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Expression and developmental regulation of two unique mRNAs specific to brain membrane-bound polyribosomes

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Translation *in vitro* of membrane-bound polyribosomal mRNAs from rat brain has shown several to be developmentally regulated [Hall & Lim (1981) Biochem. J. **196**, 327–336]. Here we describe the isolation and characterization of cDNAs corresponding to two such brain mRNAs. One cDNA (M444) hybrid-selected a 0.95 kb mRNA directing the synthesis *in vitro* of a 21 kDa pI-6.3 polypeptide, which was processed *in vitro* by microsomal membranes. A second cDNA (M1622) hybridized to a 2.2 kb mRNA directing the synthesis of a 55 kDa pI-5.8 polypeptide. Both mRNAs were specific to membrane-bound polyribosomes. Restriction maps of the corresponding genomic DNA sequences are consistent with both being single copy. The two mRNAs were present in astrocytic and neuronal cultures, but not in liver or spleen or in neuroblastoma or glioma cells. The two mRNAs were differently regulated during brain development. In the developing forebrain there was a gradual and sustained increase in M444 mRNA during the first 3 weeks *post partum*, whereas M1622 mRNA appeared earlier and showed no further increase after day 10. In the cerebellum the developmental increase in M444 mRNA was biphasic. After a small initial increase there was a decrease in this mRNA at day 10, coincident with high amounts of M1622 mRNA. This was followed by a second, larger, increase in M444 mRNA, when amounts of M1622 mRNA were constant. The contrasting changes in these two mRNAs in the developing cerebellum are of particular interest, since they occur during an intensive period of cell proliferation, migration and altering neural connectivity. As these mRNAs are specific to differentiated neural tissue, they represent useful molecular markers for studying brain differentiation.

INTRODUCTION

Many genes expressed in the brain are developmentally regulated, resulting in a greater diversity of gene transcripts in the adult brain than in any other tissue (Grouse *et al.*, 1972; Ryffel & McCarthy, 1975; Chikaraishi *et al.*, 1978). During brain development there is extensive cellular differentiation, with the formation of synaptic connections and the generation of specialized membranes, including myelin and synaptic plasma membranes. Membrane proteins play a key role in mediating cellular interactions in the brain, both in synaptic transmission and during development when cell contacts are being established. Many membrane and secretory proteins are synthesized by polyribosomes of the rough endoplasmic reticulum, and they may be co- and post-translationally modified by cleavage and/or glycosylation. Relatively few of the numerous brain-specific gene products have yet been identified. One approach to the characterization of novel brain membrane or secretory proteins is to isolate specific cDNAs synthesized from membrane-bound polyribosomal mRNA, for use as molecular probes of brain gene expression and investigation of individual protein sequences.

Brain membrane-bound and free polyribosomes contain in common several abundant mRNAs coding for proteins including tubulin, actin, creatine kinase, neuron-specific enolase and a 68 kDa microtubule-associated protein now identified as the heat-shock cognate protein (HSC 70) (Hall & Lim, 1981; Hall *et al.*, 1984; Lim *et al.*, 1984, 1986). These proteins were found to be components of brain synaptic plasma membranes, where the enzymes are involved in ATP generation, as well as having a cytosolic location (Lim *et al.*, 1983; Whatley *et al.*, 1986). However, other mRNAs are exclusive to membrane-bound polyribosomes, including those encoding 21 kDa and 33 kDa polypeptides. The latter is co-translationally modified (Hall *et al.*, 1984). Some of these polypeptides may be extensively glycosylated *in vivo*, and native brain protein counterparts have not yet been located. The mRNA for the 21 kDa polypeptide was found to be developmentally regulated (Hall & Lim, 1981; Hall *et al.*, 1984). We have now isolated cloned cDNA copies of two developmentally regulated, relatively abundant, mRNAs which are specific to membrane-bound polyribosomes, as a first step towards identifying novel brain membrane proteins/glycoproteins, expressed during the process of brain differentiation. By using the cDNAs, the mRNAs were found to be specific to differentiated neural tissue and to display different developmental profiles in the forebrain and in the cerebellum. These cDNAs therefore represent useful molecular markers which are invaluable for studying brain differentiation.

Abbreviation used: poly(A)$^+$ RNA, polyadenylated RNA.

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MATERIALS AND METHODS

Preparation of polyribosomes and RNA

Free and membrane-bound polyribosomes were isolated from forebrain and cerebellum of rats (aged from foetal day 17 to 55 days *post partum*), and polyribosomal RNA was prepared as previously described (Hall & Lim, 1981). Total cellular RNA from brain and all other tissues was prepared as described by Whatley *et al.* (1984). Preparation of poly(A)$^+$ RNA and size fractionation on sucrose density gradients (15–30%) were as previously described (Hall & Lim, 1981).

cDNA synthesis and cloning

Size-fractionated mRNA was used to synthesize cDNA (Monahan *et al.*, 1976), and, after second-strand synthesis and S1-nuclease treatment (Goodman & MacDonald, 1979), cDNA was tailed with 15–20 dC residues as described by Craig *et al.* (1981), except that $MnCl_2$ replaced $CoCl_2$. cDNAs were annealed to *Pst*1-cut dG-tailed plasmid pAT153 and used to transform *Escherichia coli* RR1 (Hanahan, 1985). A second cDNA library, with longer cDNA sequences, was constructed from membrane-bound polyribosomal poly(A)$^+$ RNA by using RNAase H and DNA ligase with Klenow polymerase in second-strand synthesis (Gubler & Hoffman, 1983). This cDNA was cloned in the *Pst*1 site of plasmid pBR322 [with poly(dG)·poly(dC) homopolymer tailing] in *E. coli* HB101.

