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Effect of homopolyribonucleotides on messenger ribonucleoprotein particles

Bo Sundquist† and Torgny Persson

Department of Microbiology, The Wallenberg Laboratory, Uppsala University, Uppsala, Sweden

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ABSTRACT

A discrete set of polypeptides copurify with and appear to be specifically attached to mRNA from polysomes of eukaryotic cells. This report describes the effect of homopolyribonucleotides on mRNA-protein complexes separated from ribosome subunits by oligo(dT)-cellulose chromatography. It is shown that poly(U) and poly(A) can release mRNA-protein complexes adsorbed to oligo(dT)-cellulose, whereas poly(C) and poly(I) are much less effective in this process. Analysis of polyribonucleotide released material showed that poly(U) effectively dissociated the mRNA-protein complexes while poly(A) caused no or only partial derangement of these particles. The specificities seen in the polyribonucleotide effects in turn suggest a high degree of specificity in the interaction between the proteins and mRNA.

INTRODUCTION

Messenger RNA-protein complexes can be isolated by affinity chromatography on oligo(dT)-cellulose (1, 2, 3). We have utilized this technique for further characterizing mRNA-protein complexes. The preceding report (4) described evidence strongly suggesting that the interaction between mRNA and protein in such complexes is of a highly specific nature. It was confirmed that a 78,000 molecular weight polypeptide is associated with the poly(A) containing 3'end of mRNA and also demonstrated that there is an additional small fraction of the mRNA which is associated with protein.

Messenger ribonucleoprotein particles recovered from oligo(dT)-cellulose with the use of formamide containing buffers is unable to direct translation in an *in vitro* protein synthesizing system. Most likely this is due to adverse effects of formamide on mRNA associated proteins since active mRNA can be recovered from these inactive particles (unpublished results), and since globin mRNP is readily translatable (5, 6, 7, 8, 9).

We have looked for alternative conditions to release mRNP from oligo (dT)-cellulose. It was reported recently that poly(U) prevents heterogeneous nuclear ribonucleoproteins from adsorbing to oligo(dT)-cellulose (3). This led us to test whether polyribonucleotides in general or with specificity could displace already adsorbed mRNP complexes from oligo (dT)-cellulose columns. We have found that mRNA plus protein are eluted from oligo(dT)-cellulose by poly(U) and poly(A), but not by poly (C) and poly(I). Furthermore, poly(U) efficiently dissociates the mRNA-protein complexes, whereas poly(A) does not. At low concentrations of poly(A) the eluted mRNP appears intact and only limited dissociation occurs at high concentrations of this polyribonucleotide. These results give further support to the idea that the messenger ribonucleoprotein particles are highly specific structures.

MATERIALS AND METHODS

Cells were grown, infected with adenovirus type 2 (Ad2), labeled and fractionated as outlined in the accompanying paper (4). Polysomes were prepared as described by Kumar and Lindberg (10) and mRNA-protein complexes adsorbed on oligo(dT)-cellulose under the conditions given previously (1). The effect of synthetic polyribonucleotides (Sigma Chem. Corp.) was examined in 0.2 M NaCl, 50 mM Tris-HCl pH 7.8, 10 mM EDTA at the polymer concentrations given in the text and in figure legends. Material eluted from oligo(dT)-cellulose with polyribonucleotides was analyzed by centrifugation in 15-30% sucrose gradients in 10 mM NaCl, 10 mM Tris-HCl pH 7.8, 10 mM EDTA. The gradients were centrifuged in a Spinco SW27 rotor at 22,000 rpm for 15 hours (+4°C) and the fractions collected were analyzed for TCA insoluble radioactivity.

Electrophoresis in slab gels (1.5 mm thick, 70 mm long) was run in the discontinuous system described by Maizel (11) containing 10% acrylamide, 0.35% bisacrylamide and a buffer containing 0.1% sodium dodecylsulphate. The electrophoresis equipment of Pharmacia (Uppsala, Sweden) was used under conditions described by Everitt and Philipson (12). After electrophoresis gels were dried under vacuum and analyzed by autoradiography using Kodak X-ray film, type XG-14.

The buoyant density of mRNP particles fixed with glutaraldehyde (6%) was determined in CsCl gradients as described by Baltimore and Huang (13). Samples were altered on preformed gradients and centri-

fuged in a Spinco SW56 rotor at 35,000 rpm for 24 hours (+4°). Radioactivity of fractions was analyzed on Whatman 3 MM filter paper discs as described before (1).

