IN VITRO FORMATION OF INFORMOFER-LIKE PARTICLES BY ISOLATED RAT LIVER NUCLEI

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1. Introduction

Nuclear 30 S ribonucleoprotein particles (informofers) carrying heterogenous nuclear RNA(HnRNA) were first studied by Samarina et al. [1], and these studies were extended by others [2–10]. From these investigations, it has been suggested that the informofers could participate in the detachment of newly synthesized RNA(mRNA) from its DNA template and the transport of this RNA from nucleus to the cytoplasm where protein synthesis takes place.

In this paper, we describe evidence for the formation of informofer-like particles carrying RNA which has been synthesized by isolated rat liver nuclei. It was found that the addition of cytosol (Fc) and nuclear sap (Fn) fractions to the RNA synthesizing system of isolated nuclei facilitated the incorporation of synthesized RNA into a 30 S structure with a density of 1.413 g/cm^3.

2. Materials and methods

Rat liver nuclei were isolated according to Chauveau et al. [11] with slightly modified conditions. Isolated nuclei were suspended in a buffer containing 50 mM Tris–HCl, pH 8.0, 4 mM $MgCl_2$, 1 mM DTT and 20% glycerol to a DNA concentration of 200 A_{260} units/ml. Crude RNA polymerase was prepared from isolated nuclei as described by Steggles et al. [12]. The cytosol fraction (Fc) was prepared from rat liver as follows. Rat liver was homogenized in 2.5 vol of a buffer containing 50 mM Tris–HCl, pH 7.6, 25 mM KCl, 5 mM $MgCl_2$ and 250 mM sucrose and centrifuged at 150 000 g for 2.5 h at 3°C. The upper 3/4 of postribosomal supernatant was concentrated by ammonium sulfate precipitation at 0 to 70% and dissolved in a buffer containing 20 mM Tris–HCl, pH 7.6, 40 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 0.2 mM EDTA and 10% glycerol, and dialysed against the same buffer at 0°C for 17 h. The nuclear sap fraction was obtained by the incubation of isolated nuclei (120 A_{260} units) for 20 min at 23°C in a 1 ml solution of 7.5 mM Tris–HCl, pH 8.0, 140 mM NaCl, 5 mM $MgCl_2$, 3.5 mM DTT, 3 mM ATP and 15% glycerol. After the incubation, the mixture was centrifuged at 10 000 rev/min for 10 min and the supernatant was designated as 'Fn'.

The reaction for the formation of informofer-like particles was carried out in two steps. The reaction mixture for the first step contained the following in a final vol of 0.1 ml: 30 mM Tris–HCl, pH 8.0, 80 mM KCl, 2 mM $MnCl_2$, 4 mM $MgCl_2$, 2 mM DTT, 10 mM creatine phosphate, 10 μg of creatine kinase (Boehringer Mannheim), 2 mM ATP, 1 mM CTP, 1 mM GTP, 0.1 mM [^{3}H] UTP (4 μCi) (Radiochemical Centre, Amersham), 100 μg protein of Fc, 4 μg of RNA polymerase and 2 A_{260} units of isolated nuclei. The mixture was incubated for 60 min at 30°C. For the second step, 0.1 ml of Fn was added to the mixture of the first step and the reaction was continued for additional 20 min at 23°C. At the end of the reaction, the mixture was centrifuged at 10 000 rev/min for 10 min and the resulting supernatant was layered onto 11 ml of a 15–30% sucrose gradient containing 10 mM Tris–HCl, pH 8.0, 140 mM NaCl and 1 mM $MgCl_2$. The tube was centrifuged at 25 000 rev/min for 17 h at 3°C in a Spinco SW-41 rotor. After the centrifugation, 5-drop fractions were collected from the bottom of the tube. Each fraction

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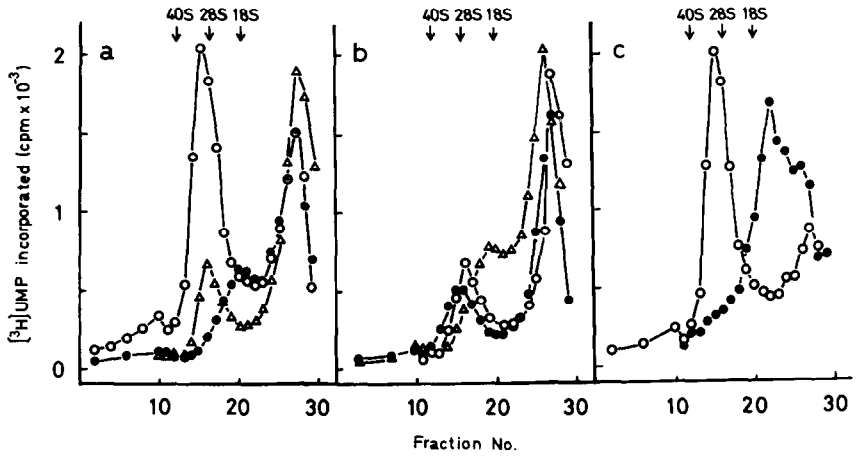

