Biochem. J. (1984) **220**, 179–187
Printed in Great Britain

Alterations in the relative amounts of specific mRNA species in the developing human brain in Down's syndrome

Stephen A. WHATLEY, Christine HALL, Alan N. DAVISON and Louis LIM
Miriam Marks Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

(*Received 31 October 1983/Accepted 8 February 1984*)

Total cellular polyadenylated RNA [poly(A)$^+$ RNA] was prepared after guanidinium thiocyanate extraction of frozen brain tissue from age-matched normal and Down's-syndrome (trisomy 21) human foetuses. Poly(A)$^+$ RNA populations were analysed by translation *in vitro*, followed by two-dimensional gel analysis by using both isoelectric focusing (ISODALT system) and non-equilibrium pH-gradient electrophoresis (BASODALT system) as the first-dimension separation. The relative concentrations of poly(A)$^+$ RNA species coding for seven translation products were significantly altered in Down's syndrome, as determined by both visual comparisons of translation-product fluorograms from normal and Down's-syndrome samples and by quantitative radioactivity determination of individual translation products. The relative concentrations of mRNA species coding for two proteins (68kDa and 49kDa) were increased in Down's syndrome and may represent genes located on chromosome 21. The relative concentrations of mRNA species coding for five proteins (37kDa, 35kDa, 25.5kDa, 24.5kDa, 23kDa) were decreased in Down's syndrome, these probably representing secondary effects of the trisomy. Six Down's-syndrome-linked translation products (49kDa, 37kDa, 33kDa, 25.5kDa, 24.5kDa, 23kDa) did not migrate with appreciable amounts of cellular proteins on two-dimensional gels and hence may represent either proteins of high turnover rates or those that are post-translationally modified *in vivo*. One translation product (68kDa) co-migrated with a major cellular protein species, which was identified as a 68kDa microtubule-associated protein by limited peptide mapping. The significance of these changes is discussed in relation to the mechanisms whereby the Down's-syndrome phenotype is expressed in the human brain.

Down's syndrome (trisomy 21) is the most common viable human aneuploidy and results in mental retardation as well as a wide variety of physiological defects (Crome & Sterne, 1972). Although the genetic basis for the syndrome is known (Lejeune *et al.*, 1959) the molecular mechanisms governing expression of the phenotype are poorly understood, although it is generally accepted that the syndrome arises from dosage-dependent transcription of qualitatively normal genetic material (Kurnit, 1979). Evidence for this proposal comes from the demonstration that the activity and synthesis of specific proteins with structural loci on chromosome 21 are dependent on gene dosage (Tan, 1975; Feaster *et al.*, 1977; Bartley & Epstein, 1980; Brown *et al.*, 1981). In addition, dosage-dependent transcription of chromosome-21 sequences has been shown to occur in human fibroblast strains (Kurnit, 1979). However, the demonstration of changes in the activities of enzymes with structural loci outside chromosome 21 (Hsai *et al.*, 1968) indicates that a combination of primary and secondary effects is responsible for the phenotypic expression of the trisomy. Such interactions may have the effect of amplifying the original gene-dosage effect, particularly when the primary changes either represent or interact directly with regulatory elements within the cell. The observed hyper-responsivity of trisomic fibroblasts to interferon (Tan *et al.*, 1974) and β-adrenergic stimulation (McSwigan *et al.*, 1981) indicate that amplification processes can occur in trisomic fibroblasts, and such 'cascade' effects may

Abbreviations used: SDS, sodium dodecyl sulphate; poly(A)$^+$ RNA, polyadenylated RNA.

represent key features in the contribution to the Down's-syndrome phenotype.

Widespread secondary changes in either protein synthesis or steady-state concentrations of proteins have not been detected in comparisons of trisomic with normal fibroblasts (Weil & Epstein, 1979; Klose *et al.*, 1982; Van Keurin *et al.*, 1982). This lack of observed changes may be in part due to the choice of cell type, particularly since fibroblasts are relatively undifferentiated cells. It is clear that the effect of trisomy 21 on specific cell function will vary between tissues and that these effects may be more important at 'critical' periods of organ development, e.g. in the brain when interneuronal connections are being made. In addition, it is important to characterize qualitative as well as quantitative changes in transcription in trisomy 21, since this both reflects primary changes in genetic expression and also represents a cellular control point at which the trisomy may exert secondary effects.

With these considerations in mind, we have analysed mRNA populations in normal and trisomy-21 human brains during the second trimester of foetal development. This is a period of rapid development in the human brain (Howard *et al.*, 1969), the function of which is greatly impaired in Down's syndrome. We have also analysed steady-state contents of proteins in the brain during this period.

