Volume 4 Number 4 April 1977 **Nucleic Acids Research**

Characterization of mRNA-protein complexes from mammalian cells

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Received 27 January 1977

ABSTRACT

In a previous report we described the use of oligo(dT)-cellulose for the isolation of mRNA-protein complexes from EDTA-dissociated polysomes extracted from normally growing or adenovirus infected KB-cells (1). Experiments presented here provide evidence that proteins involved in these complexes bind specifically to mRNA since: a) the proteins and mRNA cosediment through sucrose gradients, b) they adsorb and elute from oligo(dT)-cellulose together, and C) analysis of the products from ribonuclease digestion experiments show that the poly(A) end and a separate small fraction of the mRNA are resistant to the enzymes and attached to protein.

INTRODUCTION

Polysomes of eukaryotic cells can be dissociated in various ways: in vivo by incubating cells with puromycin (2,3,4), in aminoacid depleted media (5), at elevated temperature (6), under hypertonic conditions (7,8), in the presence of dimethyl sulfoxide (9), in vitro by incubating polysomes with EDTA (10,11,12,13,14,15), under conditions where ribosomes run off the mRNA without reinitiation (16,17), with puromycin at high salt concentration (18,19, 20). Wherever it has been tested such dissociations of polysomes have been shown to release mRNA in the form of ribonucleoprotein particles. Two polypeptides with molecular weights of 78,000 and 50,000 are commonly found in combination with mRNA-protein particles prepared under high salt conditions (18,21,22,23,24). Analysis of ribonuclease resistant structures prepared by enzyme digestion of whole polyribosomes suggested that one of these polypeptides (mw 78,000) is associated with the poly(A) containing 3'end of mRNA (25). This observation has been confirmed and some of the characteristics of the poly(A)-protein complex has been reported (26,27). Additional polypeptides appear together with the mRNA if isotonic conditions are used during preparation of the ribonucleoproteins (21,1,28,

29). However, only little information is available about the possible presence of other protein binding sequences in mRNA (25,30). Some of the proteins involved in mRNP-complexes have been shown to be phosphorylated (31,21,32), or able to bind adenosine-3´, 5´-monophosphate (33). No or only slight differences in the efficiency of translation of mRNP as compared to naked mRNA added in a cell-free protein synthesizing system have been observed (34,35,36,37,38).

We have tried to develop methods to purify bulk mRNP from eukaryotic cells hoping that a detailed characterization of the mRNP particles would define the specificity of the RNA-protein interactions and possibly reveal features of functional importance to mRNP in general. In a previous report it was shown that oligo(dT)-cellulose can be used for the isolation of mRNA-protein complexes from EDTA dissociated polysomes (1). The mRNA-protein preparations obtained were free of ribosome subunits and shown to contain a specific set of a limited number of major polypeptides.

This paper describes the further characterization of messenger ribonucleoprotein particles from normally growing and adenovirus infected cells. The polypeptides of the mRNP-fraction mentioned above both cosediment in sucrose gradients and cochromatograph on oligo(dT)-cellulose with mRNA after EDTA dissociation of polysomes. Ribonuclease digestion experiments suggest that proteins involved in mRNP-complexes only bind to limited regions of the mRNA. One centrum of protein interaction is found in the poly(A) containing 3´end of the mRNA, and a second protein binding sequence class was identified but its localization within mRNA is not yet known.

MATERIALS AND METHODS

Labeling of cells and cell fractionation: HeLa cells were grown in spinner cultures and infected with adenovirus type 2 (Ad2) as described elsewhere (39). Cells were labeled with (^{3}H)-uridine (25 Ci/mmole) or (^{3}H)-adenosine (25 Ci/mmole) and/or (^{35}S)-methionine (100 Ci/mmole) obtained from Radiochemical Centre, Amersham. Infected cells were labeled at 14-16 hours after infection with (^{3}H)-uridine or (^{3}H)-adenosine (15 µCi/ml) or at 10-16 hours after infection with (^{3}H)-adenosine and (^{35}S)-methionine, and uninfected cells for the corresponding time as described previously (1). Cells were lysed with 0.65% Nonidet P40 (Shell Oil Co.) in 0.15 M NaCl, 10 mM Tris-HCl pH 7.8, 1.5 mM $MgCl_2$ and lysates were centrifuged 10 min

at 16,000 x g to remove cell nuclei and debris. Polysomes were then prepared from the supernatant lysate by sucrose gradient centrifugation as described by Kumar and Lindberg (19).

