THE PROTEINS ASSOCIATED WITH mRNA FROM UNINFECTED AND ADENOVIRUS TYPE 5-INFECTED KB CELLS

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1. Introduction

It has been shown that mRNA in polyribosomes is associated with protein [1–4]. Up to now little is known about the function of these proteins and their diversity is a matter of dispute [5].

In the highly differentiated cells, which have been studied so far and which synthesize only one or a few proteins, polysomal mRNP* sediments more slowly than the small ribosomal subunit. In less differentiated cells the sedimentation values of polysomal mRNPs and ribosomal subunits overlap [2]; thus purification of mRNP is hard to achieve. A few reports concerning the protein composition of the mRNP in these cells have been published [6–8]. Some of the analyses involved destruction [6], others high salt treatment of the particles [7]. In one case affinity chromatography was used [8], which does not leave the particles fully intact. Even in the relatively simple systems of the highly differentiated cells reports about the composition of the protein components are not unanimous [9–11].

In this communication a new isolation technique is described, which leaves mRNP intact. It depends on differences in electrophoretic mobility in polyacrylamide agarose composite gels between ribosomal subunits and mRNP complexes.

Protein moieties of pure mRNP from uninfected HeLa and KB cells and from KB cells 18 hr after infection with Adenovirus type 5 (Ad 5) were analyzed on SDS-urea polyacrylamide gels. In both uninfected HeLa and KB cells 6–7 distinct proteins with mol. wts ranging from 45–95 $\times 10^3$ were found. Infected KB cells contained one additional, possibly virus induced protein with a mol. wt of approx. 105×10^3.

* *Abbreviations*: mRNP–messenger ribonucleoprotein. SDS–sodium dodecylsulphate. rRNA–ribosomal RNA.

2. Materials and methods

HeLa cells were grown on monolayers; one day before an experiment they were brought into a spinner culture at an initial density of 2.5×10^5 cells/ml. KB cells were grown in spinner cultures at densities ranging from $1–3 \times 10^5$ cells/ml.

Cell culture media were based on minimal essential medium according to Eagle [12] and supplemented with extra glucose and vitamins; for spinner cultures 5% heat-inactivated horse serum and for monolayers 10% calf serum were included. Penicillin and streptomycin were added to the culture media.

KB cells were infected with a clarified lysate of of Ad 5-infected cells. Infection was carried out at a multiplicity of 100 PFU.

Labelling of cells with [^{3}H]uridine was done in the presence of a low dose (0.04 μg/ml) actinomycin D to inhibit rRNA* synthesis.

After labelling cells were poured out on crushed, frozen saline and harvested by centrifugation. Cytoplasmic extracts from HeLa cells were obtained by incubating the resuspended cell pellet during 10 min in 8 vol TEAMK (20 mM Triethanolamine, pH 7.8; 50 mM KCL; 1 mM $MgCl_2$) containing 0.1% Triton X-100. Cytoplasmic extracts from uninfected and

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infected KB cells were prepared in the following way: after suspending the cell pellet in 8 vol TEAMK, the cells were allowed to swell for 10 min; they were then homogenized by 10 strokes with a tight fitting Dounce homogenizer. After disposal of nuclei and cell debris by centrifugation the extracts were layered on discontinuous sucrose gradients consisting of 1.6 and 2.0 M sucrose in TEAMK and centrifuged at 226 000 g_{max}. The pellets consisting of polysomes, ribosomes and some ribosomal subunits were suspended in TEAMK, layered on 15–30% sucrose gradients on top of a 2 M sucrose cushion and centrifuged. Fractions containing polysomes were pooled and centrifuged. Pellets were resuspended in a small volume of buffer G (40 mM Triethanolamine, pH 7.6; 20 mM NaAc; 6 mM EDTA) or stored at −70°C.

The methods described above resulted in polysomal preparations virtually free of nuclear contamination, as shown by CsCl centrifugation [13].