Materials and methods

Materials

Oligo(dT)–cellulose type T3 was from Collaborative Research (supplied by Uniscience, Cambridge, U.K.). The reticulocyte-lysate translation system was obtained from New England Nuclear, Southampton, U.K., and was supplemented with L-[^{35}S]methionine (6.6mCi/ml; sp. radioactivity 932.5Ci/mmol). [^{14}C]Methylated protein mixture was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Sucrose (grade 1) was obtained from Sigma Chemical Co., Kingston upon Thames, Surrey, U.K. *Staphylococcus aureus* V8 protease was obtained from Miles Laboratories, Slough, U.K. Guanidinium thiocyanate was obtained from Merck (Suppliers, Hopkin and Williams, Dagenham, U.K.). Ampholines were obtained from LKB, Croydon, U.K. All other chemicals were obtained as A.R. grade from BDH, Poole, Dorset, U.K. Glassware or apparatus was heat-sterilized (140°C, 4h) to destroy ribonuclease activity. All solutions were either autoclaved before use or prepared in autoclaved distilled water.

Brain samples

Human foetuses of both sexes, aged between 18 and 25 weeks of gestation, were obtained at various times (up to 16h) after prostaglandin termination of pregnancy, from the Institute of Obstetrics and Gynaecology, London; their forebrains were dissected and stored at −70°C until use. Brains from prostaglandin-induced terminations were also obtained from the foetal tissue bank, Royal Marsden Hospital, London, and frozen at −70°C collection. All Down's-syndrome foetuses were diagnosed by the existence of trisomy for chromosome 21. Three frozen Down's-syndrome brains were generously supplied by Dr. Adinolfi, Guy's Hospital, London.

RNA extraction and isolation of poly(A)$^+$ RNA

RNA was extracted from frozen brains essentially as described by Kaplan *et al.* (1979). Briefly, frozen brain tissue was broken into approx. 1cm^3 pieces, and immediately homogenized (1min at setting 5, VirTis homogenizer) in 4vol. (v/w) of 5.0M-guanidinium thiocyanate/50mM-Tris/HCl (pH7.6)/10mM-EDTA/5% (w/v) 2-mercaptoethanol. The homogenate was made 4% (w/v) with respect to *N*-lauroylsarcosine, and solid CsCl was added to 0.15g/ml. After gentle mixing, the suspension was layered over a cushion of 5.7M-CsCl/0.1M-EDTA and centrifuged at 100000*g* for 18–24h at 20°C (MSE 75 centrifuge, swing-out rotor). After centrifugation, the homogenate was removed by aspiration and the upper portion of the tube washed twice with sterile distilled water. After removal of the CsCl cushion, the RNA pellet was washed briefly twice in sterile water and resuspended in 10mM-Tris/HCl (pH8.5)/0.1M-NaCl/0.5% (w/v) SDS/5mM-EDTA/5% (w/v) phenol with a glass Teflon homogenizer. After brief warming to 65°C, RNA was further purified by phenol extraction. Ethanol-precipitated RNA was dissolved, applied to an oligo(dT)–cellulose column and chromatographed as described previously (Hall & Lim, 1981). Unbound RNA was washed from the column by high-salt buffer [0.5M-NaCl/1mM-EDTA/10mM-Tris/HCl (pH7.5)/0.1% SDS]. Bound RNA was eluted from the column by washing with intermediate-salt buffer [0.1M-NaCl/1mM-EDTA/10mM-Tris/HCl (pH7.5)/0.1% SDS], followed by low-salt buffer (10mM-Tris/HCl, pH7.5).

Size estimation of poly(A)$^+$ RNA

Poly(A)$^+$ RNA (1 A_{260} unit) in 10mM-Tris/HCl (pH7.6)/0.1M-NaCl/1mMEDTA/0.2% SDS was heated to 65°C for 5min before rapid cooling and centrifugation in 15–30% (w/v) sucrose gradients prepared in the same buffer (4h, 23°C, MSE 6×4.2Ti rotor). Size markers (28S, 18S and 4S RNA) were centrifuged in parallel gradients.

Serial fractions (0.2ml) were collected and precipitated with ethanol as described previously (Hall & Lim, 1981).

Protein synthesis

Poly(A)$^+$ RNA (low-salt fraction; 20μg/ml unless stated otherwise) was used to direct the synthesis *in vitro* of proteins in the reticulocyte-lysate system (Pelham & Jackson, 1976), with [^{35}S]methionine as the radiolabelled precursor. The lysate was already micrococcal-nuclease-treated when supplied. The incorporation of radioactivity into protein was dependent on the concentration of mRNA, saturating conditions being observed above 20μg/ml for all preparations studied. The stimulatory activity of different poly(A)$^+$ RNA populations was 2–5-fold above the blank value.

One- and two-dimensional gel electrophoresis

One-dimensional SDS/polyacrylamide-gel electrophoresis of translation products was as described by Laemmli (1970) in 18cm × 20cm vertical slab gels with a combination of 5% (w/v) acrylamide stacking gel with a linear 10–20% (w/v) separating gel.

Two-dimensional gel electrophoresis of translation products and human brain proteins was performed as described by O'Farrell (1975) (for isoelecric focusing, ISODALT system) and O'Farrell *et al.* (1977) (for non-equilibrium pH-gradient analysis, BASODALT system) with minor modifications. Lysis buffer and first-dimension separating gels contained 2% (w/v) pH3.5–10 Ampholines. Translation products were solubilized for isoelectric focusing in lysis buffer containing additionally 0.5% SDS. The second-dimension gel was as described for one-dimensional SDS/polyacrylamide-gel analysis.