RESULTS

Effect of different polyribonucleotides on messenger ribonucleoprotein particles adsorbed to oligo(dT)-cellulose.

To investigate if polyribonucleotides can affect mRNP complexes adsorbed to oligo(dT)-cellulose the following experiments were performed. Polysomes labeled with (^{3}H)-uridine were prepared from adenovirus infected cells and dissociated with EDTA. The polysomal mRNP was adsorbed to oligo(dT)-cellulose columns at +4°C, and the columns were thoroughly washed to remove ribosome subunits (1). After warming the columns to room temperature the polyribonucleotide containing buffers were passed through the columns. Material remaining on the columns after this treatment was removed from the resin with formamide-high salt buffer as described in the legend to Fig. 1. The results obtained with four different homopolyribonucleotides are shown in Fig. 1 A-D. Both poly(U) (panel A) and poly(A) (panel B) eluted the major part (70-80%) of bound labeled mRNA. With poly(C) and poly(I), however, panels C and D, 20 and 50% of adsorbed radioactivity respectively was released. This should be compared with the 20% of adsorbed material released by merely warming the columns to room temperature. Analysis of phenol extracted RNA from poly(A) released material by electrophoresis on composite polyacrylamide-agarose gels (data not shown) gave rise to patterns of labeled RNA characteristic of the polysomal mRNA from adenovirus infected cells as described earlier (14,1).

The experiments illustrated in Fig. 1 were performed at high concentrations (2 mg/ml) of the polyribonucleotides. The dependence of the eluting capacity of poly(U) and poly(A) on the concentration of the polyribonucleotides was also investigated. It was found that both these polyribonucleotides caused significant displacement of bound mRNA and protein already at 20 µg/ml, and that the fraction released increased with increasing polyribonucleotide concentration up to a plateau reached at 0.1 mg/ml. Closely similar concentration dependence curves were obtained for the two polyribonucleotides (data not shown).

The relationship between chain length of the polymer and its eluting power was also examined. For this purpose poly(U) was hydro-

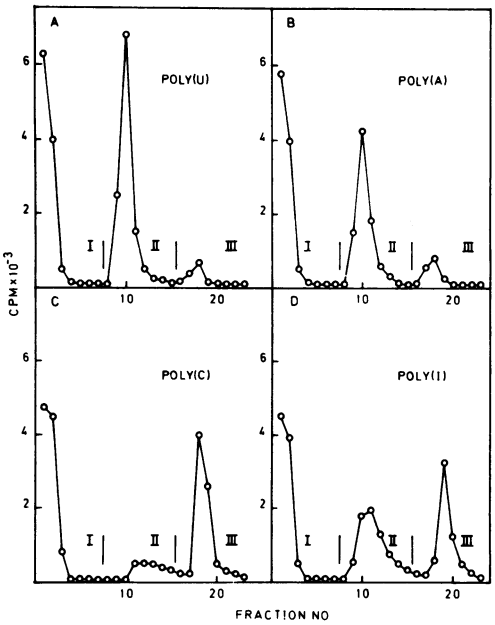

Fig. 1 : Effect of homopolyribonucleotides on mRNP adsorbed to oligo (dT)-cellulose. Polysomes were prepared from Ad2 infected cells labeled with (^{3}H)-uridine 14-16 hours after infection. Polysomal components dissociated with EDTA were applied to oligo(dT)-cellulose columns, and the columns were washed with 7x1 ml of 0.2 M NaCl, 50 mM Tris-HCl, pH 7.8, 10 mM EDTA (fraction I) and eluted with 8x1 ml of the same buffer now containing 2 mg/ml of either polyribonucleotide (fraction II), and with 8x1 ml of 1% sodium dodecylsulphate in 10 mM Tris-HCl pH 7.8 (fraction III). Samples from the fractions were precipitated and counted on Whatman 3 MM filters. The homopolyribonucleotides used were poly(U), poly(A), poly(C) and poly(I) respectively in the experiments illustrated in panels (A), (B), (C), and (D).

lyzed in alkali (0.3 M NaOH, 20 minutes, at room temperature), and resulting oligonucleotides of different chain lengths were separated by chromatography on Sephadex G-50. Two size classes, 5-15 and 15-30 nucleotide long oligonucleotides, were tested and compared with the commercially available poly(U) (10-15S, approx. 1000 nucleotides in length). It was found that oligo(U)$_{5-15}$ only released 50% of the amount eluted with high molecular weight poly(U), whereas oligo(U)$_{15-30}$ was nearly as effective as the poly(U).

