Developmental changes in the protein and ribonucleic acid components of rat brain messenger ribonucleic acid–protein particles isolated from free polyribosomes by oligo(dT)–cellulose chromatography

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A study has been made of the developmental changes that occur in the RNA and protein moieties of mRNA–protein particles isolated from newborn and adult rat forebrain free polyribosomes. mRNA–protein particles were isolated by oligo(dT)–cellulose chromatography from salt-washed polyribosomes dissociated by puromycin/0.5 M-KCl treatment as two fractions (E_1 and E_2) by using Tris/HCl/NaCl eluting buffers containing respectively 25 and 50% (v/v) formamide. Isopycnic centrifugation on CsCl gradients showed that the newborn-derived fractions E_1 and E_2 has buoyant densities of 1.48–1.50 and 1.41–1.43 g/cm³. Adult-derived E_1 and E_2 fractions had corresponding values of 1.47 and 1.42 g/cm³. The pooled mRNA–protein particles from the E_1 and E_2 fractions after deproteinization with proteinase K sedimented with a mean size of approx. 18S on a sucrose gradient containing 85% formamide with little differences between mRNA molecules from newborn and adult. The mean lengths of the poly(A) segments were similar, being about 130 nucleotides long. Distinct changes were found in the protein composition of the mRNA–protein particles. Fractions E_1 and E_2 from the newborn contained two major proteins of mol.wts. 74000 and 52000 with differences in the relative proportions in each fraction. In contrast, adult fractions E_1 and E_2 contained predominantly the larger protein. However, the adult fraction E_2 contained a more heterogeneous population of minor bands of proteins, including that of mol.wt. 52000. These findings are discussed briefly in relation to other changes in the developing brain.

There is considerable evidence that eukaryotic mRNA molecules are associated with proteins (Blobel, 1972, 1973; Bryan & Hayashi, 1973; Morel *et al.*, 1973; Liautard *et al.*, 1976; Barrieux *et al.*, 1976), and that mRNA–protein particles from different tissues and species commonly appear to contain two major proteins of mol.wts. 49000–52000 and 72000–78000. The heavier protein appears to be associated with the poly(A) segment (Blobel, 1973; Kwan & Brawerman, 1972). The lighter protein was reported to have properties similar to those of one of the subunits of the initiation factor IFMP or eIF-2 (Hellerman & Shafritz, 1975; Barrieux *et al.*, 1976). However, it has now been shown that the lighter protein and the β -subunit

of eIF-2 are not identical (Barrieux & Rosenfeld, 1979). The role of this protein remains unestablished although it has also been suggested that proteins associated with mRNA may play a role in the transport of mRNA from the nucleus to the cytoplasm (Schwartz & Darnell, 1976).

Differences in the metabolism of high-molecular-weight RNA from newborn and adult rat forebrain have been reported (Berthold & Lim, 1976*a,b*). The transition from the neonatal replicative phase to the non-replicative phase of the adult is associated with a decrease in the synthesis of mRNA relative to rRNA and in the nucleo-cytoplasmic transport of poly(A)–RNA. A developmental increase in transcriptional diversity has also been observed in rodent brains (Grouse *et al.*, 1972). In the present paper, we report on developmental changes that occur in the polyribosomal mRNA–protein particles isolated

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from the forebrains of neonatal and adult rats, particularly in the protein components. A brief description of the present work has been published (Elliott *et al.*, 1977).

Experimental

Materials

Analytical-reagent-grade chemicals were used in all cases. Glassware and centrifuge tubes were all treated with 0.1 M-NaOH before being thoroughly rinsed with deionized water. [32 P] P_i (sp. radioactivity 30–100 mCi/mg) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., as a sterile solution, pH 2–3 (radioactivity 10 mCi/ml). [3 H]Poly(U) (sp. radioactivity 200 Ci/mol of P) was obtained from Miles Laboratories, Slough, Bucks., U.K. Puromycin dihydrochloride, ribonuclease A (50–100 Kunitz units/mg; type III A proteinase-free), deoxyribonuclease I from bovine pancreas (2000 Kunitz units/mg), ribonuclease T1 grade III from *Aspergillus oryzae* (300 000–500 000 units/mg) and sucrose grade I (nuclease-free) were obtained from Sigma Chemical Co., Kingston upon Thames, Surrey, U.K. Poly(A) of mean size A_{164} was obtained from BDH Chemicals, Enfield Road, Middlesbrough, U.K. Oligo(dT)–cellulose of average length (dT) = 10 came from Collaborative Research supplied through Uniscience, Cambridge, U.K.

