The EMBO Journal vol.4 no.11 pp.2765–2768, 1985

Demonstration of immunochemical identity between the nerve growth factor-inducible large external (NILE) glycoprotein and the cell adhesion molecule L1

Elisabeth Bock, Christiane Richter-Landsberg[1], Andreas Faissner[2] and Melitta Schachner[2]

The Protein Laboratory, University of Copenhagen, 34 Sigurdsgade, DK 2200 Copenhagen N., Denmark, [1]Department of Biology, University of Bremen, MW2, 2800 Bremen 83, and [2]Department of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 504, 6900 Heidelberg, FRG

Communicated by M.Schachner

The nerve growth factor-inducible large external (NILE) glycoprotein and the neural cell adhesion molecule L1 were shown to be immunochemically identical. Immunoprecipitation with L1 and NILE antibodies of [^{3}H]fucose-labeled material from culture supernatants and detergent extracts of NGF-treated rat PC12 pheochromocytoma cells yielded co-migrating bands by SDS-PAGE. NILE antibodies reacted with immunopurified L1 antigen, but not with N-CAM and other L2 epitope-bearing glycoproteins from adult mouse brain. Finally, by sequential immunoprecipitation from detergent extracts of [^{35}S]methionine-labeled early post-natal cerebellar cell cultures or [^{3}H]fucose-labeled NGF-treated PC12 cells, all immunoreactivity for NILE antibody could be removed by pre-clearing with L1 antibody and *vice versa*.

Key words: nerve growth factor-inducible large external (NILE) glycoprotein/cell adhesion molecule L1 (L1)/neural cell adhesion molecule (N-CAM)/immunochemistry

Introduction

Several cell adhesion molecules have been implicated in cell surface interactions during development of the mammalian nervous system. The neural cell adhesion molecule (N-CAM) (for reviews, see Edelman, 1984; Rutishauser, 1984) is expressed at early developmental stages (Thiery *et al.*, 1982) and on all major neural cell types in the central and peripheral nervous system (Keilhauer *et al.*, 1985; Langley *et al.*, 1983; Noble *et al.*, 1985; Silver and Rutishauser, 1984). N-CAM mediates aggregation of single cells (Thiery *et al.*, 1977) and histotypic deployment in neural tissue culture (Buskirk *et al.*, 1980; Grumet *et al.*, 1982; Rutishauser and Edelman, 1980; Rutishauser *et al.*, 1978a, 1978b).

In contrast, the cell adhesion molecule L1 (Rathjen and Schachner, 1984) appears later during development (Fushiki and Schachner, 1985) and has a restricted set of roles. It is involved in migration of granule cell neurons in the early post-natal mouse cerebellar cortex (Lindner *et al.*, 1983) and fasciculation of neurites (Fischer *et al.*, 1985), but not in formation of the neuromuscular junction (Mehrke *et al.*, 1984) or synaptogenesis in long-term re-aggregate cultures of mouse cerebellum (Lindner *et al.*, 1985). In the central nervous system, L1 antigen expression is restricted to post-mitotic neurons (Rathjen and Schachner, 1984; Fushiki and Schachner, 1985). In the peripheral nervous system it is additionally present on undifferentiated and non-myelinating Schwann cells (Faissner *et al.*, 1984b; Nieke and Schachner, 1985). When isolated from adult mouse brain, L1 antigen consists of two major glycoprotein bands at 140 and 200 kd (Faissner *et al.*, 1984a, and in preparation; Rathjen and Schachner, 1984). In cell culture, the antigen is synthesized in its high mol. wt. form which ranges from 200 to 230 kd depending on the neural cell type and tumor studied (Faissner *et al.*, in preparation; Rathjen and Schachner, 1984).

L1 exhibits several features in common with the nerve growth factor-inducible large external (NILE) glycoprotein originally characterized on the rat PC12 pheochromocytoma cell line (Lee *et al.*, 1981; McGuire *et al.*, 1978; Salton, 1983a, 1983b). NILE glycoprotein is also preferentially localized on the processes, but not cell bodies of post-mitotic neurons (Stallcup *et al.*, 1985). It is expressed by some, but not all Schwann cells (Salton *et al.*, 1983a, 1983b; Stallcup *et al.*, 1983) and promotes neurite fasciculation in primary cultures of rat brain (Stallcup and Beasley, 1985). It is synthesized in cultures as a single glycoprotein in the 200–230 kd range (Salton *et al.*, 1983a, 1983b; Stallcup *et al.*, 1983; Stallcup and Beasley, 1985).