After electrophoresis, gels containing non-radioactive proteins were stained by a commercial silver-staining procedure (Bio-Rad Silver Stain kit; Bio-Rad, Watford, Herts., U.K.). Radioactive protein species were detected by fluorography as described by Burckhardt *et al.* (1979). To measure radioactivity in individual translation-product species, fluorograms were aligned with the original gel, and the areas corresponding to particular proteins were excised and shaken overnight in 10ml of scintillation fluid for subsequent scintillation counting (Wilson *et al.*, 1977).

Preparation of microtubule-associated proteins

Microtubule protein was prepared from the brains of 65-day-old rats by temperature-dependent polymerization/depolymerization as described by Fellous *et al.* (1977). Microtubule-associated proteins were separated from tubulin by phosphocellulose chromatography as described (Fellous *et al.*, 1977).

V8-protease cleavage mapping of proteins

Staph. aureus V8 protease digestion of proteins was performed as described by Cleveland *et al.* (1977). [^{35}S]Methionine-containing translation products (5×10^5 c.p.m.) were run on two-dimensional gels together with human brain proteins (400μg of protein). After electrophoresis, proteins were identified by brief surface staining (Cleveland *et al.*, 1977) and cut from the gels. Microtubule-associated proteins (40μg) were separated by equilibrium two-dimensional gel electrophoresis, and the 68kDa microtubule-associated protein was identified by surface staining and cut from the gels. Protease digestion was performed *in situ* during re-electrophoresis of the excised proteins as described by Cleveland *et al.* (1977).

Results and discussion

Extraction of RNA from brain samples

The samples used for these studies, the recovery of RNA and poly(A)-enriched RNA from these samples are shown in Table 1. Recovery of RNA from the tissue was variable between different samples, although consistent with previously published values (Morrison & Griffin, 1981; Sajdel-Sulkowska *et al.*, 1983). This variability may reflect differences in trauma before death among individuals, as well as differences in the time interval between death and freezing of the brains, since these may affect RNA yield. Similarly, the percentage of RNA that was eluted with low salt from the oligo(dT)–cellulose columns was also very variable, in one case down to 0.15% (Table 1). This also may reflect mRNA breakdown *pre* or *post mortem*, or shortening of their poly(A) tails, since molecules with shorter poly(A) tracts would bind to oligo(dT)–cellulose less strongly and may be eluted with the intermediate-salt wash. In support of this suggestion, the samples that yielded low recoveries of RNA in the low-salt fractions yielded high recoveries in the intermediate-salt fractions. The low- and intermediate-salt fractions were equally active in stimulating protein synthesis in the reticulocyte-lysate cell-free system, and there were no observable qualitative or quantitative differences in the composition of translation products from the two fractions.

Size analysis of poly(A)$^+$ RNA

Fig. 1 shows size distribution analysis of poly(A)$^+$ RNA isolated by one cycle of binding to oligo(dT)–cellulose. Contaminating rRNA makes it difficult to determine the size of poly(A)$^+$ RNA simply on the basis of absorbance. However, the

Table 1. *RNA yields from human brain samples*
RNA was prepared from frozen foetal brains as described in the Materials and methods section, and was estimated by measurements of the A_{260} (Hall & Lim, 1981). Key: M, male; F, female; D, Down's-syndrome foetus.

Sample	Age (weeks gestation) and sex	RNA recovery (μg/g wet wt.)	Recovery of RNA from oligo(dT)–cellulose (% of total)	
			Intermediate-salt fraction	Low-salt fraction
HT45	19 M	119	1.85	4.97
HT46	19 M	104	2.60	2.63
HT37	20 F	75	10.6	1.30
HT36	21 F	86	7.40	0.49
HT38	21.5 M	306	2.30	0.25
HT42	21.5 M	224	4.41	0.15
HT12(D)	19.5 M	314	1.61	0.71
HT10(D)	20.5 M	243	0.95	3.5
HT34(D)	21 M	327	0.79	0.31
HT11(D)	21.5 F	225	0.87	2.79
HT15(D)	24 F	787	0.80	2.04

biological activity of the RNA in the different gradient fractions may be used to determine the size distribution of the poly(A)$^+$ RNA [assuming that different size classes of brain poly(A)$^+$ RNA are translated with the same efficiency]. Maximal stimulatory activity of the poly(A)$^+$ RNA was associated with RNA of 16S, with a secondary peak at 9–12S. This size distribution is in general agreement with previous analysis of poly(A)$^+$ RNA isolated from rat brain under similar conditions (Hall & Lim, 1981) and under denaturing conditions with 85% (v/v) formamide (Elliott *et al.*, 1980). The composition of translation products from the serial gradient fractions is also shown in Fig. 1(*b*). The size of synthesized proteins varies with the corresponding increase in size of RNA as estimated by sedimentation. The size distributions of translation products of both this size-fractionated RNA and total unfractionated RNA (results not shown) were very similar to that for the corresponding fractions of rat brain RNA (Hall & Lim, 1981).

