METABOLICALLY STABLE MESSENGER-LIKE 28 S RNA FRACTION IN THE NUCLEI OF PIGEON-BONE MARROW CELLS

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1. Introduction

Messenger-like RNA present in the nuclei of higher organisms is known as 'heterogeneous nuclear RNA' (HnRNA) [1–3]. This RNA class is extremely polydisperse in molecular weight and metabolically unstable its half-life does not exceed 30 min [2,4,5]. This presumes that in about 3 h not more than 1% of the newly formed HnRNA should remain in the nuclei. However, this is hardly compatible with the fact that spectrophotometric analysis often reveals a rather large portion of cellular RNA localized in nuclei [2,5,6]. As shown in this work, for example, at least 35% of the total RNA is found in the nuclei of pigeon-bone marrow cells. It is also a puzzling point that the sedimentation profile of the optically measured nuclear RNA looks more like that of ribosomal RNA [2, 6–8] than of HnRNA. The suggestion that the bulk of nuclear RNA is represented only by extremely unstable HnRNA and pre-rRNA [2] seems hardly probable.

Our results demonstrate that the bulk of nuclear RNA of pigeon-bone marrow cells, which sediments as a homogeneous 28 S fraction and constitutes over 15% of the total cellular RNA, is metabolically stable and messenger-like despite its great similarity to 28 S rRNA.

2. Materials and methods

2.1. *Preparation and labeling of cells*

Bone marrow cells (83% erythroblasts, 17% myeloid cells) and immature cells of peripheral blood (about 75% reticulocytes, 8% erythroblast-like cells, 15% erythrocytes) were obtained from pigeons rendered anaemic by phenylhydrazine [9]. To prepare labeled nuclear and polysomal metabolically stable RNAs the pigeons were injected intraperitoneally with [^{3}H] uridine (1–2 mCi 100 g body wt) and left for an appropriate time. The animals were then decapitated and the cell populations prepared as described earlier [9].

2.2. *Extraction and fractionation of nuclear and cytoplasmic RNAs*

The cells were suspended in 0.01 M Tris–HCl (pH 7.5), 3 mM Mg-acetate, 0.25 M sucrose, 0.1% diethylpyrocarbonate and lysed with Triton X-100 (final concentration 0.5%). The lysate was centrifuged at 4°C for 5 min at 4000 rev/min. RNA from the nuclear pellet was extracted by the 'hot phenol–SDS' method [10]. Polysomal RNA was obtained from the reticulocyte cytoplasm as described by Brawerman et al. [11]. 28 S Fractions were obtained from the total nuclear RNA of bone marrow cells and from the polysomal RNA of reticulocytes after their centrifugation in a 5–20% sucrose gradient [9]. The fraction of pulse-labeled >45 S RNA was obtained from the bone marrow cells incubated for 50 min with [^{3}H] uridine [9]. The fraction of RNA molecules carrying long poly(A)-stretches was isolated by the chromatography on poly(U)-Sepharose (Pharmacia) [12]. The amount of the material resistant to RNAases (hairpin-like structures) in RNA preparations was estimated according to Ryskov et al. [13].

2.3. *Preparation of DNA fractions for hybridization*

Three DNA fractions were prepared as described previously [14].

1. The fraction of the unique DNA sequences was obtained by annealing of the total denatured DNA to $C_0t = 1500$. The non-reassociated fraction separated on hydroxyapatite represented purified unique DNA sequences.

2. The reassociated portion of the DNA was denatured and reannealed to $C_0t = 1$; the non-reassociated fraction separated on hydroxyapatite was used as the crude preparation of the intermediate repeats class which included rDNA.

3. From this DNA a family of sequences repeating about 35 times/haploid genome ('rare repeats') was isolated. This fraction consisted of the purified 'interspersed' repeats devoid of rDNA.

DNA–RNA hybridization was conducted essentially according to Melli et al. [15] with some modifications [14].

