PROTEINS ASSOCIATED WITH mRNAs FROM MEMBRANE BOUND AND FREE POLYRIBOSOMES

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

KAREN ANN RICHARD

1977

ABSTRACT

PROTEINS ASSOCIATED WITH mRNAs FROM MEMBRANE BOUND AND FREE POLYRIBOSOMES

By

Karen Ann Richard

Membrane bound and free polyribosomes were isolated from MOPC-21 cells. The polysomes were treated with 0.5M NaCl to reduce the nonspecific binding of cytoplasmic proteins and the polysomes were pelleted by centrifugation at 300,000 x g. The mRNPs were dissociated from the polysomes by the addition of EDTA and the poly(A+) mRNA and associated proteins were isolated by stepwise elution from oligo(dT)-cellulose. The proteins thus obtained were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Comparison of the proteins associated with membrane bound and free polysomal mRNPs reveals both qualitative and quantitative differences.

PROTEINS ASSOCIATED WITH mRNAs FROM MEMBRANE BOUND AND FREE POLYRIBOSOMES

By

Karen Ann Richard

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

1977

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Ronald J. Patterson for his encouragement and guidance throughout this project. Most of all I want to thank Lyle, Debbie and David, for the sacrifice and support that made this all possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

REVIEW OF THE LITERATURE

In the mid-1960s Spirin and co-workers (review 1969) noted a component present in the cytoplasmic extract of fish embryos that was rapidly labeled in the presence of ^{14}C-uridine or ^{14}C-adenine. Since new ribosomal subunits are not synthesized during the embryonic stages studied and the particles also could be labeled with radioactive amino acids, they suggested that the nucleic acid was messenger ribonucleic acid (mRNA) and that it existed complexed with protein. The ribonucleoprotein complexes (RNPs) were termed informosomes. RNP complexes were also found in sea urchin cytoplasm (Spirin and Nemer, 1965; Monroy et al., 1965) and mouse L cells (Perry and Kelly, 1966).

Doubt about the physiological existence of RNP complexes in cells resulted from the observation by Girard and Baltimore (1968) that many types of RNA will increase in sedimentation rate when added to a cytoplasmic extract from HeLa cells. Further study of the phenomenon suggested that such artificial RNP complexes lose some of their proteins upon sucrose gradient centrifugation (Spirin, 1969; Olsnes, 1971) and are not stable in 0.5M NaCl (Baltimore and Huang, 1970), conditions under which polysomal RNPs retain their complexed proteins (Nudel et al., 1973).

Commonly used methods of isolation of polysomal mRNP complexes relied on sucrose gradient centrifugation. Typically, the postnuclear (mitochondrial) supernatant (PNS) derived from disrupted cells is centrifuged on a sucrose gradient designed to separate the polyribosomal region from the native ribosomal subunits. Alternately, the PNS is subjected to a high speed centrifugation through 2M sucrose, thus pelleting the polysomes from contaminating proteins, small RNA/RNP and membranous material. The isolated polysomes are then used as a source of mRNA or mRNP.

Using rat liver polyribosomes dissociated with ethylenediaminetetraacetate (EDTA), Henshaw (1968) demonstrated that the radioactive distribution of mRNPs was heterogeneous and sedimented on sucrose gradients with sedimentation coefficients ranging from 20S to 100S, considerably heavier than mRNAs from the same cells (6S to 35S). Henshaw suggested that polysomal mRNA was in the form of an RNP complex. RNP complexes also were found to be released from polysomes in KB cells (Kumar and Lindberg, 1972), mouse L cells (Perry and Kelly, 1968), rabbit reticulocytes (Burney et al., 1968), and sheep thyroid tissue (Cartouzou et al., 1969).

Using cells labeled in the presence of low levels of actinomycin D to inhibit rRNA synthesis, Spohr et al. (1970) studied free cytoplasmic and polysome-associated RNPs. Their data supported the findings of Henshaw and others, and indicated that mRNAs exit from the nucleus into

the cytoplasm associated with proteins and that mRNAs are complexed with protein when in polyribosomal structures.

Olsnes (1970) examined the proteins associated with polysomal mRNPs and compared them to the proteins associated with nuclear RNP complexes. He used either high salt (0.5M NaCl) or sodium deoxycholate to release the proteins from polysomes without disrupting the ribosomal subunits. Polyacrylamide gel electrophoresis (PAGE) of the released proteins revealed 10 (high salt) and 5 (DOC) protein bands. Nuclear RNPs contained 2 protein bands. One protein appeared to be present in all three preparations. In order to determine if the protein observed in all three preparations was "informofer" protein, Olsnes and co-workers (Lukanidin et al., 1972) prepared an antiserum to the 30S nuclear RNP from which the two proteins were released in the earlier set of experiments. The antibodies did not appear to react with EDTA-released polysomal mRNPs, indicating that the proteins were dissimilar.

Matringe and Jacob (1972) compared the proteins from nuclear and cytoplasmic RNP particles in rat brain. They analyzed the proteins by PAGE at pH 4.5 in urea or in the presence of sodium dodecyl sulphate (SDS). Fewer bands were seen in the gels electrophoresed at pH 4.5. The SDS gels gave a more complex protein pattern, with markedly fewer protein bands in nuclear RNP preparations than in cytoplasmic RNP complexes. The two major nuclear RNP proteins were 35,000 and 40,000 daltons. Most of the cytoplasmic RNP proteins were in the 40,000 to 70,000 dalton

region. Nuclear RNP proteins from HeLa cells examined by Ducamp and Jeanteur (1973), however, displayed a complex pattern with proteins ranging in size from 35,000 to 150,000 daltons.

Analysis of nuclear RNP in mouse ascites cells (Quinlan et al., 1974), HeLa, mouse L cells, Vero and rat liver (Pederson, 1974) indicated that (1) there were a limited number of proteins associated with the RNPs, (2) the proteins ranged in size from 40,000 to 130,000 and (3) the major proteins tended to be different in each of the cell types.

The predominant synthesis of a single protein has made the reticulocyte cell a particularly good system for mRNP analysis, since the globin mRNPs sediment at a distinct rate separate from the ribosomal subunits. Lebleu et al. (1970) reported finding two proteins of molecular weight 68,000 and 130,000 associated with globin mRNAs isolated from rabbit reticulocyte polysomes. Working with the same system Blobel (1972) found two proteins of 78,000 and 52,000 daltons. Morel et al. (1971) reported two proteins in duck erythrocyte mRNPs of 49,000 and 73,000 daltons.

Gander et al. (1973) have examined the RNP complexes in duck erythroblasts. They isolated two cytoplasmic RNPs that sedimented on sucrose gradients with values of 12S and 20S. The 20S particle contained the mRNAs for the globin polypeptides. The RNP released from polyribosomes also displayed a 20S particle that contained globin mRNAs.

Comparison of the protein moieties of the cytoplasmic and polysomal RNPs indicated that the two groups of proteins were markedly different. In fact, polysomal RNP proteins were larger in molecular weight than cytoplasmic RNP proteins, thus ruling out a product-precursor relationship. Another cell system that allows the isolation of mRNPs which sediment at a rate distinct from ribosomal subunits is the ewe lactating mammary gland. The proteins α-casein and β-lactoglobulin are synthesized on membrane bound polysomes (MBP) in these cells and when released the mRNPs sediment at about 22S. Assairi et al. (1976) isolated the proteins associated with the mRNPs from MBP in these cells. They found two major proteins of 52,000 and 72,000 daltons, and two minor proteins of 58,000 and 60,000 M.W. and a protein of 86,000 daltons that was occasionally present.

Chen et al. (1976) isolated two mRNP complexes released from calf lens polysomes by EDTA dissociation. The mRNPs sedimented at 13S and 19S on sucrose gradients and could be obtained free of ribosomal subunits. The major proteins associated with each mRNP as resolved by SDS-PAGE were reported to be 64,000 and 40-42,000 daltons with minor proteins in the 90,000 to 130,000 region. Two major proteins of molecular weight 48,000 and 78,000 were reported by Bryan and Hayashi (1973) to be associated with chick embryo cerebral polysome mRNPs. These authors also reported the presence of minor bands displaying a wide range of sizes.

The polyadenylated [poly(A)] segment at the 3' end of mRNA molecules was postulated to be complexed with protein (Kwan and Brawerman, 1972). Using mouse L cells, Blobel (1973) reported that two predominant proteins of molecular weight 78,000 and 52,000 were associated with the polysomal mRNPs and that the 78,000 dalton protein was associated with the poly(A) segment.

The proteins associated with the poly(A) segment of HeLa cell polysomal RNPs were isolated by Schwartz and Darnell (1976). Using a technique that involved mild nuclease treatment, they found two predominant proteins of 75,000 and 50,000 daltons tightly bound to isolated poly(A) segments. In addition, three minor proteins of 68,000, 86,000 and 100,000 daltons also were found associated with the poly(A) regions.