Preparative electrophoresis of mRNP and ribosomal subunits was carried out as follows: to about 1 mg of polysomes suspended in 100 μl of buffer G, 50 μl of 20% sucrose in H_2O and 2 μl of bromophenolblue solution were added. This mixture was layered on top of a pre-electrophorized 1 X 7 cm 1% polyacrylamide 0.5% agarose composite gel; the ratio acrylamide: methylenebisacrylamide was 19:1. Running buffer consisted of buffer G with 0.1% 2-mercaptoethanol. Electrophoresis was carried out at 4°C for approx. 20 hr at a voltage of 40 V, using a tube equipped with an elution chamber. This chamber was a slightly modified version of the model originally described by Popescu [14]. Eluted material was continuously pumped away and fractionated. Absorbance at 260 nm was either measured continuously before fractionation on a LKB Uvicord II or afterwards manually on a Zeiss PMQ II spectrophotometer. Samples from every fraction were taken for calculating radioactivities.

Appropriate fractions were pooled, treated with 2 vol of ethanol at −20°C overnight and centrifuged for 2 hr at 12 000 g_{max}. Pellets were resuspended in 50 μl of sample buffer (10 mM sodium phosphate, pH 7.0; 1% SDS; 1% 2-mercaptoethanol in 6 M urea).

Proteins were analyzed on 10% polyacrylamide gels with a length of 10 cm and a diameter of 6 mm. Electrophoresis was carried out as described by Weber and Osborn [15]. The samples were heated for 1 min at 100°C before application. After electrophoresis gels were fixed and stained with Coomassie Brilliant Blue. After destaining the gels were scanned at 600 nm on a Gilford spectrophotometer equipped with a linear transport mechanism.

3. Results and discussion

In fig.1 the sedimentation behaviour of mRNP and ribosomal subunits is shown. A major part of the mRNPs, as indicated by the [^{3}H]radioactivity, sediments in the region of the 50S and 30S ribosomal subunits. The heterogeneity in mRNPs sedimenting from 20S to more than 80S is apparent. It is clear that separation of mRNPs from ribosomal subunits on base of differences in sedimentation values will yield only a very limited part of all mRNPs.

A good separation between mRNPs and ribosomal subunits is obtained by centrifugation in CsCl density gradients [1,16]. The particles have to be fixed prior to centrifugation, however, to prevent dissociation. This treatment prevents further analysis of the proteins.

Differences in buoyant densities in CsCl between particles consisting of RNA and protein reflect differ-

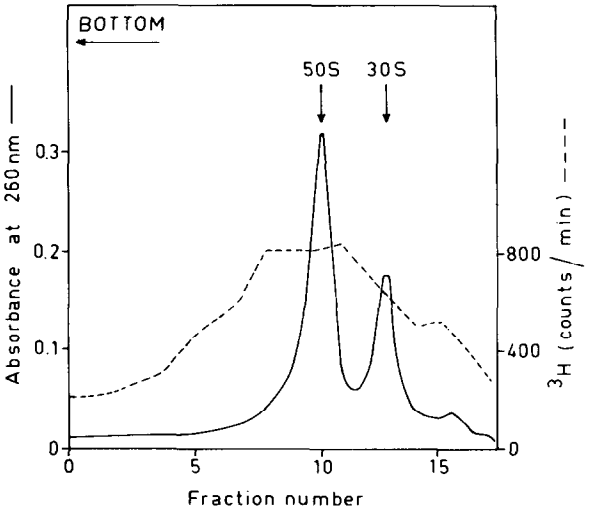

Fig.1. Sedimentation of mRNP and ribosomal subunits in a 15–30% sucrose gradient in buffer G. Centrifugation occurred in a SW 50.1 rotor at 50 000 rev/min for 1.5 hr.