Analysis of translation products by two-dimensional gel electrophoresis

Poly(A)$^+$ RNA populations isolated from each of the brain samples shown in Table 1 were used to direct protein synthesis *in vitro*, and the translation products were analysed by two-dimensional SDS/polyacrylamide-gel electrophoresis.

Fig. 2 shows typical comparisons of translation products of poly(A)$^+$ RNA from normal (HT36) and Down's-syndrome (HT11D) brains by two-dimensional SDS/polyacrylamide-gel electrophoresis. Both non-equilibrium pH-gradient electrophoresis (BASODALT system) and equilibrium pH-gradient isoelectric focusing (ISODALT system) were used as the first-dimension separation and are included in Fig. 2. There is some overlap between the protein species that are detected in the different separation systems. Major common protein species are marked on the gels, including actin and tubulin, which were identified by co-migration with their purified cellular proteins.

Over 400 individual radiolabelled protein species could be detected by using both gel systems, their molecular masses ranging from 200000Da to below 14000Da. The qualitative and quantitative patterns of translation products were highly reproducible in different translations of the same poly(A)$^+$ RNA sample. In addition, although the yields of poly(A)$^+$ RNA from different samples were variable (Table 1), translation-product patterns of mRNA from different brain samples were very similar. This is in agreement with earlier authors (Morrison & Griffin, 1981), who showed that, although biological activity and RNA yield decreases with time after death, the patterns of polypeptide synthesis appear unchanged. Most of the translation products co-migrated with unlabelled total cellular proteins from the brain, translation-product patterns being very similar to the protein patterns of human brain proteins (results not shown). This indicates that translation of poly(A)$^+$ RNA *in vitro* gave rise to products that were very similar to cellular proteins.

When patterns of translation products between normal and Down's-syndrome samples were compared, several differences could be observed in the relative fluorographic intensities and hence synthesis of individual species (these are arrowed in Fig. 2). However, visual comparison of fluorograms of translation products between the six normal and five Down's-syndrome samples revealed only eight translation products whose

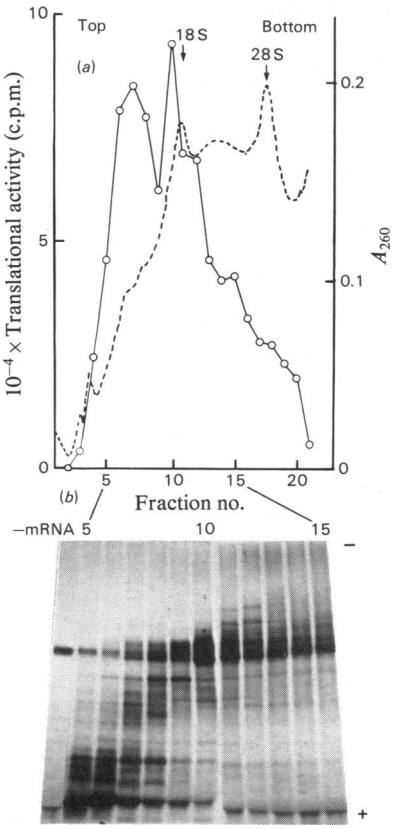

Fig. 1. *Sucrose-density-gradient analysis of poly(A)⁺ RNA*
Poly(A)⁺ RNA isolated from human total cellular RNA (sample HT6, 18-week foetus) was centrifuged on linear 15–30% (w/v) sucrose gradients, and RNA in serial gradient fractions was precipitated with ethanol and dissolved in 10 μl of sterile water as described in the Materials and methods section. Portions (1 μl) of RNA were added to 12 μl of the reticulocyte-lysate system and incubated for 1 h at 37°C. The translation products of these fractions were analysed on one-dimensional polyacrylamide gels as described in the Materials and methods section. (*a*) Analysis of gradients by absorption (----) and translational activity (O——O); (*b*) fluorographic analysis of translation products of serial fractions.

relative synthesis differed consistently between normal and Down's-syndrome samples. These differences are contained within the boxed areas in Fig. 2, three of these being seen in both ISODALT and BASODALT fluorograms and five being seen only in ISODALT fluorograms. The relevant portions of ISODALT fluorograms for five pairs of comparisons between normal and Down's-syndrome samples are shown in detail in Figs. 3 and 4, those translation products that showed a consistent change in synthesis being arrowed.

Quantitative evaluation of translation-product synthesis

In view of the semi-quantitative nature of fluorography, with possible variations in fluor impregnation, these apparent differences in translation-product synthesis between Down's-syndrome and normal control brains were confirmed by direct scintillation counting of the labelled protein species after excision of the relevant portions of the processed gels. Table 2 summarizes the radioactivity determinations of individual translation products of poly(A)⁺ RNA isolated from the six normal and five Down's-syndrome brains used for this study, and also describes the migration co-ordinates of the relevant protein species. These data confirm a statistically significant change in the synthesis of translation products a, c, d, e, f, g and h, and hence indicate changes in the relative concentrations of mRNA species coding for these translation products in Down's syndrome. Two of these (species a, 68 kDa; species c, 49 kDa) were increased in Down's syndrome, the magnitude being of the same order as the gene dosage ratio. These may represent products of genes present on chromosome 21. Other changes involved decreases in relative mRNA concentrations and hence probably reflect effects secondary to the primary trisomic effects. Total cellular poly(A)⁺ RNA represents cytoplasmic and nuclear poly(A)⁺ RNA species, hence the changes observed here could be caused by alterations in the rates of synthesis, processing, nucleo-cytoplasmic transport or turnover of poly(A)⁺ RNA in the brain in Down's syndrome. The apparent decrease in synthesis of species b was not confirmed as statistically significant by these means. Synthesis of this protein was variable in both normal and Down's-syndrome samples (see Fig. 3). Further samples are needed to confirm whether the relative concentrations of mRNA coding for this protein are linked to the Down's-syndrome phenotype.