Animals

Newborn and adult Wistar rats (Cob strain) were bred either in our laboratory or purchased when required from Charles River, Margate, Kent, U.K. Rats obtained from this latter source were allowed to recover in our animal house for at least 24 h before being used in any experiment. 32 P-labelled polyribosomes were isolated from newborn and adult rats 24 h after intracranial injection of iso-osmotic neutralized [32 P] P_i (for details see Berthold & Lim, 1976a,b) at a dosage of 30 μ Ci and 1 mCi/rat for newborn and adult rats respectively. Rats were killed by decapitation and their forebrains were homogenized in 0.25 M-sucrose/TKM buffer [50 mM-Tris/HCl (pH 7.6)/25 mM-KCl/5 mM-MgCl $_2$]/1 mM-dithiothreitol. About five to 15 strokes of a loose-fitting Teflon/glass Elvehjem homogenizer (clearance 0.15–0.23 mm) were sufficient to homogenize the tissue from both newborn and adult rat brain. Dissection and homogenization were carried out in a cold room maintained at 4°C, as were all subsequent procedures, except where stated.

Isolation of cerebral free polyribosomes

Free polyribosomes from the cerebrum were isolated by the method of Bloemendal *et al.* (1974), excepting that the homogenizing medium and

'heavy' sucrose solutions (1.5 and 2.0 M) were made up in TKM buffer including 1 mM-dithiothreitol.

Salt-washed polyribosomes

About 25–30 A_{260} units of polyribosomes were suspended in 2.5–3.0 ml of 0.25 M-sucrose/TKM buffer containing 1 mM-dithiothreitol. An equal volume of the same sucrose buffer containing 1 M-KCl was then added dropwise with stirring. The suspended polyribosomes were kept for 40 min with stirring before they were centrifuged on an MSE65 titanium rotor at 53 000 rev./min (200 000 g) for 3 h at 2–4°C. The salt-extracted polyribosome-associated proteins were decanted off, and the polyribosomal pellet was rinsed several times with deionized water and either used immediately or stored at –20°C after freezing on crushed solid CO $_2$.

Isolation of mRNA–protein particles

Salt-washed polyribosomes (25–30 A_{260} units) were suspended in 0.5 ml of ice-cold TKM buffer (pH 7.6). To this suspension was added slowly and with mixing an equal volume of ice-cold TKM buffer (pH 7.6) containing in addition 1 M-KCl, 0.2 M-puromycin dihydrochloride and 2 mM-dithiothreitol. After incubating at 30°C for 40 min the dissociated polyribosomes were layered on the oligo(dT)–cellulose column, and the column was washed with high-salt buffer [50 mM-Tris/HCl (pH 7.6)/0.5 M-KCl] until the background radioactivity was decreased to a fairly low constant value. Fractions (5 ml) were collected from the column and monitored by measurement of the Čerenkov radiation. Two buffers were used consecutively to elute the two mRNA–protein fractions. The first buffer (for fraction E $_1$) contained 50 mM-Tris/HCl (pH 7.6), 0.2 M-NaCl and 25% (v/v) formamide, and the second (for fraction E $_2$), the same buffer in which the formamide content was increased to 50% (v/v) (Irwin *et al.*, 1975).