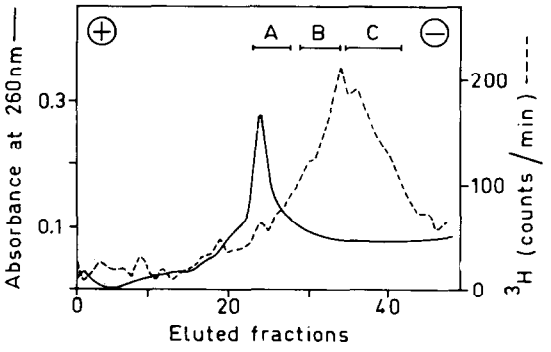

Fig.2. Separation of mRNP and ribosomal subunits by preparative electrophoresis. (The baseline always rises to some extent, even when blank gels are run). For details see the text.

ences in their ratio RNA: protein; mRNP particles contain more protein than ribosomal subunits [17].

Based on these considerations we tried to achieve a separation between mRNPs and ribosomal subunits by subjecting them to electrophoresis under conditions where mobility largely depended on the charge of the particles. When applied to a 1–1.5% polyacrylamide–0.5% agarose composite gel the two ribosomal subunits moved with nearly the same mobility; the group of heterogeneous mRNP also showed about the same mobility, which was lower than that of the subunits. mRNP obtained in this way was analyzed on CsCl density gradients. It had a density of approx. 1.43 g/ml, the value also obtained for untreated material. The eluted mRNP contained mRNA with a similar length distribution as untreated material when analyzed on polyacrylamide gels (data not shown). We conclude that our procedure leaves mRNP intact.

To obtain amounts of mRNP sufficient for analysis of the proteins we used electrophoresis on preparative scale with continuous elution of material from the gel. In fig.2 the results of this method are shown. Fractions indicated by bars were pooled and used for further analysis. As can be seen from the figure fraction A contained most of the ribosomal material, but only a very small amount of mRNP. In fraction B the quantity of mRNP has increased greatly, whereas very little ribosomal material has been left. Fraction C ultimately contains only mRNP. In this way over 50% of the mRNP can be obtained free from contaminating ribosomal subunits.

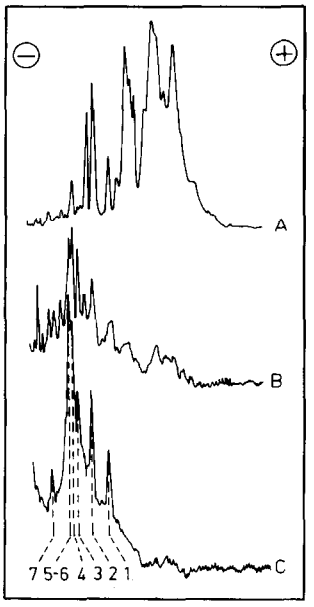

Fig.3. Electrophoretic analysis of mRNP proteins from uninfected KB cells in SDS-urea polyacrylamide gels. A, B and C correspond to the pooled fractions, as indicated in fig.2. Electrophoresis was carried out at approximately 4 V/cm during 12 hr. Absorbance range for A is 3.0 OD units, for B and C 0.5 OD units. For further details see the text.

Proteins present in the pooled fractions A, B and C were analyzed on SDS-urea polyacrylamide gels. The results from an experiment where particles of uninfected KB cells were analyzed, are shown in fig.3 and table 1.

The mol. wts of the great majority of the proteins in gel A are from 15 000–60 000, in agreement with published values for ribosomal proteins of human origin [18]. In gel B the amount of ribosomal proteins is greatly reduced. (Note the difference in scale of the absorbance plots.) On the other hand some proteins mainly in the zone of higher mol. wts, have become predominant. In gel C only these 6–7 predominant components are discernable, whereas the amount of ribosomal proteins is virtually zero. From these results it is concluded that the proteins in gel C are mRNP specific. Our results suggest a more complex composition of the protein moiety of mRNP than has been described by Blobel [6] and Bryan and Hayashi [7]. However, their results have been

Table 1
Mol. wts of proteins associated with mRNA from three cell types

Protein number	Mol. wts X 10^{-3}		
	HeLa	KB	Ad 5 infected KB
1	45 ± 3	46 ± 3	42 ± 3
2	58 ± 3	56 ± 3	55 ± 3
3	67 ± 3	66 ± 3	63 ± 3
4		72 ± 3	71 ± 3
5	71–78 ± 3	74–78 ± 3	75 ± 3
6			78 ± 3
7	92 ± 4	93 ± 4	93 ± 4
8			105 ± 5

Protein numbers correspond to those indicated in figs.3 and 4. Mol. wts were determined using bovine serum albumin, carbonic anhydrase and myoglobin as markers.

