Neurocan, a nervous tissue-specific chondroitin sulfate proteoglycan of the aggrecan family which has been shown to interact with neural cell adhesion molecules and tenasin, could be visualized by rotary shadowing electron microscopy as two globular domains interconnected by an extended flexible filament of 60–90 nm. Several recombinant neurocan fragments generated in the human embryonic kidney cell line 293 represent as observed by electron microscopy the expected parts of this structure, which indicates a correct folding of these molecules. Biological activity of the recombinant N-terminal globular domain could be demonstrated by its coelution with hyaluronan in gel permeation chromatography. In addition, the modification of the recombinant fragments with certain carbohydrate structures was analyzed. High mannosylated oligosaccharides could be mapped to the N-terminal globular domain of the brain-derived molecule. Only recombinant fragments containing parts of the central region of the molecule were modified with chondroitin sulfate chains and with the HNK-1 epitope, and could be considerably altered in their migratory behavior on SDS-polyacrylamide gel electrophoresis by neuraminidase treatment. These findings and the electron microscopic shape indicate a mucin-like character for the central neurocan region.

For the development of the nervous system, which is characterized by the migration of individual cells and axonal outgrowth, the ability to modulate cell interactions is of great importance. A molecule which may be particularly involved in this process is the nervous tissue-derived chondroitin sulfate proteoglycan neurocan (1). Neurocan has been found to colocalize in brain with the neural cell adhesion molecules N-CAM and Ng-CAM and with tenasin during certain developmental stages and to interact with these morphoregulatory proteins in binding and aggregation inhibition assays (2, 4).

Neurocan is a member of the aggrecan family of chondroitin sulfate proteoglycans (1). These proteoglycans are characterized by hyaluronan binding domains at their N termini and C-type lectin-like domains at their C-terminal ends. The central regions of these proteins have no apparent homology with each other and are considered to represent those parts of all proteins of this family where glycosaminoglycan chains are attached. The analysis of the carbohydrate content of neurocan and the size of its glycosaminoglycan chains suggested the presence of 2–3 chondroitin sulfate chains. The carbohydrate analysis also indicated the presence of 30–40 sialylated O-linked oligosaccharides, and considerable sialylation of the core protein has been shown by treatment with neuraminidase (5). During the first postnatal month neurocan is increasingly proteolytically processed in the central region of the molecule resulting in the generation of a distinct C-terminal fragment, neurocan-C (6). Neurocan-C can be selectively isolated from the brain of rats one month of age or older. This fragment has been shown to retain the ability to interact with the neural cell adhesion molecules and to inhibit their homophilic interaction (2, 4).

A significant reduction of the binding activity of neurocan to these molecules was observed after enzymatic removal of the glycosaminoglycan chains with chondroitinase ABC. However, the resulting core proteins still bind to the neural cell adhesion molecules and also inhibit their homophilic interaction.

To elucidate in more detail the structure of neurocan and the sites of interactions with other molecules, recombinant neurocan fragments were produced in a eukaryotic cell line. As an indication for a correct folding of the recombinant molecules their shapes were compared to brain-derived molecules by rotary shadowing electron microscopy and the hyaluronan binding activity of the N-terminal fragment was analyzed. The modification with two carbohydrate structures which are considered to modulate neural recognition molecule functions, the HNK-1 epitope and oligomannosidic glycans, was also investigated.

### MATERIALS AND METHODS

**Monoclonal Antibodies**—The monoclonal anti-neurocan antibody 1D1 (6) was purified from medium conditioned by the corresponding hybridoma cells grown in 1% fetal calf serum in a bioreactor (Integra, Fernwald). 50 ml of medium were adjusted to pH 5 and passed over a 2-ml protein G-Sepharose column (Sigma) equilibrated with 0.1 M sodium acetate, pH 5. The-column was washed with 15 ml of 0.1 M sodium acetate, pH 5, and the antibody was eluted with 0.1 M glycine/HCl, pH 2.7, and immediately neutralized with 1 M Tris, pH 9. The purified antibody was dialyzed into sodium hydrogen carbonate, pH 8.3, and coupled to CNBr-activated Sepharose 4B according to the manufacturer’s protocol (Pharmacia, Freiburg, Germany). The monoclonal antibody recognizing the HNK-1 epitope was obtained from Sigma (Munich). The monoclonal antibody 8A4 raised against rat link protein has been shown to recognize a structurally related peptide epitope in rat neurocan (1). This antibody was developed by Bruce Caterson and obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Science, Johns Hopkins University School of Medicine, Baltimore, MD 21205, and the Department of Biological Science, University of Iowa, Iowa City, IA 52242, under contract NO1-HD-2-3144 from the National Institute of Child Health and Human Development. Polyclonal rabbit serum raised against the entire neurocan proteoglycan was provided by Dr. Richard U. Margolis, New York. Polyclonal rabbit serum raised against a recombinant rat brevican fragment was provided by Dr. Eckart D. Gundelfinger, Magdeburg.