Isolation of mRNA-protein complexes: For the isolation of mRNA-protein complexes polysomes were dissociated with 0.03 M EDTA and the components were either fractionated directly by affinity chromatography on oligo(dT)-cellulose, or first centrifuged on 15-30% sucrose gradients in 10 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl pH 7.8 (18 hours, 19,000 rpm, +4°C, Spinco SW27 rotor), after which mRNP containing fractions (from 20-40 A_{260} units of polysomes) were chromatographed on 2x3 cm columns of T-2 or T-3 oligo-(dT)-cellulose (Collaborative Research Inc., Mass.). Samples were introduced in 0.2 M NaCl, 10 mM EDTA, 50 mM Tris-HCl pH 7.8, 0.5% Nonidet P40, and the columns were washed free of unadsorbed material with the same buffer minus Nonidet P40. Messenger ribonucleoprotein containing fractions were released from the oligo-(dT)-cellulose with 25% formamide in 0.2 M NaCl, 10 mM EDTA, 50 mM Tris-HCl pH 7.8 and residual material with 50% formamide also in this latter buffer (1).

Affinity chromatography on poly(U)-Sepharose: The RNA fragments of RNase resistant mRNP-cores were deproteinized by phenol-urea extraction according to Holmes and Bonner (40), and fractionated on poly(U)-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by Lindberg et al. (39).

Gel electrophoresis: The polypeptide composition of different fractions was analyzed by electrophoresis on SDS - polyacrylamide gels according to Maizel (41). Polypeptide gels were stained with Coomassie blue and scanned in a Gilford spectrophotometer.

Size distribution of RNA fragments obtained after chromatography on poly(U)-Sepharose was analyzed by electrophoresis on 15% polyacrylamide gels as described in detail by Adesnik and Darnell (42).

RESULTS

Fractionation of EDTA-treated polysomes by sucrose gradient centrifugation and oligo(dT)-cellulose chromatography

The preceding report (1) dealt exclusively with a fraction containing

mRNA-protein complexes isolated by direct oligo(dT)-cellulose chromatography of EDTA dissociated polysomes. To make the differentiation clear between polysomes and EDTA resistant components cosedimenting with polysomes (e.g. contaminating virus particles or precursors) the following experiment was performed.

Samples of polysome preparations from normally growing and adenovirus infected cells were treated with EDTA (0.03M) and fractionated on 15-30% sucrose gradients as shown in Fig. 1. The fractions of the gradients containing the separated ribosome subunits (between bars in Fig. 1) also contained 80-90% of the mRNA. This material was subsequently fractionated by oligo(dT)-cellulose chromatography to separate ribosome subunits from mRNA-protein complexes. The polypeptide patterns obtained by analysis of the different fractions from the sucrose gradients and oligo(dT)-cellulose chromatographies are shown in Fig. 2 A-F. Panel A and B show the polypep-

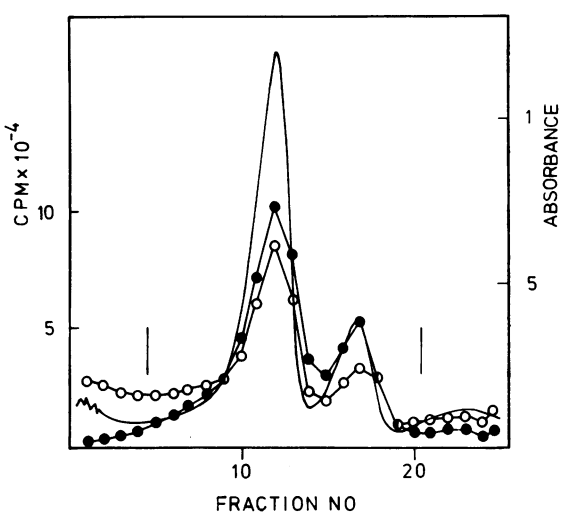

Fig. 1: Fractionation of polysomal ribonucleoprotein component by sucrose gradient centrifugation and affinity chromatography. Polysomes from Ad2 infected cells were dissociated with EDTA and subjected to sucrose gradient centrifugation (see Materials and Methods). Sedimentation is from right to left. Absorbance at 260 nm over the gradient was monitored using a recording spectrophotometer euqipped with a flow cell, and TCA insoluble radioactivity, (^{3}H)-uridine o--o: (^{35}S)-methionine o--o was determined on collected fractions. Pellets, fractions 1-4, fractions 21-25 were pooled separately and TCA precipitated and taken to polypeptide analysis directly (see Fig. 2A, B and F). Fractions within bars were pooled and chromatographed on oligo(dT)-cellulose and the separated components were then analyzed for polypeptide composition (Fig. 2C, D and E) as described under Materials and Methods.

tides of material, whose sedimentation properties did not change on EDTA treatment. Analysis of samples from infected cells harvested late in infection often show a prominent peak presumably containing adenovirus particles cosedimenting with the heaviest polysomes. In concordance with this several of the polypeptides in both panel A and B coincide with the positions of virion polypeptides (adenovirus marker was analyzed in parallel; data not shown), and these polypeptides were not present in the correspondding analysis of material from uninfected cells. Panel C and D, respectively, display the polypeptides of the ribosome and mRNP fractions from infected, and E those of the mRNP fraction from uninfected cells. The same general patterns of major polypeptides were obtained with the mRNA containing fractions from uninfected and infected cells except for the extra 110K polypeptide present in the material from infected cells. Slight variations in the relative amounts of the corresponding polypeptides in the two cases are also evident.

