EVIDENCE FOR THE EXISTENCE OF A STRUCTURAL RNA COMPONENT IN THE NUCLEAR RIBONUCLEOPROTEIN PARTICLES CONTAINING HETEROGENEOUS RNA

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SUMMARY: The structural organisation of nuclear ribonucleoprotein particles carrying the HnRNA has been investigated. Experiments are presented which indicate the existence in the RNP particles of two different RNA species: (1) the rapidly labelled HnRNA and (2) stable, low molecular weight RNA, probably located in the interior of the protein moiety, which may serve a structural role for the arrangement of the proteins within the RNP particle.

Nuclear ribonucleoprotein particles carrying heterogeneous nuclear RNA (HnRNA) of animal cells have been described by Samarina et al. (1) in rat liver and subsequently detected by other groups in various tissues and species (2-11). The name informoferes has been coined by Samarina et al. (1) for these particles present in the nucleus, to differentiate them from particles of similar nature, found exclusively in the cytoplasm, named informosomes by Spirin (12). According to Samarina et al. (1) (see Fig. 1a) the informoferes are composed solely of approximately twenty molecules of one main protein, having a molecular weight of 40.000 daltons, arranged by virtue of

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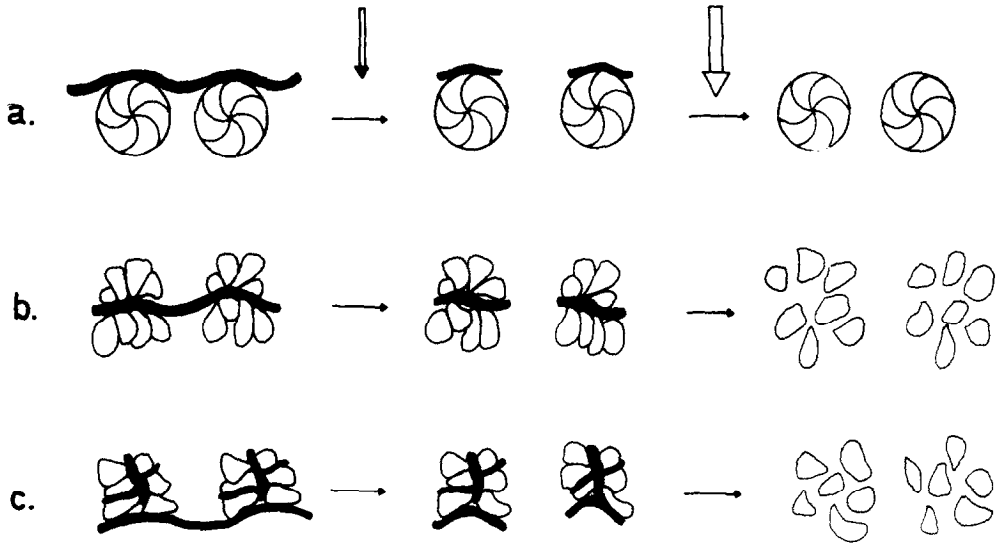

Fig. 1: Models of informoferes structure
a) original model proposed by Samarina et al (1). The monomer is composed solely of one main protein with a M.W. of 40,000 daltons, repeated 20 times. The monomers are arranged in polysomal structures by attachment to the heterogeneous RNA. In this model, mild RNase digestion should transform the polymeric structures to monomers. High RNase concentrations should have no additional effect.
b) In this model the particle proteins are held together by the heterogeneous RNA. Mild RNase digestion should transform the polymers to monomers, digestion with high RNase concentrations should destroy the monomer structure.
c) Two types of RNA are foreseen in this model of informofere structure: the heterogeneous RNA, responsible for the formation of the polymeric structures and a structural RNA, on which the proteins are assembled to form the monomeric unit. Digestion with RNase leads to similar changes as in (b). The proteins of the particles are heterogeneous and are depicted as such in Models b and c.

⇩ treatment with 5-50 µg/ml RNase, 60 min at 0°.

