Biosynthesis of the Neural Cell Adhesion Molecule: Characterization of Polypeptide C

OLE NYBROE, MERETE ALBRECHTSEN, JAN DAHLIN, DORTE LINNEMANN, JOAN M. LYLES, CLAUS J. MØLLER, and ELISABETH BOCK
The Protein Laboratory, University of Copenhagen, 2200 Copenhagen N, Denmark

ABSTRACT The biosynthesis of the neural cell adhesion molecule (N-CAM) was studied in primary cultures of rat cerebral glial cells, cerebellar granule neurons, and skeletal muscle cells. The three cell types produced different N-CAM polypeptide patterns. Glial cells synthesized a 135,000 M₉ polypeptide B and a 115,000 M₉ polypeptide C, whereas neurons expressed a 200,000 M₉ polypeptide A as well as polypeptide B. Skeletal muscle cells produced polypeptide B. The polypeptides synthesized by the three cell types were immunochemically identical. The membrane association of polypeptide C was investigated with methods that distinguish peripheral and integral membrane proteins. Polypeptide C was found to be a peripheral membrane protein, whereas polypeptides A and B were integral membrane proteins with cytoplasmic domains of ~50,000 and ~25,000 M₉, respectively. The affinity of the membrane binding of polypeptide C increased during postnatal development. The posttranslational modifications of polypeptide C were investigated in glial cell cultures, and it was found to be N-linked glycosylated and sulfated.

The neural cell adhesion molecule (N-CAM) is a cell surface glycoprotein that is involved in cell-cell adhesion (1). N-CAM has been shown to be identical to two molecules called the D2-cell adhesion molecule (D2-CAM; references 2-4) and brain surface protein-2 (BSP-2; reference 5). In postnatal tissues N-CAM has been demonstrated on three cell types: neurons, glial cells, and skeletal muscle cells (6, 7). The joining together of axons to form fascicles and the apposition of neurons to glial cells during early development suggest that neuron-neuron and neuron-glial interactions are important in defining nerve tracts. The addition of N-CAM antibodies to cultures of spinal ganglia disrupt the side to side adherence of growing neurites during fasciculation (2, 8, 9). In addition, antibodies to N-CAM interfere with the in vivo guidance of optic nerve axons by neuroepithelial endfeet (10). Likewise, the initial establishment of a physical contact between neurites and muscles depends on N-CAM-mediated adhesive interactions (11, 12).

N-CAM from postnatal rat brain consists of three distinct polypeptides: 190,000–200,000 M₉ (polypeptide A), 135,000–140,000 M₉ (polypeptide B), and 115,000–120,000 M₉ (polypeptide C; 13). (Polypeptides A–C will be referred to by letter.) Biosynthetic studies have shown that the N-CAM polypeptides are differentially expressed in different experimental systems. In cultured fetal rat brain neuronal cells A and B are synthesized (3, 14) whereas astrocytes in culture synthesize B and C (4). In studies of cell-free translation of membrane-bound polysomes from whole rat brain in a rabbit reticulocyte lysate system, the A-B-C triplet is observed (13). No interconversion between A, B, and C is seen during biosynthesis (3, 13, 14). These results and data obtained by recombinant DNA techniques (15, 16) suggest that there are at least three mRNAs coding for N-CAM.

Synthesis studies using neuronal cell cultures have shown that A and B undergo extensive co- and posttranslational modifications (3, 14). During postnatal development the pattern of N-CAM synthesis changes from an embryonic to an adult one. This conversion involves changes in the posttranslational modifications of the polypeptides as well as a change in the pattern of polypeptides synthesized (3, 13). The relative proportion of A and B polypeptides synthesized decreases, whereas that of C increases. These changes could reflect changes in the proportions of different cell types in the brain.
or could be due to modulation of N-CAM expression within one cell type. Since the adhesiveness of N-CAM increases with age (17), the function of N-CAM in cell-cell adhesion may well be modulated by developmental changes in its synthesis.