Determination of buoyant densities on CsCl gradients

Salt-washed [32 P] P_i -labelled mRNA–protein particles were characterized by their buoyant densities on preformed linear 30–55% (w/w) CsCl gradients by using a modification of the method described by Baltimore & Huang (1968). mRNA–protein particles isolated from oligo(dT)–cellulose were either dialysed or precipitated with 2.5 vol. of ethanol before centrifugation on CsCl gradients. Dialysis of mRNA–protein fractions was against three changes of 10 mM-triethanolamine/HCl (pH 7.0) buffer containing 0.5% (w/v) Brij 58 at 2–4°C for about 4 h. Ethanol-precipitated mRNA–protein fractions, however, were collected by centrifugation, the ethanol decanted off and finally removed in a vacuum

desiccator. The pellet obtained was dissolved in 0.8 ml of the triethanolamine/Brij buffer. mRNA-protein fractions (0.8 ml) were fixed by addition of 0.2 ml of freshly neutralized 33% (v/v) glutaraldehyde and then centrifuged on a 5 ml linear preformed CsCl gradient after filling most of the remaining volume of the centrifuge tube with mineral oil. Centrifugation was for 5 h on an MSE 6 × 14 titanium rotor so as to give 170 000 g at the mid-point of the gradient (34 000 rev./min). After fractionation of the gradient, density measurements were made with a pycnometer, and the radioactivity of each fraction was determined after addition of 10 ml of deionized water by measurement of the Cerenkov radiation.

Isolation of brain mRNA molecules from mRNA-protein particles and analysis of the mRNA molecules by sucrose/formamide gradients

The method of MacNaughton *et al.* (1974) was used to deproteinize the combined (E_1 and E_2) mRNA-protein fractions with proteinase K before analysis of the mRNA on 2–10% (w/v) sucrose gradients containing 85% (v/v) formamide. Centrifugation was in polypropylene tubes with an MSE 6 × 15 aluminium rotor for 42 h at 24 000 rev./min (70 000 g) at 25°C. 32 P-labelled rRNA (18S and 28S) fractions were treated in the same way and run in parallel as markers for sizing the mRNA.

Lengths of poly(A) segments of mRNA-protein fractions

The poly(A) lengths of the combined (E_1 and E_2) mRNA-protein fractions were determined by the method of Cabada *et al.* (1977), after the deproteinization step, and subsequent removal of protein by chloroform/isopentanol extraction (Lebleu, 1974). Briefly, the method consisted of an enzymic degradation of the crude mRNA fraction with deoxyribonuclease I followed by ribonuclease I. The poly(A) fractions were then electrophoresed on a 10% polyacrylamide/0.25% bisacrylamide gel at pH 7.8 together with markers 5S rRNA, 4S tRNA and Bromophenol Blue. An additional marker of sized poly(A) was also used. The marker gels were scanned at 260 nm and poly(A)-containing fractions were sliced into 1 mm fractions and 2 × 1 mm slices were homogenized together in 2 × SSC buffer [0.15 M-NaCl/0.015 M-sodium citrate (pH 6.8)]. After elution of the poly(A) by warming to 37°C with this buffer, the gel slices were re-extracted with a further volume of 2 × SSC buffer and the two extracts of the poly(A)-containing material were combined. This poly(A)-containing solution (0.9 ml) was annealed with excess [3 H]poly(U) (18 000 c.p.m.) for 30 min at 45°C. Blanks containing no poly(A) were also included. After hybridization the samples were digested with 0.5 ml of

ribonuclease A (in 2 × SSC buffer, 40 µg/ml) at 0°C for 20 min. The nuclease-resistant hybrids were precipitated by addition of 0.5 ml of 4% (w/v) cetyltrimethylammonium bromide and 0.5 ml of 1 M-sodium acetate, pH 5.0, containing 2 mg of yeast RNA carrier and radioactivity was determined. Controls from samples containing no poly(A) but with [3 H]poly(U), all had low amounts of radioactivity (approx. 120 c.p.m.).

Polyacrylamide-gel electrophoresis of mRNA-protein particles

The E_1 and E_2 mRNA-protein fractions from the oligo(dT)-cellulose column were collected by precipitation with 2.5 vol. of ethanol and centrifugation after leaving overnight at -20°C. The mRNA-protein fractions were suspended in a small volume of 50 mM-phosphate buffer (pH 7.4), containing 2% sodium dodecyl sulphate, and reduced with 2-mercaptoethanol before electrophoresis on 10% polyacrylamide gels, as described by Weber & Osborn (1969).

