Homophilic and Heterophilic Binding Activities of Nr-CAM, a Nervous System Cell Adhesion Molecule

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Abstract. Nr-CAM is a membrane glycoprotein that is expressed on neurons. It is structurally related to members of the N-CAM superfamily of neural cell adhesion molecules having six immunoglobulin-like domains and five fibronectin type III repeats in the extracellular region. We have found that the aggregation of chick brain cells was inhibited by anti-Nr-CAM Fab' fragments, indicating that Nr-CAM can act as a cell adhesion molecule. To clarify the mode of action of Nr-CAM, a mouse fibroblast cell line L-M(TK$^-$) (or L cells) was transfected with a DNA expression construct encoding an entire chicken Nr-CAM cDNA sequence. After transfection, L cells expressed Nr-CAM on their surface and aggregated. Aggregation was specifically inhibited by anti-Nr-CAM Fab' fragments. To check the specificity of this aggregation, a fusion protein (FGTNr) consisting of glutathione S-transferase linked to the six immunoglobulin domains and the first fibronectin type III repeat of Nr-CAM was expressed in *Escherichia coli*. Addition of FGTNr to the transfected cells blocked their aggregation. Further analysis using a combination of cell aggregation assays, binding of cells to FGTNr-coated substrates, aggregation of FGTNr-coated Covaspheres and binding of FGTNr-coated Covaspheres to FGTNr-coated substrates revealed that Nr-CAM mediates two types of cell interactions: a homophilic, divalent cation-independent binding, and a heterophilic, divalent cation-dependent binding. Homophilic binding was demonstrated between transfected L cells, between chick embryo brain cells and FGTNr, and between Covaspheres to which FGTNr was covalently attached. Heterophilic binding was shown to occur between transfected and untransfected L cells, and between FGTNr and primary chick embryo fibroblasts; in all cases, it was dependent on the presence of either calcium or magnesium. Primary chick embryo glia or a human glial cell line did not bind to FGTNr-coated substrates. The results indicate that Nr-CAM is a cell adhesion molecule of the nervous system that can bind by two distinct mechanisms, a homophilic mechanism that can mediate interactions between neurons and a heterophilic mechanism that can mediate binding between neurons and other cells such as fibroblasts.

SINCE the early demonstration of the adhesive properties of the neural cell adhesion molecule (N-CAM)[1], a number of related molecules have been described (see reviews in 19, 20). These cell surface glycoproteins are structurally similar in that they contain one or more extracellular domains related to immunoglobulins (Ig) and are associated with the cell membrane either via a transmembrane domain or a lipid anchor. Some of these molecules also contain extracellular segments resembling the type III repeats in fibronectin. The patterns of expression of these N-CAM-related molecules change during development, usually from a more general distribution early in development to a more restricted distribution later. Such patterns and the various means by which their expression is modulated and perturbed (reviewed in 20) suggest that these molecules have important roles throughout development and during regeneration.

Primary CAMs, such as N-CAM, are expressed early in development and subsequently on derivatives of all three germ layers (11, 43, 55). Secondary CAMs, such as the neuron-glia cell adhesion molecule (Ng-CAM) (9, 29), appear later in development and have a more restricted tissue distribution. Expression of Ng-CAM, for example, is localized to postmitotic neurons and Schwann cells (14, 56). Several members of the N-CAM-related family are nervous system specific (4, 8, 10, 17, 26, 27, 34, 38, 42, 45, 48-50, 54, 57). Many of these molecules bind by a homophilic mechanism that does not require divalent cations (e.g., 21, 32, 54). Other CAMs, such as the myelin associated glycoprotein (MAG), bind by heterophilic mechanisms (1). Some of these are cation independent and others are cation dependent (3,

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1. *Abbreviations used in this paper*: CAM, cell adhesion molecule; FGT, glutathione S-transferase portion of FGTNr; FGTNr, Nr-CAM fusion protein, consisting of glutathione S-transferase linked to an extracellular region of Nr-CAM; FNr, Nr-CAM portion of FGTNr; N-CAM, neural cell adhesion molecule; Ng-CAM, neuron-glia cell adhesion molecule; Nr-CAM, Ng-CAM related cell adhesion molecule.

32, 40). Ng-CAM binds neurons to each other by a homophilic mechanism and binds neurons to glia by a heterophilic mechanism (31).

In a search for molecules related to Ng-CAM (33), we identified a cDNA clone for a structurally similar molecule, Nr-CAM (Ng-CAM related CAM). Sequence analysis of this clone indicated that it encoded a protein (see Fig. 1) similar to chick Ng-CAM (9), mouse L1 (45), and insect neuroglian (5). All of these molecules contain six Ig domains, five fibronectin type III repeats, a transmembrane domain and a cytoplasmic domain, and they are more closely related to each other in amino acid sequence than to any of the other N-CAM-related molecules. Nr-CAM has at least two alternatively spliced segments, one of 19 amino acids between the second and third Ig domains and another of 93 amino acids that includes the entire fifth fibronectin type III repeat (33). A chick molecule called Bravo has been partially characterized (16) and appears to be identical to Nr-CAM.

Both Ng-CAM and L1 are involved in neuron–neuron interactions, particularly in neurite fasciculation. In addition, Ng-CAM binds neurons to glia in regions such as the cerebellum (32, 36). The insect neural protein neuroglian mediates adhesion and at least two mutations of this gene are lethal in *Drosophila* at the late embryonic to larval stages but have no obvious effect on the development of the structure of the nervous systems (5).

The structural similarities between Nr-CAM and the Ng-CAM/L1 subgroup of molecules suggests that Nr-CAM has similar functions. Western and Northern blot analysis indicate that it is nervous system specific (33) and in situ hybridization and immunocytochemical studies (L. Krushel, A. Prieto, K. Crossin, B. Cunningham, and G. Edelman, unpublished data) show that it is restricted to neurons in both the central and peripheral nervous systems.

We report here that Nr-CAM has both homophilic and heterophilic binding activities. Antibodies to Nr-CAM blocked the aggregation of chick brain cells in the absence of divalent cations. Mouse L cells transfected with an Nr-CAM cDNA construct expressed the molecule on the cell surface and aggregated both in the presence and absence of divalent cations. Aggregation of the transfected cells was more extensive in the presence of calcium or magnesium and under these conditions the transfected cells bound to untransfected cells. More detailed studies using a fusion protein consisting of glutathione S-transferase linked to an extracellular portion of Nr-CAM confirmed these binding properties. The combined data indicate that Nr-CAM is a cell adhesion molecule that can mediate cation-independent homophilic binding between Nr-CAM expressing cells, and calcium- or magnesium-dependent heterophilic binding between Nr-CAM expressing cells and fibroblasts.

Materials and Methods

Nr-CAM cDNA Construct

The Nr-CAM construct (see Fig. 1, *a* and *b*) was prepared using cDNA clones for chicken Nr-CAM (33). λgt11 clones 714, 717, 721, 761, 704, and 730 (see Fig. 1 *b*) were all subcloned into the Bluescript (KS) vector (pBS) (Stratagene, La Jolla, CA), and were linked together using the following strategy: the EcoRI/XhoI fragment of clone 714 and the XhoI/SalI (pBS polylinker site) fragment of clone 717 were cloned into pBS vector that had been digested with the restriction endonucleases EcoRI and SalI. The resulting clone was digested with ClaI (pBS polylinker site) and partially with HindII, and was used as a vector to accept the partial HindII/ClaI (pBS polylinker site) fragment of clone 721. The BamHI (pBS polylinker site)/KpnI fragment of this clone, along with the KpnI/NarI fragment of clone 761 and the NarI/SacI (pBS polylinker site) of a clone generated by cloning the PstI/PstI fragment of clone 730 into the PstI/PstI (pBS polylinker site) of clone 704 were cloned into pBS vector that had been digested with BamHI/SacI. The complete construct was removed from the pBS vector using BamHI and ligated into the BglII site of the pKSV-10 vector (Pharmacia Fine Chemicals, Piscataway, NJ) downstream of the SV-40 early promoter.

The FGTNr Fusion Protein: cDNA Construct and Expression

FGTNr (see Fig. 1 *c*) was constructed from a cDNA subclone that had the λ717 and λ721 inserts linked together in pBS. The XhoI/EcoRI (pBS polylinker site) fragment of this subclone was inserted into pGEX-2T (Pharmacia Fine Chemicals) giving a construct that had the glutathione S-transferase gene fused to the six Ig-domains and first FnIII repeat of Nr-CAM. Because we used the XhoI site, the DNA encoding the amino terminal 31 amino acids of Nr-CAM was excluded from the construct (see Fig. 1).