**Construction of Expression Vectors**—All functional cDNA sequences contained the 5′-untranslated sequence and the signal peptide of human BM-40 terminating in an artificial NheI site (7) followed by rat...
Structural Analysis of Neurocan

Diagram of the domain structure of tissue-derived neurocan forms and of recombinant neurocan fragments expressed in 293 cells. Domains with homology to other proteins and potential N-glycosylation and glycosaminoglycan attachment sites are indicated. Designations of the recombinant fragments are based on the first or last amino acid of the original rat neurocan sequence (1), leucine 639 (L639), aspartic acid 925 (D925), threonine 950 (T950), histidine 359 (359H), and methionine 773 (773M), contained in these fragments.

Recombinant fragments:
- L639
- 359H
- D925
- T950

Potential N-glycosylation site
- Neurocan signal peptide
- Proteoglycan attachment site
- Potential glycosaminoglycan site
- BM-40 signal peptide
- 8A4-epitope
- C-type lectin-like domain
- Ig-like domain
- EGF-like domain
- Complement regulatory-like domain

Fig. 1. The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; MOPS, 4-morpholinepropanesulfonic acid.

Potential N-glycosylation site
- Neurocan signal peptide
- Proteoglycan attachment site
- Potential glycosaminoglycan site
- BM-40 signal peptide
- 8A4-epitope
- C-type lectin-like domain
- Ig-like domain
- EGF-like domain
- Complement regulatory-like domain

The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; MOPS, 4-morpholinepropanesulfonic acid.
RESULTS

Expression and Purification of Recombinant Neurocan Fragments—Initial attempts to express the entire neurocan molecule using the 5'-untranslated region and the coding region of the rat neurocan cDNA failed. No recombinant protein could be detected in the cell culture supernatant, although neurocan-specific mRNA could be detected in several transfected cell lines (data not shown). After changing the endogenous neurocan 5'-untranslated region and signal peptide sequences against the BM-40 sequences, fragment 773M was obtained. This fragment represents the N-terminal globular domain and about 400 amino acids of the central filamentous domain followed by an unrelated amino acid sequence of 25 residues. All other recombinant fragments were also linked to the BM-40 5'-untranslated region and signal peptide sequences (Fig. 1). Fragment T950 represents the C-terminal globular domain. Fragment D925 contains in addition the last 25 amino acids of the central region with a potential glycosaminoglycan attachment site which is, compared to other parts of the central region, highly conserved between rat and mouse neurocan. The recombinant fragment L639 corresponds to neurocan-C, a proteolytic fragment that can be isolated from the brain of adult rats. Fragment 359H contains only the N-terminal globular part of neurocan and terminates, like link protein (17), five amino acids after the last disulfide bridge. This fragment could be strongly enriched by concanavalin A affinity chromatography. As shown by Coomassie Blue staining of the protein preparations, a reasonably pure preparation of the N-terminal fragment 773M could be achieved by ion exchange chromatography, whereas the C-terminal fragments L639, D925, and T950 could be purified by immunoaffinity chromatography with the monoclonal antibody 1D1 (Fig. 2). The identity of fragments 359H and 773M, which were not recognized by the monoclonal 1D1 antibody, was confirmed by immunostaining with a polyclonal rabbit serum raised against tissue-derived neurocan (results not shown).