The proteins of Ehrlich ascites tumor cell polysomal mRNPs examined by Barrieux et al. (1976) included two major proteins of 78,000 and 52,000 daltons and two minor proteins of 34,000 and 130,000. The 78,000 dalton protein appeared to form a stable complex with poly(A), while the other proteins seemed to associate with other portions of the mRNA molecules.

Oligo(dT)-cellulose chromatography has been used to isolate mRNA by its retention on the column via interaction between the poly(A) segment and the immobilized oligo(dT) (Aviv and Leder, 1972). This method allows the isolation of mRNAs from cells that synthesize an array of proteins where the mRNA sizes overlap the rRNAs. A method utilizing

the same type of interaction was reported for the isolation of mRNPs (Lindberg and Sundquist, 1974). They compared the proteins associated with polysomal poly(A+) mRNPs in KB cells before and after infection with adenovirus. A relatively simple pattern of four proteins of molecular weight 56,000, 68,000, 78,000 and 130,000 was seen in uninfected cells. The same four proteins plus one at 110,000 daltons were observed in the mRNP protein profile from adenovirus-infected cells.

Using KB cells uninfected or infected with adenovirus, VanDer Marel et al. (1975) isolated polysomal mRNPs by preparative electrophoresis on polyacrylamide agarose composite gels. They found 7 proteins of molecular weight from 46,000 to 93,000. The adenovirus infected cells again showed one additional band at 105,000 daltons, in close agreement with Lindberg and Sundquist (1974). To check the cell specificity of the mRNP proteins, HeLa cell polysomes were dissociated and electrophoresed in the same manner. Again 6 to 7 proteins with the same mobility on SDS-PAGE were observed.

The poly(A+) mRNPs released from HeLa cell polysomes (Kumar and Pederson, 1975) contained three major proteins of molecular weight 52,000, 76,000 and 120,000 plus minor bands. Heterogeneous nuclear RNP complexes (hnRNP) were also subjected to oligo(dT)-cellulose chromatography. SDS-PAGE of the proteins in the nonpolyadenylated fraction showed the existence of a single protein band with a molecular weight of 40,000. A large number of proteins

were associated with the poly(A) containing nuclear RNPs; their molecular weights ranged between 40,000 and 180,000 daltons.

The mRNP released from kidney polysomes were isolated by oligo(dT)-cellulose chromatography by Irwin et al. (1975). They observed 6 proteins of molecular weight 69,000, 75,000, 80,000, 100,000, 109,000 and 118,000 daltons associated with the poly(A) containing mRNP. They also found two proteins of molecular weight 84,000 and 90,000 that appeared in both the poly(A) containing and the unbound mRNPs.

When polysomes are dissociated with EDTA, fixed and analyzed by CsCl centrifugation, mRNPs sediment as a heterogeneous peak with an average density of 1.45 g/cm^3. Ribosomal subunits exhibit a sharp peak at a density of 1.59 g/cm^3 (Henshaw, 1968; Perry and Kelly, 1968; Spohr et al., 1970). This technique has been used widely to confirm the presence of RNA and protein in complexes released from polyribosomes. Once the complex is fixed with formaldehyde it is not possible to subject either the protein or the mRNA to further analysis. Alternative methods, i.e., sucrose gradient centrifugation and oligo(dT)-cellulose chromatography, have therefore been required in order to examine the protein and the mRNA moieties.

Recently, Greenberg (1977) reported the isolation of mRNPs released from L cell polyribosomes using $CsSO_4$ density gradient centrifugation in the absence of formaldehyde fixation. The mRNAs were broadly distributed between 1.3

to 1.5 g/cm^3, but separated from both rRNA and ribosomal proteins. Greenberg found three different classes of RNA/RNP in the mRNAs thus isolated: poly(A) containing mRNPs, histone mRNAs and non-histone poly(A)-lacking mRNPs. SDS-PAGE of the proteins from the 1.3 to 1.5 g/cm^3 mRNP band revealed 5 major polypeptides of molecular weight 30,000, 36,000, 76,000, 87,000 and 103,000 daltons.

In all systems examined there appears to be a limited number of proteins associated with mRNAs. Two proteins with approximate molecular weights of 50,000 and 75,000 daltons occur in most of the cell types studied. The 75,000 dalton protein appears to be associated with the poly(A) segment; however, the portions of the mRNA that are associated with the other proteins have yet to be defined. Functional roles have been postulated, but experimental evidence supporting such functions has not been presented. The significance of the similarities and differences in protein content of mRNP complexes awaits further study.

LIST OF REFERENCES

LIST OF REFERENCES

Assairi, L., Gaye, P. and Houdebine, L. M. 1976. Isolation and Characteristics of mRNPs Associated with Bound Polyribosomes from the Ewe Lactating Mammary Gland. Biochimie 58:325-331.

Aviv, H. and Leder, P. 1972. Purification of Biologically Active Globin Messenger RNA by Chromatography on Oligothymidylic Acid-Cellulose. Proc. Natl. Acad. Sci. U.S.A. 69:1408-1412.

Baltimore, D. and Huang, A. S. 1970. Interaction of HeLa Cell Proteins with RNA. J. Mol. Biol. 47:263-273.

Barrieux, A., Ingrahm, H. A., Nystul, S. and Rosenfeld, M. G. 1976. Characterization of the Association of Specific Proteins with Messenger Ribonucleic Acid. Biochemistry 15:3523-3528.

Blobel, G. 1972. Protein Tightly Bound to Globin mRNA. Biochem. Biophys. Res. Commun. 47:88-95.

Blobel, G. 1973. A Protein of Molecular Weight 78,000 Bound to the Polyadenylate Region of Eukaryotic Messenger RNAs. Proc. Natl. Acad. Sci. U.S.A. 70:924-928.

Bryan, R. N. and Hayashi, M. 1973. Two Proteins are Bound to Most Species of Polysomal mRNA. Nature New Biol. 244:271-274.

Burny, A., Huez, G., Marbaix, G. and Chantrenne, H. 1969. On a Messenger Ribonucleoprotein Complex From Rabbit Reticulocytes. Biochim. Biophys. Acta 190:228-231.

Cartouzou, G., Poeree, J. C. and Lissitzky, S. 1969. Rapidly Labelled Ribonucleic Acid-Protein Complexes of the Thyroid Tissue. Eur. J. Biochem. 8:357-369.

Chen, J. H., Lavers, G. C. and Spector, A. 1976. Calf Lens Messenger Ribonucleoprotein Complexes: Characterization and Comparison of Template Activity with Corresponding mRNAs. Biochim. Biophys. Acta 418:39-51.

Ducamp, C. and Jeanteur, P. 1973. Characterization of Nuclear RNP Particles from HeLa Cells. Analysis of Protein and RNA Constituents. Presence of Poly(A). Biochimie 10:1235-1243.

Gander, E. S., Stewart, A. G., Morel, C. M. and Scherrer, K. 1973. Isolation and Characterization of Ribosome-Free Cytoplasmic Messenger-Ribonucleoprotein Complexes from Avian Erythroblasts. Eur. J. Biochem. 38:443-452.

Girard, M. and Baltimore, D. 1966. The Effect of HeLa Cell Cytoplasm on the Rate of Sedimentation of RNA. Proc. Natl. Acad. Sci. U.S.A. 56:999-1002.

Greenberg, J. R. 1977. Isolation of Messenger Ribonucleoproteins in Cesium Sulfate Density Gradients: Evidence that Polyadenylated and Non-Polyadenylated Messenger RNAs are Associated with Proteins. J. Mol. Biol. 108:403-416.

Henshaw, E. C. 1968. Messenger RNA in Rat Liver Polyribosomes: Evidence that it Exists as Ribonucleoprotein Particles. J. Mol. Biol. 36:401-411.

Irwin, D., Kumar, A. and Malt, R. A. 1975. Messenger Ribonucleoprotein Complexes Isolated with Oligo(dT)-Cellulose Chromatography from Kidney Polysomes. Cell 4:157-165.

Kumar, A. and Lindberg, U. 1972. Characterization of Messenger Ribonucleoprotein and Messenger RNA from KB Cells. Proc. Natl. Acad. Sci. U.S.A. 69: 681-685.

Kumar, A. and Pederson, T. 1975. Comparison of Proteins Bound to Heterogeneous Nuclear RNA and Messenger RNA in HeLa Cells. J. Mol. Biol. 96:353-365.

Kwan, S. and Brawerman, G. 1972. A Particle Associated with the Polyadenylate Segment in Mammalian Messenger RNA. Proc. Natl. Acad. Sci. U.S.A. 69:3247-3250.

Lebleu, B., Marbaix, G., Huez, G., Temmerman, J., Burney, A. and Chantrenne, H. 1971. Characterization of the Messenger Ribonucleoprotein Released from Reticulocyte Polyribosomes by EDTA Treatment. Eur. J. Biochem. 19:264-269.