The fusion protein was produced by transforming *Escherichia coli* NM522 (Stratagene) cells with the plasmid, growing the cells to mid-log phase in ampicillin-containing medium and inducing fusion protein expression with 0.1 mM isopropyl-β-D-thiogalactoside (Pharmacia Fine Chemicals) for 20 h at 25°C. Extracts were prepared from cell pellets resuspended in 50 mM Tris (pH 7.5), 25% sucrose, 0.5% Nonidet P-40 (Sigma Chemical Co.), 5 mM $MgCl_2$, freeze-thawed twice, and sonicated five times for 60 s. The extracts were then centrifuged at 10,000 *g* for 5 min at 4°C. The supernatant was purified by affinity chromatography using glutathione Sepharose 4B (Pharmacia Fine Chemicals). Fusion protein FGTNr was recovered by elution with 10 mM glutathione (pH 9.6). SDS-PAGE of FGTNr gave two bands that immunoblotted with anti-Nr-CAM antibodies, a major component of 106 kD, corresponding to the predicted size of the glutathione S-transferase segment plus the Nr-CAM insert, and a very minor component of 68 kD (data not shown). Because both bands immunoblotted with anti-Nr-CAM polyclonal and anti-peptide antibodies (33), the 68-kD product was assumed to be a degradation product of the 106-kD product. The 106-kD component was cleaved by thrombin to 82 kD, as was expected with the removal of the 26-kD glutathione S-transferase component. An additional component of ~65 kD did not react with anti-Nr-CAM antibodies and did not appear to contribute to the binding of FGTNr.

FGTNr was cleaved by 0.2% thrombin in 50 mM Tris (pH 8.0), 150 mM NaCl, and 2.5 mM $CaCl_2$ for 1 h at 25°C to produce the glutathione S-transferase (FGT) and the Nr-CAM fragment (FNr). FNr was separated from FGT by binding the FGT to glutathione Sepharose 4B.

Cell Culture and Transfection

The Nr-CAM cDNA construct was co-transfected into mouse L-M(TK^-) cells (CCL13; American Type Culture Collection, Rockville, MD) with pSV2NEO DNA using calcium phosphate precipitation of the DNA (15).

Clones were initially selected using G418 (Gibco Laboratories, Grand Island, NY) at 400 μg/ml (207 μg/ml active). Clones resistant to G418 were then selected for cell surface expression of Nr-CAM by binding them to rabbit anti-chicken Nr-CAM antibodies immobilized on polystyrene (33). Nr-CAM-expressing cells selected in this way were cloned up to two times by limiting dilution in 96-well tissue culture plates. 27 clones were obtained that varied in Nr-CAM surface expression from very weak to strong as determined by immunofluorescent staining. All of the aggregation and binding studies were carried out using two different transfected cell lines that had different insertion sites as determined by Southern analysis of their genomic DNA.

Other cell lines used in binding assays were mouse NIH-3T3 and NCTC929, from the American Type Culture collection. The human U251MG glioma cell line was a generous gift from Dr. Wolfgang Rettig (Memorial Sloan-Kettering Cancer Center, New York). Mouse CCE embryonic stem cells were a generous gift from Dr. Elizabeth Robertson (Columbia University, New York).

Immunofluorescent Staining

Cells growing on 26-well heavy teflon coated slides (Cel-line Associates, Newfield, NJ) were fixed and stained (21) using anti-Nr-CAM rabbit antibodies at a concentration of 20 μg/ml and FITC goat anti-rabbit IgG (VECTOR Labs) at a 1:100 dilution. A Zeiss Universal microscope (Carl Zeiss,

Oberkochen, Germany) was used for viewing and photography. Some cells were cultured for up to 12 h with medium containing 10 mM sodium butyrate to enhance expression of the transfected gene product (28).

Cell Adhesion Assays

In aggregation and binding assays, subconfluent cultures of cells were released from tissue culture dishes by incubation in PBS/2% FCS/5 mM EDTA (21). Cells were collected in minimal essential medium, modified for suspension cultures (SMEM) (Gibco Laboratories) containing 10 μg/ml DNase (SMEM-DNase). The cells were centrifuged and resuspended in SMEM-DNase. 2×10^5 cells in 600 μl SMEM/20 mM Hepes (pH 7.4)/1 mM $CaCl_2$ were used in aggregation assays. Aggregations were done at 37°C/100 rpm for 30 min in 24-well bacteriological dishes that had been blocked for 2 h with PBS/2% BSA and then stopped and fixed in PBS/1% glutaraldehyde. Divalent cation-free experiments were done using Ca^{2+}- and Mg^{2+}-free HBSS (Gibco Laboratories)/20 mM Hepes (pH 7.4)/5 mM EDTA. Cell suspensions were preincubated on ice for 30 min before being assayed for aggregation, with or without anti-Nr-CAM Fab' fragments (0.1 μg/μl) or FGTNr (0.08 μg/μl) as indicated in the Tables and figure legends. The anti-Nr-CAM antibodies used were specific for Nr-CAM; they did not cross-react with any other proteins in immunoblots of various chicken tissues or with Ng-CAM (33). Cell–cell binding was monitored by measuring the disappearance of single cells using a Coulter counter (6).

In the binding assay (25), 3.5-cm bacteriological dishes (Falcon 1008) were marked in a circular dot pattern with 2 μl of FGTNr (100 μg/ml). The positive binding control was 2 μl polylysine (100 μg/ml). Plates were incubated for 30 min at room temperature. The dots were aspirated, washed twice with PBS/2% BSA, and blocked for 60 min at room temperature with 250 μl PBS/2% BSA. The blocking solution was aspirated and cells were added, prepared as described above, and incubated for 60 min at 37°C. The plates were washed with PBS or SMEM/1 mM $CaCl_2$ and fixed with 1% glutaraldehyde/PBS. The number of cells bound to the FGTNr or to the polylysine was determined by viewing the spots at a magnification of 160× and using an eyepiece grid to count the number of cells bound in each of four specific grid areas per spot. Two FGTNr or polylysine spots were used per dish. The percentage of cells bound was calculated using the number of cells bound to the polylysine as the reference value for 100% cells bound.

A modification of the cell binding assay was carried out with MV-Covaspheres (0.5 μm green; Duke Scientific Corp., Palo Alto, CA) covalently bound to FGTNr, FGT or FNr. Each binding experiment used 5 μl of Covaspheres (850 cm^2/ml) bound with 2.5 μg protein; any untreated sites remaining on the Covaspheres were blocked with 100 mM Tris HCl (pH 7.8) and 2% BSA. The Covaspheres were substituted for cells in the binding assay described above, the only modification being a decrease in assay volume from 250 to 100 μl per dish.

Co-aggregation Experiments

Cells were labeled either with 3 μg/ml diI or 10 μg/ml diO (Molecular Probes Inc., Junction City, OR) for 12 h and were removed from dishes and aggregated as described above, except that the aggregation samples contained 3×10^5 of each cell type. After fixation in 1% glutaraldehyde, cell aggregates were viewed and photographed with filters appropriate for rhodamine (diI) or fluorescein (diO). Black and white images are displayed in pseudocolor by the use of red and green filters during transfer of the images to color film.

Preparation of Primary Chicken Embryo Cells

Primary cultures of chick embryo fibroblasts were prepared using eviscerated 10-d chick embryo body cavities. Body cavities were digested in 0.25% trypsin with 1 mM EDTA for 30 min at room temperature with shaking at 70 rpm. Cells were recovered and cultured in DME medium (Gibco Laboratories) supplemented with 2% heat-inactivated chicken serum and 2% tryptose phosphate broth. The cells were passaged once before use (37). These cells were characteristically bipolar in shape in dense cultures and were arranged in parallel arrays and whorls. Brain cells were prepared from 8-d-old chick embryo brains by trypsinization in the presence of calcium (TC) or EDTA (LTE) (7). A large proportion of these cells stained with anti-Nr-CAM antibodies. Primary chicken glial cells were prepared from 9-d-old chick embryo brains (30). From the method of isolation and their morphology, they are most likely related to astroglia (30). Mouse lymphocytes were prepared from mouse spleens (12).

Results

The ability of Nr-CAM to mediate cell adhesion was demonstrated in aggregation assays using cells dissociated from 8-d embryonic chicken brains with trypsin in the presence of EDTA (see Materials and Methods). The dissociated cells stained with anti-Nr-CAM antibodies and they aggregated in the absence of divalent cations; this aggregation was inhibited by anti-Nr-CAM Fab' fragments (Table I). To analyze Nr-CAM binding activity in more detail, two cDNA clones were used, one to generate an Nr-CAM fusion protein and the other to transfect cells that do not normally express the molecule; neither construct contained the alternatively spliced inserts (Fig. 1).

Interaction with Nr-CAM Fusion Protein FGTNr

For the fusion protein, FGTNr, a segment of cDNA that encoded the bulk of the extracellular region of Nr-CAM was fused to the carboxyl terminal portion of the glutathione S-transferase gene. The fusion protein included all six Ig domains and the first fibronectin type III repeat of Nr-CAM but lacked the amino terminal 31 amino acids of the protein (Fig. 1, *a* and *c*). FGTNr was expressed in *E. coli* and purified by affinity chromatography on glutathione Sepharose (52).

The purified fusion protein inhibited the aggregation of the dissociated brain cells made from 8-d chick embryos (Table I). Furthermore, these same cells bound to FGTNr when the fusion protein was applied to a plastic substrate and the cells were allowed to settle under gravity. In this assay, the binding of dissociated chick brain cells took place in the absence of divalent cations and was inhibited by anti-Nr-CAM Fab' fragments (Table II).