DNA hybridization and screening of clones

Replica-plated tetracycline-resistant colonies, grown on nitrocellulose filters and amplified by using chloramphenicol (Maniatis *et al.*, 1982), were lysed (Grunstein & Hogness, 1975) and duplicate filters were hybridized to ^{32}P-labelled cDNA synthesized either from free or from membrane-bound polyribosomal mRNA. Hybridization in 10× Denhardt's solution/3×SSC (0.15 M-NaCl/0.15 M-sodium citrate)/0.1% SDS containing 100 μg of sheared denatured salmon testis DNA/ml and 10 μg of poly(A)$^+$ RNA/ml was at 65 °C for 20 h. Filters were washed in 2×SSC/0.1% SDS at 65 °C and in 0.1×SSC at 55 °C.

Hybrid-selected translation

Recombinant plasmid DNAs were isolated and purified (Lis, 1980; Maniatis *et al.*, 1982; Lim *et al.*, 1984), digested with *Eco*RI, denatured and bound to nitrocellulose filters. Hybrid-selection of mRNA (Ricciardi *et al.*, 1979) was as previously described (Lim *et al.*, 1984). Translation products of hybrid-selected RNAs were analysed by two-dimensional gel electrophoresis and fluorography (Hall *et al.*, 1984). Hybrid-selected translation products synthesized in the presence (or absence) of dog pancreas microsomal membranes were digested with trypsin in the presence or absence of 1.0% Triton X-100 as previously described (Hall *et al.*, 1984).

Analysis of RNA

Preparations of brain free and membrane-bound polyribosomal RNA and poly(A)$^+$ RNA, as well as total cellular RNA and poly(A)$^+$ RNA from a variety of tissues and cell cultures, were denatured in formaldehyde, and equivalent amounts electrophoresed in each lane of agarose gels (Maniatis *et al.*, 1982). All RNA concentrations were determined by A_{260} measurements. For developmental profiles 10 μg or 2.5 μg of total polyribosomal RNA was used, and larger amounts of mRNA [up to 5 μg of poly(A)$^+$ RNA] were used for greater sensitivity of detection in investigations of tissue distribution. After transfer to nylon filters (Hybond N; Amersham International) (Thomas, 1980), RNA was stained with Methylene Blue (0.04%) (Maniatis *et al.*, 1982) to ensure that equal amounts were present in each lane and as a check of RNA integrity. Hybridization to nick-translated cDNAs (10^7 c.p.m./μg) was as described above for DNA hybridization, except that 50% formamide was used and hybridization was at 42 °C. X-Omat AR X-ray film was exposed for various time intervals (2–14 days) to ensure that the hybridization signal did not exceed the linear responsiveness of the film. In all cases hybridizations were performed with different cDNAs either sequentially with the same filter-bound RNA or separately on duplicate filters.

Restriction mapping of genomic DNA

Rat brain DNA was digested with a variety of restriction endonucleases, electrophoresed in agarose gels, and after transfer to nitrocellulose filters was hybridized to ^{32}P-labelled cDNA (Cooper *et al.*, 1985). *Hin*dIII-digested λ-phage DNA was electrophoresed in parallel to determine sizes of hybridizing fragments.

Source of cells and cDNA clones

RNA was prepared from rat peripheral neurotumour RT4AC cells (Imada & Sueoka, 1978), neuroblastoma NE115 and neuroblastoma/glioma hybrid NG108/15 (Klee & Nirenberg, 1974), glioma C6 cells (Benda *et al.*, 1968) and mouse A9 fibroblasts (Tan *et al.*, 1973) cultured in minimal Eagle's medium with 10% (v/v) foetal-calf serum. Primary cultures of rat forebrain astrocytes 14 days *in vitro* (Patel & Hunt, 1985) and rat cerebellar neurons 8 days *in vitro* (Thangnipon *et al.*, 1983) were generously given by Tony Hunt and Anne Kingsbury respectively.

α-Tubulin cDNA clone pT25 (Ginzburg *et al.*, 1981) was kindly provided by Irith Ginzburg, and all other cDNA clones were isolated and characterized in our own laboratory.

RESULTS

mRNA isolated from rat brain membrane-bound polyribosomes was size-fractionated on sucrose density gradients (Fig. 1*a*). Pooled mRNA fractions (approx. 10–18 S) were used to synthesize cDNA. This fraction included mRNAs encoding 14–55 kDa polypeptides, several of which were membrane-bound, polyribosome-specific and developmentally regulated (Hall & Lim, 1981; Hall *et al.*, 1984). A two-dimensional gel-electrophoretic analysis of the translation products of this mRNA, which was used to generate a cDNA library, is shown in Fig. 1(*b*). α- and β-tubulin, actin, creatine kinase, neuronal protein 14.3.3 and calmodulin translation products were identified by comparison of two-dimensional gel-electrophoretic co-ordinates and peptide maps with those of the purified proteins (Hall *et al.*, 1984). Heat-shock cognate protein HSC70, previously designated '68K MAP' (Hall *et al.*, 1984; Lim *et al.*, 1984; Whatley *et al.*, 1986), and glyceraldehyde-3-phosphate dehydrogenase translation products were similarly identified, and these results were subsequently confirmed by DNA-sequence analysis of the corres-