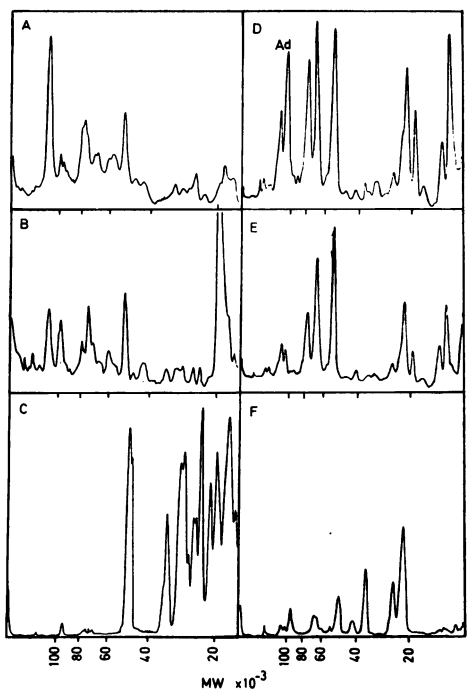

Fig. 2: Analysis of polypeptide composition of separated components of EDTA dissociated polysomes of uninfected and adenovirus infected cells. Polysomes were dissociated with EDTA and the components were separated by sucrose gradient centrifugation and oligo(dT)-cellulose chromatography as described in Fig. 1. Material from the different fractions obtained (see legend to Fig. 1) was TCA-precipitated and analyzed by electrophoresis on SDS-polyacrylamide gels. The gels were stained with Coomassie blue and scanned in a Gilford spectrophotometer (Materials and Methods).

Panel (A) displays polypeptides of pelleted material. (B) fractions 1-4, (C) unadsorbed material from oligo(dT)-cellulose chromatography of fractions pooled as indicated by bars in Fig. 1 (infected cells), (D) and (E) material from infected and uninfected cells respectively adsorbed and eluted from oligo(dT)-cellulose, (F) fractions 21-25. Polypeptide labeled Ad appeared only in mRNA from infected cells.

Ribonuclease digestion of messenger ribonucleoprotein particles adsorbed to oligo(dT)-cellulose

To estimate the proportion of mRNA which is intimately associated with proteins, the ribonuclease resistance of mRNA in mRNP complexes was determined. After sucrose gradient centrifugation of EDTA dissociated polysomes, mRNP containing fractions were pooled as indicated in Fig. 1 and applied to oligo(dT)-cellulose. The chromatogram was developed as in Fig. 3. After washing the columns to remove adsorbed ribosome and tRNA containing material (peak 1) a buffer containing ribonuclease A and T1 was introduced. The major part of the (^{3}H)-adenosine labeled material

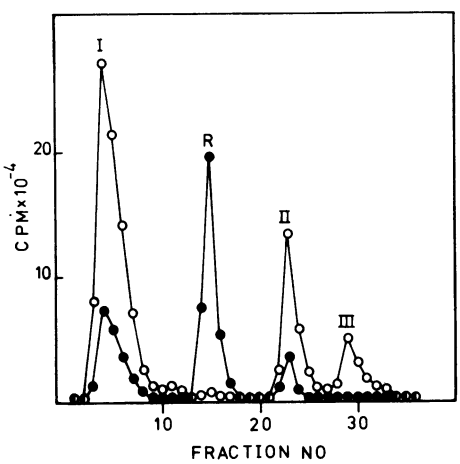

Fig. 3. Effect of ribonucleases on mRNA-protein complexes adsorbed to oligo(dT)-cellulose. Polysomes isolated from cells labeled with (^{3}H)-adenosine and (^{35}S)-methionine 10-16 hours after infection with Ad2 were dissociated with EDTA, and the constituent mRNA-protein complexes were adsorbed to oligo(dT)-cellulose as described under Materials and Methods. The columns were washed extensively to remove unadsorbed material (peak I). Ribonuclease A (25 µg/ml) and ribonuclease T_1 (10 units/ml) in 0.2 M NaCl, 10 mM EDTA, 50 mM Tris-HCl pH 7.8 were passed through the column (peak R), and RNase resistant material was subsequently eluted with buffers containing 25% formamide and 50% formamide (Materials and Methods), peak II and III, respectively. Total radioactivity of the fractions was determined by counting samples directly in toluene/methanol based scintillation liquid: (^{3}H)-adenosine ●--●, and (^{35}S)-methionine o--o. More than 95% of the RNA label in peak R was acid soluble, whereas of that present in the unadsorbed fraction (peak I) and that released with formamide containing buffers (peaks II, III) all was acid precipitable. The total recoveries of (^{35}S)-methionine and (^{3}H)-adenosine in the chromatogram were 80% and 95% respectively.