Identification of 68 kDa microtubule-associated protein as a translation product

Of these radiolabelled species listed in Table 2, only species a and b exactly co-migrated with detectable species present in patterns of total cellular proteins of foetal brains (results not shown). This suggests that the remaining translation-product species either (1) represent proteins with high turnover rates or (2) are post-translationally modified *in vivo* in such a way as to alter their migration co-ordinates. It is possible that protein species corresponding to these translation products may be enriched in specific subcellular fractions and hence may not contribute significantly to total cellular protein patterns. The use of such fractions may therefore enable a more

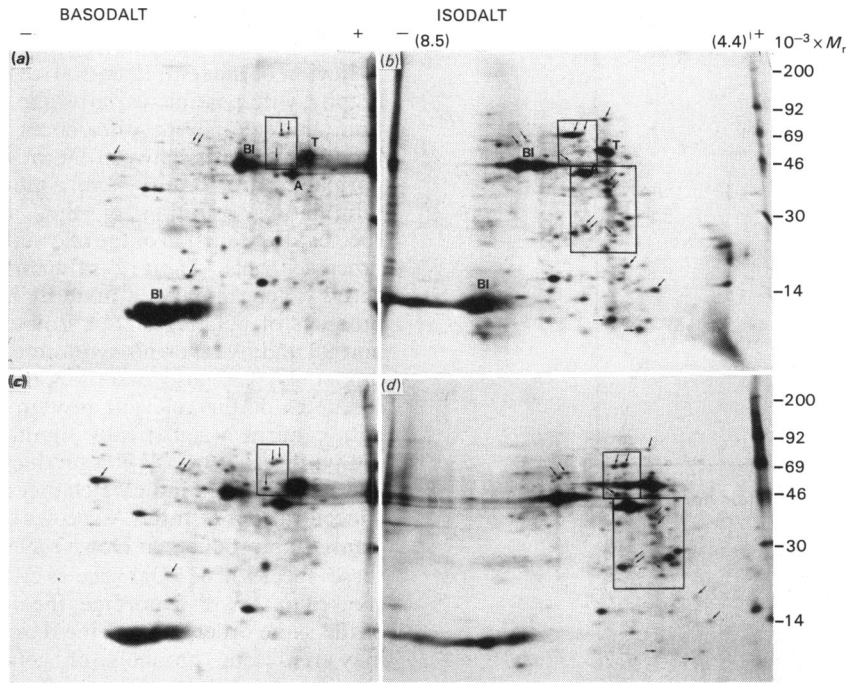

Fig. 2. *Two-dimensional polyacrylamide-gel analysis of translation products from normal and Down's-syndrome brains*
Poly(A)$^+$ RNA was purified and used to direct incorporation of [^{35}S]methionine into protein in the reticulocyte lysate as described in the Materials and methods section. Translation products (5×10^5 acid-precipitable c.p.m.) were analysed by two-dimensional polyacrylamide-gel electrophoresis and fluorography as described in the Materials and methods section. Key: *a*, *c*, BASODALT separations; *b*, *d*, ISODALT separations; *a*, *b*, translation products of poly(A)$^+$ RNA isolated from control brain (HT36); *c*, *d*, translation products of poly(A)$^+$ RNA isolated from Down's-syndrome brain [HT11(D)]. Abbreviations: A, actin; T, tubulin; Bl, [^{35}S]methionine-binding proteins of the lysate system. Arrows indicate protein species whose fluorographic intensities changed in comparisons of control and Down's-syndrome gels; boxed areas contain product species which consistently changed in comparisons of products of poly(A)$^+$ RNA from normal and Down's-syndrome brains; right-hand scale indicates migration of standard molecular-mass markers, which were run on the right-hand side of each gel. Numbers in parentheses indicate the pH of the ends of the isoelectric-focusing gels.

definitive identification of these translation products.