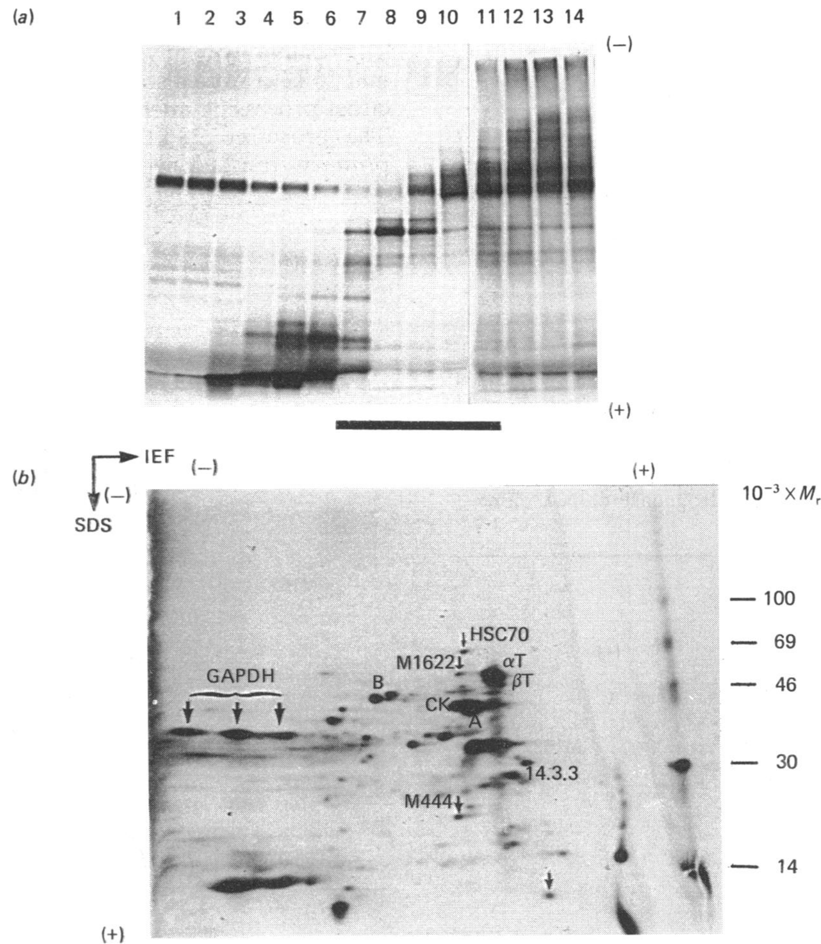

Fig. 1. Translation products of size-fractionated mRNA from membrane-bound polyribosomes

(*a*) SDS/polyacrylamide-gel analysis of [^{35}S]methionine-labelled translation products of serial poly(A)$^+$ RNA fractions separated by centrifugation on 4.2 ml sucrose density gradients (15–30%). RNA from 0.2 ml fractions was recovered by ethanol precipitation before translation in reticulocyte lysates (Hall & Lim, 1981). Translation products of the top two-thirds of the gradient (fractions 1–14) are shown. Pooled poly(A)$^+$ RNA fractions (shown by horizontal bar; 7–11 inclusive, approx. 10–18 S in size) were used for cDNA synthesis. (*b*) Two-dimensional gel-electrophoretic analysis of translation products of the pooled size-fractionated poly(A)$^+$ RNA. Two-dimensional gel electrophoresis was as previously described (Hall *et al.*, 1984). The pH range of the isoelectric-focusing (IEF) gel was approx. 4–9. Identified products are indicated: calmodulin, C; α- and β-tubulin, αT, βT; creatine kinase, CK; actin, A; neuronal protein 14.3.3, 14.3.3 (Hall *et al.*, 1984); glyceraldehyde-3-phosphate dehydrogenase, GAPDH (Leung *et al.*, 1987); constitutively expressed heat-shock cognate protein, HSC70 (Lim *et al.*, 1984; Whatley *et al.*, 1986). Translation products corresponding to cDNAs which were isolated, including M1622 and M444, are arrowed. The reticulocyte lysate 46 kDa background protein is indicated (B). ^{14}C-labelled protein M_r markers were co-electrophoresed.

ponding cDNAs (Leung *et al.*, 1987; T. K. C. Leung, C. Hall, C. Monfries & L. Lim, unpublished work). Translation products encoded by cDNAs that have been isolated and characterized are indicated in Fig. 1 by arrows.

Cloned cDNAs complementary to mRNAs specific to membrane-bound polyribosomes were identified by colony hybridization (Grunstein & Hogness, 1975), by using ^{32}P-labelled cDNA copies of free and membrane-bound polyribosomal poly(A)$^+$ RNA as differential probes (Fig. 2). Many colonies hybridized with equal intensity to both probes; this group would include cDNA copies of abundant brain mRNA sequences such as tubulin and actin that are common to both polyribosomal fractions (Hall & Lim, 1981; Hall *et al.*, 1984). Others correspond to sequences which, although present in membrane-bound polyribosomes, were more abundant in the free-polyribosomal fraction, e.g. glyceraldehyde-3-phosphate dehydrogenase (Leung *et al.*, 1987). Of the colonies which gave a hybridization signal, 27% were exclusively membrane-bound polyribosomal sequences.