Transfection of the Nr-CAM cDNA Construct into L cells

For transfection studies, the full length Nr-CAM cDNA (Fig. 1, *a* and *b*) was expressed under control of the SV-40 early promoter and was transfected into L cells by calcium phosphate precipitation. Permanently transfected cell lines were identified by immunofluorescent cytochemistry (Fig. 2) with antibodies raised against purified Nr-CAM protein (33). Consistent with cell surface expression, the predominant pattern was ring staining. 27 transfected cell lines were iso-

Table I. Aggregation of Cells in the Absence of Divalent Cations

	Addition	Percent aggregation	Percent inhibition
Chick embryo brain cells*	–	43 ± 1	–
	anti-Nr-CAM Fab'	23 ± 9	47
	FGTNr	21 ± 7	51
Nr-CAM/L cell transfectants‡	–	14 ± 2	–
	anti-Nr-CAM Fab'	4 ± 3	71
	FGTNr	5 ± 1	64
L cells	–	1 ± 1	–

The results are averages ± SD, of a minimum of three separate experiments.
* Brains from 8-d chick embryos were used to prepare cells by LTE.
‡ Cells were removed from plates using 5 mM EDTA/2% FCS/PBS, aggregations were done at 37°C/100 rpm for 30 min. Aggregations were done in Ca^{2+}/Mg^{2+}-free medium.

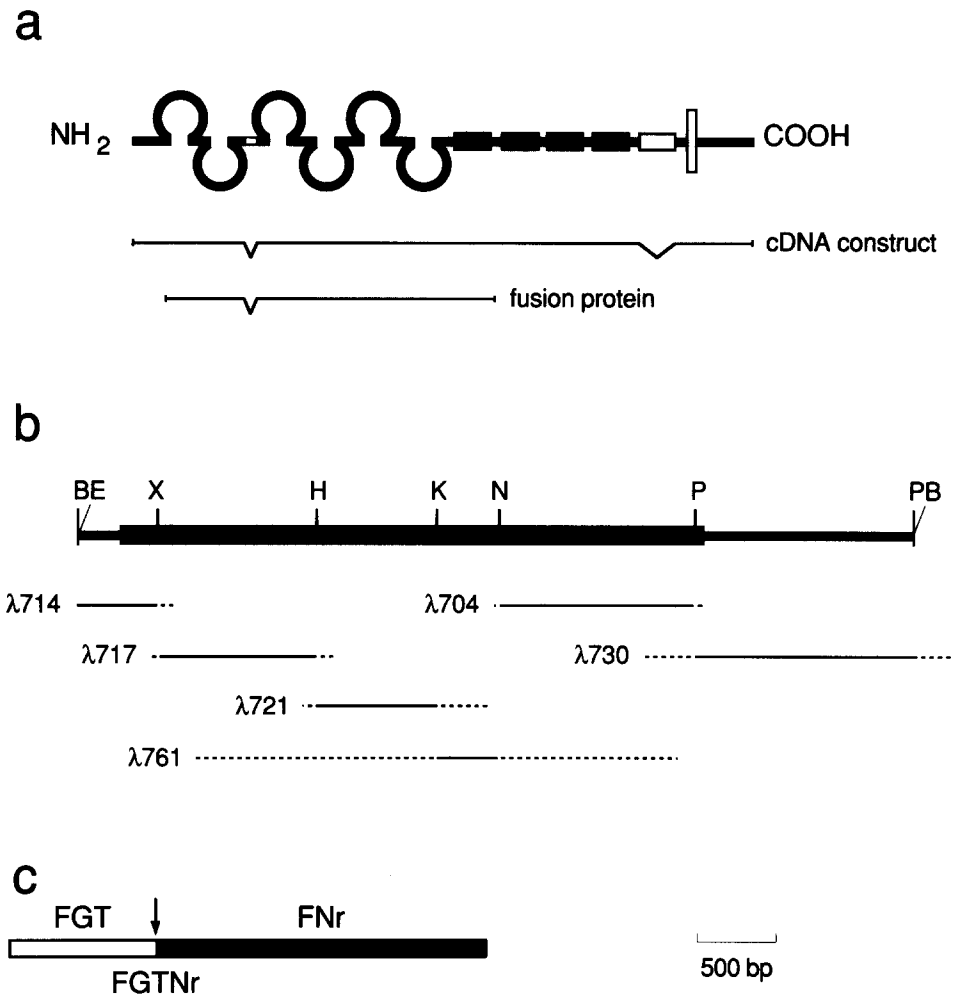


Figure 1. Structure of Nr-CAM and DNA constructs. (*a*) Model of the domain structure of Nr-CAM. The six immunoglobulin-like domains in the amino-terminal portion of the molecule are shown as loops and the five fibronectin type III repeats as boxes. The transmembrane region is shown as a vertical bar and the two alternatively spliced segments as open boxes. The regions of Nr-CAM included in the cDNA (*b*) and fusion protein (*c*) constructs are indicated as lines below the model; as indicated by the Vs, neither construct contained the alternatively spliced segments, so the representations of these constructs in *b* and *c* do not align precisely with this model. (*b*) The full-length cDNA used for transfection experiments with restriction sites shown above: *B*, BamHI; *E*, EcoRI; *H*, HindII; *K*, KpnI; *N*, NarI; *P*, PstI; *X*, XhoI. The thin lines are 5′- and 3′-untranslated sequences, the thick line is the open reading frame. The cDNA clones used to make the construct are shown below with the solid lines representing the fragments used in the construct and the dotted lines indicating the remainder of each EcoRI insert. (*c*) The fusion protein FGTNr was generated from a DNA construct that encoded the immunoglobulin-like domains and the first fibronectin type III repeat of Nr-CAM (■) coupled to the carboxyl terminal portion of glutathione S-transferase (□). For ease of construction, the Nr-CAM cDNA that encoded the amino terminal 31 amino acids was not included. The glutathione S-transferase (*FGT*) and Nr-CAM (*FNr*) fragments could be separated from each other by cleavage with thrombin (*arrow*).

lated and, in all cases, the Nr-CAM was found at the cell membrane although it was expressed at varying levels. The individual cell lines showed a variety of morphological patterns, but these same patterns were also seen in populations of untransfected L cells. After incubation in butyrate for 6–12 h, all of the transfected cell lines substantially increased their levels of expression of Nr-CAM (the cell line shown in Fig. 2 was so induced). Control untransfected cells, with or without butyrate induction, did not stain with anti-Nr-CAM antibodies (Fig. 2 *b*).

Evidence for Homophilic Binding

Aggregation of transfected cells and specific inhibition were quantitated by measuring the disappearance of single cells in the presence or absence of univalent anti-Nr-CAM Fab′ fragments or the fusion protein FGTNr. Nr-CAM transfected cells aggregated in the absence of divalent cations (Table I) and the aggregation was specifically inhibited by anti-Nr-CAM Fab′ fragments.

Only the transfected L cells bound to the Nr-CAM fusion protein in the absence of divalent cations; untransfected L cells did not bind (Table II). This binding was specifically inhibited by either anti-Nr-CAM Fab′ fragments or by FGTNr.

Homophilic binding of Nr-CAM was further investigated by covalently linking FGTNr or the Nr-CAM portion of the fusion protein (*FNr*, Fig. 1 *c*) to 0.5 μm Covaspheres and using them in binding assays. Both FGTNr- and FNr-Covaspheres bound to FGTNr that had been immobilized on the plastic dish (Fig. 3, *a* and *b*) as well as binding to themselves in solution (Fig. 3, *c* and *d*). This binding occurred in the absence of divalent cations and was inhibited by anti-Nr-CAM Fab′ fragments (Fig. 3, *b* and *d*). Control Covaspheres linked with the glutathione S-transferase segment (FGT) showed no specific binding. These results are consistent with the data obtained from the cellular aggregation studies and from the binding of cells to FGTNr and indicate that Nr-CAM can bind homophilically in the absence of divalent cations. Other experiments however, suggested that Nr-CAM can also bind by a heterophilic, divalent cation–dependent mechanism.

Table II. Binding of Cells to FGTNr

	Addition	Percent cells binding	Percent inhibition
Chick embryo brain cell (LTE)*	–	55 ± 11	–
	anti-Nr-CAM Fab'	1 ± 0.5	98
Nr-CAM/L cell transfectants‡	–	13 ± 1	–
	Ca^{2+}	14 ± 1	–
	Ca^{2+} + anti-Nr-CAM Fab'	3 ± 2	80
	Ca^{2+} + FGTNr	4 ± 2	69
L cells‡	–	2 ± 3	–
	Ca^{2+}	22 ± 6	–
	Ca^{2+} + anti-Nr-CAM Fab'	10 ± 3	55
	Ca^{2+} + FGTNr	13 ± 5	41
	Mg^{2+}	28 ± 7	–
Chick embryo fibroblasts‡	–	11 ± 1	–
	Ca^{2+}	67 ± 11	–
	Ca^{2+} + anti-Nr-CAM Fab'	26 ± 13	61
	Ca^{2+} + FGTNr	25 ± 3	63
Chick embryo brain cells (TC)*	–	12 ± 6	–
	Ca^{2+}	14 ± 2	–
	Ca^{2+} + anti-Nr-CAM Fab'	3 ± 1	79
	Ca^{2+} + FGTNr	5 ± 1	62
L cells (TC)§	Ca^{2+}	27 ± 6	–
Chick embryo glia‡	Ca^{2+}	3	–
Human U251MG‡	Ca^{2+}	0	–
Mouse NCTC929‡	Ca^{2+}	0	–
Mouse NIH-3T3‡	Ca^{2+}	4	–
Mouse embryonic stem cells‡	Ca^{2+}	5	–
Mouse lymphocytes	Ca^{2+}	3	–

The results are averages of a minimum of four separate experiments ± SD, or the average of two separate experiments. Cell binding assays were done in Ca^{2+}/Mg^{2+}-free medium. Ca^{2+} experiments were done in SMEM (contains 0.8 mM $MgCl_2$)/1 mM $CaCl_2$ for 60 min at 37°C as described in Materials and Methods.