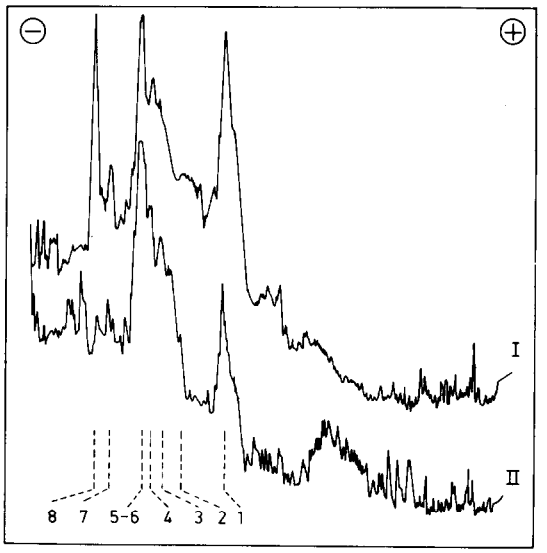

Fig.4. Electrophoretic analysis of mRNP proteins from uninfected and Ad 5 infected KB cells. I – proteins from uninfected cells; mRNP was derived from the pooled fraction C, as described in fig.2. II – proteins from Ad 5 infected cells. In this experiment the gel concentration was 7.5%. Electrophoresis was performed at approximately 3.5 V/cm for 13 hr. For further details see the text.

obtained with polysomes washed with 0.5 M KCl, a procedure which splits off part of the proteins of both ribosomal subunits and mRNP [8,13,19,20], or with polysomes treated with RNase. The latter procedure results in degradation of mRNP [6]. Only two proteins, mol. wts approx. 50 000 and 78 000, are found in their experiments. Proteins with similar mol. wts are also present in our mRNP preparations. The greater complexity of the protein component in our preparations is in agreement with that found by Lindberg and Sundquist [8] for KB cell mRNP and that observed by Morel [21] for duck erythrocyte mRNP.

Proteins of mRNP from HeLa polysomes were analyzed in the same way and compared with those of KB mRNP (table 1). In HeLa mRNP also 6–7 components with electrophoretic mobilities equal to those of the KB components were found. It appears that mRNP from HeLa and KB cells, both originating from human epithelial carcinomas, contains 6–7 proteins which behave identically during electrophoresis in SDS and urea and thus are probably of the same size. Whether they represent identical components has to await further studies.

In order to investigate the occurrence of possible species-specific proteins in mRNPs we have compared mRNP isolated from uninfected KB cells and from cells 18 hr after infection with Adenovirus type 5. The results are shown in fig.4 and table 1. mRNP from infected cells contains one additional component with a mol. wt of 105 000 possibly virus specific.

Recently Lindberg and Sundquist [8] demonstrated the occurrence of 4 different proteins in mRNP from uninfected KB cells. Their mol. wts were approx. 56 000; 68 000; 78 000; 125 000. Cells infected with Adenovirus type 2 contained one additional component with a mol. wt of approx. 110 000. Protein 1 in our experiments (mol. wt 44 000), which has not been found by Lindberg and Sundquist might originate from the 5S RNA containing RNP complex freed by dissociation of polysomes with EDTA. This has been shown to contain a protein with similar mol. wt [10]. At the present time we cannot exclude the possibility that this RNP migrates with the same rate as mRNP (fig. 2).

To which extent differences between the results of the Swedish authors and those of us are due to the totally different mRNP isolation remains to be seen. The detection by both techniques of a polypeptide of mol. wt approx. 105–110 X 10^3 in KB cells in-

fected with both Adenovirus type 2 and type 5 deserves special interest.

Acknowledgements

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