Hybrid-selected translation

Membrane-bound polyribosomal specific cDNA clones were further characterized by hybrid-selected translation (Ricciardi *et al.*, 1979; Lim *et al.*, 1984). cDNA M444 hybrid-selected an mRNA coding for a 21 kDa polypeptide (Fig. 3*b*) with the same mobility in two-dimensional gels as a specific translation product of membrane-bound polyribosomal mRNA, previously found to be developmentally regulated (Hall & Lim, 1981; Hall *et al.*, 1984; see Fig. 1). When dog pancreas microsomal membranes were present during translation, the 21 kDa product of this hybrid-selected mRNA (Fig.

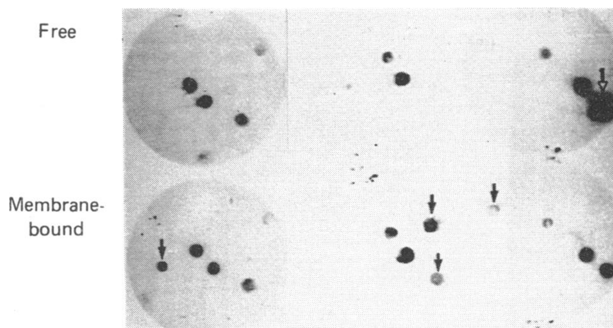

Fig. 2. Colony hybridization of ^{32}P-labelled cDNAs synthesized from free (top row) and membrane-bound (bottom row) polyribosomal poly(A)$^+$ RNA

Membrane-bound-polyribosome specific sequences (↓); glyceraldehyde-3-phosphate dehydrogenase cDNA clone (↓).

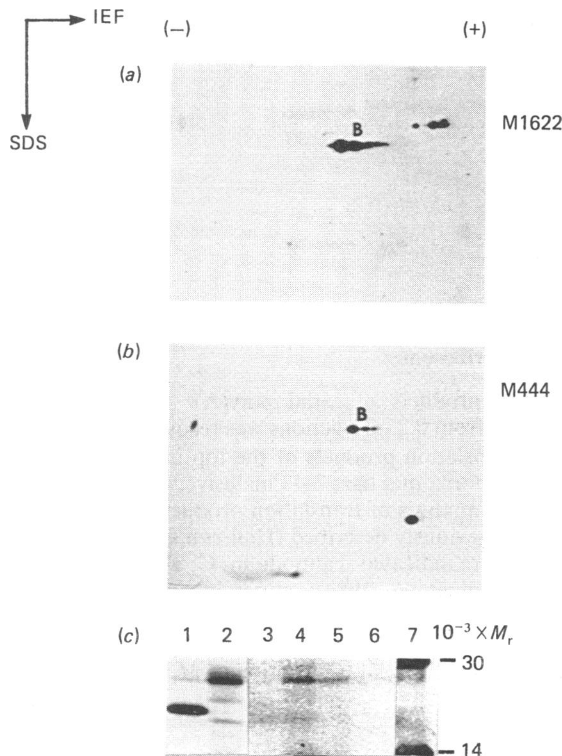

Fig. 3. Hybrid-selected translation

(*a*) and (*b*) Two-dimensional gel analysis (IEF, isoelectric focusing). mRNAs hybrid-selected by M1622 (*a*) and M444 (*b*) were translated *in vitro* and the translation products analysed on two-dimensional gels as described in the legend to Fig. 1; see also M444 and M1622 translation products in total translation products of membrane-bound polyribosomal mRNA fraction (Fig. 1*b*). Reticulocyte lysate 46 kDa background protein, B. (*c*) SDS-polyacrylamide-gel analysis of M444 hybrid-selected translation product, lane 1; synthesized in the presence of dog pancreas microsomal membranes, lanes 2 and 4; trypsin digestion of hybrid-selected M444 translation product synthesized in the absence (lane 3) or presence (lanes 5 and 6) of microsomal membranes; trypsin digestion in the presence of 1% Triton X-100, lane 6; M_r markers, lane 7.

3*c*, lane 1) was processed to 28 kDa (Fig. 3*c*, lanes 2 and 4), presumably by glycosylation. Minor bands of 20 kDa and 25 kDa were also detected, and these may represent either processing intermediates or degradation products. The processed 28 kDa product, but not the 21 kDa primary translation product, was resistant to digestion