The migration co-ordinates of species a (68 kDa, pI 5.6) on the translation-product fluorograms were very similar to those reported for one of the microtubule-associated proteins of the rat brain (Strocchi *et al.*, 1981). We prepared microtubule-associated proteins from rat brain and found that species a indeed co-migrated with the 68 kDa microtubule-associated protein in our gel system (results not shown). The co-migration of species a with this microtubule-associated protein species and the co-migration of both species a and b with proteins from the human brain strongly suggest the identities of these translation products. For a more rigorous identification, limited-digest peptide mapping was performed on these translation products and the relevant protein species (Fig. 5). Peptide maps of the human 68 kDa pI 5.6 protein, the rat 68 kDa microtubule-associated protein and translation product a were identical (Fig. 5, lanes 1, 2, 5–8), thus establishing the human protein as the human 68 kDa microtubule-associated protein and species a as its corresponding translation product. Peptide maps of species b and the corresponding human protein were also identical, and were different from that of the 68 kDa microtubule-associated protein (Fig. 5, lanes 1–4), thus establishing its identity as separate from microtubule-associated protein, despite the close proximity of the two proteins on two-dimensional gels. The peptide maps of species a and species b were identical for products from normal and Down's-syndrome samples (results not shown). The increased concentrations of the RNA species that coded for the 68 kDa microtubule-associated protein were not reflected in the steady-state concentrations of this protein in Down's syndrome, which

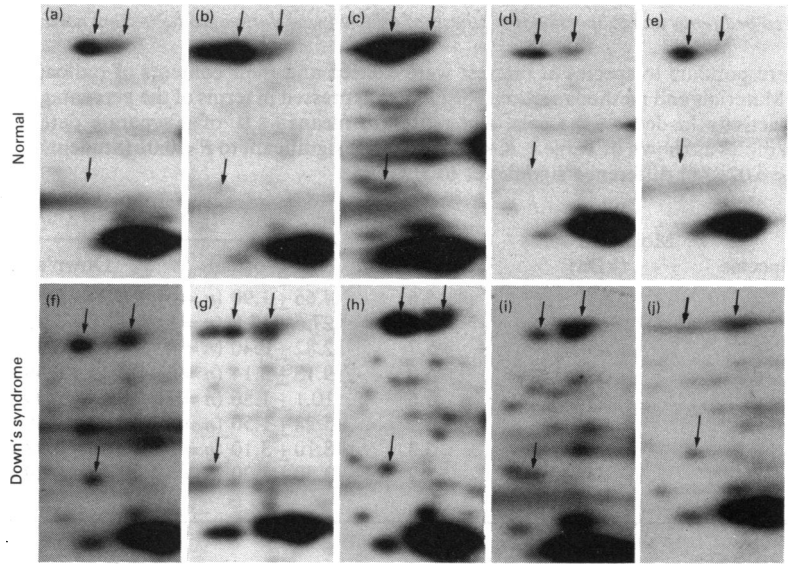

Fig. 3. *Details of 80kDa–45kDa regions of translation-product fluorograms*
Translation products (5×10^5 acid-precipitable c.p.m.) were analysed by two-dimensional gel electrophoresis as described in the legend to Fig. 2. Poly(A)$^+$ RNA used for translation *in vitro* was isolated from the following brain samples: (a) HT45; (b) HT36; (c) HT38; (d) HT37; (e) HT42; (f) HT11(D); (g) HT12(D); (h) HT15(D); (i) HT34(D); (j) HT10(D). Arrows indicate species that consistently changed in comparisons of normal and Down's-syndrome samples.

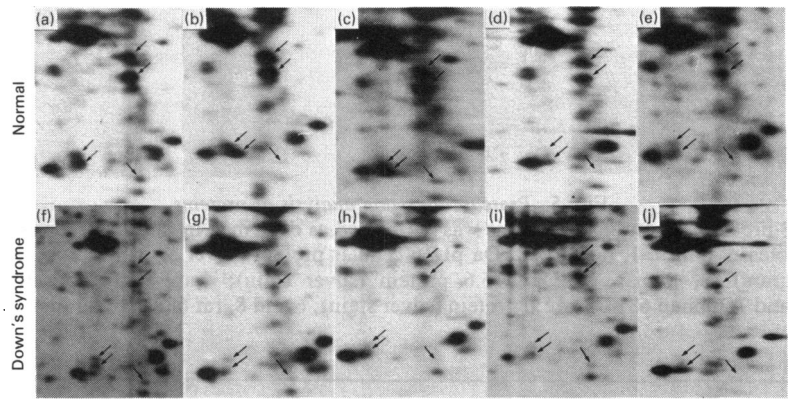

Fig. 4. *Details of 45kDa–20kDa regions of translation-product fluorograms*
Translation products (5×10^5 acid-precipitable c.p.m.) were analysed by two-dimensional gel electrophoresis as described in the legend to Fig. 2. Poly(A)$^+$ RNA used for translation *in vitro* was isolated from the following brain samples: (a) HT36; (b) HT42; (c) HT46; (d) HT38; (e) HT37; (f) HT15(D); (g) HT11(D); (h) HT10(D); (i) HT12(D); (j) HT34(D). Arrows indicate species that consistently changed in comparisons of normal and Down's-syndrome samples.

remained unchanged (results not shown). Indeed, visual comparison of the protein patterns from six normal brains with the five Down's-syndrome samples on two-dimensional gels revealed only one protein species, that corresponding to superoxide dismutase-1 by migration and peptide analysis, which was consistently different in comparisons of normal and Down's-syndrome samples (results not shown). The observation is consistent with the findings of others (Brown *et al.*, 1981; Van Keurin *et al.*, 1982). Assuming that increased concentrations of RNA reflect increased endogenous protein synthesis, this suggests that turnover of the 68kDa microtubule-associated protein is increased

Table 2. *Migration co-ordinates and proportional synthesis of selected translation products from normal and Down's-syndrome samples*

Areas of gels corresponding to species of interest were excised and their contents of radioactivity determined as described in the Materials and methods section. Results are expressed in terms of the percentage of the total mRNA-stimulated radioactivity loaded on the gels, and represent means ± S.D. of n separate determinations from the different brain samples as shown in Table 1. Key: * difference significant to $P < 0.01$ (Student's t test); ** difference significant to $P < 0.02$; *** difference significant to $P < 0.05$.