In view of the similarities between L1 antigen and NILE glycoprotein and their distinction from the neural cell adhesion molecule N-CAM, investigations on the identity of the two molecules seemed warranted. Here, we report that L1 antigen and NILE are immunochemically indistinguishable from each other.

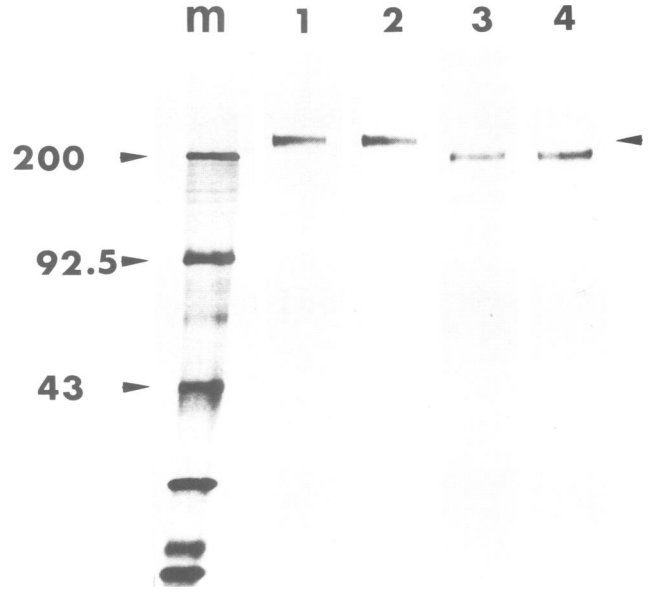

Fig. 1. Immunoprecipitation of Triton X-100 extract **(1,2)** and soluble released material **(3,4)** of NGF-treated, [^{3}H]fucose-labeled PC12 cells analyzed by SDS-PAGE (5–15% slab gel) using antibodies to NILE **(1,3)** and L1 antigen **(2,4)**. Arrowhead on the right indicates the position of cellular NILE and L1 antigen at 230 kd. The soluble L1/NILE immunoprecipitable component has a higher electrophoretic mobility than cellular NILE. Numbers on the left represent apparent mol. wts. ($\times 10^{-3}$) of radiolabeled mol. wt. markers (M).

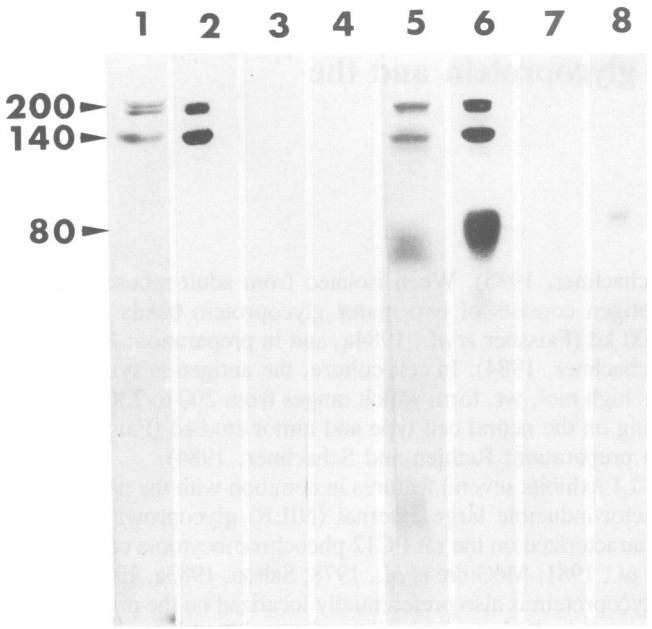

Fig. 2. Western blot analysis of crude membrane fraction of adult mouse cerebellum **(1,5)**, and L1 antigen **(2,6)**, N-CAM **(3,7)** and 'rest' L2 **(4,8)** immunopurified from adult mouse brain separated by SDS-PAGE (6–12% slab gel) using antibodies to L1 antigen **(1–4)** and NILE **(5–8)**. Apparent mol. wts. ($\times 10^{-3}$) are indicated on the left. Antibodies to NILE recognize the 80-kd component more strongly than antibodies to L1 antigen.