Polypeptide pattern of mRNP recovered from oligo(dT)-cellulose by polyribonucleotide elution.

Earlier studies showed that the mRNP fraction obtained by

affinity chromatography on oligo(dT)-cellulose is characterized by the presence of a specific set of a few major polypeptides occurring preferentially in the 50,000 - 150,000 molecular weight range. In the following experiment the polypeptide patterns of the mRNA containing material recovered from oligo(dT)-cellulose by polyribonucleotide elution was examined. Cells were labeled with (^{35}S)-methionine 10-16 hours (late) after adenovirus infection, polysomes were prepared and dissociated with EDTA, and mRNA-protein complexes were adsorbed to oligo(dT)-cellulose as described in Materials and Methods, and legend to Fig. 1. Adsorbed material was eluted with buffers containing 0.1 mg/ml of poly(U) or poly(A) and the polypeptides of the eluted material was separated by SDS-polyacrylamide slab gel electrophoresis and visualized autoradiographically as shown in Fig. 2A and B. Major polypeptide bands are seen at the 130K, 100K, 78K and 50K positions, although there are also components of lower molecular weight present. Staining of the gels with Coomassie blue showed that these major labeled polypeptides corresponded to major stained bands (data not shown) and also revealed a 56K polypeptide which apparently is poorly labeled with (^{35}S)-methionine (see also Lindberg and Sundquist, 1974). It should be noted that the polyribonucleotides cause the appearance of a 50K polypeptide in the mRNA containing fraction. This polypeptide was not seen when mRNA-protein complexes were recovered from oligo(dT)-cellulose with formamide containing buffers. However, a polypeptide of this size was then one of the major components released by subsequent washing of the oligo(dT)-cellulose under harsher conditions (4).

Analysis of polyribonucleotide eluted material by sucrose gradient centrifugation.

It was demonstrated above that the major part of labeled mRNA was eluted together with a set of polypeptides closely similar to the one found in combination with adenovirus mRNA under other conditions. (1,15,4). To investigate whether these polypeptides were associated with mRNA under the present conditions polyribonucleotide eluted mRNA-protein mixtures were analyzed by sucrose gradient centrifugation. Messenger RNA-protein complexes from cells labeled either with (^{35}S)-methionine or (^{3}H)-uridine late during the adenovirus infection were adsorbed to oligo(dT)-cellulose columns and eluted with either poly(U) or with poly(A), at high (2 mg/ml) or low (0.1 mg/ml) concentrations of the polyribonucleotides. Samples of the eluted material were layered on

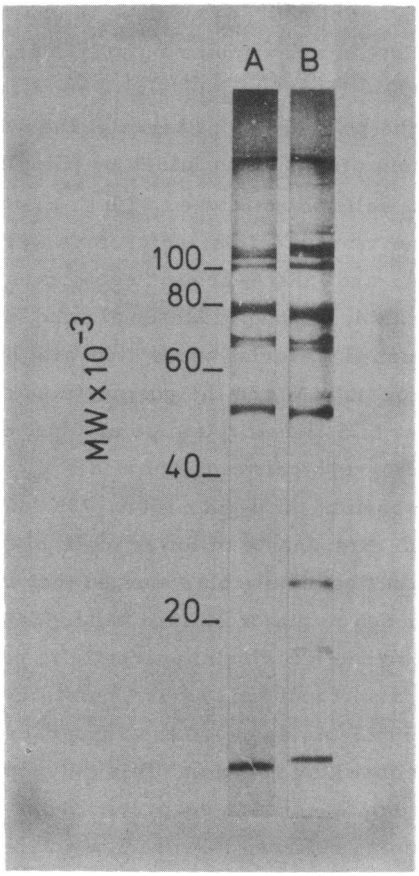

Fig. 2 : Polypeptide composition om mRNP complexes eluted from oligo (dT)-cellulose with homopolyribonucleotides. Cells were infected with Ad2 and labeled with (^{35}S)-methionine 10-16 hours after infection. Polysomes were EDTA treated and the components applied to oligo(dT)-cellulose as described in Materials and Methods. Material eluted with 0.1 mg/ml of poly(U) or poly(A) (fraction II in Fig. 1) was precipitated and analyzed by SDS-polyacrylamide gel electrophoresis (see Materials and Methods). Gel (A) shows polypeptides of material eluted with poly(U) and gel (B) those eluted with poly(A).