⇩ treatment with 100 µg/ml RNase, 60 min at 0°.

protein-protein interaction in a particle structure. The particles form polysome-like structures through their attachment to the heterogeneous RNA,the size of the polymer structures depending on the length of the HnRNA. According to this model, ribonuclease digestion should transform the polymer structures to monomers devoid of RNA component: the monomer protein particle itself should be completely resistant to RNase treatment. Some of our recent experimental data question the validity of this model.

As already reported (1) treatment of the informoferes with low doses of pancreatic ribonuclease transforms the polymer particles to monomers. The monomer particle structure, however, can be completely destroyed by digestion at high RNase concentrations. These results speak for the involvement of RNA in the organization of the monomer structure and can be accomodated by the two models b and c depicted in Fig.1. In model 1b the heterogeneous RNA is considered responsible for holding the particle proteins together. The HnRNA is partly covered by the proteins and therefore protected against digestion by low RNase concentrations. In model 1c we postulate the existence of a second type of RNA in the particles having solely a structural function.

To differentiate between these two possibilities the following experiments were performed. ^{3}H-orotic acid was injected into rats for 15 minutes and subsequently highly labelled informoferes were isolated from purified liver nuclei (7). Digestion of the particles with low concentrations of ribonuclease leads to an almost total degradation of the labelled RNA (see Fig.2) without effects on the

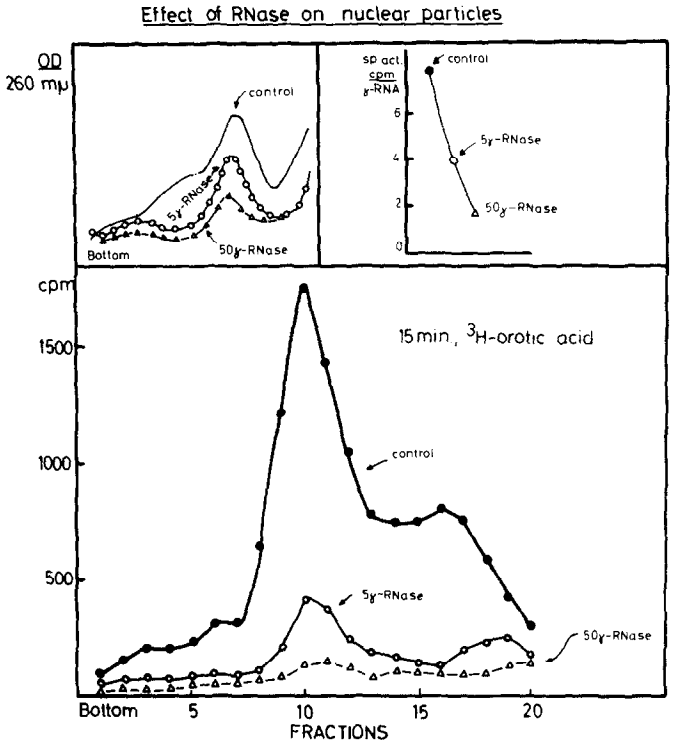

Fig. 2: Effect of pancreatic RNase on informoferes. 25 μC ^{3}H-orotic acid (sp.act. 21 C/mMol) were injected intraperitoneally, dissolved in isotonic saline to five rats weighing 140-150 g. Fifteen minutes thereafter, nuclear particles were isolated, in principle as described by Samarina et al. (1) starting with purified liver nuclei (13). Aliquots of the extracted particles were then treated with 5 and 50 μg per ml pancreatic ribonuclease at 0-4° for 1 hour. They were then layered on top of 15-30 % sucrose gradients in 0.01 M Tris-HCl, pH 8.0, containing 1 mM $MgCl_2$ and 0.14 NaCl and centrifuged in a Beckman SW 41 rotor for 15 hours at 22,000 rpm. 0.5 ml fractions were collected, the D_{260} being continuously monitored. Aliquots of the fractions were collected on filter paper discs, precipitated in 5 % CCl_3COOH and the incorporated radioactivity evaluated as described by Niessing and Sekeris (7). The specific activity of the RNA of the informoferes before and after RNase treatment is shown in the inserted figure at the top right.