* Brains from 8-d chick embryos were used to prepare cells by LTE or TC.

‡ Cells removed from plates using 5 mM EDTA/2% FCS/PBS.

§ Cells removed from plates by TC.

Evidence for Heterophilic Binding

Evidence for heterophilic binding came initially from the observation that, in the presence of divalent cations (Table III), transfected cells aggregated more extensively than in the presence of EDTA (38 vs. 14%; Tables III and I, respectively); the aggregation was still inhibited by anti-Nr-CAM Fab' fragments and FGTNr. Under these conditions, transfected cells co-aggregated with untransfected L cells (Fig. 4), whereas such co-aggregation was not observed in the presence of EDTA (not shown). Moreover, in the presence of calcium, brain cells bound untransfected L cells in an Nr-CAM-specific manner. When cells were dissociated from 8-d embryonic chicken brains by LTE (7) in order to prevent any cadherin mediated aggregation, they aggregated equally well in the presence or absence of divalent-cations (Tables III and I, respectively). Cells prepared in this manner co-aggregated extensively with L cells in the presence of divalent cations, but not in the presence of EDTA (Fig. 5). The co-aggregation was also inhibited by anti-Nr-CAM Fab' fragments or the fusion protein FGTNr.

These results suggested that Nr-CAM could bind heterophilically to a different molecule on L cells, but that the binding required either calcium or magnesium. In accord with this notion, untransfected L cells were observed to bind to FGTNr coated on plastic (see Table II and Fig. 6). This binding was specifically inhibited by anti-Nr-CAM Fab' fragments or by FGTNr and required either Ca^{2+} or Mg^{2+} at concentrations of 0.8 mM. Although transfected cells bound to FGTNr-coated substrates in the presence of calcium, the extent of binding did not increase, in contrast to the increased aggregation of transfected cells in the presence of this cation (Table III).

A variety of other cell types, including chick embryo glial cells, mouse lymphocytes, embryonic stem cells, NTCT929 and NIH-3T3 cell lines, or human U251MG cell lines did not bind to the FGTNr fusion protein (Table II). When chicken embryonic brain cells were prepared under conditions designed to preserve the cation-dependent binding activity (trypsin in the presence of calcium), they also showed no increased binding to FGTNr in the presence of divalent cations (Table II, see Materials and Methods). This method of cell preparation did not destroy the presumed Nr-CAM heterophilic receptor on L cells inasmuch as untransfected L cells prepared in this manner could bind to FGTNr in the presence of divalent cations; such binding was specifically inhibited by anti-Nr-CAM Fab' fragments (Table II). This result suggests that brain cells either lack this receptor or expose it differently on the cell surface. As indicated above (Table II), however, the binding of transfected L cells to FGTNr also did not increase in the presence of divalent cations, and thus other mechanisms may be involved.

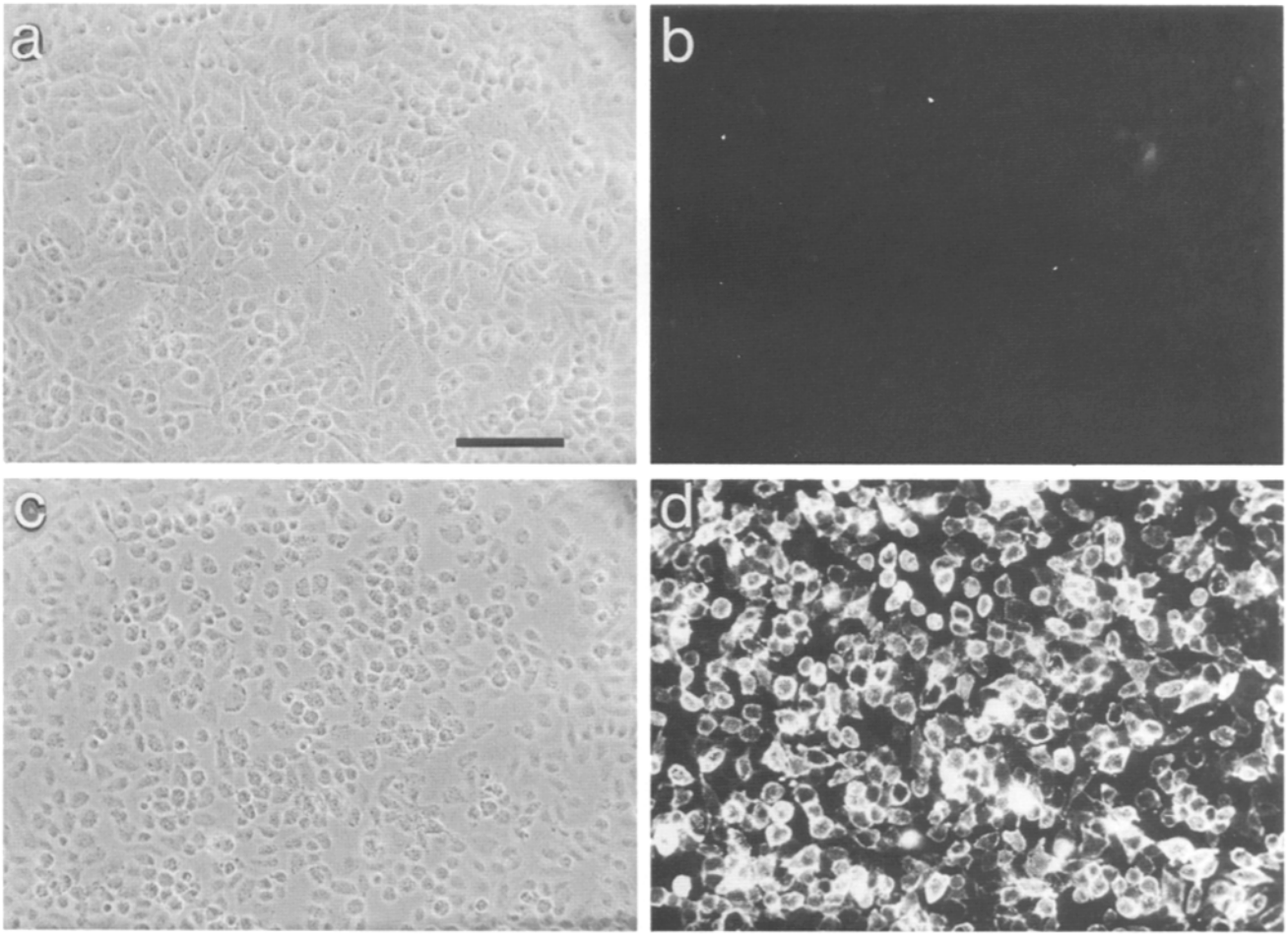


Figure 2. Cell surface expression of Nr-CAM in transfected cells. Matched phase-contrast (*a* and *c*) and fluorescence photographs (*b* and *d*) of untransfected L cells (*a* and *b*) and butyrate induced transfected cells (*c* and *d*) expressing the Nr-CAM construct, stained with rabbit antibodies to chicken Nr-CAM. Bar, 100 μm.

Because heterophilic binding was detected between chick Nr-CAM protein and mouse L cells, a fibroblast derived cell line, similar experiments were carried out with chick embryo fibroblasts. These cells did not stain with anti-Nr-CAM antibodies, but they specifically bound to FGTNr in binding assays and bound to the transfected L cells (data not shown). Binding was more extensive with these chick cells than with mouse L cells, required divalent cations, and was inhibited by anti-Nr-CAM Fab' fragments and FGTNr (Table II). The binding was specific for the Nr-CAM portion of the fusion protein and not the glutathione S-transferase segment. 57% of the cells bound the Nr-CAM fragment (*FNr*, Fig. 1 *c*) and this binding was 78% inhibited by anti-Nr-CAM Fab' fragments. The cells did not specifically bind to that portion of the fusion protein contributed by glutathione S-transferase (FGT).