15-30% sucrose gradients, centrifuged and analyzed as described under Materials and Methods and in the legend to Fig. 3. Figure 3 A and B show that if messenger ribonucleoprotein material was eluted from oligo(dT)-cellulose with a buffer containing a low concentration of poly(A) the labeled mRNA and protein cosedimented in the sucrose gradient. The use of the higher concentration of poly(A) for the elution of the mRNP fraction resulted in a partial conversion of (^{35}S)-methionine labeled material to slower sedimenting structures (Fig. 3 C). Figure 3 E and F,

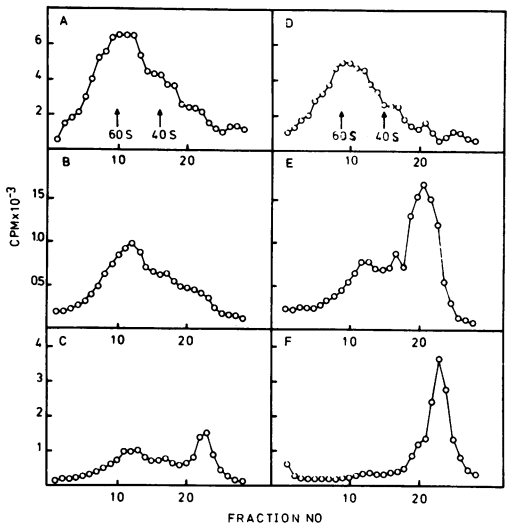

Fig. 3 : Sucrose gradient centrifugation on polyribonucleotide eluted mRNP. Polysomes were prepared from cells labeled with either (^{3}H)-uridine or (^{35}S)-methionine 10-16 hours after infection with adenovirus. The polysomes were then dissociated with EDTA and chromatographed on oligo(dT)-cellulose columns, messenger ribonucleoproteins were eluted with different polyribonucleotides as described in Fig. 1 , and samples were analyzed by sucrose gradient centrifugation as described under Materials and Methods. Material from the fractions was precipitated with TCA collected and analyzed for radioactivity on Millipore filters. Sedimentation is from right to left. The samples analyzed were labeled and eluted at polymer concentrations as follows: panel(A), (^{3}H)-uridine, 0.1 mg/ml of poly(A) ; panel(B)(^{35}S)-methionine, 0.1 mg/ml of poly(A) ; panel(C)(^{35}S)-methionine, 2 mg/ml of poly(A) ; panel(D) (^{3}H)-uridine, 0.1 mg/ml of poly(U) ; panel (E)(^{35}S)-methionine , 0.1 mg/ml of poly(U) ; panel (F)(^{35}S)-methionine, 2 mg/ml of poly(U).

respectively, show the analysis of (^{35}S)-methionine labeled material obtained by eluting the oligo(dT)-cellulose in the presence of 0.1 and 2 mg/ml of poly(U). Here even in the case of the lower concentration of the polyribonucleotide the sedimentation pattern of (^{35}S)-methionine labeled protein diverged significantly from that of (^{3}H)-uridine labeled material (Fig. 3 D). After exposure to 2 mg/ml of poly(U) (Fig. 3 E) virtually all of the protein appeared as a slow sedimenting peak. This rather homogenous peak was characterized by a sedimentation coefficient of 15-20S and appeared in the front part of an UV-absorbing peak containing the poly(U).

Figure 4 shows the polypeptide analysis performed on poly(A) and poly(U) eluted material fractionated on sucrose gradients. In the