Results and discussion

Characterization of mRNA-protein particles

(a) *Isolation.* The isolation of brain mRNA-protein particles from free polyribosomes was preceded by a 0.5 M-KCl/salt washing of the polyribosomes. This salt-washing step was necessary because a number of the extracted 'salt-wash proteins' were found to bind strongly to oligo(dT)-cellulose under the high-salt conditions used for the affinity chromatography. These proteins were also eluted by the Tris/formamide buffers used to elute the mRNA-protein fraction (Fig. 1). Most of the protein was eluted in the first fraction (E_1), with a major component of approx. 40 000 mol.wt. common to both newborn and adult brain preparations. In the preparations from the newborn-animal protein of mol.wt. 52 000 was found, which did not appear to be present to the same extent in the adult rat preparation. Much less protein was eluted with the second (E_2) buffer. The protein of mol.wt. 52 000 appeared to be the major component in the newborn-animal fraction, whereas the adult E_2 fraction was apparently composed of the same population of proteins as that of the adult E_1 fraction, although present in decreased amounts. It is possible that some of these proteins are derived from mRNA-protein particles; however, the contribution of these particles would be minimal since the KCl treatment released very small amounts of the particles.

After the salt-wash step brain polyribosomes were completely dissociated to subunits, by using a combined puromycin/0.5 M-KCl treatment. This means of dissociation does not result in release of

Fig. 1. Binding of the 0.5M-KCl-extractable proteins of polyribosomes to oligo(dT)-cellulose

Brain polyribosomes were extracted with buffer containing 0.5M-KCl as described in the Experimental section. The salt-washed polyribosomes were pelleted by centrifugation at 20000g for 3h at 2–4°C. The top two-thirds of the supernatant containing the salt extract was then passed down an oligo(dT)-cellulose column that had previously been washed in high-salt buffer (50mM-Tris/HCl (pH 7.6)/0.5M-KCl). Elution was continued with a large volume of the high-salt buffer until well after no absorbance at 280nm could be detected. The column was then eluted with the buffer for fraction E₁ (containing 25% formamide; see the Experimental section) and the material eluting in the first 5ml was collected and precipitated with 2.5vol. of ice-cold ethanol. Another 30ml of the buffer for fraction E₁ was used to wash the column before elution with the buffer for fraction E₂ (containing 50% formamide; see the Experimental section). Again the first 5ml was collected and precipitated with 2.5vol. of ethanol. After being left at –20°C overnight, the precipitated proteins were collected by centrifugation at 26000g for 1h at 2–4°C. The precipitated proteins were then dissolved in 50mM-phosphate buffer (pH 7.4) containing 2% (w/v) sodium dodecyl sulphate, reduced with 2-mercaptoethanol and layered on to pre-run cylindrical 10% polyacrylamide gels containing 0.2% (w/v) sodium dodecyl sulphate. Electrophoresis was for 6h at 8mA/gel. Gels were stained with Coomassie Blue and photographed after de-staining with methanol/acetic acid (Weber & Osborn, 1969). Migration was from the cathode to the anode.

ribosomal proteins (Sherton *et al.*, 1974), nor does it apparently result in any loss of biological activity of the mRNA-protein particles (Liautard, 1977). The dissociated ribosomes, including the released mRNA-protein particles, were subjected to oligo(dT)-cellulose chromatography. Fig. 2 shows a typical elution of ³²P-labelled brain mRNA-protein particles from oligo(dT)-cellulose. About 2% of the total radioactivity present in the salt-washed polyribosomes was eluted in fractions E₁ and E₂ derived

Fig. 2. Oligo(dT)-cellulose chromatography of dissociated salt-washed polyribosomes

Salt-washed ³²P-labelled polyribosomes were dissociated by a combined 0.5M-KCl/puromycin treatment and subjected to oligo(dT)-cellulose chromatography. All details are described in the Experimental section under 'Isolation of mRNA-protein particles'. The arrow at E₁ represents the changeover from elution with high-salt buffer [50mM-Tris/HCl (pH 7.6)/0.2M-NaCl/25% formamide] and the arrow at E₂ the subsequent changeover to elution with the buffer for fraction E₂, which contained 50% formamide in place of 25% formamide.

from preparations from both adult and newborn animals. Most of the total polyribosomal radioactivity was not bound to the oligo(dT)-cellulose and the majority of this material was eluted in the first few fractions. Generally, the newborn-animal mRNA-protein fraction E₂ contained about 70–80% of the radioactivity of the corresponding E₁ fraction, whereas the fraction E₂ from adult animals contained only about 50% of the radioactivity of the corresponding E₁ fraction. The cause of the stronger binding that occurs in fraction E₂ is not known, though there is evidence in the case of mRNA-protein particles derived from mouse kidney, that fraction E₂ contains mRNA-protein with longer 3'-poly(adenylic acid) segments (Irwin *et al.*, 1975).

