THE PRESENCE OF SMALL MOLECULAR WEIGHT RNAs IN NUCLEAR RIBONUCLEOPROTEIN PARTICLES CARRYING HnRNA

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1. Introduction

In previous papers [1–3], we have presented evidence that the nuclear RNP-particles carrying the HnRNA (informoferes) [4] are composed, in addition to proteins and HnRNA, also of RNA species with a slower turnover. These conclusions were reached on the basis of double labelling of the RNA with precursors [1–3] and of RNAase digestion of the RNA of the informoferes [1,2] as well as on the effects of inhibitors of RNA synthesis on the RNA moiety of the RNP-particles [3]. In the present report we demonstrate by acrylamide gel electrophoresis the existence in informoferes of small molecular weight RNAs, which represent the metabolically more stable RNA species of the RNP-particles.

2. Materials and methods

2.1. *Isolation of informoferes*

Rat liver nuclei were isolated by the modified Chauveau et al. [5] procedure, used by us previously [6]. Informoferes were isolated as recently described [3]. In short, nuclei were washed with 0.25 M sucrose containing 25 mM KCl, 10 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.5 and 50 mM EDTA. The washed nuclei were suspended in a 0.14 M NaCl solution containing 1 mM $MgCl_2$ and 10 mM Tris–HCl, pH 8.0 [4,7] and submitted to ultrasonic treatment with a Branson sonifier (6–10 s bursts at 40 W). The nuclear suspension was centrifuged at 10 000 rev./min for 10 min. The supernatant obtained was layered on a 15–30% sucrose gradient with a 5 ml 50% sucrose cushion prepared in 0.14 M NaCl containing 1 mM $MgCl_2$ and 10 mM Tris–HCl pH 8.0 and submitted to centrifugation at 25 000 rev./min for 18 h in a SW 27 Beckman head.

The same extraction technic, but in the presence of RNAase-inhibitor, was performed to isolate undegraded polymeric informofere structures. In this case, centrifugation on the 15–30% gradient was performed at 25 000 rev./min for 2.5 h. For the characterization of informofere RNA, fractions 18–19 and 20–22 of fig.1a and fractions 9–12, 13–16, 17–20, 21–24 and 25–30 of fig.1b, representing monomer and polymer fractions, respectively, were pooled.

2.2. *Preparation of RNAase-inhibitor*

RNAase-inhibitor was prepared in principle according to published methods [8–10], starting from rat liver cytosol and involving ammonium sulphate precipitation, Sephadex G-25 gel filtration, DEAE-cellulose chromatography and RNAase-affinity chromatography. In the present experiments the active fractions obtained after DEAE-cellulose chromatography were used.

2.3. *Radioactive labelling and phenol extraction of RNA*

For the radioactive labelling 20 μCi [^{14}C]orotic acid (sp. act. 57 mCi/mmol) were injected i.p. per female Wistar rat weighing 100–120 g 30 min prior to sacrifice. The incorporated radioactivity into acid precipitable material was determined in aliquots of the fractions by the filter disc method [11]. RNA was isolated from the pooled fractions by phenol extraction as follows [12]: The samples were brought to

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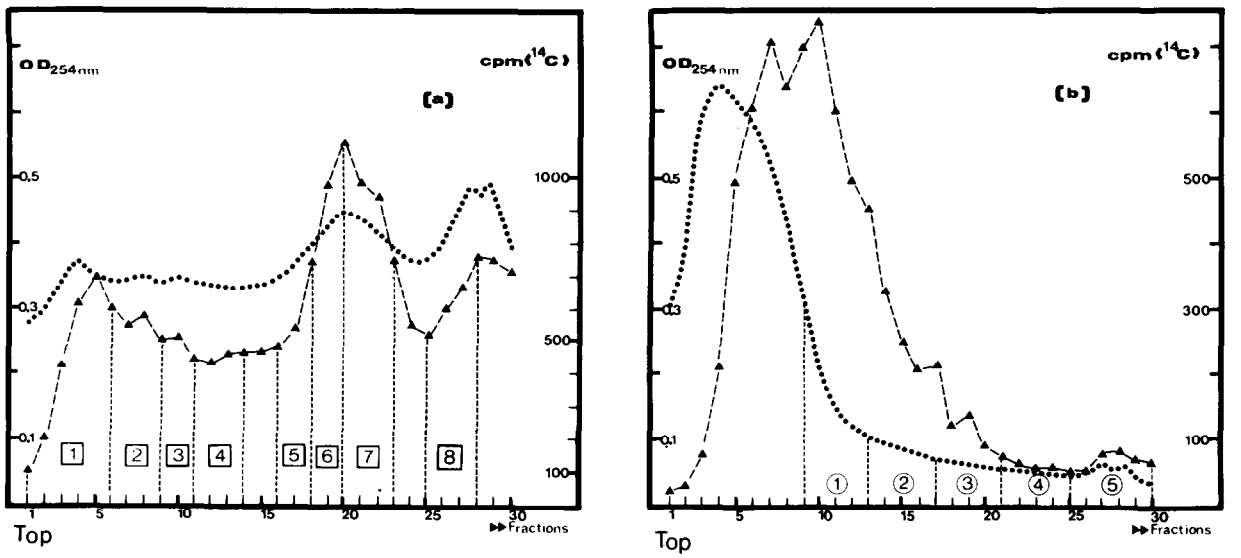

