# ARE POLYADENYLATED AND NON-POLYADENYLATED GIANT HnRNA MOLECULES TRANSCRIBED FROM DIFFERENT SITES IN THE GENOME?

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## 1. Introduction

Ten to thirty percent of the molecules in heterogeneous nuclear RNA (HnRNA) are polyadenylated post-transcriptionally [1–3]. It was supposed that the polyadenylation of HnRNA molecules is a 'signal' for their processing and transport into the cytoplasm [2]. One of the main questions in this connection is why the major part of the molecules are not polyadenylated in HnRNA and whether there is a selectivity in the process.

In this communication evidence is presented showing that polyadenylated and non-polyadenylated populations of the giant HnRNA molecules in pigeon bone marrow cells differ in some of their sequences transcribed from unique sites of genome, i.e., that a selectivity exists at polyadenylation of this RNA.

## 2. Materials and methods

Bone marrow cells were obtained from pigeons rendered anaemic by phenylhydrazine [4]. The cells were labeled and the >45 S HnRNA fraction extracted as in [4]. >45 S [$^3$H]HnRNA isolated from water sucrose gradient was additionally centrifuged in a 5–20% sucrose gradient prepared on 85% formamide (Merk)/0.01 M Tris–HCl, pH 7.0/0.01 M NaCl/0.4% sodium dodecylsulphate (SDS) on a Hitachi centrifuge (RPS 40 rotor, 16 h, at 20°C and 40 000 rev./min). The fraction which sedimentated in the heavy region of the gradient, was collected and diluted by 5 vol. 0.01 M Tris–HCl, pH 7.0/0.01 M NaCl/0.4% SDS, then concentrated by centrifugation under the above-described conditions for 7 h. The bottom fraction (0.5 ml) was collected and twice re-precipitated by ethanol. Polyadenylated (poly(A)$^+$) and non-polyadenylated (poly(A)$^-$) molecules were separated on poly(U)–Sepharose (Pharmacia) as in [5]. Total nuclear RNA was extracted from purified nuclei [6] by the 'hot phenol–SDS' method [7], whereupon the fraction with a maximum distribution near 28 S was obtained from it by centrifuging in a sucrose gradient [4,6]. The method of obtaining unique and repetitive DNA fractions and the procedure of hybridization are in [4,8].

## 3. Results and discussion

We wished to determine the extent of homology between the sequences of poly(A)$^+$ and poly(A)$^-$ subfractions of the same pulse-labeled >45 S HnRNA fraction. It can be done by competition experiments with a third RNA which should have at least partial homology with one of the two. We used the total 28 S nuclear RNA fraction (28 S nRNA) as such. Apart from rRNA, this fraction contains a messenger-like subfraction which has been shown to contain metabolically stable poly(A)$^-$ species of molecules [6]. Similar to HnRNA these non-ribosomal molecules of the 28 S nRNA fraction hybridize well both with repetitive and unique DNA [4,6].

Figure 1 shows the sedimentation of an isolated >45 S HnRNA fraction in the sucrose gradient prepared on 85% formamide and the zone of the gradient from which giant molecules were obtained for hybridization. This procedure largely liberates

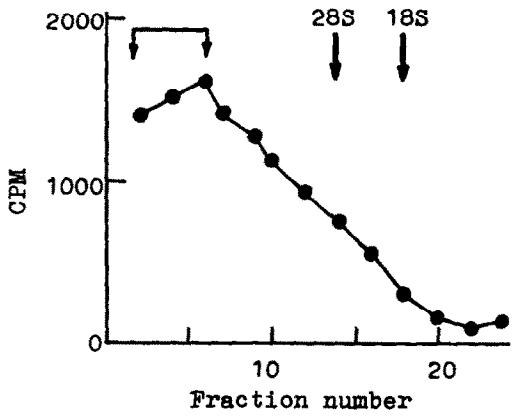


Fig.1. Sedimentation profile of > 45 S HnRNA in 5–20% sucrose gradient prepared on 85% formamide. The position of 28 S and 18 S peaks was determined by the sedimentation of reticulocyte polysomal RNA in a parallel test-tube. The gradient zone from which hybridization material was taken is indicated.

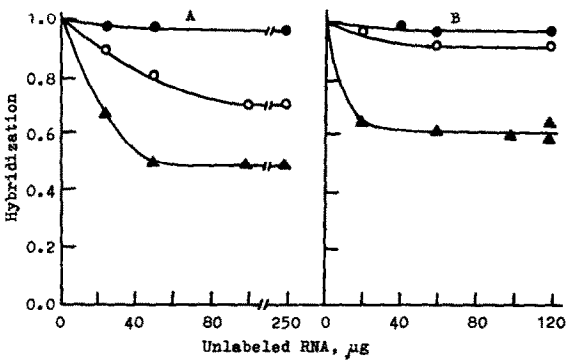


Fig.2. Competition between 28 S nuclear RNA and > 45 S [$^3$H]HnRNA for sites on a crude preparation of intermediate repeats of DNA (A) or unique DNA (B). About 1000 cpm of > 45 S RNA was mixed with 1000 μg crude preparation of intermediate repeats or 1500 μg purified unique DNA. The mixture was heated for 10 min at 98°C then brought to 62°C and incubated to $C_0t$ 1500 with a crude preparation of intermediate repeats and to $C_0t$ 30 000 with purified unique DNA. Unlabeled RNA: 28 S nuclear (▲–▲), 28 S ribosomal (○–○) and total *E. coli* (●–●).

the fraction of true > 45 S molecules from aggregated low-molecular chains. The specimens of > 45 S molecules chosen for hybridization must be free of 28 S-sized RNA molecules. Purification in a formamide-containing gradient increases maximum hybridizability of > 45 S RNA with repetitive DNA sequences from ~20–30%, while the hybridizability with unique DNA remains approximately the same.