(b) *Buoyant-density measurements.* As a first step towards the characterization of the salt-washed mRNA-protein present in fractions E₁ and E₂ their buoyant densities on CsCl gradients were determined. Isopycnic centrifugation analysis can yield information about the proportions of RNA and

Fig. 3. Determination of buoyant densities of mRNA-protein particles on CsCl gradients

^{32}P -labelled mRNA-protein particles from newborn and adult brains were isolated as described in the text. The mRNA-protein particles after fixing with 33% (v/v) glutaraldehyde were centrifuged on 30–55% (w/w) CsCl gradients containing 0.5% (w/v) Brij 58. Centrifugation was for 5 h at 170000g (mid-point). All details of the determination of buoyant densities are given in Experimental section. Symbols $\square$, density; $\circ$, ^{32}P radioactivity.

protein present in the mRNA-protein fractions and whether or not there is ribosomal contamination of the mRNA-proteins. The particles in the E_1 fractions from newborn and adult rats had buoyant densities of respectively 1.48–1.50 g/cm³ and 1.47 g/cm³ (Fig. 3). These values can be used to compute (Spirin, 1969) the composition of the newborn-animal mRNA-protein particles in E_1 fraction as approx. 58–62% protein with 42–38% RNA and the composition of the mRNA-protein particles in the adult E_1 fraction as approx. 63% protein and 37% RNA. The mRNA-protein particles from the E_2 fraction of newborn animal brains had a buoyant density of approx. 1.41–1.43 g/cm³, whereas those from adult E_2 fraction were 1.42 g/cm³, which results in a computed composition of 70% protein and 30% RNA for both.

(c) *Sizes of mRNA and length of poly(A) segments.* Because of the low yields fractions E_1 and E_2 were pooled before analysis. The pooled fractions from newborn and adult ^{32}P -labelled rat brain

mRNA-protein particles were then deproteinized and subjected to sucrose-density-gradient analyses (Fig. 4). The average size of the mRNA derived from the pooled mRNA-protein fractions of both newborn and adult brain preparations was approx. 18S with a small component of about 28S present in both. No major differences in size was found between newborn and adult rat brain mRNA molecules. Bantle & Hahn (1976) have reported a similar mean size for mouse brain mRNA on sucrose/dimethyl sulphoxide gradients.

To examine the developmental changes in size of brain mRNA further, the lengths of the poly(A) segments of the newborn and adult brain mRNA-protein particles were also determined. By using the method of Cabada *et al.* (1977), with poly(A) markers of different length for calibration, it was found that both newborn and adult brain mRNA-protein fractions had poly(A) segments ranging from 100 to 160 nucleotides with a mean length of approx. 130 nucleotides (Fig. 5). This result is in

Fig. 4. Size determination of polyribosomal mRNA molecules derived from mRNA-protein particles by proteinase K treatment

Newborn and adult rat mRNA-protein fractions derived from ^{32}P -labelled salt-washed polyribosomes were suspended in 2 ml of buffer [15 mM-KCl/10 mM-Tris/HCl (pH 7.5)/1.5% (w/v) sodium dodecyl sulphate] containing 400 μg of proteinase K. The solution was brought to 25°C for 30 min and made 0.2 M in NaCl; 50 μg of tRNA carrier was added, followed by 2.5 vol. of ice-cold ethanol. The mRNA fractions were left overnight at -20°C and collected by centrifugation at 26000g for 1 h at 2-4°C. The pelleted mRNA molecules were then dissolved in 0.5 ml of formamide buffer [10 mM-Tris/HCl (pH 7.5)/1 mM-EDTA/85% (v/v) formamide], warmed to 37°C to denature the RNA and centrifuged on a linear 2-10% (w/v) sucrose gradient containing 85% (v/v) formamide buffer. Centrifugation was at 70000g for 42 h at 25°C. Parallel gradients containing ^{32}P -labelled 28S and 18S rRNA were also run. The gradients were fractionated on an ISCO model 640 fractionator into 0.6 ml fractions, which were monitored for radioactivity by measurement of the Čerenkov radiation.

hybridization method of Cabada *et al.* (1977) as described in the Experimental section. Migration was from the cathode to the anode.