SDS-PAGE Analysis of the Recombinant Fragments—The SDS-PAGE analysis of the chondroitinase-treated recombinant fragments also revealed that the core proteins of fragments 773M and L639, which contain major parts of the central region, migrated much slower than predicted from their calculated molecular weight and the number of potential N-glycosylation sites. The apparent molecular mass values could be reduced by about 15 kDa after additional treatment with neuraminidase (Fig. 2 and Table I). In both respects the recombinant fragments resemble the core proteins of tissue-derived neurocan and neurocan-C, which also display apparent molecular mass values about twice as high as those calculated from their amino acid sequence and a similar shift in apparent molecular mass after treatment with neuraminidase (1, 5, 6). Fragments 359H, T950, and D925, which contain almost exclusively globular domains of neurocan, fit the molecular mass values expected from their amino acid sequence and potential N-glycosylation sites much better (Fig. 2 and Table I). In contrast to the larger fragments 773M and L639, the SDS-PAGE analysis of fragments 359H, T950, and D925 showed two major protein bands (for 359H, see also Fig. 5), which differ in their apparent molecular mass values by 4–5 kDa. These differences are most likely due to a modification with different numbers of N-linked oligosaccharides, since in proteins of comparable size such as decorin similar differences have been shown to correspond to different numbers of N-linked oligosaccharide units (18).

Shape of Tissue-derived Neurocan—The shape of neurocan and recombinant neurocan fragments was analyzed by rotary shadowing electron microscopy. Native neurocan isolated from rat brain appeared as two globules connected by an extended filament of 60–90 nm length (Fig. 3A). Occasionally particles with a considerably shorter connection of about 30 nm were observed. A morphometric analysis of the length of the connecting filament of 71 randomly chosen particles with two terminal globules did, however, not indicate the presence of a homogenous population of molecules (Fig. 4A). About half of the measured particles had a connecting filament with a rather uniform length of 60–67 nm, whereas the other half of the particles showed a more heterogenous size distribution with longer connecting filaments of 70–90 nm. However, within these longer connecting filaments kinks could be observed much more frequently, and consequently their length was more difficult to determine.

Shape of Recombinant Neurocan Fragments—The rotary shadowing micrographs implicate a structural model of neurocan in which the N- and C-terminal domains of neurocan, which share homologies to those of other proteoglycans, have adopted a globular shape, whereas the central region of neurocan appears to be an extended filament with limited flexibility and without an apparent globular structure. All recombinant

[Image of SDS-PAGE analysis and rotary shadowing micrographs]
Table 1
Comparison of the calculated molecular mass values for the recombinant fragments with the apparent values from SDS-PAGE analysis

| Fragment | Calculated molecular mass | Molecular mass after neuraminidase treatment |
|----------|---------------------------|---------------------------------------------|
|          | kDa                       | Observations                                |
| 359H     | 37                        | 42 (47)                                     |
| 773M     | 83                        | 185                                         |
| L639     | 68                        | 150                                         |
| D925     | 39                        | 47 (51)                                     |
| T950     | 36                        | 42 (47)                                     |

Fig. 3. Electron microscopic analysis of neurocan and recombinant neurocan fragments after rotary shadowing. Representative preparations of purified tissue-derived neurocan (A) and recombinant fragments 773M (B), L639 (C), D925 (D), and T950 (E) are shown. Some of the structures observed in A, B, and C are recapitulated by a graphic representation on the left adjacent to the respective field. A, arrows indicate molecules belonging to the particles with apparently longer central domain, arrowheads indicate molecules belonging to the particles with shorter central regions. C, arrows indicate molecules with a clearly visible glycosaminoglycan chain.