originally adsorbed was then released from the column as acid soluble products (peak R). Less than 5% (^{35}S)-methionine labeled material was removed from the oligo(dT)-cellulose during the ribonuclease treatment. A third peak (II, Fig. 3) was eluted with the buffer designed to release mRNA-protein complexes as described earlier (1). In this fraction the remainder of the originally adsorbed (^{3}H)-adenosine labeled material was recovered together with the major part of the (^{35}S)-methionine labeled components. All the RNA in this fraction was acid precipitable and as shown below consisted of poly(A) together with some other (^{3}H)-adenosine labeled RNA sequences. Additional (^{35}S)-methionine labeled material was removed from the column matrix with a buffer containing 50% formamide III (Fig. 3). The polypeptide patterns of the different fractions are shown in Fig. 4. Panel A displays the ribosomal polypeptides of the unadsorbed fraction. Panel B shows one major low molecular weight polypeptide only, presumably the ribonucleases present in the enzyme digestion fraction. Panel C demonstrates that all the major polypeptides usually found in the mRNP fraction were released together with the ribonuclease resistant fraction of mRNA (compare Fig. 2D and E). In the fourth fraction eluted

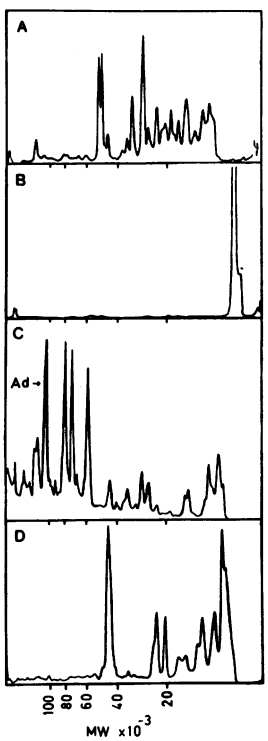

Fig. 4: Polypeptide analysis of fractions from RNase digestion of mRNA-protein complexes adsorbed to oligo(dT)-cellulose. Peak fractions from the experiment illustrated in Fig. 3 were TCA precipitated and analyzed by electrophoresis on SDS-polyacrylamide gels. Gels were stained and scanned as in Fig. 2.
(A) unadsorbed fraction containing ribosome subunits and tRNA, (B) material removed from oligo(dT)-cellulose by RNase treatment of the adsorbed mRNA-protein complexes, (C) RNase resistant core structures removed with 25% formamide and (D) with 50% formamide containing buffers (Materials and Methods and Fig. 3). Polypeptide labeled Ad appeared only in mRNP from infected cells.

with the buffer containing 50% formamide one major 50K polypeptide was found together with a set of smaller molecular weight polypeptides.

The ribonuclease resistant fraction of the mRNA in mRNP complexes was measured under various conditions using (^{3}H)-adenosine or (^{3}H)-uridine labeled material. The results from these determinations are summarized in Table I and compared with the amount of ribonuclease resistant material

Table I RNase resistant sequences in mRNA protein complexes

Sample[a]	Messenger ribonucleoprotein particles adsorbed to oligo(dT)-cellulose		
	total radioactivity (cpm)[e] before RNase	after RNase	percent resistance
mRNP-^{3}H-adenosine			
RNase A, 25 μg/ml	188,300	53,770	29
RNase A, 0.025 μg/ml[b]	149,465	44,620	30
RNase T1, 10 U/ml	156,865	48,960	31
mRNP fixed with glutaraldehyde, RNase A + T1 [c]	3,630	1,120	31
mRNA-^{3}H-adenosine[d]	440,650	60,375	14
RNase A + T1	435,275	63,000	15
mRNP-^{3}H-uridine			
RNase A, 25 μg/ml	1.049,748	97,710	9
RNase A, 0.025 μg/ml	678,440	70,220	10
RNase T1, 10 U/ml	757,520	71,470	9
mRNA-^{3}H-uridine[d]			
RNase A, 25 μg/ml	2,356,227	10,760	0,5
	591,160	2,630	0,4

[a] In the experiments described here polysomes from infected cells labeled 14-16 hours after infection were dissociated with EDTA and the mRNA protein complexes were adsorbed to oligo(dT)-cellulose (Materials and Methods). Buffers containing ribonucleases (A and T_1) at the concentrations indicated in the table were then passed through the columns until no more radioactivity was released. Then the remaining adsorbed radioactively labeled material was removed from the resin with 1.0% SDS in 10 mM Tris-HCl pH 7.8.