Lindberg, U. and Sundquist. B. 1974. Isolation of Messenger Ribonucleoproteins from Mammalian Cells. J. Mol. Biol. 86:451-468.

Lukanidin, E. M., Olsnes, S. and Pihl, A. 1972. Antigenic Differences Between Informofers and Protein Bound to Polyribosomal mRNA from Rat Liver. Nature New Biol. 240:90-92.

Matringe, H. and Jacob, M. 1972. Brain Nuclear and Cytoplasmic Ribonucleoproteins Carrying DNA-Like RNA: Comparison of Their Proteins with Those from Soluble Nuclear and Cytoplasmic Supernatants by Polyacrylamide Gel Electrophoresis. Biochimie 54: 1169-1178.

Monroy, A., Maggio, R. and Rinaldi, A. M. 1965. Experimentally Induced Activation of the Ribosomes of Unfertilized Sea Urchin Eggs. Proc. Natl. Acad. Sci. U.S.A. 54:107-111.

Morel, C. M., Kayiband, B. and Scherrer, K. 1971. Proteins Associated with Globin Messenger RNA in Avian Erythroblasts: Isolation and Comparison with the Protein Bound to Nuclear Messenger-Like RNA. FEBS Letters 18:84-88.

Nudel, U., Lebleu, B., Zehavi-Willner, T. and Revel, M. 1973. Messenger Ribonucleoprotein and Initiation Factors in Rabbit-Reticulocyte Polyribosomes. Eur. J. Biochem. 33:314-322.

Olsnes, S. 1970. Characterization of Protein Bound to Rapidly-Labelled RNA in Polyribosomes from Rat Liver. Eur. J. Biochem. 15:464-471.

Olsnes, S. 1971. Evidence that Protein is Bound to the Rapidly Labelled RNA in Polyribosomes *in vivo*. Eur. J. Biochem. 23:248-252.

Pederson, T. 1974. Proteins Associated with Heterogeneous Nuclear RNA in Eukaryotic Cells. J. Mol. Biol. 83: 163-183.

Perry, R. P. and Kelly, D. E. 1968. Messenger RNA-Protein Complexes and Newly Synthesized Ribosomal Subunits: Analysis of Free Particles and Components of Polyribosomes. J. Mol. Biol. 35:37-59.

Perry, R. P. and Kelly, D. E. 1966. Buoyant Densities of Cytoplasmic Ribonucleoprotein Particles of Mammalian Cells: Distinctive Character of Ribosome Subunits and the Rapidly Labelled Components. J. Mol. Biol. 16:255-268.

Quinlan, T. J., Billings, P. B. and Martin, T. E. 1974. Nuclear Ribonucleoprotein Complexes Containing Polyadenylate from Mouse Ascites Cells. Proc. Natl. Acad. Sci. U.S.A. 71:2632-2636.

Schwartz, H. and Darnell, J. E. 1976. The Association of Protein with the Polyadenylic Acid of HeLa Cell Messenger RNA: Evidence for a "Transport" Role of a 75,000 Molecular Weight Polypeptide. J. Mol. Biol. 104:833-851.

Spirin, A. S. 1965. Messenger RNA in Early Sea Urchin Embryos: Cytoplasmic Particles. Science 150: 214-217.

Spirin, A. S. 1969. Informosomes. Eur. J. Biochem. 10: 21-35.

Spohr, G., Granboulan, N., Morel, C. and Scherrer, K. 1970. Messenger RNA in HeLa Cells: An Investigation of Free and Polyribosome-Bound Cytoplasmic Messenger Ribonucleoprotein Particles by Kinetic Labelling and Electron Microscopy. Eur. J. Biochem. 17:296-318.

VanDer Marel, P., Tasseron-DeJong, J. E. and Bosch, L. 1975. The Proteins Associated with mRNA from Uninfected and Adenovirus Type 5-Infected KB Cells. FEBS Letters 51:330-333.

RESULTS

ARTICLE

PROTEINS ASSOCIATED WITH mRNAs FROM MEMBRANE BOUND AND FREE POLYRIBOSOMES

submitted for publication to

Cell

PROTEINS ASSOCIATED WITH mRNAs FROM MEMBRANE BOUND AND FREE POLYRIBOSOMES

Karen A. Richard and Ronald J. Patterson

Department of Microbiology and Public Health
Michigan State University
East Lansing, MI 48824

Running Title: mRNPs from membrane bound and free polysomes

SUMMARY

A modification of oligo(dT)-cellulose chromatography for isolation of mRNPs is used to isolate the protein moieties from mRNP complexes that have been released from membrane bound and free cytoplasmic polyribosomes. Both qualitative and quantitative differences are found in the proteins associated with the two mRNP classes.

INTRODUCTION

In eukaryotic cells mRNA appears to be complexed with protein during all or most of its lifetime. Nonribosomal RNA-protein complexes have been found in the nucleus (Georgiev and Samarina, 1971; Lukanidin et al., 1972; Bhorjee and Pederson, 1973), free in the cytoplasm (Spirin, 1969; Perry and Kelly, 1966; Gander et al., 1973) and associated with polyribosomes (Henshaw, 1968; Perry and Kelly, 1969; Cartouzou et al., 1969). No unequivocal function has been demonstrated for the proteins. Postulated roles for the proteins include: (1) transport of mRNA from the nucleus to the cytoplasm, (2) protection of mRNA from nucleolytic attack, and (3) regulation of translation.

The proteins associated with specific populations of RNP complexes have been examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). It appears that nuclear RNP proteins vary with the cell type from which they are isolated (Pederson, 1974) and that they are different than the proteins complexed with polysomal mRNPs (Matringe and Jacob, 1972; Kumar and Pederson, 1975). In

addition, there are differences between the proteins of free-cytoplasmic mRNPs and polyribosomal associated mRNPs (Gander et al., 1973). Nuclease treatment of mRNPs allows the isolation of a component which contains the polyadenylate [poly(A)] segment of mRNA complexed with protein(s) (Blobel, 1973; Schwartz and Darnell, 1976; Barrieux, 1976).

Mammalian reticulocytes synthesize globin as their predominant polypeptide. Ethylenediaminetetraacetate (EDTA) or puromycin dissociation of reticulocyte polysomes followed by sucrose gradient centrifugation yields a distinct RNP particle (containing globin mRNA) with a sedimentation coefficient of 20S (Blobel, 1972; Nudel et al., 1973). Thus, globin mRNPs can be purified from contaminating ribosomal subunits. Mammalian cells that synthesize an array of proteins present a burdensome problem with regard to isolation of mRNPs, as many overlap ribosomal subunits in sedimentation rate. Oligo(dT)-cellulose chromatography can be used for the isolation of poly(A+) mRNPs released from polyribosomes by EDTA (Lindberg and Sundquist, 1974), and thus greatly reduces the problem of ribosomal subunit contamination.

Murine myeloma tumor cells produce an immunoglobulin-like molecule which constitutes 10 to 20% of the total protein synthesized by the cell (Laskov and Scharff, 1970). The Ig protein is synthesized predominantly on membrane bound polyribosomes (Cioli and Lennox, 1973). In addition, the half life of Ig mRNAs is relatively long, greater than the generation time of the cells (Storb, 1973).

Since size differences are found in the proteins associated with the RNPs isolated from various intracellular locales, we examined the proteins associated with poly(A+) mRNAs isolated from membrane bound and free polysomes from a murine myeloma cell line MOPC-21. The mRNP proteins and poly(A+) mRNA are isolated using a modification of the oligo(dT)-cellulose binding technique.

RESULTS

Isolation of poly(A+) mRNPs

Aviv and Leder (1972) showed that mRNA could be isolated free of rRNA by retention on oligo(dT)-cellulose via interaction between the poly(A) segment at the 3' terminus and the immobilized oligo(dT). Lindberg and Sundquist (1974) utilized the same type of interaction for the isolation of mRNPs. RNP complexes require the addition of formamide to the elution buffer in order to release the bound mRNPs. There is variability in the capacity of different preparations of oligo(dT)-cellulose to bind mRNA or mRNP (Lindberg and Sundquist, 1974; Burns and Williams, 1975). It is not known what factor(s) in the oligo(dT) or the cellulose contribute to this variability.

We tested the cellulose used to prepare the oligo(dT)-cellulose used in these studies to insure that it did not retain particles nonspecifically. EDTA-dissociated polysomes from cells labeled for 1 hr with ^{3}H-uridine were divided into two aliquots. One portion was applied to an unsubstituted cellulose column, the other to oligo(dT)-cellulose.

Three and four-tenths percent of the radioactivity was in the poly(A+) fraction from the cellulose, while 20.6% eluted as poly(A+) mRNPs from oligo(dT)-cellulose. We also examined the eluted poly(A+) mRNPs for their capacity to rebind to oligo(dT)-cellulose. The isolated poly(A+) mRNP was divided into two aliquots. One portion was reapplied to oligo(dT)-cellulose as RNP and eluted with 50% formamide buffer; this time 72.1% eluted as poly(A+) mRNPs. The second portion was made 0.1% in SDS and applied to oligo(dT)-cellulose; 88.3% of the radioactivity was in the poly(A+) mRNA fraction.