Fig.1. Sedimentation behaviors of the reacted mixture in sucrose density gradient centrifugation. The reaction and sucrose density gradient centrifugation were carried out as described in Methods. (a) (○) Complete system; (△) Fc was omitted from the reaction mixture; (●) Fn was omitted from the reaction mixture. (b) (○) Fc was treated at 60°C for 5 min and added to the reaction mixture; (●) Fn was treated at 60°C for 5 min and added to the reaction mixture; (△) Rat liver ribosomes (100 μg) which were prepared as described by Terao and Ogata [19] were added to the reaction mixture instead of Fn. (c) (○) After the reaction, the mixture was treated with 5 mM EDTA at 0°C for 30 min; (●) After the reaction, the mixture was treated wtih 1% DOC (Difco Laboratories) at 0°C for 30 min.

was precipitated with cold 10% trichloroacetic acid containing 1% sodium pyrophosphate. The precipitate was collected on Whatman GF/C-glass filter and counted in a Beckman liquid scintillation spectrometer.

For the analysis on CsCl density gradients, the supernatant from the second step was mixed with 1.7 ml of 5 mM phosphate buffer, pH 7.6, containing 100 mM NaCl, 1 mM $MgCl_2$ and 6% formaldehyde, and dialyzed against the same buffer containing 2% formaldehyde for 18 h at 0°C [1,4]. The fixed sample was mixed with a saturated CsCl solution to give a final concentration of 1.45 g/cm^3 and then banded by centrifugation at 37 000 rev/min for 46 h at 3°C in a Spinco SW-65 rotor. After the centrifugation, the radioactivity of each fraction was measured as described above.

3. Results and discussion

In the experiment shown in fig.1, in vitro RNA synthesis by isolated rat liver nuclei was carried out in two steps; Fc was present in the first step of the reaction and Fn in the second. After the reaction, the mixture was immediately subjected to the sucrose density gradient centrifugation. It can be seen in fig.1-a that [^{3}H] UMP incorporation was fairly found at the position to which 30 S particles would sediment. In contrast, the omission of Fc from the first step strongly reduced the [^{3}H] UMP incorporation into 30 S position. Similarly, no appreciable incorporation occurred into the 30 S position when Fn was absent in the second step, while a part of the radioactivity was widely spread around 18 S position. From these results, it is evident that the inclusion of Fc with Fn in the RNA synthesizing system resulted in the incorporation of [^{3}H] UMP into 30 S material. Furthermore, as shown in fig.1-b, heat treatment of Fc or Fn diminished the [^{3}H] UMP incorporation into the 30 S material. Thus, it appears that RNA synthesized by isolated nuclei was sedimented as a 30 S structure by heat-sensitive components in the cytosol and nuclear sap fractions. On the other hand, if liver ribosomes were added instead of Fn, synthesized RNA could not be detected in the 30 S position, indicating that the ribosomes did not directly participate in the process of the formation of 30 S structure.

Since it has been reported that nuclear ribonucleo-

protein (RNP) particles carrying HnRNA are resistant to EDTA treatment [3] and sensitive to sodium desoxycholate (DOC) [7], an attempt was made to determine whether the 30 S structure is affected by these reagents. After the reaction, EDTA or DOC was added to the mixture which was analysed by sucrose gradient centrifugation. As shown in fig.1-c, no appreciable alteration of the radioactive profile took place by EDTA treatment, whereas DOC produced a displacement of the peak to the region where lighter structures would sediment, indicating that the 30 S structure is resistant to EDTA treatment and sensitive to DOC. Thus, it might be concluded that under the conditions where RNA synthesis proceeded in the presence of Fc and Fn, the synthesized RNA was incorporated into a 30 S structure analogous to RNP particles or informofers.