[b] At this concentration of ribonuclease the rate of digestion was slow. Values represent the final level of digestion.

[c] Messenger ribonucleoprotein particles adsorbed to oligo(dT)-cellulose was eluted with buffer containing 25% formamide, diluted 3 fold with 10 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl pH 7.8, fixed with glutaraldehyde and banded in $CsSO_4$/DMSO gradients as described by Lindberg and Sundquist (1). Material banding at the density of 1.45 g/cm^3 = mRNP was incubated with RNases (A 25 μg/ml, T_1 10 units/ml).

[d] Adenovirus mRNA was isolated by chromatography on poly(U)-Sepharose (39) and adsorbed to oligo(dT)-cellulose at 0.5 M NaCl, 10 mM Tris pH 7.8. The oligo(dT)-cellulose was washed with the buffer used for mRNP adsorption and ribonuclease treatment was then carried out as described for mRNP i.e. in buffer containing 0.2 M NaCl.

[e] Total radioactivity was measured as described in legend to figure 3, and all the radioactivity was acid precipitable.

retained on the column when instead phenol extracted polysomal mRNA was used. The material in this experiment was exclusively from adenovirus infected cells but similar results were obtained with mRNA-protein complexes from uninfected cells as shown below (Fig. 7). With samples from (^{3}H)-adenosine labeled cells 30% of the radioactivity of the adsorbed mRNP fraction was resistant to ribonuclease as compared to 15% when phenol extracted RNA was used. The corresponding numbers obtained with (^{3}H)-uridine labeled material were 10% and 0.5% respectively. Up to 80% of (^{3}H)-uridine labeled RNA fragments from infected cells bound to adenovirus DNA in exhaustive hybridization experiments performed as described earlier (39). This clearly demonstrates that there are sequences excepting poly(A) in mRNA, which under certain conditions are ribonuclease resistant and linked to protein. As shown in Table I similar results were also obtained with glutaraldehyde fixed mRNP isolated by CsCl-gradient centrifugation.

Analysis of RNase resistant mRNP cores by sucrose gradient centrifugation

Ribonuclease resistant mRNP cores were recovered from oligo(dT)-cellulose by elution with 25% formamide in 0.2 M NaCl, 10 mM Tris-HCl, pH 7.6. The eluted material was subjected to sucrose density gradient centrifugation and the results obtained are shown in Fig. 5. Panel A demonstrates the separation of (^{3}H)-adenosine and (^{35}S)-methionine labeled material into two major peaks: one containing 40% of the protein label and 60% of the RNA label and characterized by a sedimentation coefficient of 10-20S. The major part of the (^{35}S)-methionine labeled material, however, was recovered in a slow sedimenting peak. Here only 40% of the (^{3}H)-adenosine labeled RNA was found. A (^{3}H)-uridine labeled sample was prepared in the same way and run in parallel (Panel B). Here, one major peak was seen, sedimenting as the slow sedimenting (^{35}S)-methionine labeled peak of panel A. A sample taken in parallel to that used in panel A was treated with 0.5% Sarcosyl before the sucrose gradient centrifugation. The detergent converted RNA and protein to forms which had greatly reduced sedimentation rates suggesting that dissociation of RNA-protein complexes had occurred. As shown below (Fig. 7) (^{3}H)-adenosine labeled material consisted mainly of poly(A), whereas (^{3}H)-uridine labeled RNase resistant material was shown to be separate from poly(A) and of lower molecular weight.

In an attempt to determine to which core structure (fast or slow

sedimenting) the different mRNA associated proteins belong, the material from the fractions (pooled as indicated by bars in Fig. 5) was TCA precipitated and analyzed by SDS polyacrylamide gel electrophoresis. As shown in Fig. 6A there was a 5-fold enrichment of a 78K polypeptide in the faster sedimenting poly(A) containing core structure. However, there