Fig.1. Sucrose gradient centrifugation of nuclear extracts prepared in the absence (a) and in the presence (b) of RNAase-inhibitor. Rats were injected with [^{14}C]orotic acid 30 min prior to sacrifice. Nuclei and nuclear extracts were prepared as described in Materials and methods. (a) centrifugation time 18 h at 25 000 rev./min, (b) centrifugation time 2.5 h at 25 000 rev./min. The pooled fractions 1–8 from (a) and ①–⑤ from (b) were used for the extraction of RNA. (●●●●●●●) $A_{254\ nm}$, (▲- - - -▲) incorporated radioactivity, cpm.

0.2% SDS, extracted once with 1 vol. of water saturated phenol and twice with 1 vol. of chloroform, and the RNA was precipitated overnight with 2 vol. of cold ethanol at −20°C.

2.4. *Gel electrophoresis of RNA*

SDS–polyacrylamide gel electrophoresis of the RNA fractions was performed according to the method of Loening [13]. The gels were stained with 0.2% methylene blue for 30 min and destained in a 1 : 1 methanol–water mixture.

3. Results

Informoferes were isolated from rats injected for 30 min with [^{14}C]orotic acid either in the absence or in the presence of RNAase-inhibitor, thus yielding either monomeric or polymeric structures, respectively [4,14]. The results of the experiment are shown in fig.1a and b. In the absence of RNAase-inhibitor (fig.1a) the labeled RNA is found mainly associated to the 30 S RNP-particles (monomeric structures). A small part of the RNA is bound to material present in the 50% sucrose cushion representing, in part, still undegraded informofere structures. The labeled RNA represents degraded HnRNA, which sediments on sucrose gradients heterogeneously with S values between 3 S and 10 S with a maximum at 6–8 S [4,14]. In the presence of RNAase-inhibitor the labeled RNA is associated with informoferes, sedimenting with S values of up to 300, as under these conditions the HnRNA is protected from nuclease digestion [4,14].

The fractions corresponding to the 30 S monomers (fractions 18–19 and 20–22 of fig.1a) were pooled, the RNA extracted and submitted to acrylamide gel electrophoresis (fig.2a). On the same gel we have also analyzed RNA extracted from the other regions of the gradient (fractions 1–5, 6–8, 9–10, 11–13, 16–17 and 25–27 of fig.1a) as well as RNA stemming from polymeric structures with S values of approximately 150 (fractions 9–12 of fig.1b). On a second gel (fig.2b) we have analyzed RNA extracted from polymeric particles with S values from 150–300 (fractions 9–12, 13–16, 17–20, 21–24 and 25–30 of fig.1b). Five bands a, b, c, d and e can be visualized in the slab gel of the RNA fractions derived from the