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particle structure (see Table I). RNA, however, is still associated with the monomer particles, the specific activity

Table I: Effect of pancreatic ribonuclease on informofere structure. The RNA and protein content of the pooled ^{3}H-labelled control and RNase treated particles obtained in the experiment of Fig. 2 was evaluated on the basis of the 260/280 ratio of the fractions and on the values of protein determinations (14).

	Total protein (µg/ml)	Total RNA (µg/ml)
control particles	120.1	19.4
RNase treated (5 µg/ml)	101.2	7.7
RNase treated (50 µg/ml)	85.0	5.5

Table II: Effect of pancreatic RNase on nuclear particles labelled with ^{3}H and ^{14}C-orotic acid: $^{3}H/^{14}C$ ratio of informfere RNA before and after RNase digestion.

	$^{3}H/^{14}C$ ratio
control	4.87
5 µg/ml RNase	3.77
50 µg/ml RNase	2.41
60 µg/ml DNase I	4.64

Rats weighing 140-150 g received intraperitoneally 25 µC ^{3}H- and 15 µC ^{14}C-orotic acid 15 minutes and 20 hours respectively before sacrifice. Informoferes were then prepared from liver nuclei and submitted to RNase treatment and sucrose gradient centrifugation as described in legend to Fig. 2. The fractions containing the particles were pooled and the $^{3}H/^{14}C$ ratio measured before and after treatment with pancreatic RNase and DNase I one hour at 0^{o}.

of which is much lower than that of the RNA before RNase digestion (see Fig.2). This "core" RNA can by hydrolyzed by increasing the RNase concentration with concomitant destruction of the monomer particles. These results are compatible with model c but not with b of Fig.1. The following experiments have led to the same conclusion. We have double-labelled the informofere RNA by injecting ^{3}H- and ^{14}C-orotic acid into rats 15 minutes and 20 hours respectively before sacrifice. The $^{3}H/^{14}C$ ratio of the isolated particles was then determined before and after ribonuclease treatment (see Table II). The $^{3}H/^{14}C$ ratio declines significantly after RNase treatment, supporting the existence of a rapidly labelled, RNase sensitive and a more stable, RNase resistant RNA. DNase treatment does not affect the $^{3}H/^{14}C$ quotient. The nuclease resistant RNA is obviously responsible for the retention of the particle structure, as its digestion with high RNase concentrations is accompanied by destruction of the informoferes. The existence of more stable RNA species in the informoferes is also demonstrated by the following experiment. Polymer particles, double labelled with short ^{3}H- and long ^{14}C-orotic acid pulses, were isolated from rat liver nuclei in the presence of ribonuclease inhibitor (7) (Fig.3). Under the conditions of isolation, the particles sediment with S values of up to 300. RNA was then isolated from particles sedimenting at 200-250 S (fractions 5 to 10 of Fig.3) and submitted to sucrose gradient centrifugation (Fig.4). The rapidly labelled (^{3}H) HnRNA, due to its large size (7), sediments towards the bottom of the tube. The more stable RNA species (^{14}C-labelled) sediment mainly in the 3 to 11 S region of the gradient (Fig.4). They could,

