Isolation of mouse reticulocyte globin messenger ribonucleoprotein by affinity chromatography using oligo(T)-cellulose

Alan T.H. Burns and Robert Williamson

Institute of Animal Genetics, University of Edinburgh, Edinburgh EH9 and Beatson Institute for Cancer Research, Glasgow G3, UK.

Received 17 October 1975

ABSTRACT

Mouse globin messenger ribonucleoprotein (mRNP) has been isolated from reticulocyte polysomes by affinity chromatography to oligo(T)-cellulose, using a procedure modified from that of Lindberg and Sundquist. The messenger RNA and protein moieties of the mRNP are indistinguishable from those isolated by less rapid techniques, such as zonal ultracentrifugation.

INTRODUCTION

The development of affinity chromatography techniques for the isolation of plant and animal messenger RNAs (mRNAs) represents a great saving in time and increase in purity of material over procedures relying on size fractionation. These techniques rely on the binding of the poly(A) sequence characteristic of most animal and plant mRNAs to either poly(U) or oligo(T) immobilised on a matrix; ribosomal RNAs do not contain poly(A) sequences and pass through the affinity column while mRNA is retained and later eluted by dissociating solvents or conditions.

Messenger ribonucleoprotein can also be isolated by affinity chromatography, using oligo(T)-cellulose, but to date the procedure has only been reported for normal and adenovirus infected human K3 cells and for mouse kidney cells, for which the mRNA profile is difficult to evaluate. We report similar experiments for the well characterised mouse reticulocyte polysomal mRNP. The application of this technique should permit the rapid isolation of mRNP from a variety of higher cells, which could be useful in studies of mRNA processing and mRNA-protein association.

MATERIALS AND METHODS

Mouse reticulocyte polysomes were prepared as previously described. These were then washed with high salt buffer and dissociated with puromycin exactly as described by Blobel. The puromycin-dissociated polysomes were mixed with 4 volumes of column equilibration.
buffer (0.2M NaCl, 50 mM Tris-HCl, pH 7.8, 10 mM EDTA, 0.2% NP-40) and passed through a column containing 2.5 ml of oligo(T)-cellulose equilibrated in the same buffer. After washing the unadsorbed ribosomal subunits from the column with 10 volumes of buffer, the retained material was eluted with 6 ml of elution buffer (0.2M NaCl, 50 mM Tris-HCl, pH 7.8, 10 mM EDTA, 50% formamide). Both fractions were brought to 0.4M NaCl and precipitated with 2 volumes ethanol at -20°C. Material still bound to the column after formamide elution was estimated by washing the column with 0.1M NaOH and measuring the absorbance at 260 nm.

RNA was extracted from the ethanol pellets by dissolving in NETS buffer (0.1M NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4, 0.2% sodium dodecyl sulphate) and deproteinising with an equal volume of phenol/chloroform. The RNA was analysed on 2.6% polyacrylamide gels as previously described 4,8 (Fig. 1).

Figure 1. Gel electrophoresis of RNA from bound mRNP (---) and total reticulocyte RNA (-----) run on 2.6% gels and scanned at 265 nm.
For analysis of proteins on SDS-acrylamide gels, the mRNP pellets were dissolved in 1% sodium dodecyl sulphate, 8 M urea, 1% 2-mercaptoethanol, 0.01 M Tris-HCl, pH 7.5, for 3 hours at 37°C, then dialysed overnight at room temperature against the same buffer except for 0.1% SDS. Samples at 2 mg protein/ml were applied to 15% polyacrylamide gels and electrophoresis and analysis carried out as described in MacGillivray et al.9 (Fig. 2).