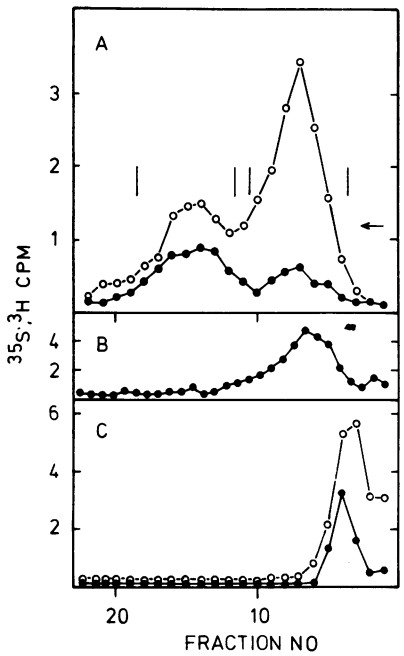

Fig. 5: Analysis of RNase resistant mRNP-cores by sucrose gradient centrifugation. Samples of RNase resistant core fraction of mRNP isolated as described in Fig. 3 were analyzed by centrifugation on 15-30% sucrose gradients in 10 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl pH 7.8 in a Spinco SW27 rotor for 36 h at 27,000 rpm and +4°C. (A) Core sample was diluted 3-fold in gradient buffer prior to centrifugation: material contained (^{3}H)-adenosine ●--● and (^{35}S)-methionine o--o (cpm x 10^{-3}). (B) Same type of preparation as in (A), but here labeled with (H)-uridine (cpm x 10^{-2}). In (C) a (^{3}H)-adenosine/(^{35}S)-methionine labeled sample, parallel to that of (A) was treated briefly with 0.5% Sarcosyl prior to centrifugation (cpm x 10^{-3}). Fractions within bars as indicated in panel (A) were pooled and further analyzed for component polypeptides by SDS-polyacrylamide gel electrophoreses as shown in Fig. 6A and B.

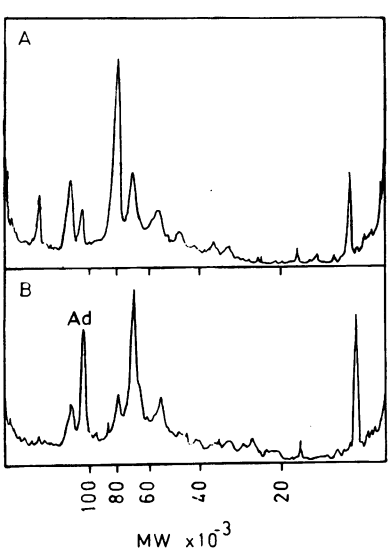

Fig. 6: Polypeptide composition of mRNP-core structures separated by sucrose gradient centrifugation. Fast and slow sedimenting mRNP core structures were prepared as described in Fig. 5. Material from fractions pooled as indicated was TCA precipitated and analyzed by SDS-polyacrylamide gel electrophoresis. Panel (A) shows the polypeptides of fraction 12-18 and (B) those of fractions 4-10. Polypeptide labeled Ad appeared only in mRNP from infected cells.

were also significant amounts of other polypeptides present which had the same mobility as those usually found in the mRNA protein fraction (compare with Fig. 2D and E). Figure 6B shows that two of these latter polypeptides were more prevalent in the slower sedimenting peak.

Analysis of RNA fragments from RNase resistant mRNP cores by chromatography on poly(U)-Sepharose and gel electrophoresis

After RNase digestion messenger ribonucleoprotein cores were eluted from oligo(dT)-cellulose with a formamide containing buffer (see legend to Fig. 3). The (^{3}H)-uridine or (^{3}H)-adenosine labeled samples were phenol extracted and fractionated by affinity chromatography on poly(U)-Sepharose as described in Materials and Methods. Only minute amounts