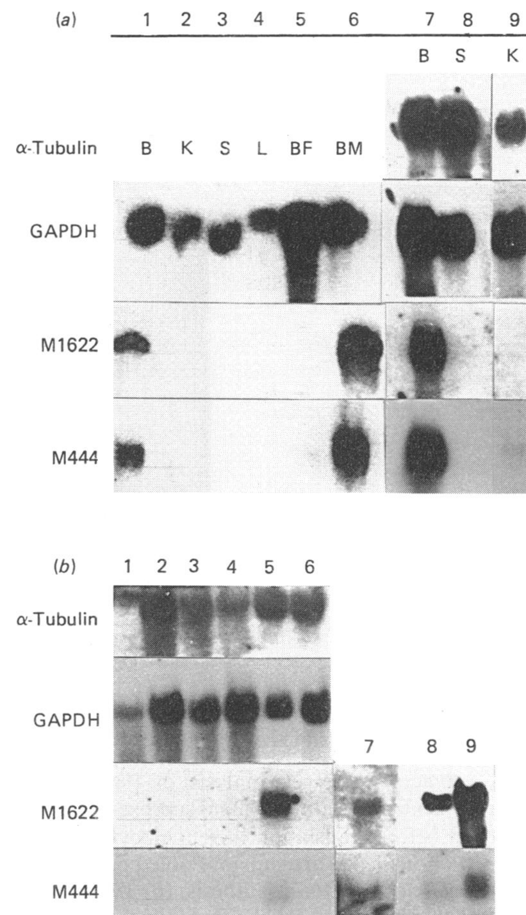

Fig. 4. Tissue specificity of mRNAs

Hybridization to α-tubulin (pT25), rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), M444 and M1622 cDNAs. (*a*) Northern analyses of: poly(A)$^+$ RNA (1 μg) from brain [total cellular poly(A)$^+$ RNA], B (lane 1), kidney, K (lane 2), spleen, S (lane 3), liver, L (lane 4); poly(A)$^+$ RNA from brain polyribosomes, free, BF (lane 5) and membrane-bound, BM (lane 6); poly(A)$^+$ RNA (5 μg) from brain, B (lane 7), spleen S (lane 8), kidney, K (lane 9). (*b*) Hybridization to total cellular RNA (25 μg) from cultured cells: C6 glioma, lane 1; rat peripheral neurotumour cell line RT4AC, lane 2; neuroblastoma NE115, lane 3; neuroblastoma/glioma hybrid NG108/15, lane 4; astrocytes, 8 days *in vitro*, lane 5; mouse fibroblast A9 cells, lane 6; RNA (3 μg, M1622; approx. 30 μg, M444) from cerebellar neuronal cultures 8 days *in vitro* containing 95% neurons as determined by immunocytochemistry (Thangnipon *et al.*, 1983), lane 7; astrocyte RNA, 10 μg (lane 8), 20 μg (lane 9). [Astrocyte cultures contained > 90% GFAP (glial fibrillary acidic protein)-positive cells; Patel & Hunt, 1985.] Hybridizations shown for all cDNAs were performed either with portions of the same RNA samples on duplicate filters (*a*, lanes 1–6; *b*, lanes 7–9) or sequentially with the same filters (*a*, lanes 7–9; *b*, lanes 1–6).