When ^{3}H-uridine labeled polysomes from MOPC-21 cells are EDTA-dissociated and subjected to sucrose gradient analysis, the radioactivity distribution follows the optical density profile of the ribosomal subunits and tRNA. Figure 1a shows the radioactivity of individual fractions plotted as a percentage of the radioactivity recovered in the gradient. Following concentration of the poly(A+) mRNP fraction, we observed by sucrose gradient analysis what appeared to be minor contamination of the poly(A+) mRNPs with ribosomal subunits. To test whether any of the isotope in the mRNPs was ribosomal subunits, cells were labeled in the presence of a low level of actinomycin D (0.04 μg/ml) to inhibit rRNA synthesis. EDTA-dissociated polysomes from actinomycin D treated cells gave a ^{3}H-uridine profile as seen in Figure 1b. Poly(A+) mRNPs isolated from the same polysomes display a similar heterogeneous distribution as mRNPs isolated from cells not treated with actinomycin D.

This indicates that with short labeling times the mRNP isotope profile represents mRNPs with little ribosomal contamination.

The proteins associated with the poly(A+) mRNPs from EDTA-dissociated polysomes were electrophoresed on SDS polyacrylamide gels. It is difficult to see the protein bands on the stained gels. Repeated attempts to improve the recovery of bound material from the columns were unsuccessful. This led us to try a new method of elution of the proteins and poly(A+) mRNAs from the oligo(dT)-cellulose.

High Salt-SDS Elution Method

Figure 2 is a flow diagram of the two methods of elution we have used in this study. The left arm of the diagram is the formamide elution method and the right arm shows the modification we made in the elution procedure. Polysomes from cells labeled for 1 hr were EDTA-dissociated, made 0.5M in NaCl buffer and divided into two equal portions. One aliquot was used for each of the elution methods. Using the formamide elution procedure, 11.9% of the radioactivity appeared in the poly(A+) mRNP fractions. In our modification of the technique, the high salt-SDS buffer removed 1% of the radioactivity and 14.9% of the ^{3}H-uridine labeled RNA was eluted as poly(A+) mRNA. We attribute the slightly higher percentage in the poly(A+) fraction using the high salt-SDS elution technique to the fact that a more complete recovery of the applied radioactivity is achieved using this method. High salt-SDS

elution consistently yields greater than 90% recovery of the applied radioactivity whereas the formamide elution gives inconsistent recoveries ranging from 65 to 95% of the applied ^{3}H-uridine labeled RNA.

We compared the proteins associated with the poly(A+) mRNPs eluted by each method. Figure 3 shows the results of SDS-PAGE of the two eluates. The protein bands seen in the formamide eluted mRNPs are present in the SDS eluate. In addition, the resolution with the SDS-eluted proteins is improved and more bands are visible.

The mRNAs eluted by the SDS method were examined by sucrose gradient centrifugation. Figure 4 shows that the size distribution is heterogeneous with a peak at about 23S. This is in agreement with the size distribution of polysomal poly(A+) mRNAs obtained by chloroform phenol extraction (Stuart et al., 1977). The inset shows that the isolated RNA was as active in protein synthesis in a wheat germ system as cytoplasmic poly(A+) RNA isolated by conventional methods.

As an additional test of the high salt-SDS elution method we isolated the proteins associated with mRNPs released from rabbit reticulocyte polysomes. SDS-PAGE (see Figure 7) reveals a number of proteins; the two major bands are about 77,000 and 48,000 daltons, in close agreement with the findings of Blobel (1972). In addition, there are prominent proteins of 100,000 and 45,000 daltons.

Membrane Bound and Free Polysomal mRNPs

In MPOC-21 cells about 75% of the polysomes, by optical density analysis, exist free in the cytoplasm and 25% are associated with the endoplasmic reticulum (unpublished observations). After 1 hr incubation in the presence of ^{3}H-uridine the distribution of radioactivity is 86% in the free polysomes and 14% in the membrane bound polysomes. Figure 5 shows the ^{3}H-uridine profile of the poly(A+) mRNPs from membrane bound and free polysomes obtained by formamide elution. The mRNPs have a heterogeneous distribution ranging from 10S to >50S with a peak at about 40S. The proteins associated with the poly(A+) mRNPs from membrane bound ($mRNP_M$) and free ($mRNP_F$) polysomes were electrophoresed on SDS acrylamide gels. The stained bands, shown in Figure 6, were only faintly visible, but it appeared that some of the proteins were dissimilar.

Next, we isolated the poly(A+) mRNP proteins and mRNAs from membrane bound and free polysomes using the high salt-SDS elution. The poly(A+) mRNAs display a heterogeneous distribution when examined by sucrose gradient centrifugation (data not shown). The profile is similar to that for total polysomal poly(A+) mRNA, with a peak at about 23S. Figure 7 shows polyacrylamide gel analysis of the poly(A+) mRNP proteins from free and membrane bound polysomes. A major protein, designated A, slightly greater than 75,000 daltons, is present in both $mRNP_F$ and $mRNP_M$ eluates. A protein at about 50,000 molecular weight, B, is a major polypeptide in the $mRNP_F$, but is only slightly evident in

the $mRNP_M$ fraction. Polypeptide C, about 40,000 daltons, is a major component in the $mRNP_M$ fraction and is barely discernible in the $mRNP_F$ proteins.

We detect other differences in relative intensities as well as proteins that are absent in one fraction but present in the other. Some of the minor bands are not consistently seen; however, the A, B and C proteins have been reproduced in several separate experiments.

DISCUSSION

Elution of bound mRNPs from oligo(dT)-cellulose by formamide does not give consistent or quantitative recovery of bound material (Lindberg and Sundquist, 1974; Burns and Williamson, 1975). Lindberg and Sundquist pointed out that batches of oligo(dT)-cellulose that work well for mRNA isolation are less suitable for mRNP isolation and vice versa. The oligo(dT)-cellulose used for the experiments presented gives quantitative elution of mRNA and recoveries of mRNPs that range between 65 and 95% of the applied ^3H-uridine labeled material. Degradation of the eluted mRNPs and losses incurred in subsequent manipulations prior to electrophoresis of the proteins made the formamide elution technique unacceptable for a comparative analysis of the proteins associated with mRNAs in membrane bound and free polysomes. Since the membrane bound polysomes constitute only 25% of the total polysomes in MOPC-21 cells, we needed a technique that would give maximum mRNP recovery and separation that was minimally contaminated with ribosomal

subunits. The technique of high salt-SDS elution of mRNP associated proteins reported here met both requirements. The major disadvantages are that intact mRNP complexes are not obtained and the mRNAs and their associated proteins cannot be reassociated.

The proteins associated with mRNPs released from polysomes have been examined in a number of cell systems. Table 1 summarizes some of the data reported. In all cell types examined there are a limited number of proteins present. Two proteins with molecular weights that range between 49,000 to 56,000 daltons and 72,000 to 78,000 daltons appear to be present in nearly all cell types studied. The mRNPs obtained from MOPC-21 polysomes also exhibit a limited number of proteins when analyzed by SDS-PAGE. We consistently observe protein A which has a molecular weight of about 75,000 daltons. Proteins B (50,000 daltons), C (40,000 daltons) and D (35,000 daltons) are also present using both elution procedures. Proteins of larger molecular weight are often seen but require further study as they are minor components and proteins of similar size are prominent bands in the unbound fractions (data not shown). Rechromatography of the nonbound fraction or extraction of the nonbound RNA followed by oligo(dT)-cellulose chromatography demonstrates that species of mRNP and mRNA that contain poly(A) segments remain in the nonbound material (Irwin et al., 1975). These authors suggest that the larger proteins may inhibit in some way the retention of some of the mRNP complexes by

oligo(dT)-cellulose and thereby account for their abundance in the proteins of the nonbound fraction.

Assairi et al. (1976) examined the mRNP proteins from membrane bound polysomes in lactating ewe mammary glands. They isolated an mRNP component that sedimented on sucrose gradients at 22S, well separated from the ribosomal subunits. They found two major proteins, 52,000 and 72,000 daltons, and two minor proteins, 58,000 and 60,000 daltons, associated with the mRNPs. The mRNAs derived from the mRNPs directed the synthesis of α-casein and β-lactoglobulin, two milk proteins. There have been no other reports of studies on proteins associated with mRNAs from membrane bound polysomes.

The method of separation used to isolate membrane bound and free polysomes results in slight contamination of the membrane fraction with free polysomes. Not enough free polysomes are present, however, to contribute significantly to the proteins in the $mRNP_M$. A detailed study of the separation will be presented elsewhere.