The A and B polypeptides have been well characterized in fetal neuronal cultures. Less is known about the modifications of C, and the nature of its membrane association has so far not been conclusively determined (18). Therefore, the aim of the present work was to examine the biosynthesis and membrane association of C. We compared the N-CAM polypeptides synthesized in primary cultures of postnatal cerebellar granule neurons, cerebral astrocytes, and skeletal muscle cells. C was predominantly a glial product. C was found to be a peripheral membrane protein, whereas A and B were integral membrane proteins with large cytoplasmic domains. The membrane association of C increased during postnatal development, and C was found to be sulfated and N-linked glycosylated.

**MATERIALS AND METHODS**

**Materials:** Micrococcous nucleae (EC 3.1.31.1), creatine phosphokinase (EC 2.7.3.2), and creatine phosphate were obtained from Boehringer Mannheim (Federal Republic of Germany). [2-13C]-trifluoroacetic acid(N)-3-[H]-iodophenol) diazirine (TID, 10 Ci/mmol), [13C]-labeled molecular weight markers, and RNaseA were obtained from Amersham International (Amersham, U.K.). [131I]-Na (carrier-free), [35S]-methionine (>800 Ci/mmol), and [35S]-sulfate (>500 Ci/mmol) were obtained from New England Nuclear (Braunschweig, FRG). Cell culture flasks were from NUNC (Roskilde, Denmark). Dulbecco's modified Eagle's medium (DME) and methionine-free DME were from Gibco Laboratories (Grand Island, NY) or Flow Laboratories, Inc. (McLean, VA). Horse serum and fetal bovine serum were purchased from Hyclone Laboratories Inc. (Ogden, UT). Micrococcal nuclease (EC 3.1.31.1), creatine phosphokinase (EC 2.7.3.2), and crude extracts of rat brain were obtained from Sigma Chemical Co. (St. Louis, MO).

**Cell Cultures:** Cultures were grown in 5% CO2 humidified atmosphere at 37°C in DME containing penicillin (100 U/ml) and streptomycin (100 #g/ml). Astrocyte cultures were prepared from newborn rat brain by mechanical dissociation as described by Chapman and Rumsby (19), with the following modifications: the medium contained 10% fetal calf serum instead of fetal calf serum, and media were changed twice a week. Cells from three brain hemispheres were seeded into each 25-cm² tissue culture flask. Glial cultures were labeled after 2 to 4 wk in vitro. Cultures of skeletal muscle cells were prepared from newborn rat thigh muscle. Cells were dissociated with trypsin, using 0.125% of trypsin in Ca2+-free Krebs buffer (120 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO4, 25 mM NaHCO3, and 13 mM glucose) at 37°C. The tissue was triturated and cell clumps were allowed to settle. Cells from the supernatant were pelleted, harvested in DME containing 10% horse serum, and muscle cultures were labeled after 4 to 8 d in vitro.

Cultures of cerebellar granule neurons were prepared from 8-d-old rats as described by Wilkins et al. (20) with the following modifications: the growth medium contained 10% horse serum instead of 10% fetal calf serum. The medium was changed after 24 and 48 h, and subsequently twice a week. Cytosine arabinoside (0.05 mM) was present during the second day in vitro. Neuronal cultures were labeled after from 2 to 10 d in vitro.

**Characterization of N-CAM Polypeptide C**

The cellular composition of the cultures was investigated by light microscopy and immunocytochemical staining. Immunocytochemistry was performed as previously described (21). Granule neurons in the neuronal cultures were identified on the basis of cellular morphology as being small, round, phase-bright cells that extended long, thin processes. Contaminating astrocytes were identified by their positive reaction with a monoclonal antibody, anti-GFAP-3, which is specific for glial fibrillary acidic protein (21). The number of astrocytes in neuronal cultures amounted to at most 3% of the number of neurons. Fibroblasts were identified by staining with an antiserum against fibronectin. Fibroblast contamination in the neuronal cultures was found to be negligible. Irr muscle cultures, the fusion of myoblasts to form myotubes was evident after 3 d in vitro and almost completed 3 d later. Spontaneous contraction of myotubes could be observed as early as day four in vitro, with contractile activity becoming intense around day six. A low number of fibronectin-positive cells were present in muscle cultures. Approximately 95% of the cells in astrocyte cultures were labeled with monoclonal antibody GFAP-3, the remainder being fibroblast positive.