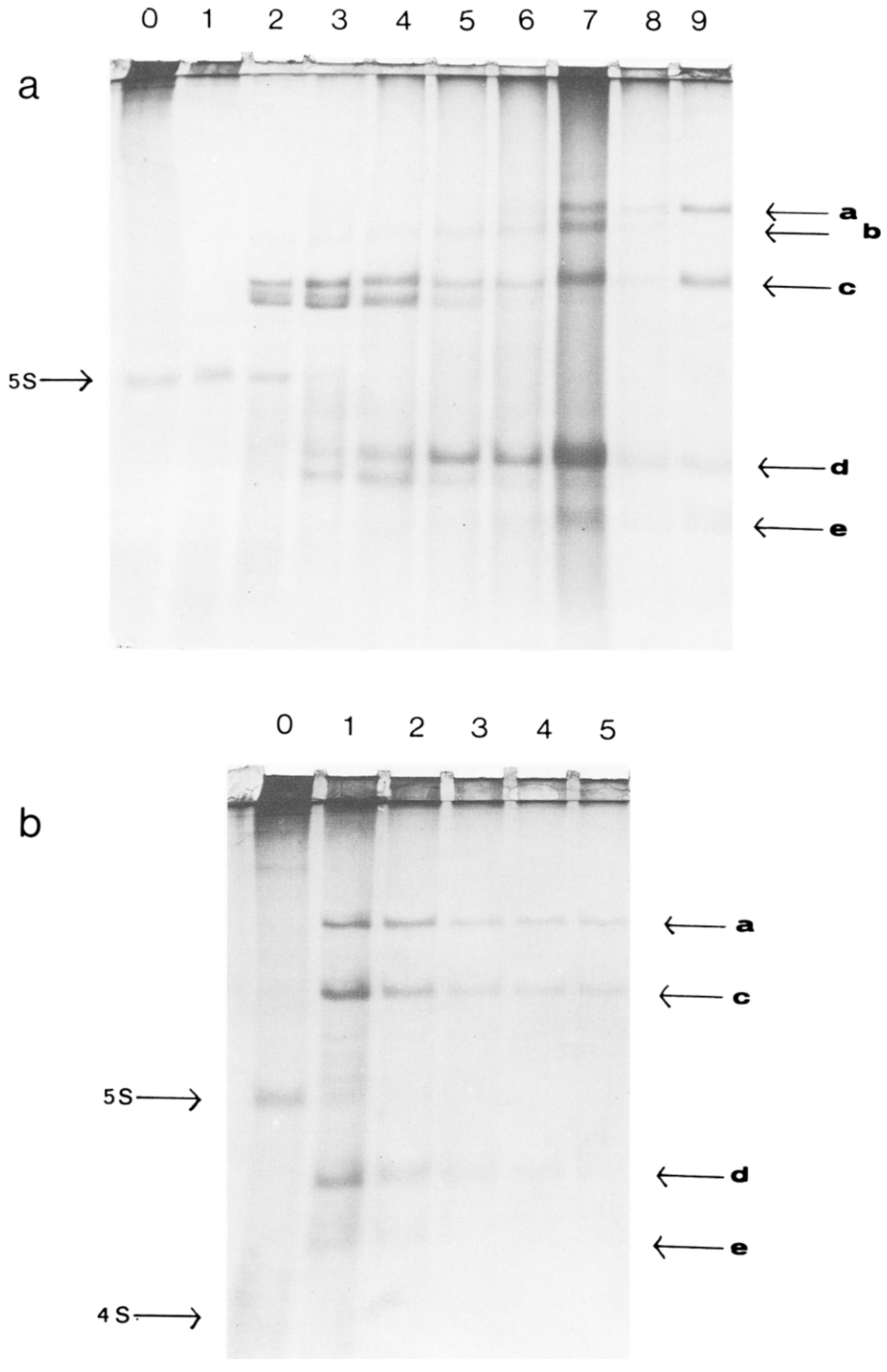

Fig. 2

monomeric RNP-particles (fig.2a). Four of these (a,c,d and e) are also present in polymeric RNP-particles (fig.2a., Nos 8 and 9 and fig.2b., Nos 2–6). In table 1 the approximate S values of the snRNAs present in the monomer and polymer informoferes are given. Quantitation of the snRNA by scanning of the gels reveals that they represent 40–70% of total RNA extracted from the informoferes, the rest being degraded HnRNA.

It seems very unlikely that the small RNAs are products of nuclease degradation of the HnRNA as they are also present in the polymeric structures which are isolated under conditions of nuclease inhibition (see fig.2a., No. 8 and fig.2b., Nos 1–5). The rapidly labeled HnRNA is visualized on gels as diffusely staining radioactive material. Under the same conditions, no radioactivity could be detected on the bands of small RNAs (results not shown).