SDS-PAGE of the proteins associated with $mRNP_F$ and $mRNP_M$ indicate that both qualitative and quantitative differences exist (see Table 2). Protein A, about 75,000 daltons, is present in both $mRNP_F$ and $mRNP_M$. This is similar in size to the poly(A) associated protein reported by Blobel (1973), Barrieux et al. (1976) and Schwartz and Darnell (1976). Protein B is a minor component of the $mRNP_F$ in the material eluted with formamide. High salt-SDS elution results in a change in the relative quantities recovered

and B becomes a prominent polypeptide in the $mRNP_F$ fraction. Trace amounts of B are seen occasionally in the $mRNP_M$ fraction but may be due to contamination of the membrane bound polysomes with free polysomes. Protein C is a prominent band in $mRNP_M$ and sometimes is present in the $mRNP_F$ fraction. We believe this too may be due to contamination at the initial separation of the membrane bound and free polysomes. Protein D (about 36,000 daltons) appears prominently in the $mRNP_F$ eluted with formamide and is a consistent yet minor component in the high salt-SDS eluted fraction. These results are difficult to explain, unless the incomplete recovery of bound mRNPs by the formamide elution technique results in protein losses which are nonrandom.

In order to study the mRNP proteins more fully, it is essential to obtain the proteins in a functional form; thus SDS must be eliminated. Studies are presently under way to test the possibility of eluting the proteins from the oligo(dT)-cellulose bound mRNPs using higher concentrations of NaCl followed by the elution of the mRNAs by low salt buffer. Such elution would allow further studies of the mRNA-protein interaction.

Differences in the protein moieties of subsets of mRNP complexes are compatible with the hypothesis that the proteins may serve a functional role in regulation of protein synthesis. Until the proteins can be obtained in a form that allows study of their interactions with mRNA, the significance of the differences remains obscure.

EXPERIMENTAL PROCEDURES

Buffers and Solutions

RSB: 10mM Tris, 10mM NaCl, 3mM $MgCl_2$, pH 7.4 at 4°C.

PB: 25mM Tris, 25mM NaCl, 5mM $MgCl_2$, pH 7.6 at 4°C.

Elution Buffer: 50 mM Tris, 10mM EDTA, 3mM $MgCl_2$, 0.5M NaCl, pH 7.6 at 4°C.

Application Buffer: 10mM Tris, 0.2mM $MgCl_2$, 0.5M NaCl, pH 7.6 at 4°C.

50% Formamide Buffer: 50% formamide in Elution buffer.

SDS Elution Buffer: 0.1% SDS in Application buffer.

Cell Culture and Labeling

MOPC-21 cells were grown in roller bottles in Dulbecco's Modified Medium (GIBCO) supplemented with streptomycin, 75 µg/ml, penicillin, 100 units/ml, mycostatin, 40 units/ml and 10% fetal calf serum. The medium containing the cells was flushed with 95% air-5% CO_2 and sealed for incubation. In these experiments cells were harvested when they had reached 5-8 x 10^5 cells/ml. RNA was labeled by the addition of ^{3}H-uridine (Amersham/Searle, 43 Ci/mmole) to a final concentration of 1 µCi/ml.

Isolation of Polysomes

Labeled cells were rapidly cooled by pouring over crushed frozen saline. The cells were pelleted by centrifugation for 10 min at 200 x g and washed twice with RSB. For isolation of total polysomes the washed cells were resuspended in RSB and lysed by the addition of one-tenth

volume 5% Nonidet P-40. After 10 min, nuclei were removed by centrifugation for 7 min at 700 x g. The supernatant was removed, the nuclei were washed once with RSB and the supernatants combined. This constitutes the postnuclear supernatant (PNS). One-tenth volume of 1% Triton X-100 was added to the PNS. The polysomes were either $MgCl_2$ precipitated according to the method of Palmiter (1974) or pelleted by centrifugation at 300,000 x g for 1 hr at 4°C in a Beckman SW50.1 rotor. To prepare membrane bound and free polysomes, twice washed cells were resuspended in RSB containing 0.25M sucrose. The cells were lysed by Dounce homogenization and the nuclei were pelleted. The PNS was layered onto a discontinuous gradient consisting of 0.5 ml 45% sucrose and 0.5 ml 65% sucrose in RSB. The gradients were centrifuged at 4°C for 45 min at 27,000 x g in the SW50.1 rotor. The supernatant above the 45% sucrose was removed and constitutes the free polysome fraction; the membrane bound polysomes form an opaque layer at the interphase between the 45% and 65% sucrose. The membrane bound polysome fraction was diluted, treated with 1% Triton X-100 and both fractions were made 0.5M in NaCl and the polysomes were pelleted as for total polysomes. The polysome pellets were rinsed twice with 2 ml RSB.

Oligo(dT)-Cellulose Chromatography

Polysomes were resuspended in RSB, dissociated by adding one-third volume 0.1M EDTA (pH 7.0) and centrifuged for 10 min at 700 x g to remove large clumps. The

suspension was made 1X in column elution buffer by addition of concentrated elution buffer.

Formamide elution: Poly(A+) mRNPs were isolated using the method of Lindberg and Sundquist (1974). Oligo(dT)-cellulose was prepared according to Gilham (1974). Three millimeter columns were formed in a disposable pipette. The dissociated polysome mixture was loaded onto the column, eluted with elution buffer followed by 50% formamide in elution buffer. One milliliter fractions were collected; 50 µl aliquots were precipitated with 10% trichloroacetic acid (TCA) using BSA as carrier. The precipitates were collected on Whatman GF/C glass fiber discs, washed with 5% TCA, dried and counted in 5 ml toluene-Omnifluor (New England Nuclear) scintillation fluid. Fractions containing mRNPs were combined and dialyzed against either RSB or PB.

SDS elution: The dissociated polysome mixture was loaded onto oligo(dT)-cellulose and eluted stepwise with application buffer, 0.1% SDS in application buffer and distilled water. The SDS buffer was removed from the column prior to elution of the poly(A+) mRNAs by washing with 20 to 25 ml application buffer. The SDS buffer fractions were combined, dialyzed against 50mM LiCl, and then lyophilized. The fractions containing poly(A+) mRNAs were combined and processed as in the figure legends.

Sucrose Gradient Analysis

mRNPs: Linear 10 to 30% sucrose density gradients were layered with dissociated polysomes or poly(A+) mRNPs

derived from oligo(dT)-cellulose chromatography. The gradients were centrifuged for 135 min at 300,000 x g in the SW50.1 rotor at 4°C. The gradients were fractionated on an ISCO density gradient fractionator and absorbance at 254 nm (A_{254}) recorded using a Gilford 2400-S recording spectrophotometer. Fractions were dissolved in toluene-Omnifluor:TX-100:H_2O (6:3:1) and counted in a Packard Tri-Carb 3320 Scintillation Spectrometer.

mRNAs: Linear density gradients, 5 to 20% sucrose in 0.1% SDS buffer, were layered with resuspended poly(A+) mRNAs that had been precipitated by the addition of 2 volumes ethanol in the presence of .2M NaCl. Gradients were centrifuged at 240,000 x g for 2 hr in the SW50.1 rotor at 20°C. The optical density profile was monitored and fractions collected and processed as for mRNPs.

SDS Polyacrylamide Gel Electrophoresis

mRNP preparations were precipitated with 10% TCA and resuspended in protein solution (10mM Tris, pH 8.0, 1mM EDTA, 1% SDS). Lyophilized poly(A+) proteins from SDS elution were resuspended in the protein solution. Ten percent polyacrylamide gels were prepared and electrophoresed according to Fairbanks et al. (1971). Gels were electrophoresed at constant voltage (90v) and were stopped when the tracking dye had migrated 8.5 cm. Gels were stained with Coomassie Blue (0.2%) in 50% methanol, 7% acetic acid and destained electrophoretically.

Wheat Germ Cell Free Protein Synthesis

Wheat germ extracts were prepared and cell free synthesis performed according to Davies and Kaesberg (1973) as modified by Astell and Ganoza (1974).

Figure 1. Sucrose Gradient Analysis of EDTA-Dissociated Polysomes and mRNP Derived from Polysomes.

MOPC-21 cells were labeled for 1 hr with ^{3}H-uridine, or were treated for 30 min with 0.04 µg/ml actinomycin D and then labeled for 1 hr with ^{3}H-uridine. The polysomes prepared from each set of cells were resuspended in RSB, EDTA dissociated and an aliquot centrifuged on 10-30% sucrose gradients. The remainder of the dissociated polysomes were chromatographed on oligo(dT)-cellulose. The poly(A+) mRNPs eluted by the formamide method were concentrated by precipitation, resuspended and layered onto gradients. The gradients were fractionated and radioactivity monitored as described in Experimental Procedures. Arrows indicate the location of small and large ribosomal subunits.

(a) Control.
(b) Actinomycin D treated.

●—● EDTA-dissociated polysomes.
○—○ Poly(A+) mRNPs.