Mapping of the Chondroitin Sulfate and Sialic Acid Attachment Region—With respect to the modular structure of neurocan, previously only consensus sequences located in the central region of neurocan have been considered as potential attachment sites for the estimated 2–3 glycosaminoglycan chains (1). The observed secretion of fragments 359H and T950 as chondroitinase-insensitive glycoproteins reveals that in fact only the central part of the protein can be modified with chondroitin sulfate chains in 293 cells (Fig. 2A), although Ser-Gly and Gly-Ser dipeptide motifs, which seem to be the most important prerequisites for the attachment of glycosaminoglycan chains (19, 20), also occur in other parts of the neurocan sequence. In accordance with previous conclusions (1) one functional glycosaminoglycan attachment site is likely to be represented by a Ser-Gly motif within the last 25 amino acids of the central region (amino acids 944 and 945 in the rat neurocan sequence). This motif is the only Ser-Gly motif present in fragment D925, a fragment which can be modified with glycosaminoglycan chains. It is, however, not present in fragment T950, which is lacking these 25 amino acids and is secreted without this modification. Treatment of the recombinant fragment L639 and 773M with neuraminidase resulted in an increased mobility of the core proteins in SDS-PAGE, whereas the migration of fragments 359H, T950, and the core protein of fragment D925 was not, or only marginally, affected by neuraminidase treatment (Fig. 2B).

Expression of the HNK-1 Epitope by the Recombinant Fragments—Neurocan and neurocan-C derived from rat brain have previously been shown to be modified with a carbohydrate...
structure recognized by the monoclonal antibody HNK-1. In various biological assays this antibody and also the carbohydrate structure itself have been found to interfere with interactions of neural cells. The core proteins of the two recombinant fragments containing potential N-glycosylation sites located in the central region, L639 and 773M, were recognized by the HNK-1 antibody (Fig. 7A). The two fragments 359H and T950, which represent the globular domains of neurocan with no contribution of the central region, were HNK-1-negative, whereas the core protein of fragment D925, containing 25 amino acids of the central region but no additional N-glycosylation site in comparison to fragment 950, was modified with the HNK-1 epitope (Fig. 7A). Thus, the fragments which carried the HNK-1 epitope were the same fragments which could be modified with glycosaminoglycan chains. A separate analysis of the two variants of fragment D925 revealed that only those fragments which were not modified with glycosaminoglycan chains were modified with the HNK-1 epitope (results not shown).

Expression and Mapping of Oligomannosidic Glycans—One of the identified ligands of neurocan, N-CAM, has been shown to have a particular affinity to oligomannosidic glycans (21). Therefore, brain-derived neurocan was probed with concanavalin A, a lectin that specifically recognizes these oligosaccharides. Since oligomannosidic glycans may represent biosynthetic intermediates in the synthesis of more complex types of N-linked oligosaccharides, the modification with these oligosaccharide structures can certainly not be considered to be as specific as a modification with HNK-1 epitopes. However, staining of rat brain-derived neurocan with concanavalin A revealed that only the 250-kDa form is recognized by this lectin, whereas the 150-kDa band, corresponding to neurocan-C, is not (Fig. 7B). Neurocan-C contains all but the first two N-glycosylation sites located in the N-terminal globular domain, indicating a specific modification of the N-terminal neurocan domain with oligomannosidic glycans in rat brain (see also Fig. 1). Analysis of the recombinant fragments with concanavalin A revealed that the recombinant fragments 359H and 773M, which both contain the N-terminal globular domain, strongly bound to this lectin. No staining could be detected with fragment L639, corresponding to neurocan-C. However, considerable binding to the two C-terminal fragments representing parts of fragment L639, fragment T950, and fragment D925, was apparent (Fig. 7B).

Differential Processing of N-Linked Oligosaccharides in Recombinant Fragment 773M—A comparison of the staining patterns of the recombinant fragment 773M preparation with concanavalin A and the HNK-1 antibody reveals that the HNK-1 antibody recognizes only the predominant protein band of 185 kDa, whereas also smaller core proteins of about 130 and 90 kDa were recognized by concanavalin A (Fig. 7). These smaller core proteins were also observed in a DEAE-purified preparation of the recombinant fragment 773M after silver staining (Fig. 8). To evaluate whether these fragments have been generated by proteolytic degradation of fragment 773M close to the N- or the C-terminal end, the monoclonal antibody 8A4 was employed. The epitope recognized by this antibody has previously been found to be located in the central region of neurocan around amino acids 754–758 (1). This is close to the C-terminal end of fragment 773M, in the vicinity of potential N-glycosylation sites present in the central region of neurocan (see also Fig. 1). With the 8A4 antibody only the undegraded recombinant fragment was recognized (Fig. 8). This staining pattern indicates that the observed degradation products are produced by processing events in the central region of neurocan and that only the N-glycosylation sites located in this region of fragment 773M are modified with the HNK-1 epitope. It also indicates that, like in brain-derived neurocan, the globular domain of this fragment is modified with oligomannosidic glycans.

