# THE ISOLATION OF INFORMOFERS FREE FROM dRNA

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## 1. Introduction

It was shown previously that the nuclear dRNA (DNA-like RNA, heterogeneous nuclear RNA) is complexed with special macromolecular protein particles, denoted as "*informofers*" [1–3]. Each informofer was assumed to consist of a number ($\geqslant 20$) of identical protein subunits.

However, the isolation of informofers in a pure state has not yet been achieved, as the destruction of RNA by RNase leads to aggregation of the protein particles. In this paper a method is described which allows complete removal of dRNA from informofers without destruction of the particles. Informofers survive the procedure and remain soluble.

## 2. Material and methods

Ribonucleoprotein 30 S particles containing dRNA were isolated from rat liver nuclei or Ehrlich ascites carcinoma cells. The rats were injected with $^{14}$C-orotic acid 45 min before killing. Crude particle preparations were obtained by gel filtration through Sephadex G-200 [4]. For labeling of the protein moiety the samples obtained after gel filtration were treated with $^{125}$I (153 mCi/ml) according to Bale et al. [5]. After this the material was centrifuged through a sucrose density gradient and the fraction of double-labeled RNP 30 S particles was collected.

Ehrlich ascites carcinoma cells were labeled with a $^{14}$C-algal protein hydrolysate (1 Ci/mmole) for 48 hr *in vivo*. The nuclear RNP particles were then isolated as described previously [1, 2].

To dissociate the RNA from the protein samples of the 30 S RNP particles were dialyzed against 2 M NaCl [6]. The dissociated material was directly layered onto a 15–30% sucrose gradient in 2 M NaCl and centrifuged in an SW-25 bucket rotor (for 12–15 hr at 24,000 rpm). From 20 to 25 fractions were collected and the aliquots taken from them were treated with trichloroacetic acid and the precipitates washed on nitrocellulose filters and their radioactivity measured: $^{14}$C in an SL-40 Intertechnique counter (France), and $^{125}$I in an USS-1 counter (USSR). Sometimes $^{125}$I was counted directly in solution in the same counter.

The other samples of material obtained from sucrose gradients, before or after 2 M NaCl treatment, were fixed with 2% formaldehyde and then ultracentrifuged in a CsCl density gradient [7]. In some experiments formaldehyde fixation was omitted.

In reconstruction experiments fractions of nuclear dRNA obtained by hot phenol fractionation [8] were used. RNA was mixed with the suspension of informofers in 2 M NaCl and dialyzed against 0.1 M NaCl, 0.001 M $MgCl_2$, 0.025 $K_2HPO_4$, pH 7.5. After this the material was centrifuged through a sucrose density gradient or fixed with formaldehyde and banded in a CsCl density gradient.

## 3. Results and discussion

One can see from fig. 1A that $^{125}$I-protein is present in the same ~30 S peak as dRNA (optical density; $^{14}$C-label). In the CsCl density gradient the distribu-