**Bioisotopic Labeling of Cell Cultures:** When labeling with [35S]-methionine was performed, cell cultures were preincubated with methionine-free DME for up to 30 min before [35S]-methionine was added. Labeling with [35S]-sulfate was performed similarly except that sulfate-free basal medium was added. Cells labeled in the presence of tunicamycin were preincubated with 20 #g/ml of tunicamycin for 3 h before the addition of [35S]-methionine. Tunicamycin was present at 20 #g/ml during the labeling period. To avoid proteolytic breakdown of N-CAM, the protease inhibitor aprotinin was employed at 100 #g/ml during labeling.

**Preparation of Microsomes:** Rough microsomes were prepared from newborn rat brain by centrifuging the homogenate at 10,000 g for 15 min. Pellets were resuspended in 100 mM sodium carbonate, pH 11.0, and incubated at 4°C for 30 min (25). The incubation mixtures were then neutralized by the addition of 1 M potassium phosphate buffer, pH 7.1. Microsomes were pelleted by centrifugation for 1 h at 100,000 g and N-CAM was immunoprecipitated from both the membrane fraction and the 30% supernatant.

**Posttranslational Proteolysis of Rat Brain Microsomes:** After translation of microsomes in vitro for 60 min at 30°C, translation mixtures were incubated for 5 min at 20°C in the presence of 1.6 mM tetracaine to stabilize microsomal membranes. Assays were then adjusted to 10 mM calcium chloride. Tryptsin was added to 25 #g/ml, and enzymatic digestion was carried out for 30 min at 4°C. The reaction was terminated by the addition of tannin (100 mg/ml) and phenylmethylsulfonyl fluoride (2 #g/ml). Digestions were performed in the presence of 4% Triton X-100. After termination of the digestion, the incubation mixtures were immediately submitted to immunospecific labeling with the chloramine-T procedure.

**[35S]-Labeling with the Chloramine-T Procedure:** Brains of rats aged 4, 25, or 40 d were homogenized 1:10 (wt/vol) in 116 mM phosphate buffer, pH 7.1, containing aprotinin (100 U/ml). Membranes were obtained by centrifuging the homogenate at 10,000 g for 15 min. Some mixtures were then diluted to 10 mM calcium chloride. Tryptsin was added to 25 #g/ml, and enzymatic digestion was carried out for 30 min at 30°C. The reaction was terminated by the addition of Triton X-100. After termination of the digestion, the incubation mixtures were immediately submitted to immunospecific labeling with the chloramine-T procedure.

**[35S]-TID-Labeling:** Washed membranes were prepared as described above. Aliquots corresponding to 25 mg original tissue weight were suspended in 1 ml of 50 mM sodium phosphate buffer, pH 7.6. Membranes were iodinated by photoinactivation for 30 min in a 1:1 ml cuvette with constant stirring, using 50 #Ci [35S]-TID per ml membrane suspension. The light source was a mercury lamp focused at a quartz lens. Samples were quenched by the addition of BSA (1% wt/vol) and cooling on ice (see reference 3).

**Immunospecific Isolation of N-CAM:** Radiolabeled N-CAM was immunospecifically bound by crossed immunoelectrophoresis as described by Hansen et al. (13). The N-CAM content of cell-free translation assays, gial cell cultures, and muscle cell cultures was too low to produce visible immunoprecipitates. Therefore, soluble rat brain membranes containing unlabelled N-CAM were used as carrier. Visible immunoprecipitates were excised from the agarose gels and submitted to SDS PAGE.