Discussion

The results presented here indicate that Nr-CAM, like other N-CAM related molecules, can act as a cell adhesion molecule. Chick brain cells that express Nr-CAM and mouse L cells transfected with a cDNA construct encoding chick Nr-CAM aggregated in an Nr-CAM-specific manner. The aggregation of both types of cells was inhibitable with anti-Nr-CAM Fab' fragments or with the fusion protein, FGTNr, which contained the major part of the extracellular portion of Nr-CAM. By using a binding assay with FGTNr as a substrate and exploiting differences in the cation dependence of binding, we were able to distinguish two independent binding activities: a homophilic, divalent cation-independent mechanism whereby Nr-CAM on one cell binds to Nr-CAM on another, and a heterophilic, divalent cation-dependent mechanism whereby Nr-CAM on brain cells or transfected cells binds to a different molecule on fibroblasts. Other studies (Krushel, L., A. Prieto, K. Crossin, B. Cunningham, and G. Edelman, unpublished data) have revealed that surface expression of Nr-CAM is restricted to neurons, indicating that Nr-CAM mediates homophilic binding between neurons and heterophilic binding between neurons and other cell types such as fibroblasts.

We anticipated the homophilic divalent cation-independent binding of Nr-CAM on the basis of the structural similarities of Nr-CAM to other N-CAM related molecules that bind by homophilic mechanisms. N-CAM itself binds by such a mechanism (21, 35), as do the structurally related molecules

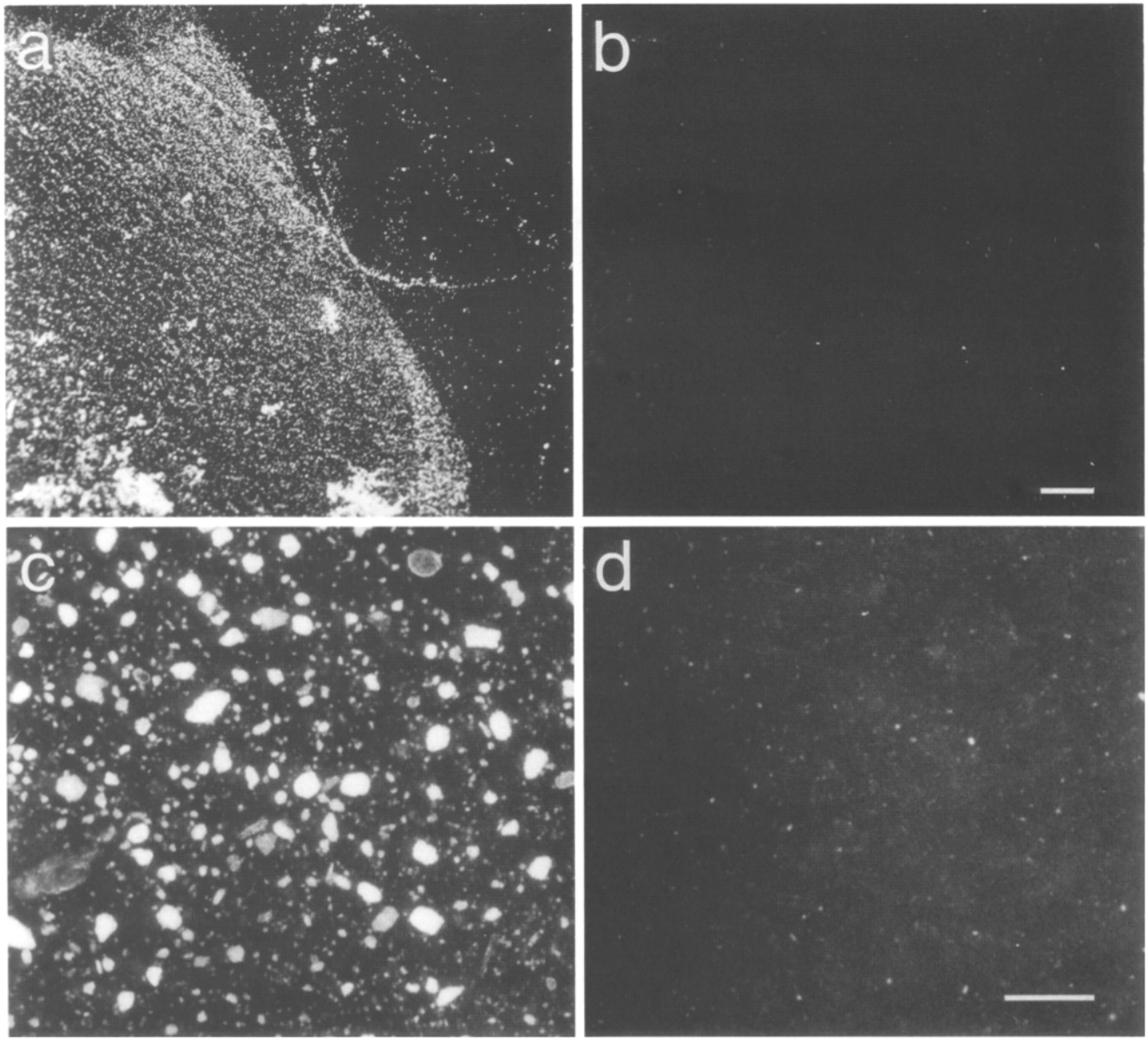


Figure 3. Binding of FGTNr bound Covaspheres to FGTNr coated on plastic and aggregation of FNr bound Covaspheres. Fluorescent photographs of Covaspheres bound to Nr-CAM fusion protein in the presence of 5 mM EDTA (*a*), was inhibited from binding by anti-Nr–CAM Fab′ fragments (*b*). FNr-bound Covaspheres aggregated in the presence of 5 mM EDTA (*c*), and the aggregation was inhibited by anti-Nr–CAM Fab′ fragments (*d*). Bars (*a* and *b*) 20 μm; (*c* and *d*) 50 μm.

Ng-CAM (9, 31) and L1 (39, 45), and the neural proteins SC1 and P_o (23, 51, 54) both of which contain Ig-like domains but no fibronectin type III repeats.

The ability of antibodies to Nr-CAM to inhibit aggregation of neural cells which express a number of different CAMs resembles the effects observed with antibodies to N-CAM and Ng-CAM on these cells. Anti-N–CAM or anti-Ng–CAM Fab′ fragments used independently block neuron–neuron adhesion almost completely, even though the cells express both molecules. Similarly the Nr-CAM fusion protein, FGTNr, inhibited ~50% of the adhesion of brain cells. These observations raise the possibility that there may be interactions among the various CAMs on the same cell (32). Alternatively, the different CAMs may interact through or compete for a common cytoskeletal or cytoplasmic element so that blocking one CAM modulates the actions of the others (18). The variety of cell adhesion molecules present on chick brain cells and the potential for multiple interactions may explain why these cells aggregated more extensively than the L cells expressing only Nr-CAM.

The finding that Nr-CAM can also mediate heterophilic binding is consistent with its structural similarity to Ng-CAM and other related molecules that have both homophilic and heterophilic binding activities. Ng-CAM binds neurons to each other homophilically but its heterophilic binding of neurons to glia is mediated by a calcium-independent mechanism (31). Heterophilic cation–independent binding has also been detected between Ng-CAM and axonin-I (40). Two

*Table III. Aggregation of Transfected and Untransfected Cells in the Presence of Divalent Cations**

	Addition	Percent aggregation	Percent inhibition
Nr-CAM/L cell transfectants	–	38 ± 5	–
	anti-Nr-CAM Fab'	24 ± 3	37
	FGTNr	15 ± 6	61
L cells	–	5 ± 3	–
	anti-Nr-CAM Fab'	5 ± 2	–
	FGTNr	4	–
Chick embryo brain cells‡	–	48	–

The results without SDs are the averages of two separate experiments. All other results are averages ± SD of a minimum of four separate experiments for the L cells and 12 separate experiments for the transfected cells.
* Aggregations were done in SMEM (contains 0.8 mM $MgCl_2$) and 1 mM $CaCl_2$.
‡ Brains from 8-d chick embryos used to prepare cells by LTE.

other members of the N-CAM family, the carcinoembryonic antigens W272 and NCA, also bind to each other in a heterophilic, calcium-independent manner (47).