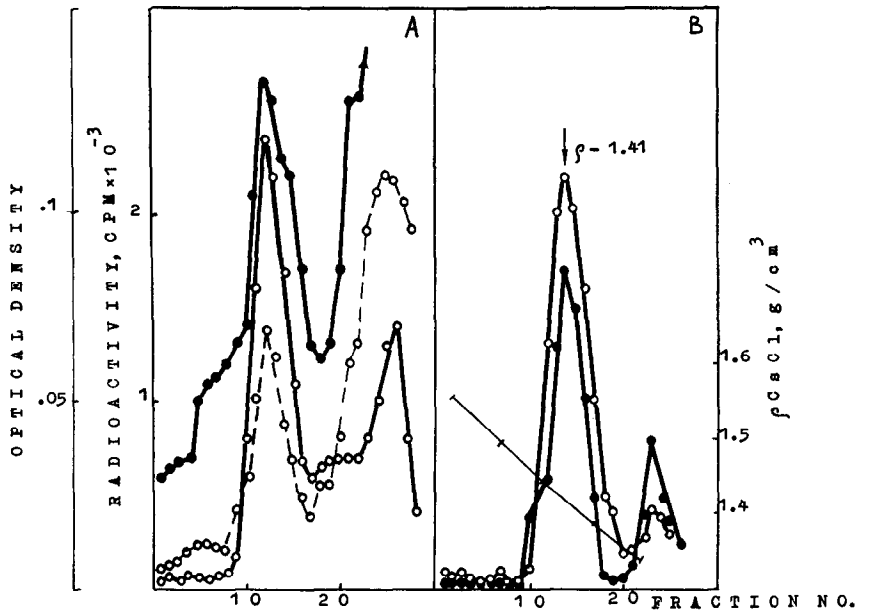


Fig. 1. Sedimentation and density distribution of non-treated double-labeled ribonucleoprotein 30 S particles. (A) centrifugation through a 15–30% sucrose gradient in 0.1 M NaCl, 0.001 M $MgCl_2$, 0.025 M phosphate, pH 7.5 for 14 hr at 24,000 rpm in an SW-25 rotor of a Spinco L2 at 2°. ○ - - - - - ○: optical density at 260 nm. ●——●: $^{125}I$, cpm. ○——○: $^{14}C$, cpm. (B) The peak from the sucrose gradient (fig. 1A) was collected and fixed with 2% formaldehyde; 1 ml was layered onto 4 ml of a preformed CsCl density gradient ($\rho$ from 1.3 to 1.6 g/cm$^3$), and centrifuged in an SW-50 rotor of a Spinco L2 ultracentrifuge for 18 hr at 45,000 rpm, 2°. ●——●: $^{125}I$, cpm. ○——○: $^{14}C$, cpm.

tion of protein also follows that of dRNA (fig. 1B), indicating that the labeled protein is really part of the RNP particles.

After treatment with 2 M NaCl RNA is completely separated from the particles and sedimented as a broad peak with a maximum in the 6–10 S region. On the other hand the main part of the protein migrates as a single homogeneous peak with the same velocity as the original non-dissociated RNP particles. The same result was obtained with nuclear particles from both rat liver and Ehrlich carcinoma cells (fig. 2), indicating that the result with rat liver particles is not due to protein iodination.

Thus, the removal of RNA does not destroy the particle and the informofer survives the treatment mentioned above.

The buoyant density of free informofers is equal to ~ 1.34 g/cm$^3$ (fig. 3). No labeled RNA is present in this region of the CsCl density gradient. Formaldehyde fixation does not influence the results of CsCl ultra-centrifugation.

The addition of urea dissociates informofers into sub-units; in polyacrylamide gel electrophoresis more than 90% of the material may be found in one component with a molecular weight of ~ 40,000 (as determined in the presence of SDS) (see [9]).

Free informofers easily interact with dRNA after removal of the dissociating agent. Fig. 4 illustrates the results of reconstitution experiments. Depending on the molecular weight of dRNA added either single 30 S particles (fig. 4B, C) or poly-particles (fig. 4C) were obtained. In CsCl density gradient they have a buoyant density of about 1.41 g/cm$^3$ (fig. 4D) equal to that of the original RNP particles.

The results presented demonstrate the possibility of obtaining informofers free from dRNA in a soluble state. In the electron microscope free informofers are very similar to the original 30 S particles and look like globules of 200 Å in diameter [10].

This supports the model of the structure of nuclear RNP particles containing dRNA described previously [2, 3]. According to this model dRNA is distributed on the surface of preexisting protein macromolecular globular particles.

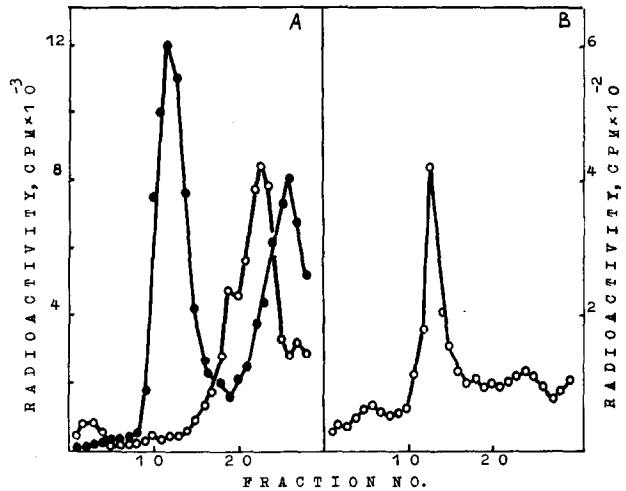


Fig. 2. Sedimentation distribution of free informofers. Ribonucleoprotein 30 S particles were dialyzed against 2 M NaCl; the mixture was layered onto 15–30% sucrose gradient in 2 M NaCl and ultracentrifuged for 14 hr at 24,000 rpm in SW-25 rotor of a Spinco L2 ultracentrifuge at 2°. (A) double-labeled particles from rat liver containing $^{14}$C-RNA and $^{125}$I-protein. (compare with fig. 1) ●——●: $^{125}$I, cpm. ○——○: $^{14}$C, cpm. (B) Ehrlich carcinoma particles containing $^{14}$C-labeled protein. ○——○: $^{14}$C, cpm.