Figure 2 shows the control experiments on competition with 28 S rRNA purified from polysomes, which demonstrated that a part of the sequences in > 45 S fraction used in the experiments was represented by the rRNA precursors. So, in all the subsequent experiments parallel competition with 28 S rRNA was created so as to take into account the contribution of rRNA to hybridization. Figure 2 shows which part of non-ribosomal > 45 S HnRNA sequences hybridizing with repetitive (fig.2A) and unique (fig.2B) DNA was represented by homologous sequences in the 28 S nRNA. 20% and 30% homology has been found between them for sequences transcribed from non-ribosomal repeats and unique DNA, respectively. It was of interest to find out whether this homology is characteristic to the same extent of the poly(A)$^+$ and poly(A)$^-$ subfractions of > 45 S HnRNA.

The results of the competition of 28 S nRNA with separate poly(A)$^+$ and poly(A)$^-$ subfractions of > 45 S RNA are presented in fig.3. As regards poly(A)$^-$ subfraction, polysomal 28 S rRNA competed with it only when hybridization was with repetitive DNA, while the 28 S nRNA was a competitor both for the hybridization with repetitive and unique DNA. As

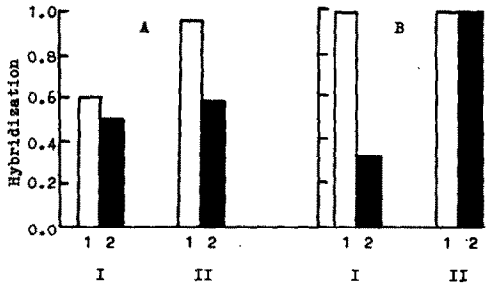


Fig.3. Competition between poly(A)$^-$ (A) and poly(A)$^+$ (B) subfractions of > 45 S [$^3$H]HnRNA and excess (100 μg) unlabeled 28 S ribosomal RNA (1) or 28 S nuclear RNA (2) for sites on a crude preparation of intermediate repeats of DNA (I) or unique DNA (II). Maximum hybridizability in the presence of 100 μg *E. coli* RNA (taken for 1) was achieved at hybridization with repeats of: poly(A)$^+$ [$^3$H]RNA, 30%; poly(A)$^-$ [$^3$H]RNA, 28%; and at hybridization with unique DNA of the same RNAs, 46% and 39%, respectively. The average results of 2–3 experiments are given. The average error of the competition experiments is ±0.064 for 28 S nuclear RNA and ±0.028 for 28 S ribosomal RNA.

for the poly(A)$^+$ fraction, the formation of hybrids was never suppressed in the presence of polysomal rRNA. 28 S nRNA offered considerable competition in the case of hybridization between poly(A)$^+$ > 45 S HnRNA and repeats, while it was not a competitor where hybridization with unique DNA was concerned.

Thus the main fact ensuing from a purely qualitative evaluation of these results is that no measurable competition is observable between the sequences of pulse-labeled poly(A)$^+$ > 45 S HnRNA and the total 28 S nuclear non-ribosomal RNA transcribed from unique DNA sites. This fact points to the existence of a certain class of copies homologous to unique DNA which are represented both in the total 28 S nuclear RNA and the pulse-labeled poly(A)$^-$ subfraction of giant molecules, while being practically absent from the poly(A)$^+$ subfraction.

A more detailed treatment of the above-cited data leads to some valuable conclusions. Among other things, we proceeded from the assumption [9,10] that sequences transcribed from repeats and unique DNA alternate in giant HnRNA molecules and their derivatives.

1. At least some of the individual poly(A)$^-$ species of > 45 S HnRNA molecules containing copies of unique DNA sequences are absent from the poly(A)$^+$ subfraction. If correct, this can be considered as an experimental evidence of a selectivity in post-transcriptional polyadenylation of the giant transcripts in these cells.
2. The post-transcriptional fates of at least a part of the poly(A)$^+$ and poly(A)$^-$ molecules of HnRNA are different: we find in the 28 S fraction strongly different concentrations of the poly(A)$^+$ and poly(A)$^-$ > 45 S HnRNA derivatives transcribed from unique DNA sites (see fig.3). A higher concentration of unique class 28 S RNA sequences formed from the poly(A)$^-$ chains of > 45 S HnRNA as compared with the unique sequences of the nuclear 28 S fraction derived from poly(A)$^+$ giant molecules, points to selective stabilization of some derivatives of the poly(A)$^-$ subfraction of the > 45 S HnRNA in the nuclear 28 S RNA fraction. This independent indication is in accordance with our earlier observations to the effect that the nuclear RNA fraction of these cells with sedimentation coefficient of about 28 S contains stable non-ribosomal ('messenger-like') molecules free of long poly(A) [6].
3. The poly(A)$^-$ molecules of HnRNA eligible for stabilization contain unique sequences that are absent from poly(A)$^+$ HnRNA molecules, while they also include some repeated DNA copies that are represented both in the poly(A)$^+$ and poly(A)$^-$ subfractions of > 45 S HnRNA.

The results obtained accord with the evidence on the existence of certain differences between the poly(A)$^+$ and poly(A)$^-$ subfractions of HnRNA molecules reported [11] from analysis of the 5′-ends of these subfractions. This conclusion is also supported by our previous observations [5] as to the different 'hairpin' content in the subfractions of HnRNA.

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