Species	Molecular mass (kDa)	Approx. pI	$10^2 \times$ Synthesis (%) Normal	Down's syndrome
a	68	5.6	4.66 ± 1.90 ($n = 6$)	9.23 ± 2.32 ($n = 5$)*
b	65	5.8	27.6 ± 18.8 ($n = 6$)	12.8 ± 13.2 ($n = 5$)
c	49	5.7	2.82 ± 0.40 ($n = 4$)	4.02 ± 0.98 ($n = 5$)***
d	37	5.6	9.13 ± 3.14 ($n = 6$)	5.15 ± 0.69 ($n = 5$)***
e	35	5.6	10.1 ± 1.86 ($n = 6$)	4.64 ± 2.04 ($n = 5$)*
f	25.5	5.3	5.28 ± 1.50 ($n = 6$)	2.49 ± 1.55 ($n = 5$)**
g	24.5	5.3	8.10 ± 3.10 ($n = 6$)	3.80 ± 0.98 ($n = 5$)***
h	23	5.2	3.50 ± 1.20 ($n = 6$)	1.85 ± 0.29 ($n = 5$)***

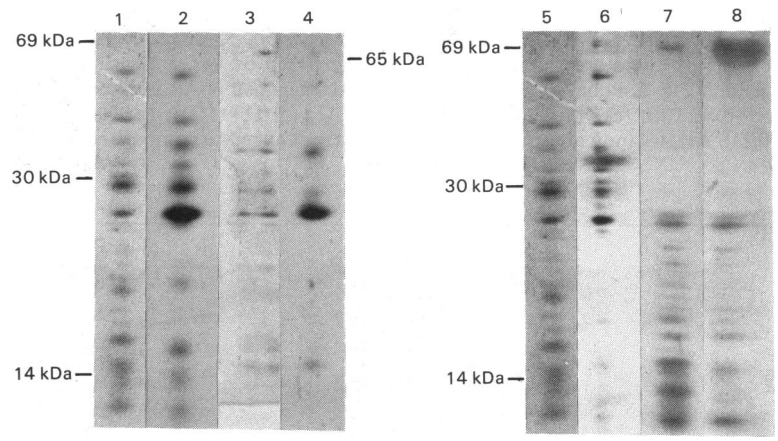

Fig. 5. *Protease-digestion analysis of proteins*

Staph. aureus-V8-protease digestion of proteins was performed as described in the Materials and methods section with 50 ng of protease. Lanes: 1, human 68 kDa pI 5.6 cellular protein (silver stain); 2, 68 kDa pI 5.6 translation product (fluorogram); 3, human 65 kDa pI 5.6 protein (silver stain); 4, 65 kDa pI 5.6 translation product (fluorogram); 5 and 7, human 68 kDa pI 5.6 protein (silver stain); 6 and 8, rat 68 K microtubule-associated protein (silver stain).

in Down's-syndrome brains. It remains to be discovered whether the increased mRNA concentrations observed here are a cause, or an effect of such increased protein turnover.

Conclusion

In common with others (Morrison & Griffin, 1981), we could find no evidence of either selective loss of specific size classes of poly(A)$^+$ RNA in material obtained *post mortem* or loss of specific poly(A)$^+$ RNA species in samples of low RNA yield. In particular, there was no correlation between the amounts of translation products a–h and RNA breakdown as judged by lower RNA yield. Hence, although RNA breakdown *pre* and *post mortem* potentially represents the most important limitation in the usefulness of the techniques used here, it is unlikely that the changes seen in this study in comparisons of normal and Down's-syndrome translation products simply reflect such effects.

With these considerations in mind, we have found seven poly(A)$^+$ RNA species whose relative concentrations were consistently altered in comparisons between age-matched normal and Down's-syndrome brains, out of over 400 species detectable by translation *in vitro*. One of the species of poly(A)$^+$ RNA coded for the 68 kDa microtubule-associated protein. This protein, which is a constituent of brain cell membranes (Strocchi *et*

al., 1981), is a major component of synaptic plasma membranes in the rat, being tightly associated with the membrane, as were other specific protein species (Lim *et al.*, 1983; L. Lim, C. Hall, T. Leung & S. Whatley, unpublished work). The interaction of this protein, and hence of microtubules, with the synaptic membrane may have functional significance for both membrane properties and cell shape (Strocchi *et al.*, 1981). Such a protein may have special significance in brain function, considering the high degree of structural order in the neuron and the unique properties of the neuronal membrane. Alterations in the metabolism of this protein could be envisaged to contribute to the observed changes in neuronal morphology (Marin-Padilla, 1976; Takashima *et al.*, 1981) and neuronal electrical properties (Scott *et al.*, 1982) seen in Down's syndrome.