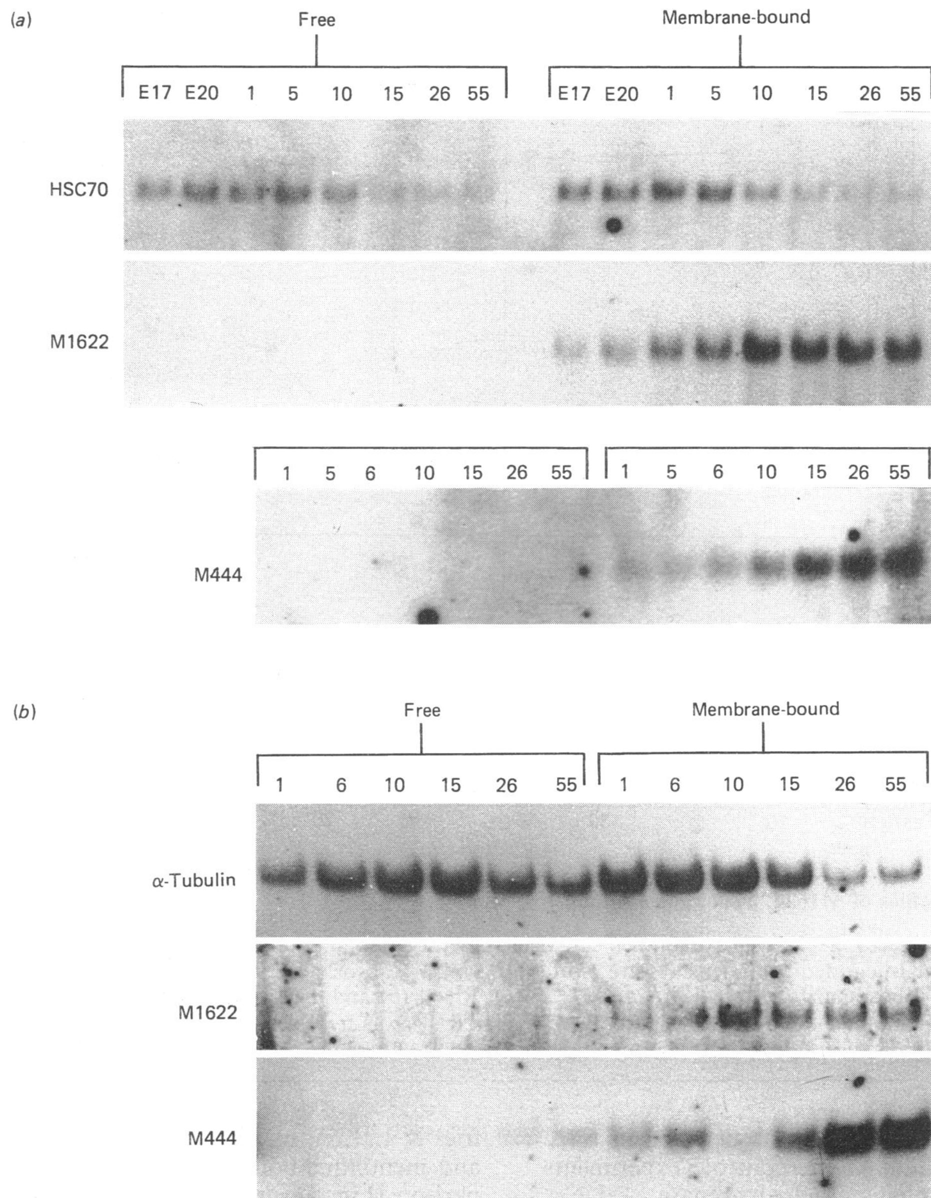

Fig. 5. Developmental changes in M444 and M1622 mRNAs

(*a*) Rat forebrain: RNA (either 10 μg or 2.5 μg per gel lane) from free and membrane-bound polyribosomes from embryo day 17 (E17) to 55 days *post partum* (55) as indicated above gel lanes was analysed on formaldehyde/agarose gels and hybridized to heat-shock cognate (HSC70), M1622 and M444 cDNAs. (*b*) Rat cerebellum: polyribosomal RNA (10 μg) from postnatal day 1 (1) to postnatal day 55 (55) as indicated above gel lanes was hybridized to α-tubulin, M1622 and M444 cDNAs. Each cDNA hybridized to a single mRNA band, and only the relevant portion of each Northern blot is shown. For all cDNAs, hybridization analyses were performed both sequentially on the same nitrocellulose filter and separately on duplicate filters. Several polyribosomal RNA samples were analysed, and typical results are shown.

by trypsin (Fig. 3*c*, lane 5 and lane 3 respectively), indicative of its incorporation into the microsomal membranes (Sabatini & Blobel, 1970). In the presence of Triton X-100 to solubilize membranes, the 28 kDa product was digested by trypsin (Fig. 3*c*, lane 6).

A second cDNA sequence specific to the membrane-bound polyribosomal fraction, M1622, hybridized to an mRNA specifying the synthesis of a 55 kDa polypeptide (Fig. 3*a*; see also Fig. 1*b*). The size of the primary translation product of mRNA hybrid-selected by M1622 cDNA was unaltered by the presence of dog pancreas microsomal membranes.

Tissue distribution of M444 and M1622 mRNAs

The mRNAs corresponding to both M444 and M1622, which were respectively 0.95 kb and 2.2 kb in size, were relatively abundant in brain (Fig. 4*a*, lanes 1, 6 and 7) and specific to membrane-bound polyribosomes (Fig. 4*a*, cf. lanes 5 and 6; Fig. 5). Neither mRNA was present in liver or spleen poly(A)$^+$ RNA (Fig. 4*a*, lanes 3 and 4), nor were they detected in muscle or testis RNA. Trace amounts of M444 mRNA (but not M1622 mRNA) were detected in kidney when larger amounts of poly(A)$^+$ RNA (5 μg) were used (Fig. 4*a*, lanes 2 and 9). M1622

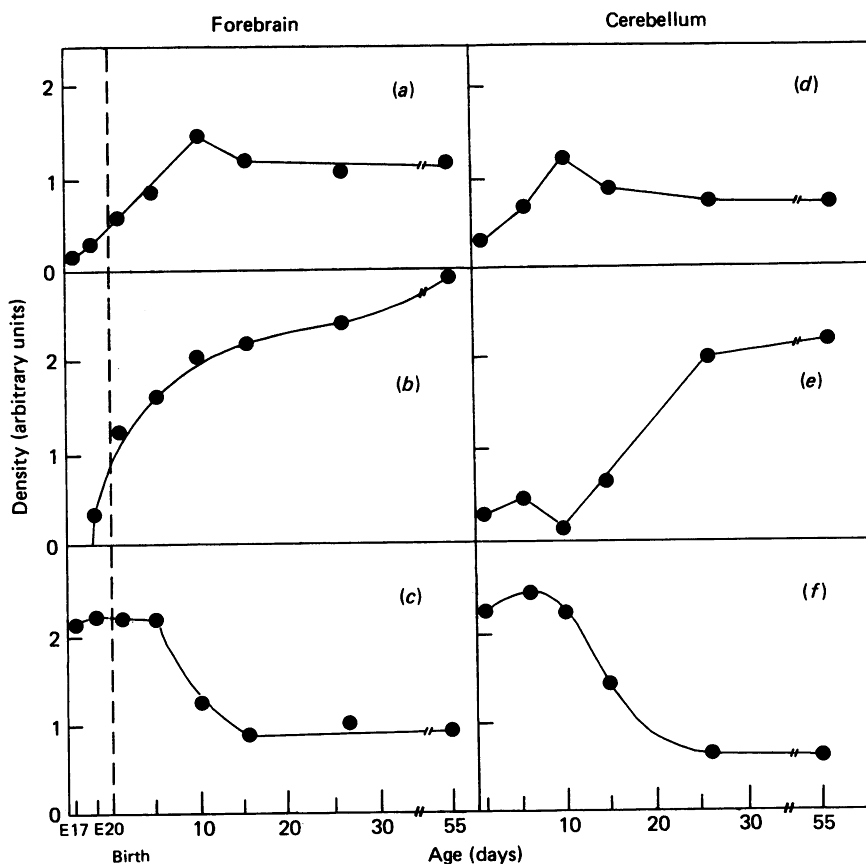

Fig. 6. Developmental profiles of M1622, M444 and α-tubulin mRNAs

Hybridization signals (see Fig. 5) were quantified by densitometric scanning of autoradiographs with a Joyce–Loebl Chromoscan 3 scanner. Integrated peak densities are plotted as arbitrary absorbance units. Data shown for hybridization of membrane-bound polyribosomal forebrain RNA to M1622 (*a*), M444 (*b*) and α-tubulin (*c*) cDNAs were derived by serial hybridizations to the same filter-bound RNA. Similarly cerebellar RNA was hybridized successively to M1622 (*d*), M444 (*e*) and α-tubulin (*f*). Hybridization to duplicate RNA samples on separate filters produced qualitatively equivalent profiles.