DISCUSSION

Fragments of the brain-derived chondroitin sulfate proteoglycan neurocan have been produced in a mammalian kidney
cell line, and some of their structural and functional features have been compared to the tissue-derived molecules. It could be shown that the shape and most of the modifications of tissue-derived material are retained in the ectopically expressed fragments. The observed interaction of the recombinant N-terminal neurocan domain with hyaluronan is an additional indication that 293 cells are able to produce correctly folded and modified molecules, suitable for functional studies. However, the detailed analysis of the carbohydrate modifications also revealed that the processing of the oligosaccharide structures in some fragments might have been terminated at premature stages or might depend on other elements than the modified protein domain itself. In general, the expression system used in this study seems to be appropriate to analyze the structure and molecular interactions of specifically designed parts of extracellular matrix proteins, even of proteins with highly glycosylated mucin-like structures. Neurocan could be expected to contain such structures, since the carbohydrate analysis (6) and various glycosidase treatments (5) of neurocan isolated from rat brain indicated the presence of many sialic acid containing saccharide structures. Therefore, in the comparison of the recombinant fragments with the tissue-derived proteins much attention was attributed to characteristic features of mucins.

Mucin-type proteolytic glycoprotein fragments obtained from tumor cells have a relatively stiff, rod-like appearance (22), and mucin domains often segregate protein domains from each other, for example by extending extracellular domains of membrane proteins to gain optimal exposure above the glyocalyx of the outer cell surface (23). The electron microscopic shape of tissue-derived neurocan, two terminal globules separated by a 60–90-nm long extended filament, indicates a mucin-like structure of the central region of neurocan. Although this segment appears to be more flexible than the glycoprotein fragments mentioned above, it was able to efficiently separate the globular domains in all observed molecules. Occasionally observed molecules with a considerably shorter central region of around 30 nm might represent brevican, a related proteoglycan with a much shorter central region, since some staining around 140 kDa could be observed with anti-brevican antiserum in immunopurified neurocan preparations from brain.\(^2\) The N- and C-terminal domains of neurocan are homologous to similar globular domains of the cartilage-derived proteoglycan aggrecan (24). The central region of neurocan, however, appears to be considerably more compact than the central region of aggrecan. From rotary shadowing electron micrographs of aggrecan it can be estimated that the 1200 amino acids of the glycosaminoglycan attachment region of aggrecan extend over a distance of 300 nm (24), resulting in only 4 amino acids/nm. This would not be compatible with tertiary structures of the polypeptide strand. In the 60–90-nm long central region of neurocan, composed of about 600 amino acids, a higher structural organization would be possible. The kinked appearance of molecules with an apparently long central region could possibly even be due to tertiary structures, which became unfolded. The length of the central protein region in its native conformation might therefore be much closer to 60 nm than to 90 nm.

A characteristic feature of mucins is their unpredictable behavior in SDS-PAGE (25). Unusually low mobilities, as previously observed with tissue-derived neurocan and neurocan-C, could be observed only with those recombinant fragments which contained major parts of the central region, fragments 773M and L639, but not with the fragments containing only globular parts of the protein. A considerable decrease in the apparent molecular weight of fragments 773M and L639, but not of fragments 359H and T950, could be observed after treatment with V. cholerae neuraminidase. This observation supports experimentally earlier suggestions about the predominant occurrence of sialylated O-linked oligosaccharides within the serine-, threonine-, and proline-rich central region of neurocan (1, 26). This glycosylation might also protect major parts of this region leaving only small stretches of amino acids accessible for proteolytic degradation. The major degradation products of fragment 773M of 130 and 90 kDa have about the same size as N-terminal neurocan fragments found in rat brain (27, 28), indicating a conservation of protease accessible sites in the recombinant fragments.