Results

Mol. wts. of immunoprecipitated glycoproteins from detergent extract and culture supernatants of rat PC12 pheochromocytoma cells

Antibodies to L1 antigen and NILE glycoprotein precipitate a single glycoprotein band with apparent mol. wt. of 230 kd from Triton X-100 extracts of nerve growth factor (NGF)-treated, [^{3}H]fucose-labeled PC12 cells (Figure 1). Soluble released material obtained from the culture supernatant of PC12 cells contains an L1 and NILE antibody-immunoprecipitable component with higher electrophoretic mobility than the cellular counterpart of NILE as described previously (Richter-Landsberg *et al.*, 1984) (Figure 1).

Western blot analysis of crude membrane fractions from adult mouse cerebellum and L1 antigen, N-CAM and 'rest' L2 immunopurified from adult mouse brain

In crude membrane fractions of adult mouse cerebellum L1 and NILE antibodies react with bands co-migrating at apparent mol. wts. of 140 and 200 kd. The 80-kd component, probably a proteolytic degradation product, is prominently recognized by NILE, but not, or weakly, by L1 antibodies (Figure 2). Immunopurified N-CAM and 'rest' L2 from adult mouse brain is neither recognized by L1 nor NILE antibodies (Figure 2). 'Rest' L2 consists of an as yet unknown number of neural cell surface glycoproteins, including L1 and N-CAM, which carry the L2 carbohydrate epitope (Kruse *et al.*, 1984). Since these glycoproteins are a family

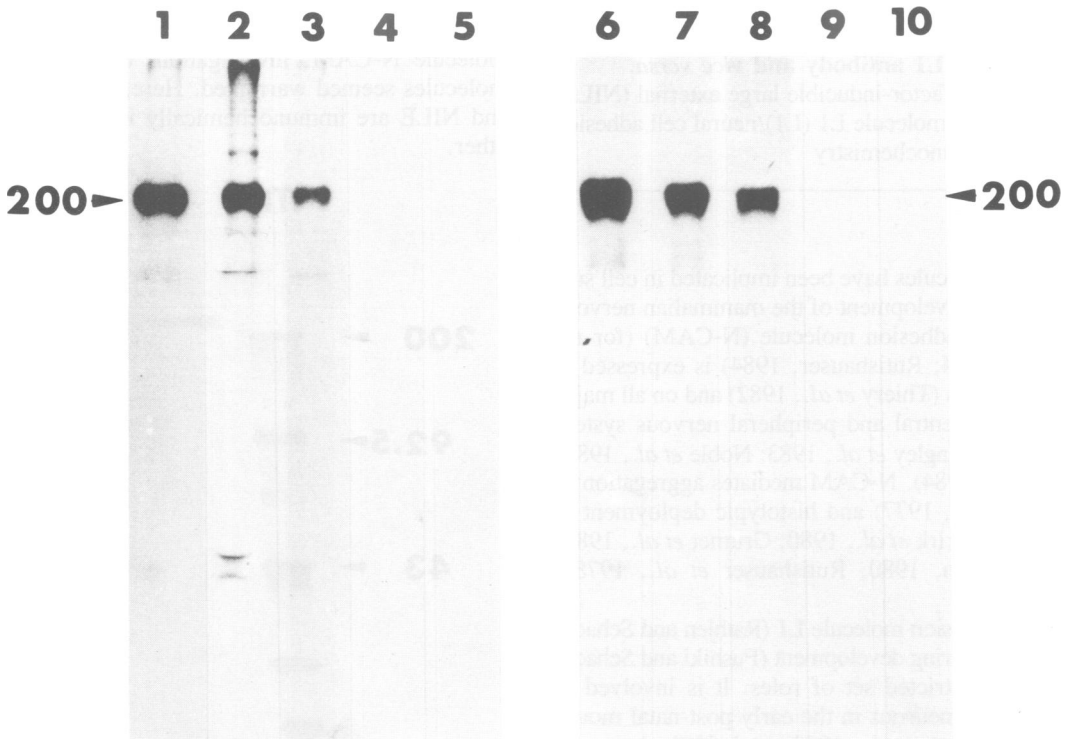

Fig. 3. Sequential immunoprecipitation of Nonidet P-40 extract of [^{35}S]methionine-labeled early post-natal cerebellar cell cultures analyzed by SDS-PAGE (6–12% slab gel) using L1 antibodies for the first immunoprecipitations **(1–4**, four cycles of pre-clearing were necessary for depletion) followed by NILE antibodies **(5)**, or using antibodies to NILE for the first immunoprecipitations **(6–9**, four cycles of pre-clearing were necessary for depletion) followed by L1 antibodies **(10)**. Apparent mol. wt. ($\times 10^{-3}$) of the immunoprecipitated band is indicated on both sides.