The data presented here are broadly in agreement with the results of others, who noted no widespread consistent changes in the patterns of steady-state protein contents (Klose *et al.*, 1982; Van Keurin *et al.*, 1982) or polypeptide synthesis over long (Weil & Epstein, 1979) or short (Van Keurin *et al.*, 1982) precursor labelling periods between fibroblast strains derived from normal and Down's-syndrome patients. It is therefore likely that in the developing brain, as in undifferentiated cell strains, the Down's-syndrome phenotype is the result of a limited number of alterations affecting specific gene products. However, the protein products observed to change in the present study are distinct from the proteins whose synthesis has been reported to change in fibroblasts strains (Weil & Epstein, 1979; Van Keurin *et al.*, 1982). The contribution of individual changes to the trisomic phenotype is likely to be different in distinct cell types (as suggested by the results of Klose *et al.*, 1982). These present changes may therefore represent specific contributions to the expression of Down's syndrome in the brain. The functional significance in alterations in the relative concentrations of these mRNA species and their possible specificity to the brain needs to be investigated further in studies of their expression in other human foetal tissues in Down's syndrome and the nature of their corresponding protein products.

We thank the Wellcome Trust and the Brain Research Trust for support. The excellent assistance provided by Miss Diana Menzies and Mr. Mark Habershon is gratefully acknowledged.

References

Bartley, J. A. & Epstein, C. J. (1980) *Biochem. Biophys. Res. Commun.* **93**, 1286–1289

Brown, T. W., Dutrowski, R. & Darlington, G. J. (1981) *Biochem. Biophys. Res. Commun.* **102**, 675–681

Burckhardt, J., Telford, J. & Birnsteil, M. L. (1979) *Nucleic. Acids Res.* **6**, 2963–2971

Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106

Crome, L. & Sterne, J. (1972) *Pathology of Mental Retardation*, pp. 200–224, Churchill-Livingstone, Edinburgh

Elliott, R. M., Davison, A. N. & Lim, L. (1980) *Biochem. J.* **190**, 215–223

Feaster, W. W., Kwok, L. W. & Epstein, C. J. (1977) *Am. J. Hum. Genet.* **29**, 563–570

Fellous, A., Francon, J., Lennon, A. & Nunel, J. (1977) *Eur. J. Biochem.* **78**, 167–174

Hall, C. & Lim, L. (1981) *Biochem. J.* **196**, 327–336

Howard, E., Granoff, D. M. & Bujnovszky, P. (1969) *Brain Res.* **14**, 697–706

Hsia, D. Y., Nadler, H. L. & Shih, L. (1968) *Ann. N.Y. Acad. Sci.* **171**, 526–536

Kaplan, B. B., Bernstein, S. L. & Gioio, A. E. (1979) *Biochem. J.* **183**, 181–184

Klose, J., Zeindl, E. & Sperling, K. (1982) *Clin. Chem.* **28**, 987–992

Kurnit, D. M. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2372–2375

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Lejeune, M., Gautier, M. & Turpin, R. (1959) *C.R. Hebd. Seances Acad. Sci.* **248**, 1721–1722

Lim, L., Hall, C., Leung, T., Mahadevan, L. & Whatley, L. (1983) *J. Neurochem.* **81**, 1177–1181

Marin-Padilla, M. (1976) *J. Comp. Neurol.* **167**, 63–82

McSwigan, J. D., Hanson, D. R., Lubiniecki, A., Heston, L. L. & Sheppard, J. R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7670–7673

Morrison, M. R. & Griffin, W. S. (1981) *Anal. Biochem.* **113**, 318–324

O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021

O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12**, 1133–1142

Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256

Sajdel-Sulkowska, E., Coughlin, J. F. & Marotta, C. A. (1983) *J. Neurochem.* **40**, 670–680

Scott, B. S., Petit, T. L., Becker, L. E. & Edwards, B. A. V. (1982) *Dev. Brain Res.* **2**, 257–270

Strocchi, P., Brown, B. A., Young, J. D., Bonventre, J. A. & Gilbert, J. M. (1981) *J. Neurochem.* **37**, 1295–1307

Takashima, S., Becker, L., Armstrong, D. L. & Chan, F. W. (1981) *Brain Res.* **225**, 1–22

Tan, Y. H. (1975) *Nature (London)* **253**, 280–282

Tan, Y. H., Schneider, E. L., Tischfield, J., Epstein, C. J. & Ruddle, F. H. (1974) *Science* **186**, 61–63

Van Keurin, M. L., Goldman, D. & Merril, C. R. (1982) *Ann. N.Y. Acad. Sci.* **396**, 55–67

Weil, J. & Epstein, C. J. (1979) *Am. J. Hum. Genet.* **31**, 474–488

Wilson, D. E., Hall, M. E., Stone, G. C. & Rubin, R. W. (1977) *Anal. Biochem.* **83**, 33–44