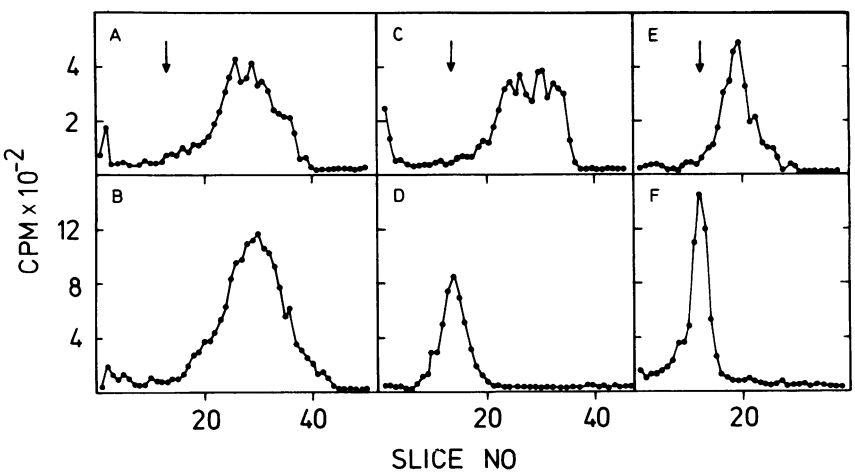

Fig. 7: Size distribution of RNA fragments derived by RNase digestion of mRNA-protein complex. Ribonuclease resistant cores from polysomal mRNP were prepared, deproteinized by phenol extraction, and RNA fragments were fractionated by chromatography on poly(U)-Sepharose as described under Materials and Methods and Fig. 3. Ethanol precipitable RNA fragments were then analyzed on 15% polyacrylamide gels. Panel (A) shows RNase resistant RNA-fragments from mRNP prepared from (^{3}H)-uridine labeled uninfected cells. Only 0.2% of (^{3}H)-uridine labeled RNA fragments were retained by poly(U)-Sepharose. (B) Material from Ad2 infected cells labeled with (^{3}H)-uridine 14-16 hours after infection and prepared as in (A). Panels (C) and (D) show RNA fragments from mRNP of uninfected cells labeled instead with (^{3}H)-adenosine, where (C) shows fragments passing unadsorbed and (D) fragments retained by poly(U)-Sepharose. Panels (E) and (F) respectively represent the corresponding analysis on (^{3}H)-adenosine labeled RNA fragments from Ad2 infected cells; (E) material unadsorbed and (F) adsorbed by poly(U)-Sepharose. Arrows in figures A, C and E indicating the position of poly(A) isolated by high salt RNase digestion from phenol extracted polysomal mRNA (48). Direction of migration - left to right.

(0.2%) of (^{3}H)-uridine labeled RNA had affinity for poly(U)-Sepharose whereas 60-70% of the (^{3}H)-adenosine labeled RNA was retained by the resin. The size distribution of the RNA fragments in the different fractions were analyzed by polyacrylamide gel electrophoresis.

As shown in Fig. 7 (^{3}H)-uridine labeled RNA fragments or (^{3}H)-adenosine labeled fragments not retained by poly(U)-Sepharose were distributed as if they were 50-100 nucleotides long. The (^{3}H)-adenosine labeled RNA fragments retained by poly(U)-Sepharose migrated at the position of the poly(A) marker prepared by ribonuclease digestion of phenol extracted polysomal mRNA run in parallel (data not shown). The analysis of mRNA cores from uninfected cells gave the same general type of results.

DISCUSSION

Proteins found in combination with mRNA could bind either generally to the ribose phosphate backbone or to specific regions of the polyribonucleotide. If the function of the proteins were to protect the mRNA against nuclease attack or to fix the whole mRNA molecule wound in a certain configuration one could expect to find the proteins bound without regard to nucleotide sequence and one or a few kinds of polypeptides could be involved in this type of function. Such binding of proteins to mRNA, however, would be difficult to distinguish from artefactual binding to mRNA during preparation of proteins with a generalized tendency to stick to negatively charged polymers. If the proteins served specific functions executed at specific steps in the life history of mRNA, e.g. in processing of precursor mRNA in the nucleus, in transport of matured mRNA from nucleus to cytoplasm or in translation of mRNA one would expect the binding to be directed towards specific sequences of the mRNA or specific traits in its three dimensional structure. The most likely regions of an mRNA molecule to contain specific binding sites for proteins are at or 5´terminal to the initiation codon, or at or 3´terminal to the termination codon. The possible functions implied here would be basic mechanisms also expected to employ a limited number of protein factors common to all or classes of mRNA. Interesting features of these regions of mRNA which might be of importance for protein binding has been described recently e.g. the 'CAP'-structure and the methylated bases of the 5´terminal end of mRNA (for references see 43,44). Moreover the domain of mRNA located in juxtaposition to the 3´terminal poly(A) is of interest in this context since it seems to contain sequences capable of forming hairpin loops. Analysis of the nucleotide sequence of these latter regions has also revealed possible

homologies between different mRNAS (45).

When adenovirus mRNP adsorbed to oligo(dT)-cellulose was digested with ribonucleases (A and T_1) 30% of (^{3}H)-adenosine labeled and 10% of (^{3}H)-uridine labeled material remained adsorbed to and was recovered in acid precipitable form when eluted from the resin (Table I and Fig. 3). This is compared to 15% when (^{3}H)-adenosine (all in poly(A)) and 0.5% when (^{3}H)-uridine labeled, deproteinized mRNA was digested under comparable experimental conditions. Since ribonuclease digestion of glutaraldehyde fixed, (^{3}H)-adenosine labeled mRNP from virus infected cells gave results similar to those obtained with unfixed material, the limited protection of mRNA in mRNP complexes against RNase digestion which we observe can hardly be explained by protein displacement caused by the enzymes during digestion. Instead the results suggest that only limited regions of the mRNA are involved in binding the proteins. It appears from the studies of Blobel (25) and others (26,27) that the poly(A) end of the mRNA is linked to protein. Our results show that there are sequences in mRNA in addition to poly(A), which under our conditions are RNase resistant and retained on oligo(dT)-cellulose after digestion. It should be stressed again that these fragments are not seen if the mRNP fraction is deproteinized by phenol extraction before the experiment. Only a small fraction (ca 10%) of either poly(A) or non poly(A) RNase resistant fragments (like intact mRNP see ref. 1) elute from the column matrix at low salt concentration (Persson, unpubl results), whereas RNA + protein is removed by the addition of detergent to the low salt buffer (Table I). These observations strongly suggests that both structures are bound to protein and that the protein in both cases is of importance for the binding of the RNA structures to the oligo(dT)-cellulose.