In the present study, it was found that Nr-CAM bound heterophilically to mouse L cells and to chick embryo fibroblasts, but unlike Ng-CAM, it does not appear to bind neurons to glia. Heterophilic binding between chick Nr-CAM and chick fibroblasts was more extensive than that between chick Nr-CAM and mouse L cells, but in both cases it appeared specific for Nr-CAM. Other molecules in the N-CAM family have also been shown to bind heterophilically to L cells. For example, L cells transfected with PECAM-1 bound to each other and to untransfected L cells by a divalent cation-dependent mechanism (46). L cells transfected with MAG also bound untransfected L cells (1), but the significance of this observation is unclear because MAG-bearing Schwann cells in the peripheral nervous system and oligodendrocytes in the brain should never encounter fibroblasts in vivo (41). In contrast to Nr-CAM, PECAM-1, and MAG,

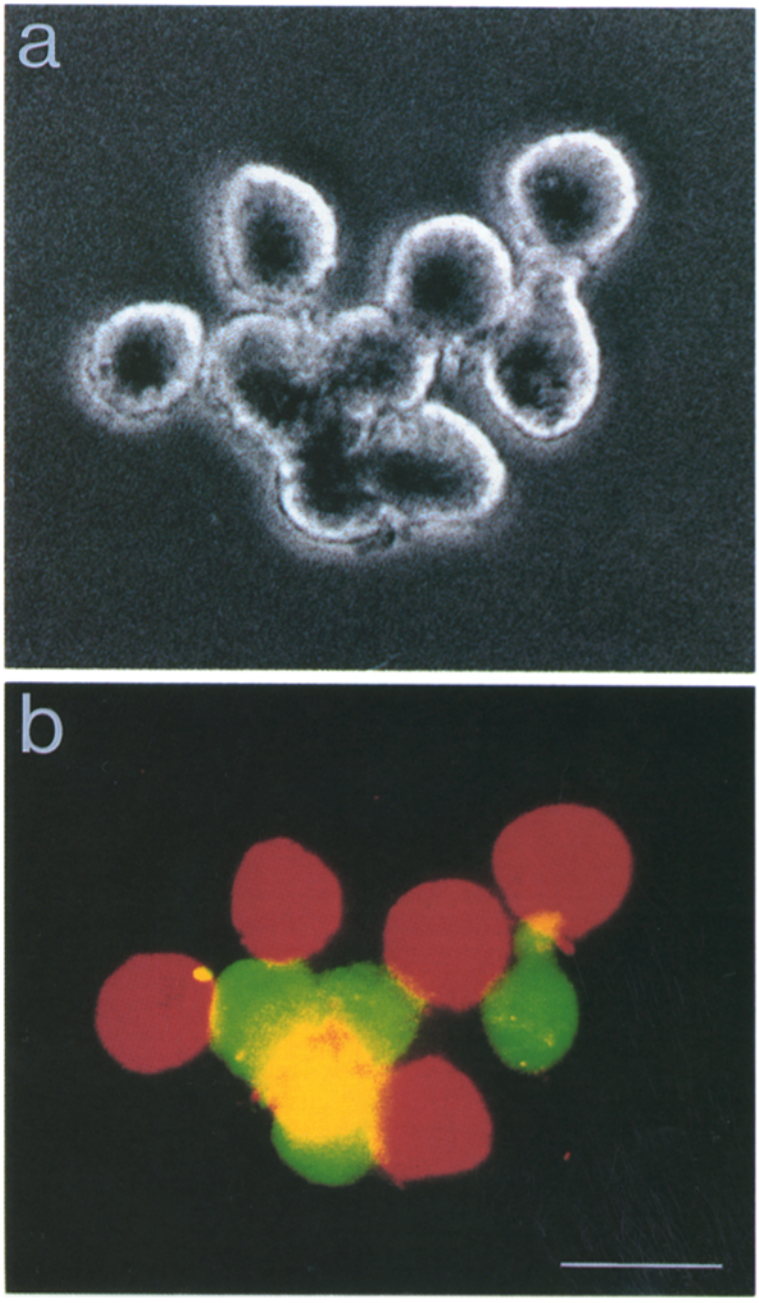


Figure 4. Coaggregation of Nr-CAM–expressing cells and untransfected L cells. (*a*) Phase contrast (*b*) matched fluorescence photographs of a cell aggregate. Transfected L cells were labeled with the fluorescent dye diO (*green*) and untransfected L cells with diI (*red*) and aggregated at 100 rpm/37°C/20 min. The yellow appears where green and red cells are superimposed. Bar, 20 μm.

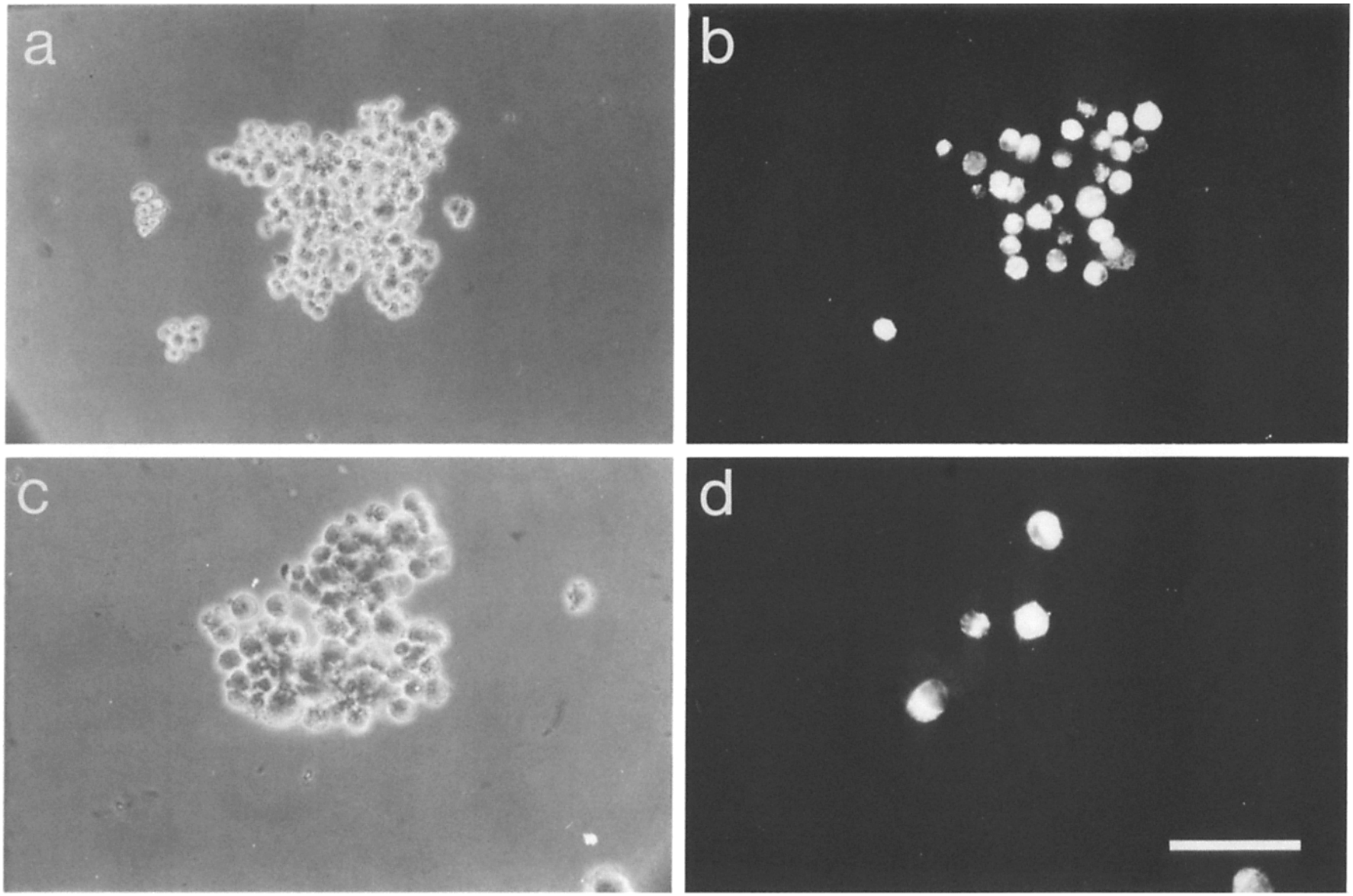
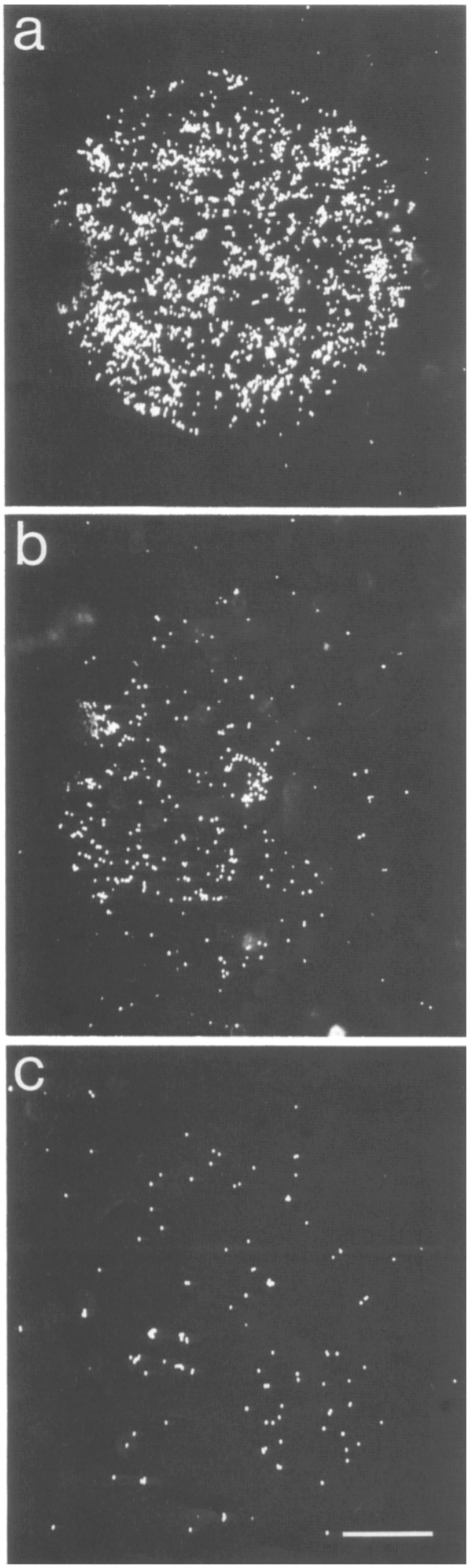


Figure 5. Coaggregation of chick embryo brain cells with mouse L cells. Brain cells were dissociated from 8-d chick embryos by LTE treatment. Mouse L cells were labeled with the fluorescent dye dil. Matched phase contrast (*a* and *c*) and fluorescence (*b* and *d*) photographs of cell aggregates in the presence of 1 mM $CaCl_2$/0.8 mM $MgCl_2$ (*a* and *b*) or 5 mM EDTA (*c* and *d*). Fluorescent L cells (*b*) are distributed throughout the aggregate. Aggregations were done at 100 rpm/37°C/20 min. Bar, 50 μm.



not all CAMs that contain Ig-like domains bind fibroblasts. For example L cells transfected with N-CAM did not coaggregate with untransfected L cells, in the presence or absence of divalent cations (data not shown).