mRNA (but not M444 mRNA) was detected in trace amounts in the adrenal. In control experiments glyceraldehyde-3-phosphate dehydrogenase cDNA (Leung *et al.*, 1987) hybridized strongly to brain [both free and membrane-bound polyribosomal poly(A)$^+$ RNA], spleen, kidney and liver poly(A)$^+$ RNAs (Fig. 4*a*, lanes 1–9) and to muscle, adrenal and testis RNAs. Both M444 and M1622 mRNAs were present in primary astrocyte (Fig. 4*b*, lanes 5, 8 and 9) and cerebellar neuronal cultures (Fig. 4*b*, lane 7), but were not detected in fibroblasts (lane 6), undifferentiated neuroblastoma (NE115 and NG108/15) (lanes 3 and 4), glioma (C6) (lane 1) or RT4AC (lane 2) cell lines (Fig. 4*b*). α-Tubulin and glyceraldehyde-3-phosphate dehydrogenase mRNAs were present in all of these cultured cells (Fig. 4*b*, lanes 1–6).

Developmental regulation

Both brain mRNAs increased during post-natal development in the rat forebrain. M1622 mRNA was present at foetal day 17 and increased up to day 10 post-natally, with no marked changes subsequently (Figs. 5*a* and 6*a*). M444 mRNA was first detectable in the forebrain at foetal day 20 (Fig. 6*b*) and increased gradually up to 55 days *post partum* (Figs. 5*a* and 6*b*). The HSC 70 mRNA (T. K. C. Leung, C. Hall, C. Monfries & L. Lim, unpublished work) present in both free and membrane-bound polyribosomes, shown for comparison (Fig. 5*a*), undergoes a developmental decrease in rat forebrain, as does tubulin mRNA (Gozes *et al.*, 1977; Ginzburg *et al.*, 1985; Fig. 6*c*).

In the cerebellum, M444 mRNA showed a biphasic developmental profile (Figs. 5*b* and 6*e*). The mRNA was present at day 1, increased by day 6 and then decreased to very low amounts at day 10, followed by a second large increase to adult values. Interestingly, M1622 mRNA amounts in the cerebellum were maximal at day 10 (Figs. 5*b* and 6*d*). M444 mRNA was also detected in the free polyribosomal fraction at days 26 and 55 (Fig. 5*b*), suggesting considerable synthesis of the mRNA at this time, with initiation of newly synthesized mRNA in free polyribosomes.

The same mRNA samples from membrane-bound polyribosomes of cerebellum showed a developmental decrease in α-tubulin mRNA (Figs. 5*b* and 6*f*). In the free polyribosomal fraction, α-tubulin mRNA increased up to 15 days and then decreased (Fig. 5*b*).

Restriction-enzyme analysis of cDNAs and rat genomic sequences

The two cDNAs, M444 (620 bp) and M1622 (750 bp), were both partial sequences, corresponding respectively