**SDS PAGE:** Immunoprecipitates containing labeled N-CAM were analyzed by SDS PAGE essentially according to Schäfer-Nielsen and Rose (27). Immunoprecipitates were boiled in SDS PAGE sample buffer containing 5% beta-mercaptoethanol and electrocoated on 4–20% gradient gels. The N-CAM protein was visualized by autoradiography.
labeled molecular weight markers employed were myosin (200,000), phosphorylase b (92,500), BSA (68,000), ovalbumin (46,000), carbonic anhydrase (30,000), and lysozyme (14,300). Gels were either developed for autoradiography on X-Omat film at ~70°C for 1-3 d, or prepared for fluorography as described by Bonner and Laskey (28) and exposed to X-Omat film at ~70°C for 1-2 wk.

Enzyme-linked Immunosorbent Assay of N-CAM: Cultures were harvested and solubilized as described above, and N-CAM was quantified by an enzyme-linked immunosorbent assay (29). The enzyme immune assay employed was an inhibition assay in which solubilized rat brain membranes are attached to a polystyrene microtiter tray. Extracts of the cell cultures to be measured were mixed with a specific rabbit anti-rat N-CAM antibody, and this mixture was incubated with the sensitized solid phase. After washing was done, enzyme-labeled anti-rabbit immunoglobulin was added, and the immunosorbent assay was developed by the addition of enzyme substrate. The titration of neuronal, glial, and muscle cell extracts was performed using twofold serial dilutions.

RESULTS
N-CAM Polypeptide Composition in Neuronal, Glial, and Muscle Cells in Culture

When radioiodinated N-CAM was immunosolated from postnatal rat brain and analyzed by SDS PAGE and autoradiography it was found to contain three distinct polypeptides, A, B, and C, of approximate Mr 190,000 (A), 140,000 (B), and 120,000 (C), (Fig. 1, lane 1).

Cultures of cerebellar granule neurons obtained from 8-d-old rats produced two N-CAM polypeptides of Mr 200,000 (A) and 135,000 (B; Fig. 1, lane 2). The A-B pattern was consistently obtained when labeling was performed after both 2 and 10 d in vitro. The N-CAM content of similar unlabeled cultures was determined by an immunosorbent assay and found to be 18 ± 2 μg/mg total protein (mean ± SD, n = 5).

Astrocytes in culture were found to produce mainly the B and C polypeptides of Mr 135,000 and 115,000, respectively (Fig. 1, lane 3). A very faint A band could occasionally be observed. Again the polypeptide pattern was unaffected by the in vitro age of the cultures during the period from 2 to 28 d. Glial cultures contained 0.8 ± 0.2 μg N-CAM/mg total protein (mean ± SD, n = 4), which is 20 times less than found in the neuronal cultures.

Finally, N-CAM isolated from muscle cell cultures appeared as one predominant polypeptide of 135,000 Mr (B; Fig. 1, lane 4). Trace amounts of A and C appeared inconsistently. The amount of N-CAM in five muscle cell cultures was determined to be 2.4 ± 0.3 μg/mg total protein.

In cell cultures the Mr's of the N-CAM polypeptides differ by ~5 kD from the Mr's of the polypeptides isolated from whole brain. This is probably due to differences in glycosylation.

The immunochemical relationship between the N-CAM polypeptides produced by the three cell types was investigated by an immunotitration experiment. In the immunosorbent assay employed, titration curves of N-CAM from all three cultures were found to be parallel (Fig. 2), indicating that neuronal, glial, and muscle N-CAM were immunologically identical.

Membrane Insertion of Polypeptide C

Although the A and B polypeptides have been shown to be integral membrane proteins (3), the membrane association of the C polypeptide has not been conclusively determined (18). We have therefore determined the nature of the membrane association of C by techniques that distinguish integral and peripheral membrane proteins.

To study the membrane association of newly synthesized N-CAM polypeptides rat brain rough microsomes were translated in vitro in a rabbit reticulocyte lysate system. After translation, microsomal membranes were treated with carbonate buffer, pH 11.0, which opens the microsomal vesicles and converts them into membrane sheets. Proteins originally sequestered into the microsomal lumen are thus released into the medium and, simultaneously, peripheral membrane proteins are detached from the membranes (25). After carbonate treatment membranes were isolated by centrifugation and N-CAM was immunosolated from both the membrane and the supernatant fractions.