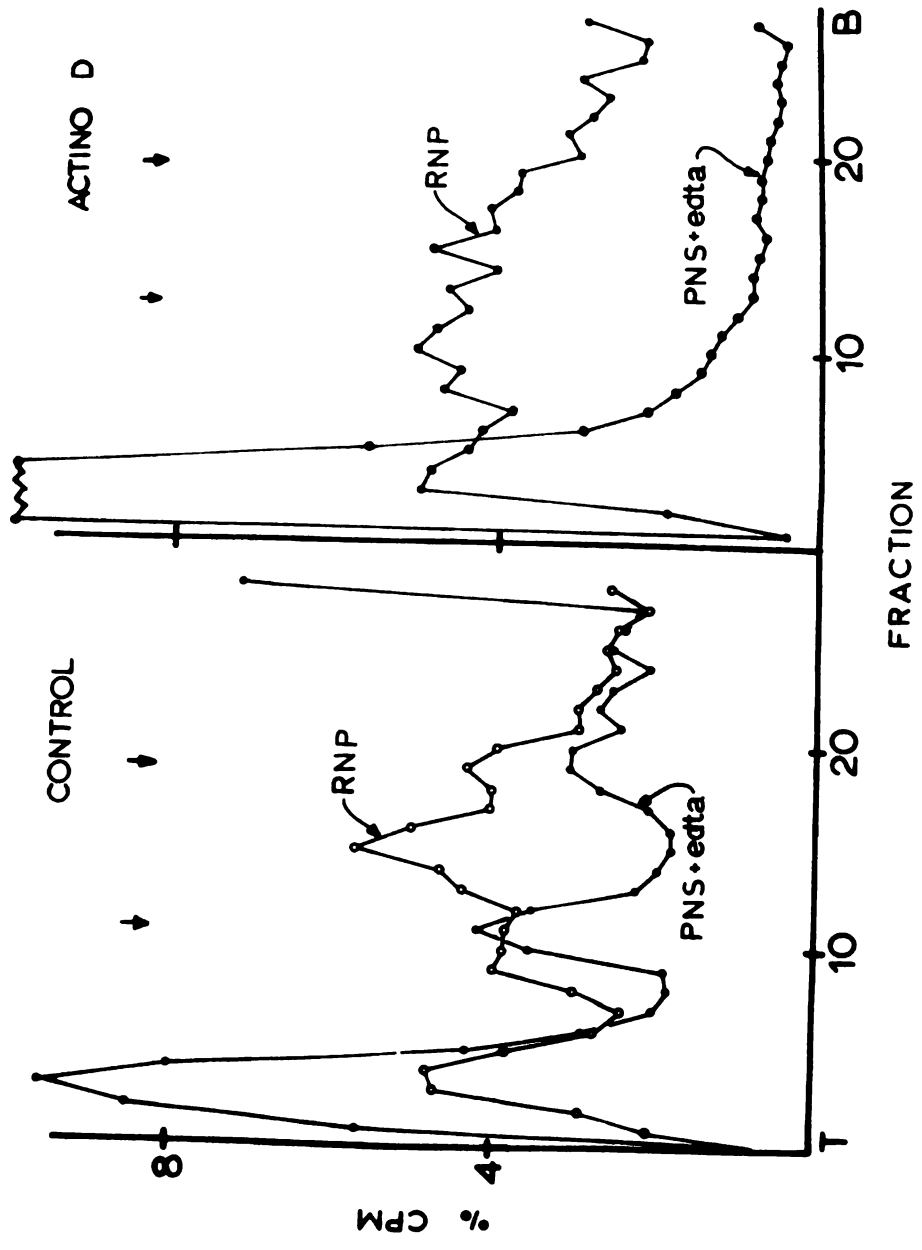
CONTROL
ACTINO D
RNP
PNS+edta
RNP
PNS+edta
% CPM
8
4
T
10
20
FRACTION
10
20
B

Figure 1

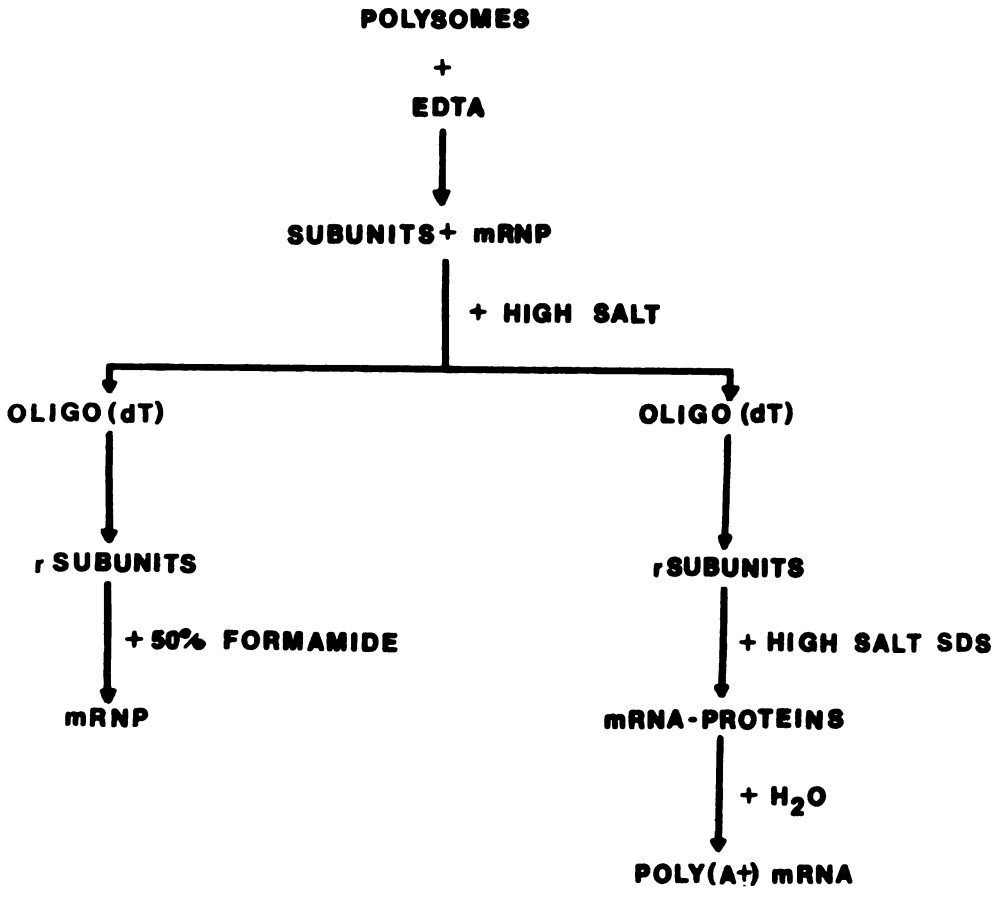

Figure 2. Flow Diagram of Oligo(dT)-Cellulose Chromatography of mRNPs.

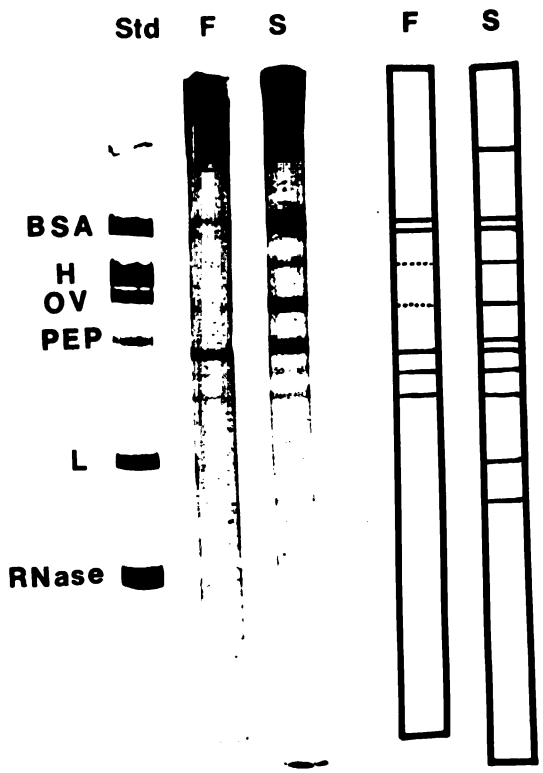

Figure 3. SDS-Polyacrylamide Gel Electrophoresis of mRNP Proteins Eluted by the Formamide and High Salt-SDS Method.

Gel Std. Standards: BSA (Bovine serum albumin), H (Heavy chain of MPOC-21 myeloma protein), OV (Ovalbumin), PEP (Pepsin), L (Light chain of MOPC-21 myeloma protein), RNase (Ribonuclease A).

Gel F. mRNP proteins isolated by the formamide method.

Gel S. mRNP proteins isolated by the high salt-SDS method.