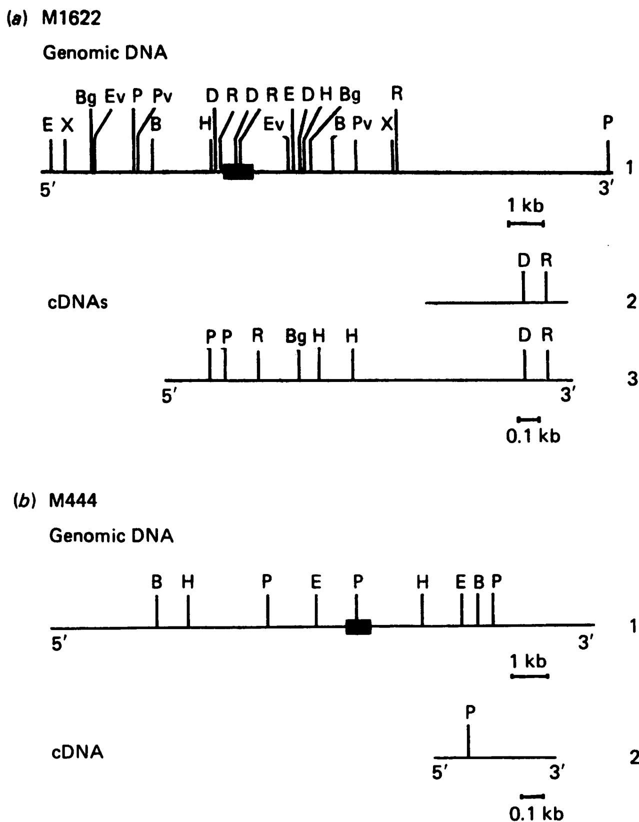

Fig. 7. Restriction-enzyme map of M1622 and M444 sequences

(*a*) Restriction map of M1622 sequence in the rat genome (1) and of short (M1622) (2) and long (M16225E) (3) cDNAs. (*b*) Restriction map of M444 sequence in the rat genome (1) and of partial M444 cDNA (2). Restriction enzymes used were: *Bam*HI, B; *Bgl*II, Bg; *Eco*RI, E; *Eco*RV, Ev; *Hin*dIII, H; *Pst*I, P; *Pvu*II, Pv; *Xba*I, X; *Dde*I, D; *Rsa*I, R. The positions of the respective cDNA sequences located in the genomic maps are indicated by black bars. (N.B. only the *Rsa*I and *Dde*I sites in M1622 cDNA, which were used for orientation of the cDNA with respect to the genomic restriction map, are shown.)

to 0.95 and 2.2 kb mRNAs. Longer cDNA sequences were synthesized from membrane-bound polyribosomal mRNA (see the Materials and methods section). Two M1622 sequences of > 2 kb were isolated (with opposite orientations in pBR322). There were two internal *Pst*I sites in these longer cDNA clones. A restriction map of the longest cDNa sequence (2.15 kb) is shown in Fig. 7(*a*).

M444 cDNA also contains a *Pst*I site 420 nucleotides from the 3′ end (Fig. 7*b*). The longest M444 sequence isolated was 700 nucleotides.

Both M1622 and M444 were used in Southern blotting experiments to probe single, double and triple restriction-enzyme digests of rat brain DNA. Restriction maps of the region around M1622 and M444, shown in Figs. 7(*a*) and 7(*b*) respectively, were derived by using the smaller cDNAs (corresponding to the 3′ portion of the gene sequence) as probes. That it was possible to deduce the genomic maps of both of these regions is good evidence for their being single-copy sequences in the rat genome. For each restriction enzyme, mapped sites in genomic DNA must necessarily correspond to those closest to the cloned sequence, since more distal sites would not have been detected with the same probe. It is nevertheless clear from a comparison of the restriction maps of the longer M1622 cDNA (M16225E) and genomic sequences (Fig. 7*a*) that the M1622 gene contains a minimum of one intron. The complexity of Southern-blot analyses using the whole M16225E sequence (D. N. Cooper, C. Hall & L. Lim, unpublished work) suggests that there may be several introns.

The partial DNA sequences of the coding regions of M444 and M1622 cDNAs show no homology with any sequence in the current GenBank database, and predicted amino acid sequences have no homologues in the PIR database (C. Hall & L. Lim, unpublished work). Thus both sequences encode novel brain proteins. Fusion proteins synthesized in *E. coli* and antibodies raised against these proteins should permit the identification of the corresponding brain proteins and their localization within the brain.

DISCUSSION

cDNA copies of two rat brain mRNAs specific to membrane-bound polyribosomes have been isolated, both of which were highly enriched in brain, if not brain-specific. It is well established that proteins hitherto considered as brain- or nervous-system-specific can occur in low amounts in other tissues (Jackson & Thompson, 1981). Thus it is not surprising that trace amounts of M1622 mRNA were also detected in the adrenal, a tissue of common embryonic origin with peripheral neural tissue. The other mRNA (M444) was present in kidney, in approx. 100-fold lower amount than in brain. Ectopic production of a brain-specific protein has been reported to occur in kidney tubules as a result of lead intoxication (Egle & Shelton, 1986).

M444 and M1622 mRNAs were not present in any other tissue examined, nor were they detected in neuroblastoma or glioma cell lines. Since both mRNAs were developmentally regulated in the brain and were present in primary neural-cell cultures (but not in established cell lines of neural origin), these sequences may serve as useful markers of neural differentiation. Both mRNAs are expressed during the peri- and post-natal period at a time of extensive cellular differentiation, including the establishment of cellular contacts, dendritic arborization and synaptogenesis. However, the temporal sequence of expression of M444 and M1622 mRNA differed. In the forebrain there was a developmental increase in both mRNAs. The change in amounts of M444 mRNA was more pronounced and continued throughout the developmental period, whereas M1622 mRNA reached maximal concentrations earlier. The changes in the cerebellum were more complex, with M444 mRNA exhibiting two distinct phases of expression, with minimal amounts at a period of elevated M1622 mRNA concentrations. This period, at around 10 days *post partum*, is characterized by cell migration, changing cellular complement and alterations in synaptic connectivity in the cerebellum (Altman, 1972). Interestingly, the cerebellar concentration of *c-myc* mRNA, whose expression has been correlated with cell proliferation (Kelly & Siebenlist, 1986), decreases postnatally, except for a period of re-expression at a time when M444 mRNA concentrations are minimal (Ruppert *et al.*, 1986). There was a subsequent large increase in M444 mRNA during

the later period of synaptogenesis and continuing elaboration of cellular processes in the cerebellum.

The two brain mRNAs, whose expression is differentially regulated and which code for novel brain-specific proteins, will clearly be invaluable in probing the molecular events underlying development of the brain and in particular of the cerebellum. Whatever the nature of the native proteins coded for, these two cDNAs assume a special significance, since isolation of their genomic counterparts will undoubtedly lead to a better understanding of regulatory sequences governing tissue-specific and developmental appearance of mRNAs.

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