In control assays microsomes were translated but not treated with carbonate. A, B, and C could be immunosolated from the whole microsomes (Fig. 3a, lane 1). After carbonate treatment only A and B were found in the membrane fraction (Fig. 3a, lane 2), whereas C was found exclusively in the supernatant (Fig. 3a, lane 3). This demonstrates that C is not an integral membrane protein but a peripheral membrane protein or a secreted protein.

The membrane association of the mature N-CAM polypep-
Age-dependent Changes in the Membrane Association of C

The possibility that C exists in a soluble form, in addition to the membrane-associated form, was investigated. Total homogenates and membranes from postnatal day 4 (P4), day 25 (P25), and day 40 (P40) were radiiodinated. Some membrane aliquots were washed in Tris-barbital buffer, pH 8.6, before iodination (see Materials and Methods).

N-CAM isolated from total homogenates exhibited the A-B-C pattern at all ages investigated (Fig. 3c, lane 1-3). When membranes were separated by centrifugation, N-CAM from P4 membranes showed only the A-B pattern, whereas P25 and P40 membranes showed an A-B-C pattern (Fig. 3c, lanes 4-6). This suggests that the membrane association of C became stronger between day P4 and day P25. Washing membranes with Tris-barbital buffer, pH 8.6, removed C from the membranes at P4 and P25, but not at P40 (Fig. 3c, lanes 7-9). Thus, C is firmly attached to membranes at the adult stage (P40). That washing of membranes with 6 M urea could not detach C at this age (not shown) indicates a very strong membrane attachment of C.

To exclude the possibility that factors in the homogenate could convert A and B into a C-like component, control experiments were performed in which a radiiodinated P4 membrane preparation was incubated with an unlabeled P4 crude homogenate. This did not change the radiiodinated A-B pattern observed, which illustrates that no factors in the P4 homogenate produce a C-like component from A or B, under the conditions employed.

Cytoplasmic Domains of N-CAM

Microsomes were treated with trypsin immediately after cell-free translation. In this procedure cytoplasmic domains are degraded, whereas domains destined to become extracellular are protected inside the microsomes (30). Fig. 4 shows that the Mr of C was not affected by trypsin digestion (compare lanes 1 and 2). Since this polypeptide was completely protected from the action of trypsin, it was apparently located inside the microsomes corresponding to a final extracellular location. Translations performed for short periods, before the synthesis of A was completed, allowed the identification of the two remaining polypeptides seen in Fig. 4, lane 2. The reduction in Mr, effected by trypsin treatment was ~50,000 for A and ~25,000 for B, representing the minimum sizes of their cytoplasmic domains.

To verify that parts of the N-CAM polypeptides were not resistant to the action of trypsin, enzymatic degradation was performed in buffer containing 4% Triton X-100, which ensured solubilization of microsomal membranes. That no N-CAM polypeptides could be isolated after this treatment (not shown) indicates that the parts of N-CAM that are normally protected by the microsomal membranes are susceptible to the action of trypsin.
[35S]methionine in the presence of tunicamycin, a decrease in $M_r$ of $\sim 10,000$ was observed for B and C as compared to pulse-labeled control cultures (Fig. 5, lanes 1 and 2). This demonstrated N-linked glycosylation of these polypeptides with four or five high mannose cores each. A similar number of high mannose cores are added to A and B in fetal neuronal cell cultures (14).

To demonstrate sulfation of the C polypeptide, astrocyte cultures were labeled with [35S]sulfate for from 3 to 4 h. The glial cultures produced sulfate-labeled B and C (Fig. 5, lane 3).