To confirm the characteristics of this 30 S structure, the reacted mixture was fixed with formaldehyde and examined by equilibrium density centrifugation in CsCl. Fig.2-a shows the presence of a radioactive peak with a buoyant density of 1.413 g/cm^3 almost corresponding to the density of the RNP particle of the informofer type (1.41 g/cm^3) [1,2]. As shown in fig.2-b and c, when either Fc or Fn was omitted from the reaction mixture, the radioactivity detected at the density of 1.413 g/cm^3 decreased markedly, confirming the participation of Fc and Fn in the reaction as described in the preceding figure. Low radioactive peaks were, however, found at densities

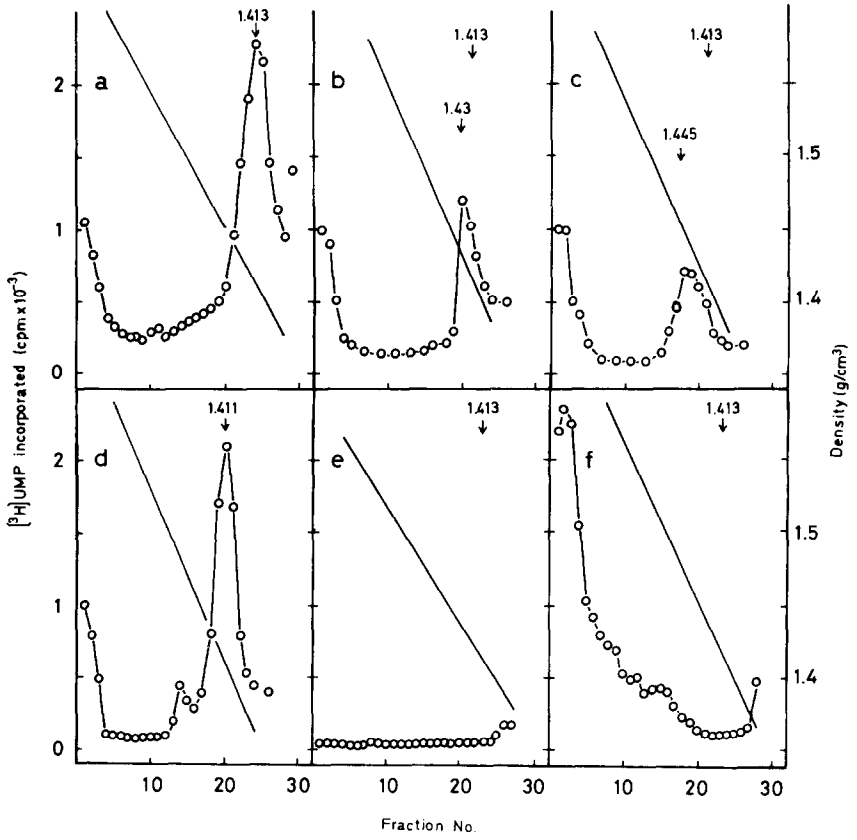

Fig.2. CsCl density profile of the reacted mixture. The reaction and analysis on CsCl density gradients were performed as described in Methods. (a) Complete system; (b) Fc was omitted from the reaction mixture; (c) Fn was omitted from the reaction mixture; (d) After the reaction, the mixture was treated with DNase 1 (20 μg/ml, bovine pancreas, Worthington Biochemical Corp.) at 37°C for 5 min; (e) After the reaction, the mixture was treated with RNase A (10 μg/ml, bovine pancreas, Schwarz/Mann) at 37°C for 5 min; (f) After the reaction, the mixture was treated with pronase (200 μg/ml, Calbiochem) at 37°C for 5 min.

of 1.43 g/cm^3 and 1.445 g/cm^3. Although the reason for this finding is not clear, they may be other forms of RNP particles [13]. That the peak (1.413 g/cm^3) was an informofer-like particle was also supported by lack of DNase degradation (fig.2-d), and susceptibility to RNase (fig.2-e) and pronase (fig.2-f) degradation.

In summary, we have demonstrated the formation of informofer-like particles using an RNA-synthesizing system of isolated rat liver nuclei. The reaction requires at least two heat-sensitive subcellular components, one of them being present in the cytosol fraction (Fc) and the other in the nuclear sap fraction (Fn). Although no clear-cut understanding of the function of these components emerged from the present studies, it is not unreasonable to suggest that the components could play an important role in the regulation of RNA synthesis and transport of mRNA species to the cytoplasm. Our system may be useful for studying the mechanism of mRNA transport and the control of genetic expression in eukaryotic cells. Evidence for the presence in cytosol of proteins with a high affinity for RNA has been accumulating [13–17]. Recently, some cytoplasmic components have been shown to be essential for maintaining high rates of RNA synthesis [18]. Their relation to our system remains to be elucidated. Experiments to find out the functional significance of the stimulatory components for the formation of informofer-like particles are in progress.

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