For mucin-like domains of cell membrane receptors, which are involved in lymphocyte homing, like MADCAM-1 (29), it has been shown that they can be specifically modified with carbohydrate epitopes participating in molecular recognition. The modification of all three recombinant fragments containing parts of the central mucin-like region with the HNK-1 epitope

\(^2\) C. Retzler and U. Rauch, unpublished observation.
is therefore an interesting observation. The same fragments were also modified, although not always quantitatively, with chondroitin sulfate chains. The expression of the N-terminal fragment 773M as a chondroitin sulfate-modified proteoglycan might be particularly noteworthy, since in chondrocytes of nanomelic chicken an unmodified truncated aggrecan molecule without a C-terminal domain has been observed that was retained in the endoplasmatic reticulum (30). Although all biochemical data indicate the attachment of all glycosaminoglycan chains to the central filamentous region of neurocan, in Fig. 3C both filamentous structures seem to extend from the globular domain of fragment L639. This impression might be caused by an association of the proximal part of the glycosaminoglycan chains with a cluster of basic amino acids (5 lysine, 9 arginine, and also 10 histidine residues within 30 residues) in this domain which does not contain any Ser-Gly motif. A comparison of the sole Ser-Gly motif of fragment D925 with attachment sites of proven function in other chondroitin sulfate proteoglycans, the invariant chain (31), decorin (32), thrombomodulin (33), betaglycan (34), proteoglycan macrophage colony-stimulating factor (35), and amyloid precursor-like protein 2 (36), reveals no particular pattern of identical or similar amino acids. However, in all these sites, like in neurocan fragment D925, there are at least two acidic and no basic amino acids among the four amino acids preceding and following the modified Ser-Gly motif.

The results of this study indicate that structural characteristics of the central region of neurocan, like the minimal length, the flexibility and possibly even the proteolytic accessibility, are, at least partially, regulated by a mucin-like glycosylation. One major function of the central region might be to facilitate the proper arrangement of the C-terminal globular domains. Leukin domains of the C-type family, which are present in these domains, are usually expressed in multimeric structures to potentiate the weak adhesive forces of single domains. Changes in the density of these domains might therefore considerably influence their biological activity (37). The observed copurification of neurocan and link protein (6, 27), which induces a dense association of proteoglycans along the hyaluronan strand (24), and other studies (38, 39) indicate that lambrush-like aggregates, similar to those which have been observed in cartilage, also exist in brain. In such aggregates the density and the distribution of the C-terminal lectin-like domains of the proteoglycans would directly depend on the properties of their central regions. Therefore, the evaluation of the molecular properties of these individual molecules can be considered to be an important prerequisite to answer the question of how the extracellular brain matrix is structurally organized.

Acknowledgments—We thank Dr. Rupert Timpl for support, suggestions and discussions, Drs. Günter Kostka, Richard Margolis, and Karlheinz Mann for reading manuscripts, and Cornelia Platte for expert technical assistance.