Fig. 5. Determination of lengths of poly(A) segments of mRNA-protein fractions

Newborn and adult rat mRNA-protein particles were deproteinized with proteinase K as described in the text. The proteinase K was then extracted with chloroform/isopentanol. The mRNA fraction was dissolved in 1 ml of buffer [10 mM-Tris/HCl (pH 7.0)/0.1 M-NaCl/1 mM-MgCl₂] and treated with DNAase I (40 μg in the same buffer) for 30 min at 37°C. The solution was then made 10 mM in EDTA, and RNAase T1 was added to a ratio of 20:1 (w/w) to the mRNA. After a further 30 min at 37°C the mixture was chilled in ice, carrier tRNA was added and RNA was precipitated by addition of 2.5 vol. of ice-cold ethanol. The RNA fraction was left overnight at -20°C, collected by centrifugation at 26000g for 1 h at 2-4°C and then dissolved in buffer containing 36 mM-Tris/HCl (pH 7.8)/30 mM-NaH₂PO₄/2 mM-EDTA/0.2% (w/v) sodium dodecyl sulphate. The poly(A) fractions were then electrophoresed on pre-run 10% (w/v) polyacrylamide gels in the same buffer at 7 mA/gel. Markers were run on parallel gels [4S RNA, 5S rRNA, poly(A)₁₆₀]. The size markers were scanned at 260 nm, and the gels containing the brain poly(A) were sliced into 1 mm fractions with a Mickle gel slicer. The presence of the poly(A) was determined by the [^3H]poly(U)-

agreement with the results of Bantle & Hahn (1976), though not with those of deLarco *et al.* (1975) or Mahoney & Brown (1975).

(d) *Polypeptides associated with mRNA.* The protein composition of the mRNA-protein E_1 and E_2 fractions of newborn and adult rats was analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 6). Two major proteins of mol.wt. 74 000 and 52 000 were found in the mRNA-protein of newborn-brain fractions E_1 and E_2 . Newborn-brain mRNA-protein fraction E_1 contained relatively more of the larger protein than the corresponding E_2 fraction protein. In contrast, the adult fractions E_1 and E_2 contained only one major protein of mol.wt. 74 000, with much smaller amounts of other proteins being also present, including the protein of mol.wt. 52 000.

The presence of the two major bands of proteins in newborn-brain mRNA-protein particles could also be demonstrated without resorting to oligo(dT)-cellulose chromatography. Salt-washed polyribosomes were dissociated by the combined KCl/puromycin treatment and the ribosomal subunits were quantitatively sedimented by high-speed centrifugation, leaving a proportion of the mRNA-protein

particles in suspension. These mRNA-protein particles were then precipitated and subjected to electrophoresis. The proteins of mol.wts. 74 000 and 52 000 (Fig. 7) were major constituents, as was previously found. In addition, there was a major protein of mol.wt. 30 000. This smaller protein was also reported by Barrieux *et al.* (1976), who found that it was not associated with Ehrlich ascites tumour cell mRNA-protein particles and was likely to be of ribosomal origin, which probably explains its absence from oligo(dT)-cellulose-derived mRNA-protein particles.

Conclusions

The oligo(dT)-cellulose method of isolating poly(A)-mRNA molecules (Aviv & Leder, 1972) has been used with some modifications to isolate mRNA-protein particles (Lindberg & Sundquist, 1974; Irwin *et al.*, 1975). Some of the non-ribosomal proteins extracted from brain polyribosomes with

Fig. 6. Protein composition of newborn and adult brain mRNA-protein particles isolated by oligo(dT)-cellulose chromatography

mRNA-protein particles were isolated from salt-washed polyribosomes by oligo(dT)-cellulose chromatography as described in Fig. 2. The particles eluting in the E_1 and E_2 fractions were precipitated, pelleted, reduced with 2-mercaptoethanol and electrophoresed on cylindrical polyacrylamide gels by the method described in Fig. 1. The positions of some of the protein bands with identical mobilities are altered because of differential expansion and decrease in size encountered with cylindrical gels during the staining/de-staining process. The positions of the 74 000- (74 K) and 52 000- (52 K) mol.wt. proteins are marked for each gel. Migration was from the cathode to the anode.