A quite different explanation of our results, that has been pointed out to us, could be that the non poly(A) RNase resistant sequences (which contain all four ribonucleotides; Sundquist, unpubl observation) belong to a separate entity not in covalent linkage with the mRNA. This entity could be bound to the mRNA molecule via protein or via oligo(U) stretches contained in them. It should be noticed that also in this model the binding of the non poly(A) sequences as well as the poly(A) to the oligo(dT)-cellulose would involve protein since none of the structures elute from the column matrix at low salt concentration. This explanation obviously is more involved since it postulates the presence of a new entity. We have looked for such RNA hydrogen bonded to mRNA by various techniques, but

been unable to find any. Therefore this interpretation seems less attractive to us.

Analyses of mRNP complexes by isopycnic gradient centrifugation suggest that the protein complement amounts to as much as 60% of the total mass of the mRNP particles (10,1). This is a surprisingly large amount of protein per RNA molecule considering that the ribosome subunits are only about 40% in protein. The results presented here suggest that although there is a lot of protein linked to mRNA, only small regions of the mRNA are in close association with this protein mass. The RNase resistant mRNP core containing poly(A) (Fig. 5) was found to be enriched 4-5 fold in a 78K polypeptide in agreement with Blobel's results (25). There was also a slight enrichment of a 130K polypeptide in this core fraction, but two of the other polypeptides normally found in the mRNP fraction appeared preferentially in slower sedimenting structures together with (^{3}H)-uridine labeled RNA fragments. The location of the non poly(A) RNase resistant sequences in mRNA and the positions of the various polypeptides are still unknown. It should be noticed, however, that there is a rather high degree of cross contamination of polypeptides between the two core fractions. This could mean that the different core structures are connected to each other in some way in the mRNP particles, maybe via protein components, but that the conditions used for their isolation lead to partial dissociation.

The polypeptides regarded as common to mRNP from different cells have molecular weights of 78,000 and 50,000. Their binding to mRNA resists high salt treatment (0.5 M KCl), and it is reasonable therefore to believe that these two polypeptides bind to the mRNA itself and that the other proteins are linked to the mRNA by protein:protein interaction. We find the 78K polypeptide eluting with bulk mRNP reproducibly. Originally we thought that the 56K polypeptide of our preparation (1) corresponded to the 50K polypeptide found in mRNP fractions by others (18,21,22). However, this appears to be wrong. There is a 50K polypeptide also in our material, but this component stays on the oligo(dT)-cellulose when bulk mRNP is eluted with the 25% formamide buffer, and is recovered only after elution with buffer containing 50% formamide (Fig. 4). Evidence for the association of this 50K polypeptide with mRNA is given in the accompanying paper, where mRNP is recovered from the oligo(dT)-cellulose by homopolyribonucleotide elution (46). In addition to its binding to mRNA appearently the 50K polypeptide has a strong affinity for oligo(dT)-cellulose and

might be the protein that contributes to the binding of mRNP to the resin as discussed earlier (1). We reported earlier that polysomal mRNA protein complexes isolated by affinity chromatography on oligo(dT)-cellulose contain four major labeled polypeptides. A set of polypeptides of similar size distribution was found labeled also in adenovirus infected cells during the late phase of infection. This is when the majority of the mRNA exported from nucleus to cytoplasm is of viral origin. In the mRNP fraction of infected cells we found in addition to the common set an extra 100K polypeptide. This polypeptide is believed to be identical to the 100K polypeptide described by others as coded for by the adenovirus genome (47). The presence of this polypeptide is confirmed here and it is shown that it indeed behaves as a polysome component changing its sedimentation properties on EDTA treatment. This polypeptide is under intensive synthesis late in the infection (1), and as demonstrated in Fig. 2 and 4 it is also one of the major stained polypeptides bands seen in the analysis of mRNP from infected cells. Similar findings have been reported by Van der Marel el al. (29) studying adenovirus mRNP isolated by different techniques. The role played by this protein in the function of mRNP in infected cells is not known. It could be a control element involved in mechanisms ensuring preferential synthesis of virus proteins in late phase of infection. It could be linked to the adenovirus mRNA giving this mRNA and advantage over host mRNA in translation or the same effect could be brought about by inhibitory binding of this protein to remaining host mRNA.

ACKNOWLEDGEMENTS

We acknowledge the excellent technical assistance of Sigbritt Eriksson and Ingegärd Svensson and secretarial work by Gunvor Lindman. We thank Dr. Lennart Philipson for continued interest and facilities. The investigation was supported by grants to U.L. from the Swedish Cancer Society.

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