![Electrophoretic pattern of the 50% formamide fraction obtained by oligo(T)-cellulose chromatography of puromycin dissociated polysomes from mouse reticulocytes. Electrophoresis in 15% polyacrylamide gels containing sodium dodecyl sulphate was performed according to the method of MacGillivray et al.9. Gels were stained for protein with 0.2% Coomassie brilliant blue in 45% ethanol - 10% acetic acid for 30 minutes at 65°C. Also shown in diagrammatic fashion is the distance of migration of marker proteins electrophoresed in parallel individual gels.](https://academic.oup.com/nar/article-abstract/2/12/2251/1099337)

The molecular weights of the proteins were calculated using the method of Weber and Osborn 10 using β-galactosidase (130,000), α-phosphorylase (94,000), bovine serum albumin (68,000), catalase (60,000), ovalbumin (43,000), aldolase (40,000), chymotrypsinogen (25,700) and cytochrome C (12,400) as markers.
RESULTS AND DISCUSSION

Several batches of oligo(T)-cellulose were used in these experiments, and some bound no mRNP at all. Those batches which bound isolated mRNA quantitatively from total polysomal RNA were in general unsatisfactory for mRNP. Dr. M. Doel, of G.D. Searle Research Laboratories, High Wycombe, Bucks, England, kindly provided several batches of oligo(T)-cellulose which were poor at binding isolated mRNA, and of these, three bound mRNP preferentially and one (Batch 14C) bound mRNP with low background binding of ribosomal subunits. There is no apparent chemical difference between the batches, and we cannot suggest an explanation for this variability, which was previously noted by others. For batch 14C, 96% of the total absorbance at 260 nm was unadsorbed to the column, 2% was retained and eluted with elution buffer, and 2% was only eluted with alkali. In agreement with Lindberg and Sundquist, we never succeeded in recovering more than approximately half of the absorbance at 260 nm retained by the column. Washing with 90% formamide buffer did not improve the eluted yield. We do not know if this tightly bound material includes mRNP or is ribosomal or other contaminants.

The profile obtained for RNA isolated from column-bound mRNP on 2.6% polyacrylamide gel electrophoresis is shown in Figure 1, with total reticulocyte polysomal RNA as marker. The RNA derived from mRNP runs primarily at 9S, in the position of intact globin mRNA, and shows little contamination with ribosomal RNA and a small amount of breakdown. Dissociation of polysomes with EDTA led, in our hands, to greater breakdown of mRNA than did dissociation with puromycin.

A 15% SDS-polyacrylamide gel analysis of mRNP proteins is shown in Figure 2. In mouse reticulocytes there are no major protein species other than globin and ribosomal proteins which could contaminate mRNP proteins. Two major protein species, migrating at positions corresponding to molecular weights of 73,000 and 49,000 were identified, as well as two minor protein moieties of minimum molecular weight approximately 140,000. Other minor bands of smaller molecular weight were occasionally observed, but were presumed to be contaminants, since only those of described components were found routinely in every preparation. The molecular weights of the two major components are in excellent agreement with the values reported for rabbit globin mRNP and duck globin mRNP.

These results with a characterised mRNP specific for globin mRNA confirm the data of Lindberg and Sundquist obtained with EDTA-
released mRNA from a cultured cell line. There are, however, differences in molecular weight for the protein moieties with the data of Irwin et al., which we cannot explain. We can confirm the absence of contamination with ribosomal RNA because of the known migration properties of globin mRNA on polyacrylamide gel electrophoresis.

Isolation with puromycin from salt-treated ribosomes decreases both adventitious binding of cytoplasmic proteins to mRNP and also RNA breakdown. A detailed study of the parameters of the affinity column giving most effective binding and release of mRHF is still required.

ACKNOWLEDGMENTS

We wish to thank Mrs. Theresa McShane, Mrs. Ruth Clayton and Dr. D.E.S. Truman for helpful discussions and Searle Research Products Ltd., High Wycombe, for the gift of batches of oligo(T)-cellulose. The Beatson Institute for Cancer Research is funded by the Medical Research Council and the Cancer Research Campaign. Mr. A.T.H. Burns was supported by the Cancer Research Campaign.

REFERENCES

6 Williamson, R. (1973) FEBS Letters 37, 1-6