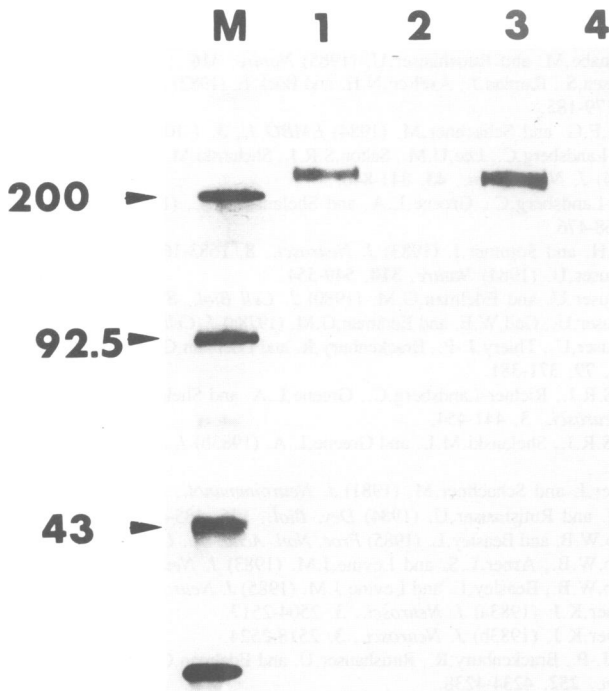

Fig. 4. Sequential immunoprecipitation of Triton X-100 extract of [^{3}H]fucose-labeled PC12 cells analyzed by SDS-PAGE (5 – 15% slab gel) using NILE antibodies for pre-clearing **(1)** followed by L1 antibodies **(2)**, and using L1 antibodies for pre-clearing **(3)** followed by NILE antibodies **(4)**. One cycle of pre-clearing was sufficient for depletion by first antibodies. Numbers on the left represent apparent mol. wts. ($\times 10^{-3}$) of radiolabeled mol. wt. markers (M).

of structurally and functionally related molecules, they serve as a valuable control for the specificity of NILE antibody.

Sequential immunoprecipitation from detergent extracts of [^{35}*S*]*-methionine-labeled early post-natal mouse cerebellar cell cultures*

To show that L1 and NILE antibodies recognize identical molecules, sequential immunoprecipitations were performed from detergent extracts of [^{35}S]methionine-labeled early post-natal mouse cerebellar cell cultures (Figure 3) and NGF-treated, [^{3}H]fucose-labeled PC12 cells (Figure 4). L1 antibodies exhaustively removed all further immunoreactivity for L1 and NILE antibodies. Conversely, NILE antibodies removed all further reactivity for NILE and L1 antibodies. Exhaustive pre-clearing of detergent extracts with N-CAM antibody in four cycles (see legend to Figure 3) did not remove any immunoreactivity for subsequent immunoprecipitation with L1 or NILE antibodies (not shown). These results show that L1 and NILE antibodies identify the same molecular species.

Discussion

Our results show that the NILE glycoprotein and the neural cell adhesion molecule L1 are identical molecular species. These findings tie together previous observations on the biochemical properties of NILE glycoprotein in the normal peripheral nervous system, adrenal medulla and a neural tumor of peripheral origin, the rat pheochromocytoma (Salton *et al.*, 1983a, 1983b; Stallcup *et al.*, 1983; Sweadner, 1983a, 1983b) and our knowledge on the functional properties of L1 antigen in the developing central nervous system (Fischer *et al.*, 1985; Keilhauer *et al.*, 1985; Lindner *et al.*, 1983, 1985; Mehrke *et al.*, 1984). In general, the electrophoretic mobilities of the individual NILE components in SDS-PAGE are lower in the peripheral than in the central nervous system. This difference in apparent mol. wts. is most likely due to differences in levels of glycosylation (Salton *et al.*, 1983b; Margolis *et al.*, 1983). Furthermore, NILE components of the peripheral nervous system with higher electrophoretic mobility are thought to derive from those with lower mobility by limited proteolysis (Sweadner, 1983a, 1983b).