3. Results

3.1. *The content, sedimentation properties and metabolic stability of bone marrow nuclear RNA*

Of the cellular RNA, 35–40% is found in the nuclear preparations isolated from these cells. There were enough reasons (microscopic examinations etc.) to think that RNA obtained from the nuclear preparations was really of nuclear origin in spite of its unusually high yield. It is clearly seen (fig.1) that the optical density profile of nuclear RNA resembles that of one typical of a polysomal RNA containing two sufficiently homogeneous peaks: 18 S and 28 S. The 18 S peak could be identified partly as the bulk of nuclear messenger RNA [16] and in part as a cytoplasmic contaminant (see [2,6]). We were concerned with the reasons for the presence in the nuclei of such a great amount of a homogeneous 28 S RNA and about its origin. As shown previously [17], a single [^{3}H]uridine injection into the anaemic pigeons provides a high radioactivity in the nucleotide pool of the bone marrow cells only within the first 5 or 6 h. Therefore 8–10 h after [^{3}H]uridine injection the amount of newly formed ^{3}H-labeled RNA must be negligible in the cells since the nucleotide pool shows little or no radioactivity. At that time and later on (up to 30 h)

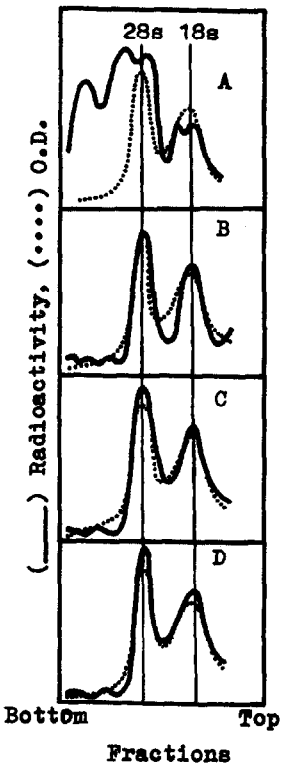

Fig.1. Sucrose-gradient sedimentation of total nuclear RNA isolated 1 h (A), 5 h (B), 15 h (C) and 40 h (D) after injection of [^{3}H]uridine. Each probe contained 5000–8000 cpm of ^{3}H-labeled RNA and 250 μg unlabeled nuclear RNA. (——) cpm, (· · · ·) optical density.

we found in the nuclei, considerable amounts of labeled RNA the distribution of which coincides with that of the total nuclear RNA (fig.1). Even more than 30 h after the cessation of RNA-labeling, about 40% of the total radioactivity is to be found in the nuclear RNA, 15–20% being in the 28 S fraction. Thus this fraction of nuclear RNA (as well as the 18 S fraction) exhibits extremely high metabolic stability.

3.2. *Sequence similarity and difference between the 28 S nuclear RNA, 28 S ribosomal RNA and pulse-labeled >45 S RNA*

The next step was to discover if this mass of 28 S nuclear RNA represents the large rRNA subunit somehow accumulated in the nuclei. This was achieved by hybridization-competition experiments. Figure 2 shows that an excess of unlabeled 28 S rRNA does not signi-

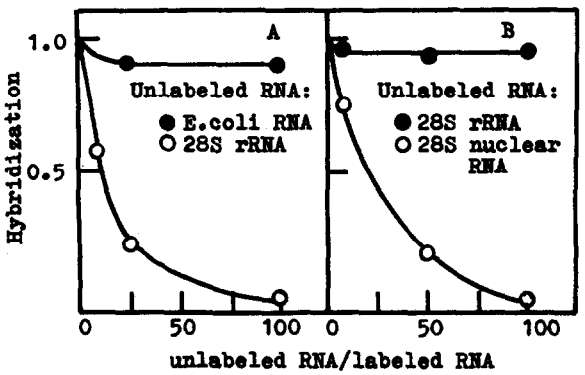

Fig.2. Competition between 28 S ^{3}H-labeled rRNA (A) and 28 S ^{3}H-labeled RNA (B) and unlabeled RNAs for sites on crude preparation of intermediate repeats of DNA. DNA : RNA ratio = 1000 : 1. 1.0 = hybridization without competitors: 24% (A) and 26% (B).

ficantly influence the level of hybridization of 28 S nuclear (labeled) RNA with a crude preparation of intermediate repeats including ribosomal genes (see table 1). These results indicate that even if 28 S rRNA sequences were present in the preparations of 28 S nuclear RNA, their content was insignificant.