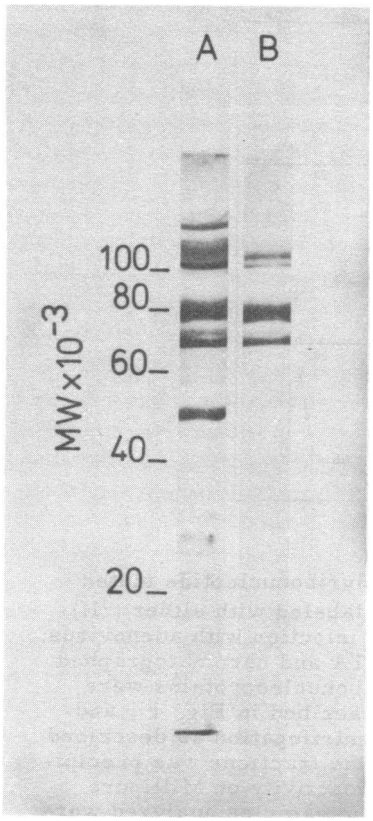

Fig. 4 : Polypeptide compositions of mRNP exposed to poly(A) and fractionated by sucrose gradient centrifugation. Messenger ribonucleoprotein complexes from polysomes prepared as described in Fig. 2 were eluted from oligo(dT)-cellulose with buffer containing 2 mg/ml of poly(A) and fractionated by sucrose gradient centrifugation as in Fig. 3. Fractions from the gradients were pooled (7-18, and 21-25 in Fig. 3A), precipitated with TCA and precipitates analyzed by SDS - polyacrylamide gel electrophoresis as described in Materials and Methods. Gel (A) shows polypeptides cosedimenting with mRNA after poly(A) elution and gel (B) polypeptides appearing in a slow sedimenting peak after exposure of mRNP to high concentration of poly (A) (Fig. 3 C).

case utilizing 2 mg/ml of poly(A) for the elution all the polypeptides (Fig. 4 A) commonly occurring in the mRNP fraction were recovered in fast sedimenting structures. The small peak, which appeared at about 15S (Fig. 3C) when mRNP complexes had been exposed to high concentrations of poly(A) contained some but not all the polypeptides characterizing the mRNP fraction (Fig. 4 B). The significance of this selective dissociation of certain polypeptides from mRNP with poly(A) is not known. When instead poly(U) was used all the polypeptides (Fig. 2A) were found in the slow sedimenting material (Fig. 3 F), apparently dissociated off from mRNA, which sedimented much faster (data not shown).

Buoyant density of poly(A) and poly(U) eluted mRNP

The mRNA-protein complexes eluted from oligo(dT)-cellulose at low concentrations of polyribonucleotides (0. 1 mg/ml) were characterized by density gradient centrifugation. Samples of the mRNP fractions were fixed with glutaraldehyde and banded in CsCl as described in Fig. 5. Ninety percent of the poly(A) eluted material was recovered in

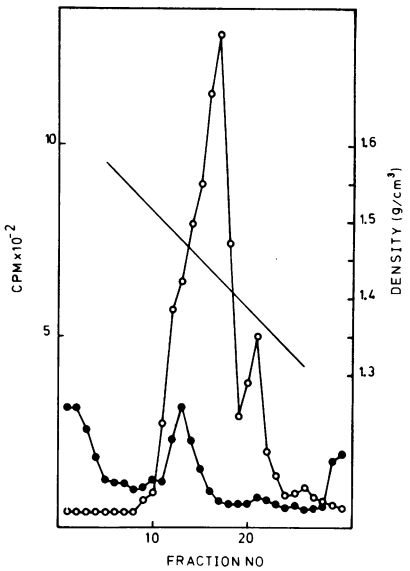

Fig. 5 : Density distribution of mRNP released from oligo(dT)-cellulose with poly(U) or poly(A). Messenger ribonucleoproteins from cells labeled and fractionated as in Fig. 3 were released from oligo(dT)-cellulose with either 0.1 mg/ml of poly(U) (closed circles) or poly(A) (open circles). Samples were fixed with 6% glutaraldehyde and analyzed by CsCl density gradient centrifugation as described in Materials and Methods.

the density range 1.35 - 1.50 g/cm^3 with the major peak at a mean density of 1.42 g/cm^3. This is close to the values reported earlier for polysomal mRNP prepared in various ways, summarized in Irwin et al. (2) and suggests that mRNP particles prepared in this way also consist of about 60% protein and 40% RNA. The poly(U) eluted material behaved quite differently. Here only a small fraction (20%) of the labeled mRNA banded at densities near those reported for mRNP in general. The main part of the mRNA banded at higher densities confirming the results concerning the dissociating effects of poly(U) on mRNA-protein complexes described above (Fig. 3 E and F).