Figure 4. Sucrose Gradient Analysis of mRNAs from Oligo(dT)-Cellulose Chromatography using High Salt-SDS Elution.

mRNA was dialyzed, lyophilized and resuspended. Two-tenths milliliter containing 55,000 cpm layered onto 5-20% linear sucrose gradient in 0.5% SDS buffer, centrifuged as described in Experimental Procedures.

Inset: Template activity of poly(A+) mRNA from MOPC-21 polysomes isolated by phenol-chloroform extraction and oligo(dT)-cellulose chromatography (A+) and mRNA from MOPC-21 polysomal mRNPs isolated by high salt-SDS elution method (RNP).

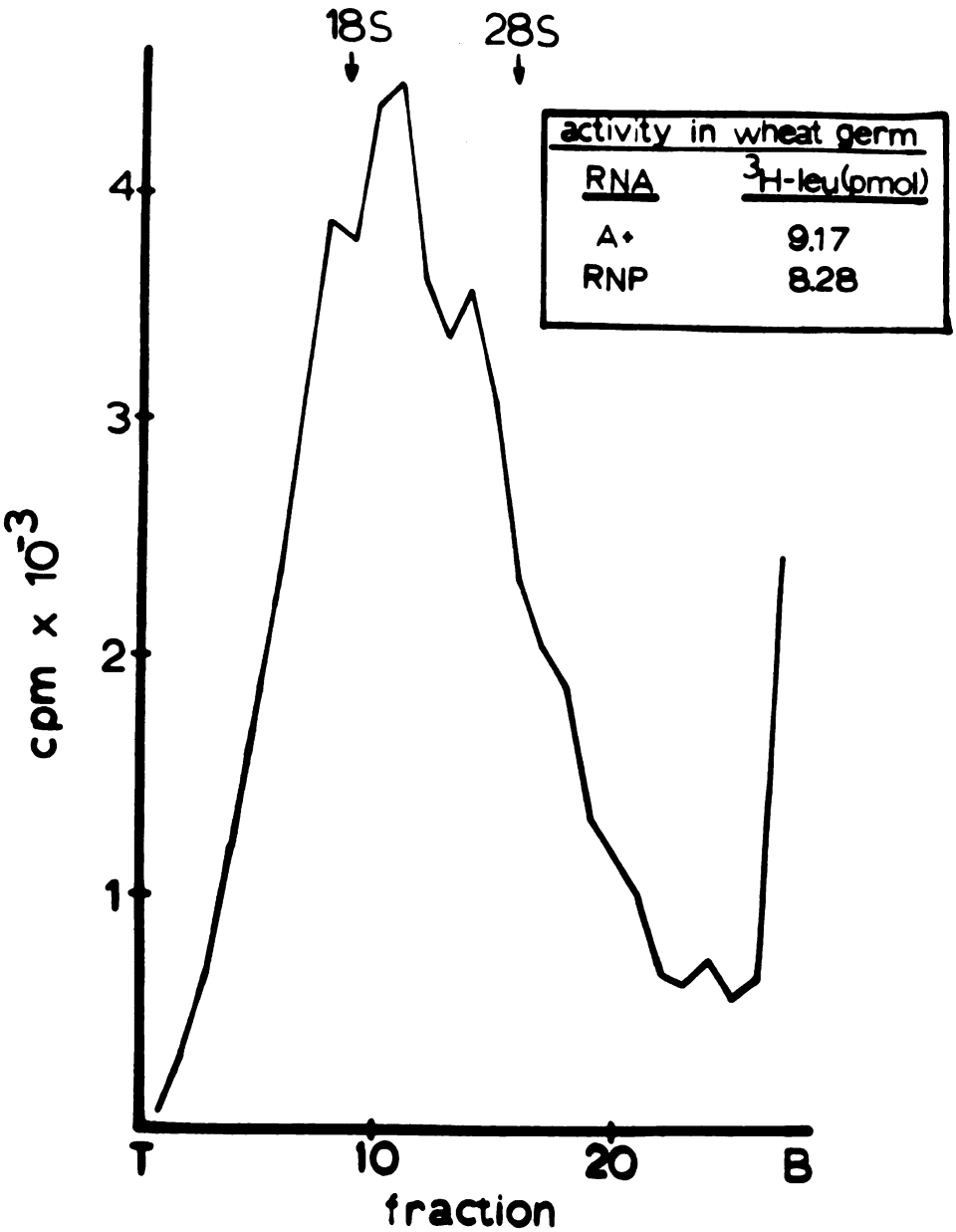
18S
28S
activity in wheat germ
RNA
^{3}H-leu(pmol)
A•
9.17
RNP
8.28
4
3
2
1
cpm x 10^{-3}
T
10
20
B
fraction

Figure 4

Figure 5. Radioactivity Distribution of mRNPs from Membrane Bound and Free Polysomes.

Polysomes were isolated from cells labeled for 1 hr. Membrane bound and free polysomes prepared as in Experimental Procedures. $mRNP_M$ was Mg-Ethanol precipitated according to Dessev and Grancharov (1973). $mRNP_F$ was dialyzed against PB and applied directly to the gradient.

●—● $mRNP_M$, 5000 cpm ^{3}H-uridine.

○—○ $mRNP_F$, 9500 cpm ^{3}H-adenine.

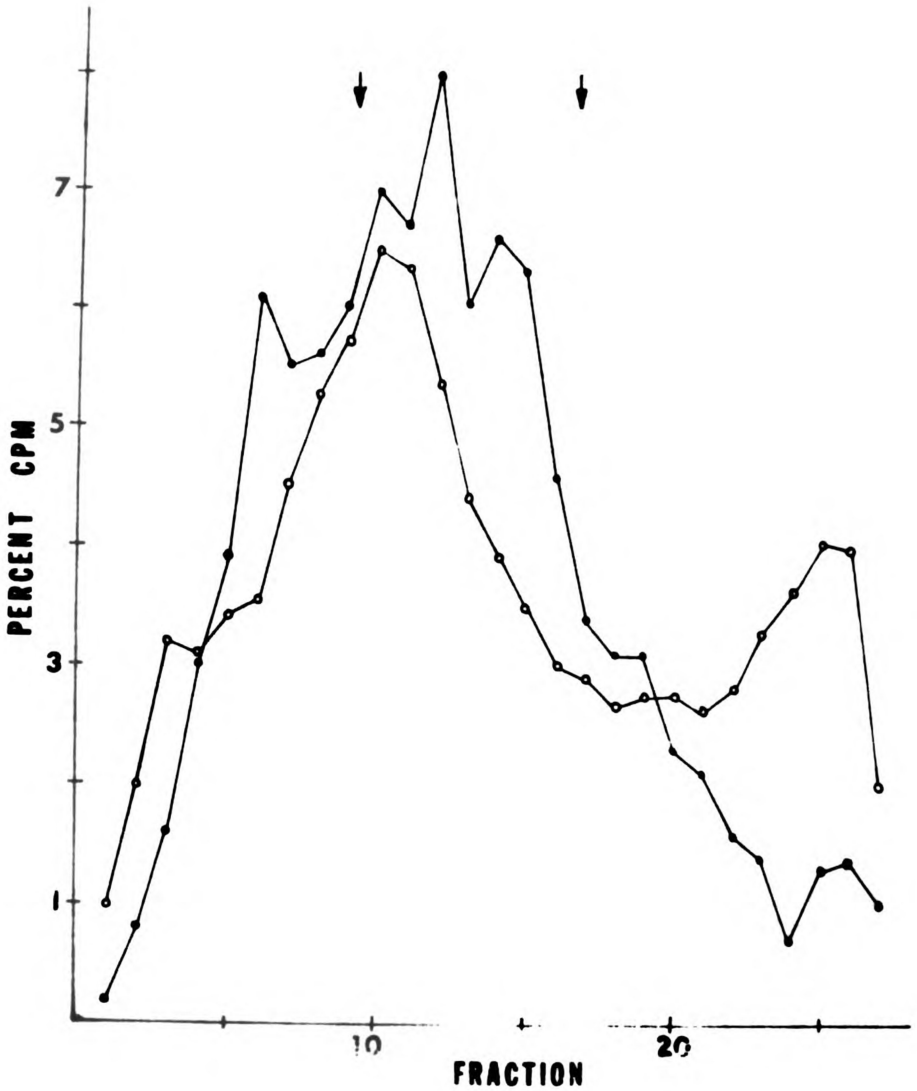

Figure 5

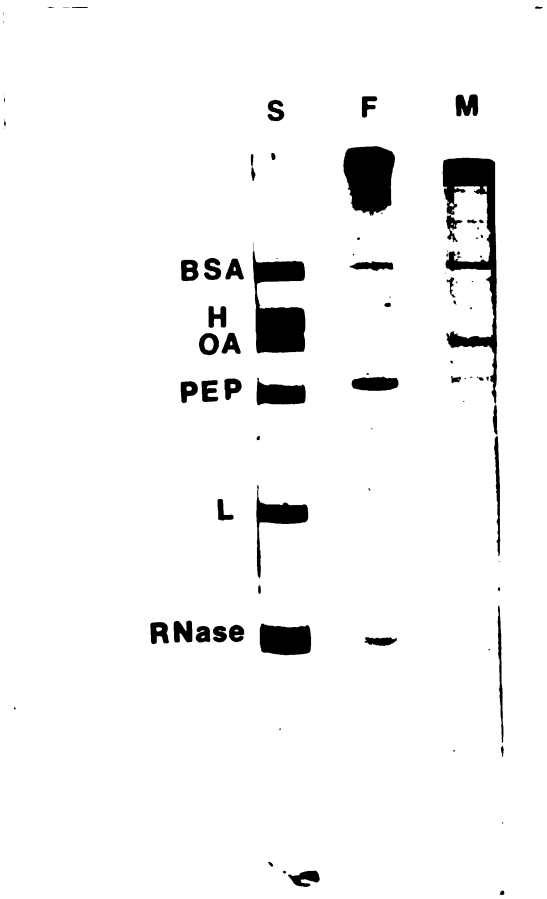

Figure 6. SDS-Polyacrylamide Gel Electrophoresis of $mRNP_F$ and $mRNP_M$ Proteins.