Nuclear RNA, 28 S (30 h labeled) is well hybridized with the preparation of rare repeats lacking in ribosomal genes (27%) as well as with the unique DNA (22%) (table 1). These DNA classes also hybridize well with HnRNA. These results show that the bulk of the 28 S RNA (at least 50%), if not the whole, accumulated in the nuclei of bone marrow cells is copied from the same classes of DNA as is pulse-labeled >45 S HnRNA.

Table 2
Amount of RNAase-resistant structures and poly(A)-containing molecules in 28 S stable nuclear RNA and > 45 S pulse-labeled RNA

RNA	RNAase-resistant material, (%)	Poly (A)-containing molecules, (%)
28 S nuclear	6.8	0.5
> 45 S Fraction	1.4	16

It was of interest to compare some other properties of these two kinds of RNAs. As seen from table 2 about 16% of the chains in the >45 S HnRNA contain long poly(A) sequences while the amount of such molecules in 28 S nuclear RNA does not exceed 0.5%. The amount of RNAase-resistant (hairpin-like) structures in 28 S nuclear RNA was found to be about 5-times more than that in the >45 S HnRNA.

4. Discussion

The results obtained in this work show that the bulk of messenger-like RNA present in the nuclei of pigeon-bone marrow cells is represented not only by typical HnRNA, or possible by a 18–20 S mRNA [16], but also to a sufficient extent by a discrete population of metabolically stable RNA molecules with sedimentation coefficients of about 28 S. This RNA population is copied from the same classes of DNA

Table 1
Hybridization of 28 S stable nuclear RNA (30 h labeled), 28 S polysomal rRNA (30 h labeled) and > 45 S nuclear RNA (50 min labeled) with repeated and unique DNA fractions

DNA fractions	Hybridization (%)		
	28 S Nuclear RNA	> 45 S RNA	28 S rRNA
Unique[a]	21.5	40	–
Rare repeats[b]	26.7	15.5	0.2
Crude preparation of intermediate repeats[a]	26.0	21.5	24.0

[a] DNA : RNA ratio = 1000 : 1
[b] DNA : RNA ratio = 100 : 1

sequences from which HnRNA is transcribed, i.e., partly from unique DNA and in part from the class of interspersed repeats. In the pigeon genome this class of repeats (which is characteristic of metazoan species [18]) is represented by sequences of about 350 nucleotides long occurring about 35 times/haploid genome ('rare repeats') [14]. The evidence presented above allows us to suggest that 28 S nuclear RNA represents a derivative of HnRNA, i.e., a fraction of HnRNA that is metabolically stable and is stored in the nucleus.

Metabolically stable small-molecular-weight (<26 S, up to 4–6 S) RNA species of undefined nature have been detected in the nuclei of some mammalian [19,20] and avian [7,17] cells. The existence in the higher organisms' nuclei of such a homogeneous and large fraction of stable messenger-like RNA with sedimentation coefficients of about 28 S has been demonstrated in the present study. To elucidate that this RNA represents a messenger-like fraction is hampered by the following:

(1) Its sedimentation properties are very close to those of the large component of rRNA.

(2) Like rRNA it does not contain long poly(A) sequences.

(3) In HnRNA labeled for a short period of time, this stable fraction is not distinguishable as it is masked by HnRNA.

The purpose of the nuclear accumulation of a messenger-like 28 S RNA remains unclear. We suggest that a fraction of HnRNA molecules contains stable pre-mRNA (about 5000 nucleotides long) and is retained in the nuclei. It undergous selected and delayed processing gradually releasing stable messengers into the cytoplasm. Thus the data presented here implies that, along with the informosomes stored in the cytoplasm [21], there exists a nuclear pool of non-processed pre-mRNA.

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