These findings raise the possibility that in vitro, Nr-CAM, PECAM-1, and MAG bind to a common receptor present on fibroblasts, and possibly other cells. Such a receptor could be either a membrane associated molecule such as an integrin or an extracellular matrix molecule associated with the cells. The dependence on divalent cations and the fact that N-CAM family members such as I-CAM-1 and V-CAM can bind to integrins support this notion (2, 22, 44, 53). Investigators studying PECAM-1 have also suggested that its receptor on fibroblasts may be an integrin (46).

Whether the binding of Nr-CAM to fibroblasts occurs in vivo and the functional significance of such binding remain to be explored. Members of the N-CAM family mediate cell interactions in the nervous system and have roles in neuron–neuron adhesion, neuron–glia adhesion, neurite fasciculation, myelination, and cell migration (13, 24, 29, 36). It is likely that Nr-CAM has one or more such functions related to its homophilic binding; a search by perturbation methods (see reviews in 19, 20) should prove fruitful in determining its biological role. In particular, Nr-CAM might mediate binding between nerve fibers and fibroblasts in the endoneurium (41), an area where individual nerve fibers move among fibroblasts. The location of the Nr-CAM receptor in peripheral tissues, and the strong expression of Nr-CAM on peripheral nerves are consistent with a possible role in nerve growth and guidance or in peripheral axon regeneration after injury. To test such functions, we are currently attempting to identify the heterophilic receptor on non-neural cells.

The efficacy in these binding studies of FGTNr, a bacterial fusion protein, suggests that neither homophilic nor heterophilic binding depends on glycosylation of Nr-CAM. Furthermore, the binding activities described here both appear to be restricted to the Ig-domains and the single fibronectin type III repeat contained within FGTNr. The additional repeats were contained in the transfection construct but neither construct contained either of the known alternatively spliced segments (33). We are currently testing these additional regions to see whether they modulate the binding described here or possibly contribute to other Nr-CAM activities.

The portion of the Nr-CAM molecules contained within the fusion protein FGTNr did not bind to either chick primary glial cells or a human glial cell line (Table II). Indeed, brain cells did not bind to FGTNr any better in the presence of calcium than in its absence. Nr-CAM transfected L cells also failed to bind FGTNr bound to plastic Petri dishes any better in the presence of calcium. In contrast to these effects, the transfected cells did aggregate more extensively with each other in the presence of calcium than in EDTA. The expression of Nr-CAM on transfected cells or its binding to FGTNr may prevent the calcium-dependent mechanism of binding of the heterotypic ligand to FGTNr linked to the plastic. Clearly, further assays to assess these variables must be carried out. Similar considerations may apply to neu-

Figure 6. Attachment of L cells to the Nr-CAM fusion protein (FGTNr) coated onto a plastic substrate. Phase-contrast photographs of cells bound to Nr-CAM fusion protein in the presence of divalent cations (*a*). Binding in the presence of divalent cations was inhibited by anti-Nr–CAM Fab′ fragments (*b*). Binding in the absence of divalent cations (*c*). Bar, 500 μm.

ron–neuron interactions and a conclusive test of whether such cells can bind heterophilically at the same time that they bind homophilically must await the design of new assay methods.

The present data and those of other workers (e.g., 21, 31, 40, 47) suggest that certain N-CAM–like molecules can mediate a variety of adhesive interactions. They are consistent with the conclusion that some CAMs can mediate both homophilic cation-independent binding and heterophilic cation-dependent or independent binding. It remains to be seen how many of these molecular interactions occur between receptors on different cells and how many occur between molecules on the same cell. In any event, the ability of cells to adhere to each other is obviously the result in each case of a specific combination of various types of interactions by more than one kind of CAM.