Microheterogeneity among the family of NILE glycoproteins has been shown using polyclonal antibodies (Margolis *et al.*, 1983; Sweadner, 1983a; Stallcup *et al.*, 1983). As microheterogeneity of N-CAM molecules is now well documented (Williams *et al.*, 1985; Goridis *et al.*, 1985), it would not be surprising if such heterogeneities would exist also for L1/NILE. The hydrodynamic properties of the NILE components indicate that they are non-globular in shape, both before and after post-translational modification and release (Sweadner, 1983b).

Observations on the NGF inducibility of L1/NILE add a new dimension to our understanding of axon elongation in the peripheral nervous system. Release of NGF from the target tissue may well induce neurite extension by induction of a particular cell adhesion molecule, such as L1/NILE. Furthermore, NGF receptors have been observed on immature Schwann cells (Rohrer and Sommer, 1983). It remains to be seen whether the population of L1 antigen-positive Schwann cells (Faissner *et al.*, 1984b; Nieke and Schachner, 1985) also express NGF receptors and whether the developmental decrease in expression of L1 antigen and NGF receptors are functionally related. The functional implications of Sweadner's observations on the Ca^{2+}-dependent release of soluble NILE components into the culture medium following chemically evoked transmitter release (Sweadner, 1983a, 1983b) remain to be interpreted in terms of possible de-adhesion or adhesion stabilizing mechanisms.

Whether the recently described neural cell adhesion molecule Ng-CAM (Grumet *et al.*, 1984a, 1984b) is immunochemically related to L1/NILE awaits further investigations. The biochemical features, developmental appearance, cell type specificity and immunocytological localization of Ng-CAM are strikingly similar to L1/NILE. However, Ng-CAM has not been observed on Schwann cells (Thiery *et al.*, 1985). Furthermore, L1 antigen has been implicated in neuron to neuron, but not in neuron to astrocyte adhesion (Keilhauer *et al.*, 1985), whereas Ng-CAM has been reported to be involved in both neuron to neuron and neuron to astrocyte adhesion mechanisms (Grumet *et al.*, 1984a, 1984b). It remains to be seen whether Ng-CAM is immunochemically similar or identical to the L1/NILE group of molecules.

Materials and methods

Preparation of antigens

L1 antigen, N-CAM and 'rest' L2 were purified by immunoaffinity chromatography as described previously (Kruse *et al.*, 1984; Rathjen and Schachner, 1984). 'Rest' L2 refers to a fraction of glycoproteins obtained when L1 and N-CAM are removed from detergent extracts of adult mouse brain membranes and residual polypeptides recovered by immunoaffinity chromatography on a monoclonal L2 antibody column (Kruse *et al.*, 1984). The L2 carbohydrate epitope is shared by L1 antigen, N-CAM, myelin-associated glycoprotein and the neural cell adhesion molecule J1 (Kruse *et al.*, 1985) and other, as yet unidentified glycoproteins (Kruse *et al.*, 1984). 'Rest' L2 contains myelin-associated glycoprotein, J1 antigen and other glycoproteins. NILE glycoprotein was prepared as described previously (Salton *et al.*, 1983a, 1983b; Richter-Landsberg *et al.*, 1984). Membrane fractions from adult mouse cerebellum were prepared for Western blot analysis as described previously (Faissner *et al.*, 1984b; Rathjen and Schachner, 1984).

Preparation and specificity of polyclonal antibodies

Polyclonal antibodies to L1 antigen from adult mouse brain were prepared in

rabbits (Rathjen and Schachner, 1984). Their specificity has been described (Rathjen and Schachner, 1984; Faissner *et al.*, 1984a, 1984b). Polyclonal antibodies to NILE from PC12 cells were prepared in guinea pigs and their specificities were described previously (Salton *et al.*, 1983a, 1983b; Richter-Landsberg *et al.*, 1984). Antibodies to N-CAM from adult rat brain were prepared in rabbits (Rasmussen *et al.*, 1982). The antibodies are specific for N-CAM isolated from mouse or rat brains (Rasmussen *et al.*, 1982).