DISCUSSION

Primary cell cultures of astrocytes, muscle, and neuronal cells synthesize N-CAM polypeptides. Although N-CAM from these three cell types is immunologically identical, differences have been noted in their polypeptide composition. Muscle cells synthesize mainly B, and glial cells produce B and C. Neuronal cells from postnatal brain express A and B. Thus, C seems to be predominantly a glial product. It has previously been shown that fetal neuronal cells synthesize A and B (3, 14).

Despite the differences in polypeptide pattern, the observed immunological identity of muscle, glial, and neuronal N-CAM indicates that the N-CAM polypeptides from these cell types are quite similar. The employed immunoadsorbant used the immunoglutinin fraction of pooled sera from four animals that had been immunized for 6 mo. Such an antibody preparation would be expected to recognize at least 5 to 10 different epitopes, corresponding to 35 to 70 amino acid residues on the N-CAM polypeptides (32). Strong similarities between the N-CAM polypeptides A and B have previously been suggested by peptide mapping and by NH$_2$-terminal amino acid sequence analysis (3, 33).

Our results indicate that the expression of the individual N-CAM polypeptides can be regulated independently. Only one (15) or a few (16) N-CAM genes are presumed to exist, yet there appear to be three or more mRNAs producing the different N-CAM polypeptides (13, 15, 16). Hence, a modulation in N-CAM expression may occur that involves differential RNA splicing or differentiation control at the translational level.

The expression of C in vivo increases relative to that of A and B during postnatal development (3, 5, 13). C is not expressed by cultured neuronal cells but is synthesized by cultured astrocytes. The late expression of C in developing whole brain might therefore reflect the later proliferation of glial cells than of neurons in brain ontogeny.

In this report we show that C is N-linked glycosylated with about four high mannose cores. This finding has been confirmed by susceptibility to endoglycosidase H treatment of N-CAM synthesized by microsomes in cell-free translation as says (Nybroe, O., unpublished results). Furthermore, C is sulfated. These modifications are also found for A and B, which supports the suggestion that the three N-CAM polypeptides have quite similar extracellular regions (18, 34). The membrane association of C is different from that of A and B. Neither the newly synthesized nor the mature C polypeptide contains membrane-spanning regions. Since C was labeled by radioiodination of brain membranes at least part of the C population is assumed to be membrane associated as a peripheral membrane protein. It has previously been proposed that C occurs as two populations: one being soluble and another being an integral membrane protein (18).

The membrane affinity of C appeared to increase during postnatal development. A large proportion of the C polypeptide was found to be soluble at P4, whereas C could not be detached from the membranes at P40, even in the presence of urea. This increased affinity may be related to the increased affinity of N-CAM to N-CAM binding that occurs during development (17).

We determined the minimal size of the A and B cytoplasmic domains to be $\sim 50,000$ and $\sim 25,000$ $M_r$, respectively. Gen- narini et al. (18) determined the maximal size of these domains using an array of trypptic fragments of mouse N-CAM incorporated into liposomes. They found domains of $M_r$ $\sim 90,000$ (A) and $M_r$ $\sim 35,000$ (B). Thus, our results, from the use of a different approach, confirm the presence of large cytoplasmic domains of the N-CAM polypeptides A and B. Since these domains seem to be phosphorylated (3, 34, 35), intracellular portions of N-CAM may be important sites of regulatory control of N-CAM function. Further work on the molecular structure of N-CAM and on the control of N-CAM expression will, we hope, make it possible to obtain a detailed understanding of the mechanism underlying N-CAM mediated-cell-cell adhesion.

We thank Lotte Hansen and Grazyna Hahn for expert technical assistance.

This work was supported by The Danish Medical Research Council (grants 12-4703, 12-4871, 12-5091, and 12-5281), Kontorechef Svend Coles Frederiksen og Hustrus Fond, Krafens bekempelse (grant 84-013), The NOVO Foundation (grants of 10 December 1984), the Linex Foundation, the Harboe Foundation (grant of 1 January 1985), and the Carlsberg Foundation (grant 204/1).

Received for publication 7 March 1985, and in revised form 21 August 1985.