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References

1. Afar, D. E. H., R. M. Marius, J. L. Salzer, C. P. Stanners, P. E. Braun, and J. C. Bell. 1991. Cell adhesion properties of myelin-associated glycoprotein in L cell fibroblasts. *J. Neurosci. Res.* 29:429–436.
2. Albelda, S. M., and C. A. Buck. 1990. Integrins and other cell adhesion molecules. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 4:2868–2880.
3. Albelda, S. M., W. A. Muller, C. A. Buck, and P. J. Newman. 1991. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): a novel vascular cell-cell adhesion molecule. *J. Cell Biol.* 14:1059–1068.
4. Arquint, M., J. Roder, L.-S. Chia, J. Down, D. Wilkinson, H. Bayley, P. Braun, and R. Dunn. 1987. Molecular cloning and primary structure of myelin-associated glycoprotein. *Proc. Natl. Acad. Sci. USA.* 84:600–604.
5. Bieber, A. J., P. M. Snow, M. Hortsch, N. H. Patel, J. R. Jacobs, Z. R. Traquina, J. Schilling, and C. S. Goodman. 1989. Drosophila neuroglian: a member of the immunoglobulin superfamily with extensive homology to the vertebrate neural adhesion molecule L1. *Cell.* 59:447–460.
6. Brackenbury, R., J.-P. Thiery, U. Rutishauser, and G. M. Edelman. 1977. Adhesion among neural cells of the chick embryo. I. An immunological assay for molecules involved in cell-cell binding. *J. Biol. Chem.* 252: 6835–6840.
7. Brackenbury, R., U. Rutishauser, and G. M. Edelman. 1981. Distinct calcium-independent and calcium-dependent adhesion systems of chicken embryo cells. *Proc. Natl. Acad. Sci. USA.* 78:387–391.
8. Brummendorf, T., J. M. Wolff, R. Frank, and F. G. Rathjen. 1989. Neural cell recognition molecule F11: homology with fibronectin type III and immunoglobulin type C domains. *Neuron.* 2:1351–1361.
9. Burgoon, M. P., M. Grumet, V. Mauro, G. M. Edelman, and B. A. Cunningham. 1991. Structure of the chicken neuron-glia cell adhesion molecule, Ng-CAM: origin of polypeptides and relation to Ig superfamily. *J. Cell Biol.* 112:1017–1029.
10. Burns, F. R., S. von Kannen, L. Guy, J. A. Raper, J. Kamholz, and S. Chang. 1991. DM-GRASP, a novel immunoglobulin superfamily axonal surface protein that supports neurite extension. *Neuron.* 7:209–220.
11. Crossin, K. L., C.-M. Chuong, and G. M. Edelman. 1985. Expression sequences of cell adhesion molecules. *Proc. Natl. Acad. Sci. USA.* 82: 6942–6946.
12. D'Eustachio, P., and G. M. Edelman. 1975. Frequency and avidity of specific antigen-binding cells in developing mice. *J. Exp. Med.* 142: 1078–1091.
13. D'Urso, D., P. J. Brophy, S. M. Staugaitis, C. S. Gillespie, A. B. Frey, J. G. Stempak, and D. R. Colman. 1990. Protein zero of peripheral nerve myelin: biosynthesis, membrane insertion, and evidence for homotypic interaction. *Neuron.* 2:449–460.
14. Daniloff, J., C.-M. Chuong, G. Levi, and G. M. Edelman. 1986. Differential distribution of cell adhesion molecules during histogenesis of the chick nervous system. *J. Neurosci.* 6:739–758.
15. Davis, L. G., M. D. Dibner, and J. F. Battey. 1986. Basic Methods in Molecular Biology. Elsevier, New York. 388 pp.
16. de la Rosa, E. J., J. F. Kayyem, J. M. Roman, Y.-D. Stierhof, W. J. Dreyer, and U. Schwarz. 1990. Topologically restricted appearance in the developing chick retinotectal system of Bravo, a neural surface protein: experimental modulation by environmental cues. *J. Cell Biol.* 111:3087–3096.
17. Dulac, C., M. B. Tropak, P. Cameron-Curry, J. Rossier, D. R. Marshak, J. Roder, and N. M. Le Douarin. 1992. Molecular characterization of the schwann cell myelin protein, SMP: structural similarities within the immunoglobulin superfamily. *Neuron.* 8:323–334.
18. Edelman, G. M. 1976. Surface modulation in cell recognition and cell growth. *Science (Wash. DC).* 192:218–226.
19. Edelman, G. M., and K. L. Crossin. 1991. Cell adhesion molecules: implications for a molecular histology. *Annu. Rev. Biochem.* 60:155–190.
20. Edelman, G. M., and B. A. Cunningham. 1991. Place-dependent cell adhesion, process retraction, and spatial signalling in neural morphogenesis. *Cold Spring Harbor Symp. Quant. Biol.* 55:303–318.
21. Edelman, G. M., B. A. Murray, R.-M. Mege, B. A. Cunningham, and W. J. Gallin. 1987. Cellular expression of liver and neural cell adhesion molecules after transfection with their cDNAs results in specific cell-cell binding. *Proc. Natl. Acad Sci. USA.* 84:8502–8506.
22. Elices, M. J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M. E. Hemler, and R. R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell.* 60:577–584.
23. Filbin, M. T., F. S. Walsh, B. D. Trapp, J. A. Pizzey, and G. I. Tennekoon. 1990. Role of myelin P_0 protein as a homophilic adhesion molecule. *Nature (Lond.).* 344:871–872.
24. Fischer, G., V. Kunemund, and M. Schachner. 1986. Neurite outgrowth patterns in cerebellar microexplant cultures are affected by antibodies to the cell surface glycoprotein L1. *J. Neurosci.* 6:605–612.
25. Friedlander, D. R., S. Hoffman, and G. M. Edelman. 1988. Functional mapping of cytotactin: proteolytic fragments active in cell-substrate adhesion. *J. Cell Biol.* 107:2329–2340.
26. Furley, A. J., S. B. Morton, D. Manalo, D. Karagogeos, J. Dodd, and T. M. Jessel. 1990. The axonal glycoprotein TAG-1 is an immunoglobulin superfamily member with neurite outgrowth-promoting activity. *Cell.* 61:157–170.
27. Gennarini, G., G. Cibelli, G. Rougon, M.-G. Mattei, and C. Goridis. 1989. The mouse neuronal cell surface protein F3: a phosphatidylinositol-anchored member of the immunoglobulin superfamily related to chicken contactin. *J. Cell Biol.* 109:775–788.
28. Gorman, C. M., and B. H. Howard. 1983. Expression of recombinant plasmids in mammalian cells is enhanced by sodium butyrate. *Nucleic Acids Res.* 11:7631–7648.
29. Grumet, M. 1992. Structure, expression and function of Ng-CAM, a member of the immunoglobulin superfamily involved in neuron-neuron and neuron-glia adhesion. *J. Neurosci. Res.* 31:1–13.
30. Grumet, M., and G. M. Edelman. 1984. Heterotypic binding between neuronal membrane vesicles and glial cells is mediated by a specific cell adhesion molecule. *J. Cell Biol.* 98:1746–1756.
31. Grumet, M., and G. M. Edelman. 1988. Neuron-glia cell adhesion molecule interacts with neurons and astroglia via different binding mechanisms. *J. Cell Biol.* 106:487–503.
32. Grumet, M., S. Hoffman, C.-M. Chuong, and G. M. Edelman. 1984. Polypeptide components and binding functions of neuron-glia cell adhesion molecules. *Proc. Natl. Acad. Sci. USA.* 81:7989–7993.
33. Grumet, M., V. Mauro, M. P. Burgoon, G. M. Edelman, and B. A. Cunningham. 1991. Structure of a new nervous system glycoprotein, Nr-CAM, and its relationship to subgroups of neural cell adhesion molecules. *J. Cell Biol.* 113:1399–1412.
34. Harrelson, A. L., and C. S. Goodman. 1988. Growth cone guidance in insects: Fasciclin II is a member of the immunoglobulin superfamily. *Science (Wash. DC).* 242:700–708.
35. Hoffman, S., and G. M. Edelman. 1983. Kinetics of homophilic binding by E and A forms of the neural cell adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 80:5762–5766.
36. Hoffman, S., D. R. Friedlander, C.-M. Chuong, M. Grumet, and G. M. Edelman. 1986. Differential contributions of Ng-CAM and N-CAM to cell adhesion in different neural regions. *J. Cell Biol.* 103:145–158.
37. Hoffman, S., K. L. Crossin, and G. M. Edelman. 1988. Molecular forms, binding functions, and developmental expression patterns of cytotactin and cytotactin-binding proteoglycan, and interactive pair of extracellular matrix molecules. *J. Cell Biol.* 106:519–532.
38. Hortsch, M., A. J. Bieber, N. H. Patel, and C. S. Goodman. 1990. Differential splicing generates a nervous system-specific form of *Drosophila* neuroglian. *Neuron.* 4:697–709.
39. Keilhauer, G., A. Faissner, and M. Schachner. 1985. Differential inhibition of neurone-neurone, neurone-astrocyte and astrocyte-astrocyte adhesion by L1, L2, and N-CAM antibodies. *Nature (Lond.).* 316:728–730.
40. Kuhn, T. B., E. T. Stoeckli, M. A. Condrau, F. G. Rathjen, and P. Sonderegger. 1991. Neurite outgrowth on immobilized axonin-1 is mediated by a heterophilic interaction with L1(G4). *J. Cell Biol.* 115:1113–1126.
41. Landon, D. N. 1976. The Peripheral Nerve. Chapman and Hall Ltd., Salisbury, England. 836 pp.
42. Lemke, G., and R. Axel. 1985. Isolation and sequence of a cDNA encoding

the major structural protein of peripheral myelin. *Cell.* 40:501–508.
43. Levi, G., K. L. Crossin, and G. M. Edelman. 1987. Expression sequences and distribution of two primary cell adhesion molecules during embryonic development of *Xenopus laevis*. *J. Cell Biol.* 105:2359–2372.
44. Marlin, S. D., and T. A. Springer. 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell.* 51:813–819.
45. Moos, M., R. Tacke, H. Scherer, D. Teplow, K. Fruh, and M. Schachner. 1988. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. *Nature (Lond.).* 334:701–703.
46. Muller, W. A., M. E. Berman, P. J. Newman, H. M. DeLisser, and S. M. Albelda. 1992. A heterotypic adhesion mechanism for platelet/endothelial cell adhesion molecule (CD31). *J. Exp. Med.* 175:1401–1404.
47. Oikawa, S., C. Inuzuka, M. Kuroki, F. Arakawa, Y. Matsuoka, G. Kosaki, and H. Nakazato. 1991. A specific heterotypic cell adhesion activity between members of carcinoembryonic antigen family, W272 and NCA, is mediated by N-domains. *J. Biol. Chem.* 266:7995–8001.
48. Prince, J. T., N. Milona, and W. B. Stallcup. 1989. Characterization of a partial cDNA clone for the NILE glycoprotein and identification of the encoded polypeptide domain. *J. Neurosci.* 9:876–883.
49. Ranscht, B. 1988. Sequence of contactin, a 130-kD glycoprotein concentrated in areas of interneuronal contact, defines a new member of the immunoglobulin supergene family in the nervous system. *J. Cell Biol.* 107:1561–1573.
50. Salzer, J. G., W. P. Holmes, and D. R. Colman. 1987. The amino acid sequences of the myelin-associated glycoproteins: homology to the immunoglobulin gene superfamily. *J. Cell Biol.* 104:957–965.
51. Schneider-Schaulies, J., A. von Brunn, and M. Schachner. 1990. Recombinant peripheral myelin protein Po confers both adhesive and neurite outgrowth-promoting properties. *J. Neurosci. Res.* 27:286–297.
52. Smith, D. B., and K. S. Johnson. 1988. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. *Gene.* 67:31–40.
53. Staunton, D. E., S. D. Marlin, C. Stratowa, M. L. Dustin, and T. A. Springer. 1988. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell.* 52:925–933.
54. Tanaka, H., T. Matsui, A. Agata, M. Tomura, I. Kubota, K. C. McFarland, B. Kohr, A. Lee, H. S. Phillips, and D. L. Shelton. 1991. Molecular cloning and expression of a novel adhesion molecule, SC1. *Neuron.* 7:535–545.
55. Thiery, J.-P., and J.-L. Duband, U. Rutishauser, and G. M. Edelman. 1982. Cell adhesion molecules in early chick embryogenesis. *Proc. Natl. Acad. Sci. USA.* 79:6737–6741.
56. Thiery, J.-P., A. Delouvée, M. Grumet, and G. M. Edelman. 1985. Initial appearance and regional distribution of the neuron-glia cell adhesion molecule, in the chick embryo. *J. Cell Biol.* 100:442–456.
57. Zuellig, R. A., C. Rader, A. Schroeder, M. B. Kalousek, F. von Bohlen und Halbach, T. Osterwalder, C. Inan, E. R. Stoeckli, H. U. Affotter, A. Fritz, E. Hafen, and P. Sonderegger. 1992. The axonally secreted cell adhesion molecule, axonin-1: primary structure, immunoglobulin-like and fibronectin-type-III-like domains and glycosyl-phosphatidylinositol anchorage. *Eur. J. Biochem.* 204:453–463.