Table 1
Approximate S values of snRNAs in monomer and polymer informoferes

Band	S Values	Present in monomers	Present in polymers
a	6.4	+	+
b	6.3	+	–
c	5.9	+	+
d	4.8	+	+
e	4.5	+	+

4. Discussion

The results presented above demonstrate the presence of small molecular weight RNAs having S values of approximately 4.5–6.4S in RNP-particles carrying the rapidly labeled HnRNA. They are present not only in the monomer RNP-structures, which derive from polymeric RNP-complexes as a result of nuclease action, but also in the polymeric structures themselves, prepared in the presence of RNAase-inhibitor. As shown previously [4,14] under these conditions, the very nuclease sensitive HnRNA is obtained in high molecular form. This excludes the possibility that the small RNAs represent degradation products of larger RNA species.

These small RNAs can also be directly isolated from nuclear extracts, independent of whether these were prepared in the absence or presence of RNAase-inhibitor (results not shown). This also strongly suggests that they are not degradation products.

It could be argued that the isolated snRNA belongs to contaminating structures sedimenting through the gradient. This argument is rendered unlikely in view of the results depicted in fig.2a demonstrating different RNA patterns in the material sedimenting in the lighter regions of the gradient.

The presence, however, of the additional snRNA b in the monomer informoferes (see fig.2a., Nos 6 and 7) could reflect, as our recent findings suggest, the existence of a RNP structure, sedimenting between 25–30 S, the nature of which is currently under consideration.

It is very probable that snRNA c corresponds to RNA U1 described by Busch et al. [15]. Raj et al. [16] have recently shown the presence of RNA U1 and U2 in nuclear extracts and in particulate material, which on the basis of the extraction procedure applied for their isolation and their sedimentation characteristics, but not their buoyant density in CsCl gradients, show similarity to the informofere structures, which we have

Fig.2. Acrylamide gel electrophoresis of RNA from various fractions of the sucrose gradients depicted in fig.1a and b. RNA was prepared from the pooled fractions as described in Materials and methods. The concentration of acrylamide was 15%.
Fig.2a: RNA from the pooled fractions of fig.1a: (0) marker 4 S and 5 S RNA from rat liver cytoplasm, (1) small molecular weight RNA (snRNA) isolated from fractions 1–5, (2) snRNA isolated from fractions 6–8, (3) snRNA isolated from fractions 9–10, (4) snRNA isolated from fractions 11–13, (5) snRNA isolated from fractions 16–17, (6) snRNA isolated from fractions 18–19, (7) snRNA isolated from fractions 20–22, (8) snRNA isolated from fractions 25–27, (9) snRNA isolated from fractions 9–12 of fig.1b (polymer structures). Fig.2b: RNA from the pooled fractions of fig.1b: (0) marker 4 S and 5 S RNA from rat liver cytoplasm, (1) snRNA from fractions 9–12, (2) snRNA from fractions 13–16, (3) snRNA from fractions 17–20, (4) snRNA from fractions 21–24, (5) snRNA from fractions 25–30.

used as starting material for the isolation of the small RNAs. RNA U2 could be the additional band b already discussed.

In a very recent report Zieve and Penman [17] have analyzed on acrylamide gels the small molecular weight RNAs from HeLa cells. From the electrophoretic mobility we conclude that RNAs SnB, C, D, G and H of Zieve and Penman [17] correspond to RNAs a, b, c, d and e described in this paper. Zieve and Penman [17] report that SnD is released from the nucleus after brief warming and is present in particles with S values greater than that of the nu bodies. SnB and SnC seem to be tightly complexed to the nuclear skeleton [17]. Faiferman and Pogo [18] have also reported the presence of small RNAs in the nuclear network.

Although the presence of small RNAs in the nucleus has been shown by various authors [19–24], their precise localization within the confines of the nucleus has been only recently attempted. The detection of some of the small RNAs in informoferes is a step towards this direction.

In previous papers we concluded on the basis of double labelling experiments with RNA precursors [1–3] and RNAase digestion of the informoferes and more recently [3] as a result of the effect of inhibitors of RNA synthesis on the RNA of the RNP-particles, that two different RNA species should be associated with the informoferes (a) the HnRNA, showing a higher turnover and (b) more stable RNA species, sedimenting between 4–11 S. The present results confirm our assumptions. We have postulated on the basis of previous and experiments to be presented, that the small RNAs serve as structural components for the attachment of the proteins to form the monomer RNP-structures. Whether this is true and whether these RNAs also serve other functions remains to be seen.

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