sephadex column.

Components of RNAse hydrolysate		Digestion with two RNAses						Digestion with pyrimidylic RNAse			
		Light dRNA (10–18 S)		Intermediate dRNA (20–30 S)		Heavy dRNA (≥35 S)		Intermediate dRNA (20–30 S)			
		(cpm)	3'-end purine oligonucleotides (%)	(cpm)	3'-end purine oligonucleotides (%)	(cpm)	3'-end purine oligonucleotides (%)	(cpm)	3'-end purine oligonucleotides (%)	(cpm)	3'-end purine oligonucleotides (%)
	Non retained material (N, PuN)*	13500	—	15000	—	45000	—	12700	—	—	—
Material eluted by NaCl gradient	I peak $(Pu)_2N$	290	9.0	350	10.5	—	—	200	6.3	210	10.3
	II peak $(Pu)_3N$	714	22.0	600	18.1	3000	32.0	230	7.3	250	12.3
	III peak $(Pu)_4N$	271	8.4	235	7.1	600	6.3	90	2.8	130	6.4
	IV peak $(Pu)_5N$	850	41.2	1320	56.2	1500	50.0	270	8.6	210	10.3
	V peak $(Pu)_6N$	480		550		3150		40	1.3	120	5.9
	VI peak $(Pu)_7N$	320	10.0	80	2.4	750	8.0	1800	73.5	180	50.3
	VII peak $(Pu)_8N$	204	6.3	90	2.7	230	2.3	530		840	
	VIII peak $(Pu)_9N$	100	3.1	100	3.0	140	1.4	10	0.2	90	4.5
Material eluted with 0.5 M KOH (or 2 M NaCl)		400	—	160	—	760	—	40	—	130	—

*Pu = purine nucleotide.
N = adenosine, uridine or guanosine.

N = A or U) and in peaks IV–V ($A_{5-6}N$). The latter comprises 40–50% of total oligonucleotides. The distribution of 3′-ends was very similar for all dRNA fractions: heavy, intermediate and light. After digestion with pyrimidylic RNAse only purine oligonucleotides remain intact. In this case the main part of 3′-ends was found in peaks VI–VII (table 1, fig. 1b), corresponding to $Pu_{7-8}N$ (again N = A or U). Enzymatic digestion for different times (1 or 18 hr) revealed no differences in elution patterns.

4. Discussion

The main conclusion from the results obtained is that about 25% of nuclear dRNA chains contain relatively short purine oligonucleotides at the 3′-end. They are heterogeneous in size. However, a clear maximum was found corresponding to compounds of the $Pu_{7-8}N$ type. After the combined action of two RNAses this peak disappears and the maximum is shifted to the peak containing the compounds of the $A_{5-6}N$ type. Thus the main part (about a half) of 3′-end purine oligonucleotides in dRNA is represented by the following sequences: Pu GAAAAA{A, U} or Pu GAAAAAA{A, U}. They comprise 10–15% of total 3′-end sequences in dRNA. About 75% of dRNA chains do not contain purine oligonucleotides joined to 3′-end nucleotide. It was found previously that dRNA molecules of different sizes have the same end nucleosides and possess the same hybridization properties. This was explained as being a result of conservation of 3′-end sequences during processing [1,2]. The results in this paper support this idea, as the nature and distribution of 3′-end purine oligonucleotides are very similar in different dRNA fractions.

Short polyA found at the 3′-ends of 20–25% of dRNA chains probably are not parts of long polyA chains (n = 100–200) found in the nuclear dRNA as well as in the cytoplasmic mRNA [7,8]. In our recent paper it was shown that the bulk (>98%) of 3′-end nucleosides of dRNA did not belong to this long polyA and it was suggested that long polyA was localized at 5′-end of the light dRNA chains [9].

It should be pointed out that recently Barr and Lingrel found short polyA blocks at the 3′-end of hemoglobin mRNA [10]. Their size is very close to that described here. Thus it seems very probable that certain service sequences (for example, terminators) localized near the 3′-end of giant nuclear precursor survive during the processing and then are transferred to polysomes, remaining as a part of functioning mRNA.

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