Cell cultures

Monolayer cultures from cerebellum of 5- to 7-day-old C57BL/6J mice were prepared and maintained as decribed by Schnitzer and Schachner (1981). Cells were cultured for 2 days before they were labeled with [^{35}S]methionine. PC12 rat pheochromocytoma cells were grown in the presence of NGF (50 ng/ml) as described by Richter-Landsberg *et al.* (1984). Cells were maintained for 6 days in culture before [^{3}H]fucose labeling was performed.

Analytical procedures

Western blot analysis on crude membrane fractions and immunopurified antigens was performed as described previously (Faissner *et al.*, 1984b; Rathjen and Schachner, 1984). For immunoprecipitation, PC12 cells were treated with [^{3}H]fucose (10 μCi/ml) for 3 days and then extracted in Tris-HCl buffer, 50 mM, pH 6.8, containing 2% Triton X-100. The cell extract was centrifuged for 30 min at 10 000 *g* at 4°C. For immunoprecipitation of antigens from detergent extracts of cerebellar cell cultures, cultures were labeled with [^{35}S]methionine (200 μCi/ml) for 4 h. Detergent solubilization of washed cells was carried out in Tris-HCl, 20 mM, 0.15 M NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM methionine, 0.5% Nonidet P-40, 0.05% NaN_3, pH 7.4, containing aprotinin, soybean trypsin inhibitor, turkey egg white trypsin inhibitor (all at 10 μg/ml), phenylmethylsulfonylfluoride (PMSF) and iodoacetamide (both at 5 mM), and pepstatin (at 2 μM) as described (Kruse *et al.*, 1984; Faissner *et al.*, in preparation). Supernatants were obtained by centrifugation at 800 *g* for 10 min and 100 000 *g* for 1 h at 4°C. Soluble released material from culture supernatants of [^{3}H]fucose-labeled PC12 cells were prepared as described by Richter-Landsberg *et al.* (1984). Immunoprecipitations from Triton X-100 extracts of PC12 cells and from culture supernatants were performed as described by Richter-Landsberg *et al.* (1985). Immunoprecipitation and sequential immunoprecipitation from detergent extracts of [^{35}S]methionine-labeled mouse cerebellar cultures were carried out according to Kruse *et al.* (1984) and Faissner *et al.* (in preparation).

Acknowledgements

The authors are grateful to Bernd Gehrig and Barbara Krech for technical assistance and The Danish Medical Research Council (grant nos. 12-4703/4871/5282), Kraeftens Bekaempelse (grant no. 84-120), and Deutsche Forschungsgemeinschaft (Scha 185/9-5) for support.