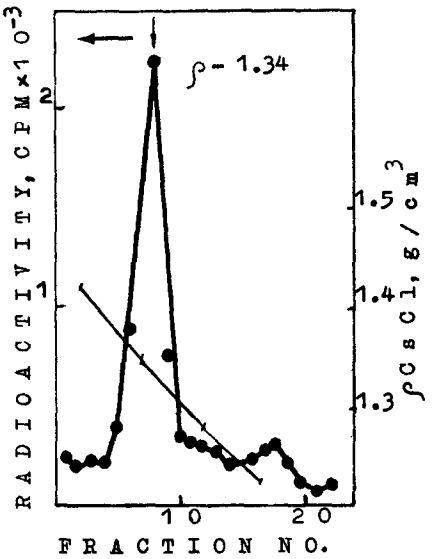


Fig. 3. Banding of free informofers in a CsCl density gradient. The peak from the sucrose gradient (fig. 2A) was collected, fixed with 2% formaldehyde (the same result may be obtained without fixation), 1 ml was layered on 4 ml preformed CsCl density gradient ($\rho$ from 1.4 to 1.2 g/cm$^3$), and centrifuged in an SW-50 rotor of a Spinco L2 ultracentrifuge for 18 hr at 45,000 rpm, 2°. ●——●: $^{125}$I, cpm.

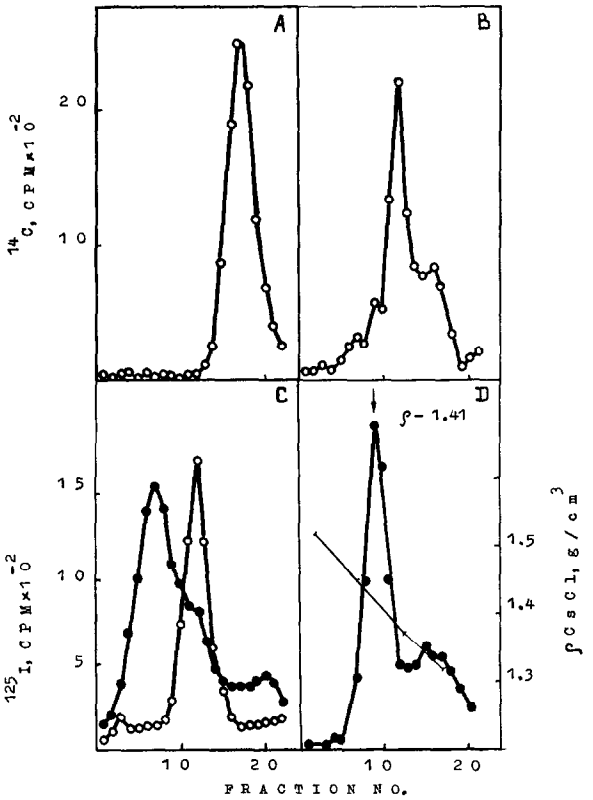


Fig. 4. Sedimentation and density distribution of the products formed as a result of mixing free informofers and dRNA. Free rat liver informofers were mixed in 2 M NaCl with purified dRNA and dialyzed against 0.1 M NaCl, 0.001 M $MgCl_2$, 0.025 M $K_2HPO_4$, pH 7.5. (A) Sedimentation distribution of $^{14}$C-dRNA used in a reconstitution experiment (rotor SW-50, 15–30% sucrose gradient, 50,000 rpm for 2 hr at 2°). (B) Sedimentation distribution of material obtained after mixing $^{14}$C-dRNA from (A) with non-labeled informofers (conditions the same as in (A)). (C) The same as (B) but after mixing non-labeled dRNA with $^{125}$I-labeled informofers. ○——○: 6–10 S dRNA (isolated from 30 S particles) was used for reconstitution. ●——●: 12–18 S dRNA obtained by the hot phenol method was used. (D) Centrifugation through a CsCl density gradient (1.3–1.5 g/cm$^3$) (SW-50, 15 hr at 45,000 rpm, 2°) of material reconstituted from informofers and 12–18 S dRNA.

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