REFERENCES

1. Edelman G. M. 1984. Modulation of cell adhesion during induction, histogenesis, and perinatal development of the nervous system. Annu. Rev. Neurosci. 7:339-377.
2. Jæger-Larsen, O. S., A. Delouwe, J.-P. Thiery, and G. M. Edelman. 1980. The nervous system-specific protein D2 is involved in adhesion among neurites from cultured rat ganglia. FEBS (Fed. Eur. Biochem. Soc.) Lett. 111:99-102.
3. Lyles, J. M., D. Linneman, and E. Bock. 1984. Biosynthesis of the D2-cell adhesion molecule: posttranslational modifications, intracellular transport, and developmental changes. J. Cell Biol. 99:2082-2092.
4. Noble, M. A., M. Nielsen, C. J. Møller, and J. M. Lyles, E. Bock, C. Goridis, M. Watanabe, and U. Rutishauser. 1985. Purified astrocytes express NCAM/D2-CAM-like molecules in vitro. Nature (Lond.), 316:725-728.
5. Hirn, M. M., S. Sambhur, H. Deagostini, and C. Goridis. 1983. Molecular heterogeneity and structural evolution during cerebellar ontogeny detected by monoclonal antibody of mouse cell surface antigen BSP-2. Brain Res. 265:87-100.
6. Langet, O. K., G. Gombos, M. Hirn, and C. Goridis. 1983. Distribution of the neural antigens BSP-2 in the cerebellum during development. J. Neurosci. 1353-401.
7. Bock, E., V. Berzin, and S. Rasmussen. 1984. Characterization of the D2-cell adhesion molecule. Protides Biol. Fluids Proc. Coll. 30:75-78.
8. Rutishauser, U., W. F. Gall, and G. M. Edelman. 1978. Adhesion among the neural cells of the chick embryo. IV. Role of the cell surface molecule CAM in the formation of neurite bundles in cultures of spinal ganglia. J. Cell Biol. 79:382-393.
9. Rutishauser, U., and G. M. Edelman. 1980. Effects of fasciculation on the outgrowth of neurites from spinal ganglia in culture. J. Cell Biol. 87:370-378.
10. Silver, J., and U. Rutishauser. 1984. Guidance of optic axons in vivo by a preformed adhesive pathway on neuroepithelial endfeet. Dev. Biol. 106:485–499.

11. Grumet, M., U. Rutishauser, and G. M. Edelman. 1982. Neural cell adhesion molecule is on embryonic muscle cells and mediates adhesion to nerve cells in vivo. Nature (Lond.) 295:693–695.

12. Rutishauser, U., M. Grumet, and G. M. Edelman. 1983. Neural cell adhesion molecule mediates initial interactions between spinal cord neurons and muscle cells in culture. J. Cell Biol. 97:145–152.

13. Hansen, O. C., O. Nybroe, and E. Bock. 1985. Cell-free synthesis of the D2-cell adhesion molecule: evidence for three primary translation products. J. Neurochem. 44:712–717.

14. Lyles, J. M., B. Norrild, and E. Bock. 1984. Biosynthesis of the D2-cell adhesion molecule: pulse chase studies in cultured fetal rat neuronal cells. J. Cell Biol. 98:2077–2081.

15. Murray, B. A., J. J. Hemperly, W. J. Gallia, J. S. MacGregor, G. M. Edelman, and B. A. Cunningham. 1984. Isolation of cDNA clones for the chicken neural adhesion molecule (N-CAM). Proc. Natl. Acad. Sci. USA 81:5585–5588.

16. Goridis, C., M. Him, M.-J. Santoni, G. Gennarini, H. Deagostini-Bazin, B. R. Jordan, M. Kiefer, and M. Steinmetz. 1985. Isolation of mouse N-CAM-related cDNA: detection and cloning using monoclonal antibodies. EMBO (Eur. Mol. Biol. Organ.) J. 4:631–635.

