POSSIBLE FUNCTION OF THE PROTEIN BOUND TO NUCLEAR COMPLEMENTARY RNA

S. OLSNES and A. PIHL

Norsk Hydro's Institute for Cancer Research, Montebello, Oslo 3, Norway

Received 17 November 1971

1. Introduction

It is well known that purified nuclear complementary RNA (cRNA) is capable of binding ribosomes *in vitro* [1, 2]. However, no evidence is available that similar binding occurs in cell nuclei. Most, if not all, of the nuclear cRNA is present in cells in the form of a complex with a single type of protein [3, 4], the function of which is not understood. In the present study it is shown that ribosomes do not bind to native nuclear ribonucleoprotein complexes, but that binding occurs when 80–90% of the protein moiety is removed. The data suggest that the protein part may play a role in preventing unspecific and premature binding of ribosomes or their precursors to cRNA.

2. Materials and methods

Ribosomes were isolated from rat livers by treating the postmitochondrial supernatant with 1% Triton X-100 and sedimenting the polysomes through 1 M sucrose, as previously described [5]. The pellet was resuspended in buffer A (0.15 M KCl, 1 mM $MgCl_2$ and 10 mM triethanolamine, pH 7.5). Sucrose density gradient centrifugation showed that the suspension, besides polysomes, contained monoribosomes as well as ribosomal subunits. In the following the preparation is referred to as ribosomes.

Nuclei were isolated from livers of rats 30 min after intraperitoneal injection of 1 mCi ^{3}H-orotic acid as earlier described [5]. cRNA was obtained from the isolated nuclei by the method of fractionated phenol extraction [6, 7], and nuclear ribonucleoprotein particles containing cRNA were extracted by the method of Samarina et al. [3] as earlier described [5]. In order to purify the ribonucleoprotein the supernatant was layered onto a discontinuous sucrose gradient consisting of 5 ml of 1 M sucrose over 0.5 ml of 2 M sucrose in buffer A and centrifuged at 101,000 *g* for 1 hr. Under these conditions the ribonucleoprotein containing cRNA accumulated at the interphase between the 2 sucrose concentrations. The lower half of the sucrose gradient was collected by puncturing the bottom of the tube and was used as the preparation of ribonucleoprotein containing cRNA.

3. Results and discussion

The binding of purified cRNA to ribosomes *in vitro* is demonstrated in fig. 1, which shows the sedimentation patterns of labelled cRNA in CsCl gradients before and after incubation with ribosomes. In the absence of ribosomes (fig. 1B) the labelled cRNA sedimented to the bottom of the tube, as expected, since pure RNA has a buoyant density of $\rho = 1.90$. On the other hand, after incubation with ribosomes a considerable part of the cRNA banded with a buoyant density very close to that of the ribosomes (revealed by the absorbance at 260 nm), as expected for a complex of cRNA and ribosomes. The results are in accordance with findings by other authors [1, 2].

Similar experiments with the nuclear ribonucleoprotein complex containing cRNA showed (fig. 2A) that after incubation with ribosomes the labelled ribonucleoprotein banded with a sharp peak at $\rho = 1.42$, like in the control (fig. 2B), where no ribosomes were present. Virtually no radioactivity banded together with the ribosomes ($\rho = 1.55$) or in the intermediate

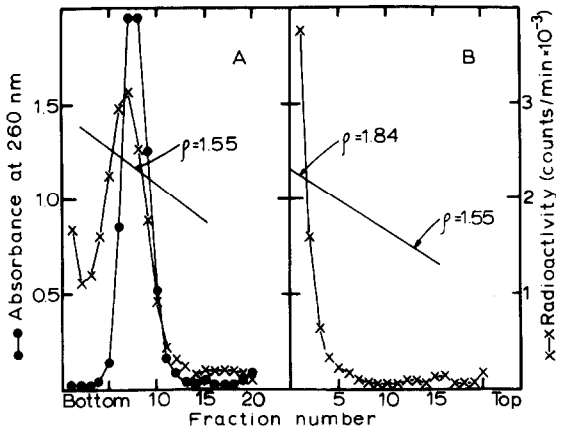

Fig. 1. Sedimentation patterns of labelled cRNA in CsCl density gradients before and after incubation with ribosomes. (A) 10 μg of labelled cRNA was added to 1 mg of unlabelled ribosomes and the mixture was incubated at 0° for 30 min and then fixed with formaldehyde. (B) 10 μg of labelled cRNA was fixed with formaldehyde. After fixation the preparations were submitted to CsCl-gradient centrifugation.

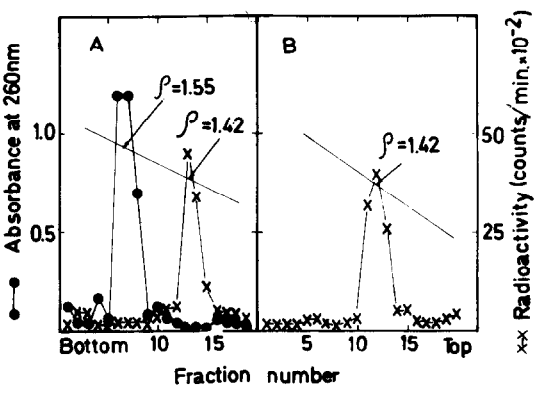

Fig. 2. Inability of nuclear cRNA-containing ribonucleoprotein to bind to ribosomes. A preparation of nuclear ribonucleoprotein containing cRNA was divided into 2 parts, to one of which (A) 1 mg of unlabelled ribosomes was added, whereas the other part (B) was used as control. After incubation for 30 min at 0° the preparations were fixed with formaldehyde and submitted to CsCl-gradient centrifugation.