Fig. 7. Protein composition of mRNA-protein particles released from polyribosomes

Newborn-brain salt-washed polyribosomes were dissociated by the combined 0.5 M-KCl/puromycin treatment at 30°C as described in the Experimental section. The ribosomal subunits were quantitatively pelleted by centrifugation at 200 000g for 3 h at 2–4°C. No ribosomal subunits could be detected in the supernatant. The top two-thirds of the supernatant were removed and the mRNA-protein particles left in suspension were precipitated by the addition of 2.5 vol. of ice-cold ethanol and left overnight at –20°C. The protein composition of the mRNA-protein particles was analysed as previously described except that in this case the positions of the stained proteins were determined by scanning the gels at 586 nm with a Gilford spectrophotometer fitted with a linear gel scanner. Migration was from the cathode to the anode.

0.5M-KCl also showed strong binding affinities towards oligo(dT)-cellulose and could not be removed by 0.5M-KCl alone (see Fig. 1). This emphasizes the importance of removing these polyribosome-associated proteins before isolation of mRNA-protein particles by oligo(dT)-cellulose chromatography. Fractions containing mRNA-protein particles isolated without a prior 0.5M-KCl/salt wash of polyribosomes displayed decreased buoyant densities on CsCl gradients and more complex protein composition of the E₁ and E₂ mRNA-protein fractions (results not shown). A number of the 0.5M-KCl-extracted polyribosome-associated proteins have also been shown to bind to poly(A)-Sephadex columns (Blanchard *et al.*, 1974) and to poly(A)-mRNA (Rosenfeld & Barrieux, 1977). However, it is not clear whether in these specific cases the 'mRNA-protein particles' represent reconstituted mRNA-protein particles or merely a fortuitous association of these proteins with mRNA molecules.

The characteristics of the mRNA component are similar to those described for polyribosomal mRNA molecules derived from a variety of tissues, consisting of a heterogeneous population with the majority sedimenting at 18S (corresponding to a molecular weight of approx. 0.7×10^6). No changes are seen with development of the brain, although it was previously reported that there was a decrease in the size of cytoplasmic poly(A)-RNA from a molecular weight of 0.8×10^6 in the newborn brain to 0.6×10^6 in the adult forebrain (Berthold & Lim, 1976b). Whether this reflects differences between total cytoplasmic mRNA and free-polyribosomal mRNA is uncertain in view not only of the small difference but also of the difficulties associated with accurate sizing of large-molecular-weight RNA molecules, related to the status of aggregation and/or degradation of different RNA samples.

With regard to the protein components, the ubiquitous presence of the protein of mol.wt. 74000 in all mRNA-protein fractions is consistent with the results of Blobel (1973), who suggested an association of this protein with the poly(A) segment of mRNA. This association may be relevant to the process of mRNA degradation (Lim & Canellakis, 1970; Lim, 1977). There are, however, developmental differences in the overall protein composition of the mRNA-protein particles (Fig. 6). The protein composition of the adult-brain mRNA-protein particles is more heterogeneous than that in the young brain. This may reflect the developmental increase in transcriptional diversity observed in rodent brains (Grouse *et al.*, 1972). A marked alteration is the decrease in the relative proportion of the 52000-mol.wt. protein in the adult-brain mRNA-protein particles. Whether this relative loss is involved in the concurrent developmental decrease

in nucleo-cytoplasmic transport of the RNA, which we have reported previously (Berthold & Lim, 1976b; Hall & Lim, 1978), remains to be established, since there is as yet no firm evidence linking this protein with mRNA transport.

Further investigations on the developmental changes in protein composition of brain mRNA-protein particles should provide valuable information on the role of these proteins associated with the mRNA molecules, particularly in view of the sensitivity of the developing brain to hormonal and metabolic agents.

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