17. Sadoul, R., M. Him, H. Deagostini-Bazin, G. Rougon, and C. Goridis. 1983. Adult and embryonic mouse neural cell adhesion molecules have different binding properties. Nature (Lond.) 304:347–349.

18. Gennarini, G., M. Him, H. Deagostini-Bazin, and C. Goridis. 1984. Studies on the transmembrane disposition of the neural cell adhesion molecule N-CAM. The use of liposome-inserted radioiodinated N-CAM to study its transbilayer orientation. Eur. J. Biochem. 142:65–73.

19. Chapman, J., and M. C. Rumsby. 1982. A syringe modification for the simple and rapid dissociation of post-natal rat cerebral tissue for preparing primary cultures of mixed glial cells. Neurosci. Lett. 34:307–313.

20. Wilkins, C. P., B. Balazs, J. B. Wilson, J. Cohen, and G. R. Dutton. 1976. Preparation of cell bodies from the developing cerebellum: structural and metabolic integrity of the isolated cells. Brain Res. 115:181–199.

21. Allbrecht, M., A. C. von Gerstenberg, and E. Bock. 1984. Mouse monoclonal antibodies reacting with human glial fibrillary acidic protein. J. Neurochem. 42:86–93.

22. Featherstone, J., and I. Boime. 1983. Synthesis of bovine luteotropin in cell-free lysates containing pituitary microsomes. J. Biol. Chem. 258:8143–8147.

23. Jackson, R. J., and J. Hunt. 1983. Preparation and use of nucleoside-treated rabbit reticulocyte lysates for the translation of eucaryotic messenger RNA. Methods Enzymol. 96:50–74.

24. Pelham, H. R. B., and R. J. Jackson. 1976. An efficient mRNA dependent translation system from rabbit reticulocyte lysates. Eur. J. Biochem. 67:247–256.

25. Fujiy, Y., A. L. Hubbard, S. Fowler, and P. P. Lazarow. 1982. Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. J. Cell Biol. 93:97–102.

26. Greenwood, F. C., W. M. Hunter, and J. S. Glower. 1985. The preparation of 125I-labelled human growth hormone of high specific radioactivity. Biochem. J. 89:114–123.

27. Schaller-Nielsen, C., and C. Rose. 1982. Separation of nucleic acids and chromatin associated proteins by hydrophobic interaction chromatography. Biochem. Biophys. Acta. 696:323–331.

28. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46:83–88.

29. Ibison, S., V. Beretzin, B. Nergaard-Pedersen, and E. Bock. 1983. Enzyme-linked immunoassay of the D2-glycoprotein. J. Neurochem. 41:356–362.

30. Morimoto, T., M. Arpin, and S. GaOani. 1983. Use of proteases for the study of membrane insertion. Methods Enzymol. 96:121–150.

31. Nakamura, K., and R. W. Compens. 1978. Effect of glucosamine, 2-deoxyglucose, and tunicamycin on glycosylation, sulfation and assembly of influenza viral proteins. Virology 84:303–319.

32. Hopp, T. P., and C. Rose. 1982. Prediction of a protein antigenic determinant from amino acid sequences. Proc. Natl. Acad. Sci. USA. 79:3824–3828.

33. Cunningham, B. A., S. Hoffman, U. Rutishauser, J. J. Hemperly, and G. M. Edelman. 1983. Molecular topography of the neural cell adhesion molecule N-CAM: surface orientation and location of sialic acid rich and binding regions. Proc. Natl. Acad. Sci. USA. 80:3116–3120.

34. Gennarini, G., G. Rougon, H. Deagostini-Bazin, M. Him, and C. Goridis. 1984. Studies on the transmembrane disposition of the neural cell adhesion molecule N-CAM. A monoclonal antibody recognizing a cytoplasmic domain and evidence for the presence of phosphoserine residues. Eur. J. Biochem. 142:57–64.

35. Sorbin, B. C., S. Hoffman, G. M. Edelman, and B. A. Cunningham. 1984. Sulfation and phosphorylation of the neural cell adhesion molecule, N-CAM. Science (Wash. DC). 224:1476–1478.