REFERENCES

1. Rauch, U., Karthikeyan, L., Maurel, P., Margolis, R. U., and Margolis, R. K. (1992) J. Biol. Chem. 267, 19536–19547
2. Grumet, M., Flaccus, A., and Margolis, R. U. (1993) J. Cell Biol. 120, 815–824
3. Grumet, M., Milev, P., Sakurai, T., Karthikeyan, L., Bourbon, M., Margolis, R. K., and Margolis, R. U. (1994) J. Biol. Chem. 269, 21142–21146
4. Friedlander, D. R., Milev, P., Karthikeyan, L., Margolis, R. K., and Grumet, M. (1994) J. Cell Biol. 125, 669–680
5. Oda, A., Matsui, F., Watanabe, E., Kushima, Y., and Maeda, N. (1994) Neuroscience 60, 145–157
6. Rauch, U., Gao, P., Janetzko, A., Flaccus, A., Hilgenberg, L., Tekotte, H., Margolis, R. U., and Margolis, R. K. (1991) J. Biol. Chem. 266, 14785–14801
7. Mayer, U., Nischt, R., Pöschl, E., Mann, K., Fukuda, K., Gerl, M., Yanada, Y., and Timpl, R. (1993) EMBO J. 12, 1879–1885
8. Vara, J. A., Portela, A., Ortín, J., and Imenez, A. (1986) Nucl. Acid Res. 14, 4617–4624
9. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
10. Nischt, R., Pottgreisser, J., Kriegl, T., Mayer, U., Aumailley, M., and Timpl, R. (1993) Eur. J. Biochem. 200, 529–536
11. Fox, J. W., Mayer, U., Nischt, R., Aumailley, M., Reinhart, D., Wiedemann, H., Mann, K., Timpl, R., Kriegl, T., Engel, J., and Chiu, M.-L. (1991) EMBO J. 10, 3137–3146
12. Laemmli, U. K. (1970) Nature 227, 680–685
13. Towbin, H., Staehlin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
14. Giese, W. F., Briggs, R. C., and Hnilica, L. S. (1981) Anal. Biochem. 115, 219–224
15. Engel, J., and Furthmayr, H. (1987) Methods Enzymol. 145, 3–78
16. Paulsson, M., Yurchenko, P. D., Ruben, G. C., Engel, J., and Timpl, R. (1987) J. Biol. Chem. 262, 19536–19547
17. Neame, P. J., and Barry, F. P. (1993) Experientia (Basel) 49, 393–402
18. Rauch, U., Gössl, J., and Kresse, H. (1986) Biochem. J. 238, 465–474
19. Bourdon, M. A., Kraus, T., Campbell, S., Schwartz, N. B., and Ruoslahti, E. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3194–3198
20. Uplat, W. B., Chandrasekaran, L., and Tanzer, M. L. (1993) Experientia (Basel) 49, 389–392
21. Horstkotte, R., Schachner, M., Magyar, J. P., Vorherr, T., and Schnitt, B. (1993) J. Cell Biol. 121, 1409–1421
22. Sjövall, H. S., and Collin, M. (1977) J. Biol. Chem. 242, 3405–3410
23. J. entoft, N. (1990) Trends Biochem. Sci. 15, 291–294
24. Mörgelin, M., Heineward, D., Engel, J., and Paulson, M. (1994) Biochim. Biophys. Acta 121, 133–138
25. Tytgat, K. M. A., Swallow, D. M., van Klinken, B. J.-W., Büller, H. A., Einerhand, A. W. C., and Decker, J. (1995) Biochem. J. 310, 1053–1054
26. Hansen, J. E., Lund, O., Engelbrecht, J., Bohr, H., Nielsen, O. J., Hansen, J.-E. S., and Brunak, S. (1995) Biochem. J. 308, 801–813
27. Meyer-Puttitz, B., Milev, P., Juncker, E., Zimmer, I., Margolis, R. U., and Margolis, R. K. (1995) J. Neurochem. 65, 2327–2337
28. Matsui, F., Watanabe, E., and Ohshima, A. (1994) Neurochem. Int. 25, 425–431
29. Berg, E. L., McEvoy, L. M., Berlin, C., Bargatze, R. F., and Butcher, E. C. (1993) Nature 366, 695–698
30. Li, H., Schwartz, N. B., and Vertel, B. M. (1993) J. Biol. Chem. 268, 23504–23511
31. Miller, J. H., Hatch, A., Simonis, S., and Cullen, S. E. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1399–1363
32. Mann, D. M., Yanaguchi, Y., Bourbon, M. A., and Ruoslahti, E. (1990) J. Biol. Chem. 265, 5317–5323
33. Lin, J.-H., Mclean, K., Morser, J., Young, T. A., Wydro, R. M., Andrews, W. H., and Light, D. R. (1994) J. Biol. Chem. 269, 25021–25030
34. Lopez-Casillas, F., Payne, H. M., Andrews, J. L., and Massague, J. (1994) J. Cell Biol. 124, 557–568
35. Kimura, F., Suzuki, S., Nakamura, Y., Wakimoto, N., Kanatani, Y., Yanai, N., Nagata, N., and Motoyoshi, K. (1994) J. Biol. Chem. 269, 19751–19756
36. Thnakanan, G., and Sisodia, S. S. (1994) J. Biol. Chem. 269, 22099–22104
37. Drickamer, K., and Taylor, M. E. (1993) Annu. Rev. Cell Biol. 9, 237–264
38. Iwata, M., and Carlson, S. S. (1993) J. Neurosci. 13, 195–207
39. Iwata, M., Wight, T. N., and Carlson, S. S. (1993) J. Biol. Chem. 268, 15061–15069