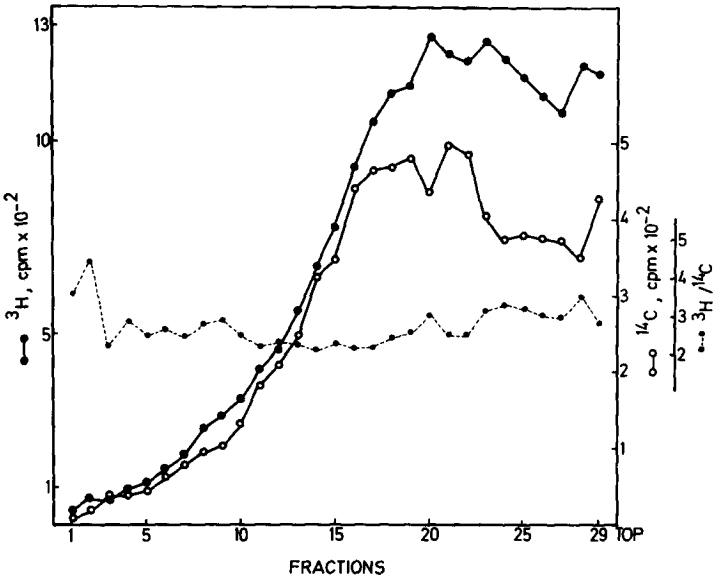

Fig. 3: Isolation of polymer particles from rat liver nuclei double labelled with ^{3}H- and ^{14}C-orotic acid. Five rats weighing 140-150 g each received intraperitoneally 100 µC ^{3}H- (sp. act. 21 C/mMol) and 20 µC ^{14}C-orotic acid (sp. act. 61 mC/mMol) one and 20 hours respectively before sacrifice. Nuclear particles were then prepared from purified nuclei in the presence of RNase inhibitor as previously described (7). The extracts containing the polymer particles were layered on 15-30 % sucrose gradients in 0.01 M Tris-HCl, pH 8.0 containing 1 mM $MgCl_2$ and 0.14 M NaCl and centrifuged in a SW 25.II Beckman rotor 150 min at 22,000 rpm. Fractions of 2 ml were collected and the ^{3}H- and ^{14}C-incorporated orotic acid into RNA evaluated by precipitating aliquots of the fractions on filter paper discs with 5 % CCl_3COOH as described previously (7). Fractions 5-10 containing polymer particles with an S value of approximately 200 - 250 S were pooled, $NaDodSO_4$ added to a final concentration of 0.5 % and the dissociated RNA precipitated with 2 volumes of ethanol. The precipitated RNA was collected by centrifugation, dissolved in 0.5 % $NaDodSO_4$ in 0.05 Tris-HCl, pH 7.4 and submitted to sucrose gradient centrifugation (see Fig. 4).

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in part, correspond to the small molecular ribonucleic acids described by Busch et al. (14) in nuclei of animal cells.

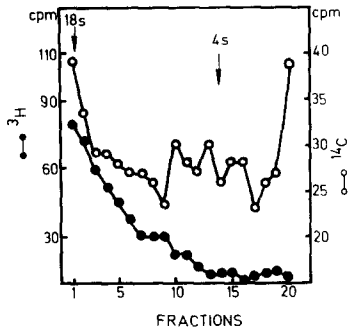

Fig. 4: SDS-sucrose gradient centrifugation of informofere RNA. The RNA obtained in the experiment described in Fig. 3 was layered on 5 to 20 % sucrose in 0.5 % SDS and 0.05 M Tris buffer, pH 7.5, and centrifuged at 36,000 rpm for 16 hours in a SW 41 Beckman Rotor at 15°. 0.5 ml fractions were collected, aliquots precipitated on filter papers with 5 % CCl_3COOH (7) and the ^{3}H- and ^{14}C-radioactivity incorporated evaluated.

Lastly, administration of α-amanitin to rats in doses inhibiting the incorporation of precursors into heterogeneous RNA up to 80-95 % leads to significant reduction of the amounts of informoferes extracted (our unpublished experiments). The RNA isolated from informoferes double labelled with ^{3}H- and ^{14}C-orotic acid (20 and 1 hour respectively) extracted from α-amanitin treated animals is almost devoid of heterogeneous RNA but still contains the stable species. Results obtained in our (15) and other laboratories (8-11) have demonstrated that the protein component of the informoferes is highly heterogeneous, encompassing polypeptides ranging in size from 25,000 to over 130,000 daltons. Among the proteins associated with the informoferes are RNA cleaving enzymes and homoribopolymer synthetases (17-19).

It is interesting to note the striking similarities in the structure of the informoferes of the nucleus and the polysomes of the cytoplasm. Both are ribonucleoproteins, the

protein moiety being composed of an heterogeneous population of polypeptides in part having enzymatic activity, partly of still unknown function. Both have structural RNA components and on both heterogeneous RNA is attached: on the informoferes the precursor of messenger RNA, on the ribosomes the functional m-RNA, in the former case very probably to be processed and completed (19) in the latter to be translated.

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