References

Buskirk,D.R., Thiery,J.-P., Rutishauser,U. and Edelman,G.M. (1980) *Nature*, **295**, 486-489.
Edelman,G.M. (1984) *Annu. Rev. Neurosci.*, **7**, 339-377.
Faissner,A., Kruse,J., Goridis,C., Bock,E. and Schachner,M. (1984a) *EMBO J.*, **3**, 733-737.
Faissner,A., Kruse,J., Nieke,J. and Schachner,M. (1984b) *Dev. Brain Res.*, **15**, 69-82.
Fischer,G., Künemund,V. and Schachner,M. (1985) *J. Neurosci.*, in press.
Fushiki,S. and Schachner,M. (1985) *Dev. Brain Res.*, in press.
Goridis,C., Hirn,M., Santoni,M.-J., Gennarini,G., Deagostini-Bazin,H., Jordan,B.R., Kiefer,M. and Steinmetz,M. (1985) *EMBO J.*, **4**, 631-635.
Grumet,M., Rutishauser,U. and Edelman,G.M. (1982) *Nature*, **295**, 693-695.
Grumet,M., Hoffman,S., Chuong,C.-M. and Edelman,G.M. (1984a) *Proc. Natl. Acad. Sci. USA*, **81**, 7989-7993.
Grumet,M., Hoffman,S. and Edelman,G.M. (1984b) *Proc. Natl. Acad. Sci. USA*, **81**, 267-271.
Keilhauer,G., Faissner,A. and Schachner,M. (1985) *Nature*, **316**, 728-730.
Kruse,J., Mailhammer,R., Wernecke,H., Faissner,A., Sommer,I., Goridis,C. and Schachner,M. (1984) *Nature*, **311**, 153-155.
Kruse,J., Keilhauer,G., Faissner,A., Timpl,R. and Schachner,M. (1985) *Nature*, **316**, 146-148.
Langley,O.K., Gombos,G., Hirn,M. and Goridis,C. (1983) *Int. J. Dev. Neurosci.*, **1**, 393-401.
Lee,V.M., Greene,L.A. and Shelanski,M.L. (1981) *Neuroscience*, **6**, 2773-2786.
Lindner,J., Rathjen,F.G. and Schachner,M. (1983) *Nature*, **305**, 427-430.
Lindner,J., Orkand,P.M. and Schachner,M. (1985) *Neurosci. Lett.*, **55**, 145-150.
Margolis,R.K., Salton,S.R.J. and Margolis,R.U. (1983) *J. Neurochem.*, **41**, 1635-1640.
McGuire,J.C., Greene,L.A. and Furano,A.V. (1978) *Cell*, **15**, 357-365.
Mehrke,G., Jockusch,H., Faissner,A. and Schachner,M. (1984) *Neurosci. Lett.*, **44**, 235-239.
Nieke,J. and Schachner,M. (1985) *Differentiation*, in press.
Noble,M., Albrechtsen,M., Moller,C., Lyles,J., Bock,E., Goridis,C., Watanabe,M. and Rutishauser,U. (1985) *Nature*, **316**, 725-728.
Rasmussen,S., Ramlau,J., Axelsen,N.H. and Bock,E. (1982) *Scand. J. Immunol.*, **15**, 179-185.
Rathjen,F.G. and Schachner,M. (1984) *EMBO J.*, **3**, 1-10.
Richter-Landsberg,C., Lee,U.M., Salton,S.R.J., Shelanski,M.L. and Greene,L.A. (1984) *J. Neurochem.*, **43**, 841-848.
Richter-Landsberg,C., Greene,L.A. and Shelanski,M.L. (1985) *J. Neurosci.*, **5**, 468-476.
Rohrer,H. and Sommer,I. (1983) *J. Neurosci.*, **8**, 1683-1693.
Rutishauser,U. (1984) *Nature*, **310**, 549-554.
Rutishauser,U. and Edelman,G.M. (1980) *J. Cell Biol.*, **87**, 370-378.
Rutishauser,U., Gall,W.E. and Edelman,G.M. (1978a) *J. Cell Biol.*, **79**, 382-393.
Rutishauser,U., Thiery,J.-P., Brackenbury,R. and Edelman,G.M. (1978b) *J. Cell Biol.*, **79**, 371-381.
Salton,S.R.J., Richter-Landsberg,C., Greene,L.A. and Shelanski,M.L. (1983a) *J. Neurosci.*, **3**, 441-454.
Salton,S.R.J., Shelanski,M.L. and Greene,L.A. (1983b) *J. Neurosci.*, **3**, 2420-2430.
Schnitzer,J. and Schachner,M. (1981) *J. Neuroimmunol.*, **1**, 429-456.
Silver,J. and Rutishauser,U. (1984) *Dev. Biol.*, **106**, 485-499.
Stallcup,W.B. and Beasley,L. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 1276-1280.
Stallcup,W.B., Arner,L.S. and Levine,J.M. (1983) *J. Neurosci.*, **3**, 53-68.
Stallcup,W.B., Beasley,L. and Levine,J.M. (1985) *J. Neurosci.*, **5**, 1090-1101.
Sweadner,K.J. (1983a) *J. Neurosci.*, **3**, 2504-2517.
Sweadner,K.J. (1983b) *J. Neurosci.*, **3**, 2518-2524.
Thiery,J.-P., Brackenbury,R., Rutishauser,U. and Edelman,G.M. (1977) *J. Biol. Chem.*, **252**, 4234-4238.
Thiery,J.-P., Duband,J.-L., Rutishauser,U. and Edelman,G.M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 6737-6741.
Thiery,J.-P., Delouvee,A., Grumet,M. and Edelman,G.M. (1985) *J. Cell Biol.*, **100**, 442-456.
Williams,R.K., Goridis,C. and Akeson,R. (1985) *J. Cell Biol.*, **101**, 36-42.

Received on 4 July 1985; revised on 29 July 1985