Poly(A+) mRNPs were isolated by oligo(dT)-cellulose chromatography using the formamide elution. The mRNPs were TCA precipitated and electrophoresed as described in Experimental Procedures.

Gel S: Standards (same as Figure 3).

Gel F: Free polysome mRNPs.

Gel M: Membrane bound polysome mRNPs.

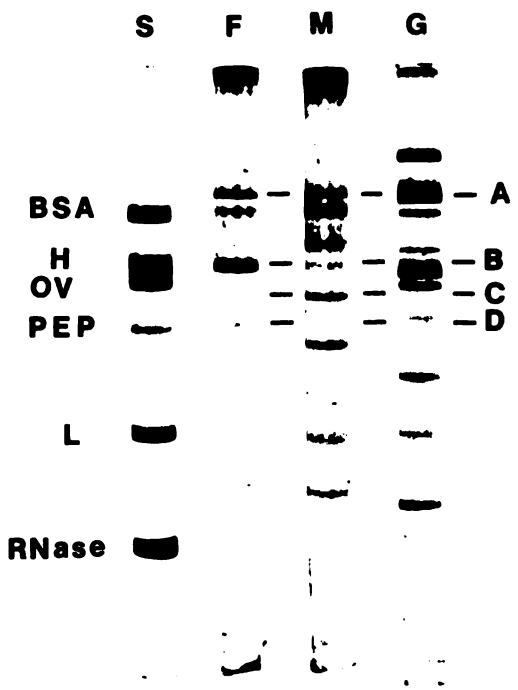

Figure 7. SDS-Polyacrylamide Gel Electrophoresis of Poly(A+) mRNP Proteins Eluted from Oligo(dT)-Cellulose by High Salt-SDS.

Proteins eluted by high salt-SDS were dialyzed, lyophilized and electrophoresed as described in Experimental Procedures.

Gel S: Standards (same as Figure 3).

Gel F: mRNP proteins from free polysomes.

Gel M: mRNP proteins from membrane bound polysomes.

Gel G: mRNP proteins from globin polysomes.

Table 1. Comparison of Sizes of Polysomal mRNP-Associated Proteins from Various Eukaryotic Cells

Cell Type	M.W. (daltons) x 10^{-3}	Reference
HeLa	52 76 120	Kumar and Pederson, 1975
	45 58 67 71 78 92	VanDer Marel et al., 1975
	50 73	Aurbach and Pederson, 1975
	50 75	Schwartz and Darnell, 1976
KB	56 68 78 130	Lindberg and Sundquist, 1974
	46 56 66 72 78 93	VanDer Marel et al., 1975
Lactating Ewe Mammary Gland	52 58 60 72	Assairi et al., 1976
Calf Lens	42 64 90 130	Chen et al., 1976
Ehrlich Ascites Tumor	34 52 78 130	Barrieux et al., 1975
Reticulocytes	52 78	Blobel, 1973
	49 73 140	Burns and Williamson, 1975
Rat Liver		Blobel, 1973
L Cells	30 36 76 87 103	Greenberg, 1977
	52 78	Blobel, 1973
Kidney	69, 75, 80, 100, 109, 118	Irwin et al., 1975

Table 2. Comparison of Major Polypeptides Isolated from $mRNP_M$, $mRNP_F$ and Globin mRNPs by Formamide or High Salt-SDS Elution from Oligo(dT)-Cellulose

Polypeptide	Formamide Elution		Approximate M.W. (daltons)	High Salt-SDS Elution		
	F	M		F	M	G
A	+	+	75,000	++	+	+++
B	±	-	50,000	++	±	++
C	-	+	40,000	±	++	
D	++	±	35,000	+	-	

F: mRNP proteins from free polysomes.

M: mRNP proteins from membrane bound polysomes.

G: mRNP proteins from rabbit reticulocyte polysomes.

-, no visible band.

±, faint band.

+, ++, +++, estimation of relative band intensities.

REFERENCES

Assairi, L., Gaye, P. and Houdebine, L. M. 1976. Biochimie 58:325-331.

Astell, C. R., and Ganoza, M. C. 1974. Mol. Biol. Reports 1:483-491.

Aurbach, S. and Pederson, T. 1975. Biochem. Biophys. Res. Commun. 63:149-156.

Aviv, H. and Leder, P. 1972. Proc. Natl. Acad. Sci. U.S.A. 69:1408-1412.

Barrieux, A., Ingrahm, H. A., Nystul, S. and Rosenfeld, M. G. 1976. Biochem. 15:3523-3538.

Bhorjee, J. S., and Pederson, T. 1973. Biochem. 12:2766-2773.

Blobel, G. 1972. Biochim. Biophys. Res. Commun. 47:88-95.

Blobel, G. 1973. Proc. Natl. Acad. Sci. U.S.A. 70:924-928.

Burns, T. H. and Williamson, R. 1975. Nuc. Acids Res. 2: 2251-2255.

Cartouzou, G., Poiree, J. C. and Lissitzky, S. 1969. Eur. J. Biochem. 8:357-369.

Chen, J. H., Lavers, G. C. and Spector, A. 1976. Biochim. Biophys. Acta 418:39-51.

Cioli, D. and Lennox, E. S. 1973. Biochem. 12:3211-3217.

Davis, J. W. and Kaesberg, P. 1973. J. Virol. 12:1434-1441.

Dessev, G. N. and Grancharov, K. 1973. Anal. Biochem. 53: 269-274.

Gander, E. S., Stewart, A. G., Morel, C. M. and Scherrer, K. 1973. Eur. J. Biochem. 38:443-452.

Georgiev, G. P. and Samarina, O. P. 1971. in Adv. in Cell Biology, eds. Prescott, D. M., Goldstein, L. and McConkey, E. (Appleton-Century-Crofts, New York), vol. 2 pp. 47-110.

Gilham, P. 1974. J. Amer. Chem. Soc. 86:4982-4985.

Greenberg, J. R. 1977. J. Mol. Biol. 108:403-416.

Henshaw, E. C. 1968. J. Mol. Biol. 36:401-411.

Irwin, D., Kumar, A. and Malt, R. A. 1975. Cell 4:157-165.

Kish, V. M. and Pederson, T. 1975. J. Mol. Biol. 95: 227-238.

Kumar, A. and Pederson, T. 1975. J. Mol. Biol. 96:353-365.

Laskov, R. and Scharff, M. D. 1970. J. Exp. Med. 131:515-541.

Lindberg, U. and Sundquist, B. 1974. J. Mol. Biol. 86: 451-468.

Lukanindin, E. M., Zalmanzov, E. S., Komaromi, L., Samarina, O. P. and Georgiev, G. P. 1972. Nature New Biol. 238:193-197.

Matringe, H. and Jacob, M. 1972. Biochimie 54:1169-1178.

Nudel, U., Lebleu, B., Zehavi-Willner, T. and Revel, M. 1973. Eur. J. Biochem. 33:314-322.

Palmiter, R. D. 1974. Biochem. 13:3606-3615.

Pederson, T. 1974. J. Mol. Biol. 83:163-183.

Perry, R. P. and Kelly, D. E. 1966. J. Mol. Biol. 16: 255-268.

Perry, R. P. and Kelly, D. E. 1969. J. Mol. Biol. 35: 37-59.

Schwartz, H. and Darnell, J. E. 1976. J. Mol. Biol. 104: 833-851.

Spirin, A. S. 1969. Eur. J. Biochem. 10:21-35.

Storb, U. 1973. Biochem. Biophys. Res. Commun. 52:1483-1491.

Stuart, S. E., Clawson, G. A., Rottman, F. M. and Patterson, R. J. 1977. J. Cell Biol. 72:57-66.

VanDer Marel, P., Tasseron-DeJong, J. E. and Bosch, L. 1975. FEBS Letters 51:330-334.