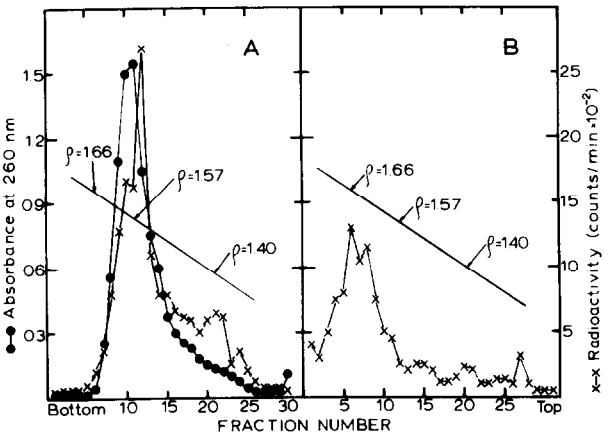

Fig. 3. Binding of cRNA to ribosomes after treatment of the nuclear cRNA containing complexes with sodium deoxycholate. Ribonucleoprotein containing cRNA was prepared as in fig. 2 and made up to contain 1% sodium deoxycholate. The preparation was then divided into 2 parts, to one of which (A) 1 mg of unlabelled ribosomes was added, whereas the other part (B) was used as control. After 30 min the preparations were fixed with formaldehyde and submitted to CsCl gradient centrifugation.

zone. Also, when the incubation was carried out at 37°, no binding of ribosomes to intact nuclear ribonucleoprotein was found (data not shown). The data indicate that the intact nuclear ribonucleoprotein containing cRNA is unable to bind to ribosomes or to their constituent parts.

Deoxycholate removes part of the protein from ribonucleoprotein complexes containing cRNA [5, 8]. It is seen in fig. 3A that after treatment of the ribonucleoprotein complex with sodium deoxycholate the main part of the radioactivity banded at ρ = 1.57, together with the ribosomes, whereas in the control there was little radioactivity at this density (fig. 3B), and the main part of the cRNA banded at a distinctly higher buoyant density (ρ = 1.63–1.66). It is therefore clear that ribosomes become bound to the ribonucleoprotein treated with deoxycholate. From the increase in buoyant density of the ribonucleoprotein from ρ = 1.42 (fig. 2B) to ρ = 1.63–1.66 (fig. 3B) it can be estimated that the deoxycholate-treatment had removed 80–90% of the protein [9]. Apparently the remaining protein did not prevent the binding of the cRNA to ribosomes.

The present results indicate that the protein of the nuclear complex inhibits the binding between cRNA and ribosomes. In contrast, the protein bound to the

mRNA in polysomes, which is different from that bound to the nuclear cRNA [10], seems to be necessary for the binding of the messenger RNA to the 40 S ribosomal subunit under similar conditions [11]. It is widely believed that nuclear cRNA is a precursor of mRNA [12–14]. The possibility should therefore be considered that the protein bound to the cRNA plays a role in the inhibition of unspecific and premature binding of ribosomes or their precursors during the maturation of the mRNA in the nucleus.

Acknowledgements

The authors wish to thank Dr. P. Eker for valuable discussion and Miss Sigrid Opstad for technical assistance. The work was supported by The Norwegian Cancer Society.

References

[1] H. Naora and K. Kodaira, Biochim. Biophys. Acta 182 (1969) 469.
[2] H. Naora and M.J. Pritchard, Biochim. Biophys. Acta 246 (1971) 269.
[3] O.P. Samarina, E.M. Lukanidin, J. Molnar and G.P. Georgiev, J. Mol. Biol. 33 (1968) 251.
[4] A. Sarasin, FEBS Letters 4 (1969) 327.
[5] S. Olsnes, Biochim. Biophys. Acta 213 (1970) 149.
[6] G.P. Georgiev and V.L. Mantieva, Biochim. Biophys. Acta 61 (1962) 153.
[7] S. Olsnes, European J. Biochem., in press.
[8] Y. Moulé and J. Chauveau, J. Mol. Biol. 33 (1968) 465.
[9] R.P. Perry and D.E. Kelley, J. Mol. Biol. 16 (1966) 255.
[10] S. Olsnes, European J. Biochem., in press.
[11] B. Lebleu, G. Marbaix, G. Huez, J. Temmerman, A. Burny and H. Chantrenne, European J. Biochem. 19 (1971) 264.
[12] G.P. Georgiev, J. Theoret. Biol. 25 (1969) 473.
[13] K. Scherrer, G. Spohr, N. Granboulan, C. Morel, J. Grosclaude and C. Chezzi, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) 539.
[14] J.E. Darnell, G.N. Pagoulatos, U. Lindberg and R. Balint, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) 555.