DISCUSSION

Protein free mRNA is released from oligo(dT)-cellulose at low salt concentration, while displacement of mRNP complexes requires a combination of salt and formamide. This implies that both the poly(A) of mRNA and one or several of the mRNP proteins contribute to the binding of mRNP to oligo(dT)-cellulose (1). The experiments described here (Fig. 1)

show that the homopolyribonucleotides poly(U) and poly(A), but not poly(C), release mRNP complexes from oligo(dT)-cellulose. Poly(I) had an intermediate effect. Poly(U) and poly(A) are the two polyribonucleotides, which would be expected to interfere with the poly(A) dependent part of the binding of mRNP to oligo(dT)-cellulose; poly(U) by hybridizing to poly(A) of mRNA and poly(A) through its affinity for oligo(dT). These results support the interpretation that under the conditions used a significant part of the poly(A) in mRNP is available for base pairing interactions and provides the main binding site on mRNP for oligo(dT) even though the poly(A) of mRNA apparently is associated with proteins (16,17). Similar conclusions have been presented by Favre et al. (18) reporting studies on the interaction of poly(U) with globin mRNA and mRNP using ethidium bromide binding to monitor hybrid formation.

The set of polypeptides released together with mRNA upon elution with poly(U) or poly(A) is closely similar to the one obtained with formamide eluted material as described previously by Lindberg and Sundquist (1) and in the accompanying paper by Sundquist et al. (4). There is one significant difference, however. The homopolymer eluted material contains a 50K polypeptide which is missing in the main mRNP fraction recovered with formamide. As shown in Fig. 4 A this polypeptide also sediments along with mRNA if the mRNP has been detached from the oligo(dT)-cellulose with poly(A) containing buffers. When formamide is used for the elution of mRNP the 50K polypeptide apparently dissociates off from mRNP and remains attached to oligo(dT)-cellulose (4). It can be recovered from fractions eluted with buffers containing higher concentrations of formamide.

Messenger ribonucleoprotein particles appear to be recovered from oligo(dT)-cellulose in intact form, when they are eluted with low concentrations of synthetic poly(A). (Fig. 3 B). At high concentrations of the polymer there is a partial dissociation of the mRNA-protein complexes. However, this dissociation affected only some of the mRNP proteins - the 68K, 78K polypeptides and polypeptides in the 100-130K range. About half the amount of these polypeptides still sedimented along with mRNA together with all the other polypeptides usually found in the mRNP fraction (compare Fig. 4 A and B, and Sundquist et al. (4)). It is possible that the polypeptides bound to poly(A) of mRNA in mRNP complexes are the ones competed off with synthetic poly(A) and that the other polypeptides are located elsewhere on the mRNA. Evidence for the associ-

ation of a 78K polypeptide with the poly(A) end of mRNA has been reported earlier (16). The results in the accompanying paper corroborate this finding and also suggests that some of the other polypeptides may by linked to the poly(A) structure.

It can be noticed that the sedimentation of poly(A)- and poly(U)-released mRNP are similar (Fig. 3 A vs. 3 D) even though much protein is released from the latter. This could be explained either by complex formation between the eluting poly(U) and the poly(A)-part of the mRNP or by a conformational change of the mRNP particle.

The main site on mRNA interacting with poly(U) is the 3´terminal poly(A). The reaction between poly(U) and poly(A) of mRNA is well characterized and forms the basis for various mRNA assays (19,20,21). A few specific mRNAs have been partially sequenced and their 5´terminal ends were found to contain regions which are relatively rich in adenosine and uridine residues (22,23). Such regions could not form stable hybrids with poly(U), although it is possible that their biological activity is affected by this polymer. The experiments described in Fig. 3 E and Fig. 5 revealed that exposure of mRNP complexes to poly(U), even at low concentrations, resulted in serious derangements of the mRNP complexes. At high concentrations of poly(U) an almost quantitative dissociation of the proteins from mRNA occurred (Fig. 3 F). The primary interaction site for poly(U) on mRNP appears to be on poly(A). However, the binding of poly(U) to mRNA seems to affect not only the poly(A)-protein linkage, but also the protein binding to the additional RNase resistant sequences of mRNP described in the accompanying paper (4). It is reasonable to assume therefore, that these additional protein binding sequences in mRNA have some connection with the 3´terminal poly(A).

We have not succeeded in translating the polynucleotide eluted mRNP. There could be several reasons for this. One is the severe derangement of the mRNP seen and another is that it is difficult to remove the polynucleotides used for elution of the mRNP from oligo(dT)-cellulose. The polynucleotides inhibit the translation of mRNA.

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†Present address: Department of Virology, The Royal Veterinary College, Biomedicum, Box 585, S-751 23 Uppsala, Sweden.

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