Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8502–8506, December 1987
Developmental Biology

Cellular expression of liver and neural cell adhesion molecules after transfection with their cDNAs results in specific cell–cell binding

GERALD M. EDELMAN, BEN A. MURRAY, RENE-MARC MEGE, BRUCE A. CUNNINGHAM, AND WARREN J. GALLIN

The Rockefeller University, New York, NY 10021

Contributed by Gerald M. Edelman, August 26, 1987

ABSTRACT **Mouse L cells, which do not express the known primary cell adhesion molecules (CAMs), were permanently transfected with vectors containing the simian virus 40 early promoter and cDNA sequences encoding chicken liver CAM (L-CAM) or each of the three major polypeptide forms of chicken neural CAM (N-CAM). Transfected cells in culture expressing the Ca^{2+}-dependent L-CAM showed uniform surface expression of the molecule. Unlike untransfected L cells, these cells aggregated readily; the aggregation was inhibited by Fab' fragments of antibodies to L-CAM but not by fragments of anti-N-CAM. These cells spread more efficiently in culture than did their untransfected counterparts, forming small colonies of flattened cells that gradually assumed morphologies resembling closely packed L cells. Transfected L cells expressing either the small or large intercellular domain polypeptide (sd or ld) chains of N-CAM aggregated specifically with each other or bound membrane vesicles from chick brain. Both types of binding were specifically inhibited by Fab' fragments of anti-N-CAM antibodies. These cells, in contrast to those transfected with vectors for L-CAM, showed rounded morphologies and spread inefficiently in culture. L cells transfected with vectors specifying the small surface domain polypeptide (ssd) chain of N-CAM showed no phenotypic changes and no evidence for linkage of ssd chains to the cell membrane by phosphatidylinositol intermediates. Instead, these cells synthesized the molecule and released it into the medium. These findings complete the demonstration that different CAMs have specific roles in ligating the cells that synthesize them, and they provide further evidence that L-CAM and N-CAM bind by homophilic mechanisms. The different phenotypic changes observed for each specific CAM are consistent with the hypothesis that CAM synthesis or differing associations of CAM carboxyl-terminal domains with the cell surface and cortex may lead directly or indirectly to specific alterations in the cells bound together by that CAM.**

The demonstration (1, 2) that cell adhesion molecules (CAMs) of different specificities play major roles in morphogenesis, histogenesis, and regeneration has opened the possibility of relating key phenotypic properties of cells with their epithelial or mesenchymal states (3). Primary CAMs of different specificities, such as the Ca^{2+}-dependent liver CAM (L-CAM) and Ca^{2+}-independent neural CAM (N-CAM), appear to bind by homophilic mechanisms—i.e., CAM on one cell to the same CAM on an apposing cell (4). The findings that these molecules (*i*) appear in defined developmental expression sequences (2), (*ii*) are specified by one or at most a few genes (5), and (*iii*) are under regulatory control related to boundaries of the condensed mesenchyme or epithelia that they help to ligate (6) suggest that specific CAM binding functions must be correlated with particular cell states.

Recent analyses (7–9) of the structures of cDNAs specifying these two CAMs and of the genomic DNA for N-CAM (10) are consistent with this view. N-CAM, which appears to be closely related evolutionarily to the precursor of the entire immunoglobulin superfamily (7, 8, 11), is expressed as three major polypeptides that arise as a result of alternative splicing of RNA transcribed from a single gene (12). Each N-CAM chain has a different carboxyl-terminal domain and a different mode of association with the cell surface or cell cortical region. Of these polypeptides, the large and small intercellular domain (ld and sd) polypeptide chains contain intracellular domains of different sequence and size, whereas the small surface-domain (ssd) polypeptide chain lacks an intracellular domain but is linked to the cell surface by a phosphatidylinositol-containing intermediate. L-CAM is structurally unrelated to N-CAM and has another kind of intracellular domain (9). This Ca^{2+}-dependent CAM and the different N-CAM polypeptides all have individually characteristic tissue distributions (2, 3, 12).

The availability of appropriate cDNA clones for all of these molecules has made it possible to examine the effects of expression of CAMs on cells that ordinarily do not synthesize them. In the present experiments, vectors constructed with cDNAs specifying L-CAM and N-CAM chains were used to transfect L cells that are free of these molecules in their ordinary state. The transfected cells expressed CAMs of the correct binding specificity that ligated the cells in a homophilic fashion. Permanent lines transfected with each type of CAM showed distinct phenotypic changes in cell shape and cell spreading in culture. Transfection experiments thus open the possibility of correlating amino-terminal CAM binding specificities with the properties and cellular effects of their carboxyl-terminal surface-bound structures or their intracellular domains. Such experiments should also facilitate the analysis of the effects of differential expression of two different CAMs in the same cell.

MATERIALS AND METHODS

DNA Constructs. All DNA constructs were prepared with cDNA clones for chicken N-CAM (7, 8, 13) and L-CAM (9). Constructs encompassing the coding sequence for each N-CAM polypeptide (ld, sd, and ssd) were inserted after the simian virus 40 (SV40) early promoter in a variant of the plasmid pCH110 (14). The *Eco*RI site downstream from the poly(A)$^+$ addition site in pCH110 was removed by partial *Eco*RI digestion, and the ends were polished with the large (Klenow) fragment of DNA polymerase and rejoined by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAM, cell adhesion molecule; N-CAM, neural CAM; L-CAM, liver CAM; SV40, simian virus 40; ld and sd, large and small intracellular domain polypeptides; ssd, small surface domain polypeptide.

blunt-end ligation. The *Hin*dIII fragment from the N-CAM plasmid (pEC254/pEC208) pGEM construct (8) containing 1800 base pairs (bp) of the 5′ end of N-CAM cDNA was then inserted into the *Hin*dIII site of pCH110 found immediately adjacent to the SV40 early promoter. The resulting plasmid was digested with *Eco*RI and ligated to *Eco*RI fragments containing the remaining sequences for the ld (pEC208), sd (pEC281; see ref. 12), and ssd (pEC151) polypeptides. The structures of these plasmids were confirmed by restriction mapping. We designate the clones pEC1401 (ld), pEC1402 (sd), and pEC1403 (ssd).

The L-CAM cDNA clones extended 5′ from the region coding for the amino terminus of the mature molecule and more than 300 bp into the coding sequence of the precursor polypeptide but did not extend to the 5′ end of the mRNA. Therefore, an initiator methionine and signal sequence were provided by constructing N-CAM/L-CAM chimeras. The L-CAM coding region, contained in the *Eco*RI/*Bam*HI fragment of L-CAM clone pEC320, was inserted into the Bluescript KS vector (Stratagene, La Jolla, CA) digested with *Eco*RI and *Bam*HI to give clone B67. pEC1301 was then prepared by ligating the 191-bp *Nae* I/*Alu* I fragment of N-CAM clone pEC254 into the *Eco*RI site of the Bluescript polylinker of B67. pEC1301 thus contains sequences coding for about 64 amino acids at the amino terminus of N-CAM. pEC1302 was prepared by ligating the 391-bp *Nae* I/*Eco*RI fragment of pEC254 into *Eco*RI/*Eco*RV-digested B67; this clone codes for about 103 amino acids of the amino terminus of N-CAM. The structure and orientation of pEC1301 and pEC1302 were confirmed by DNA sequencing.

pEC1301 and pEC1302 were digested with *Sal* I, the ends were polished with Klenow fragment, and *Bam*HI linkers were added. After digestion with *Bam*HI, these inserts were purified on agarose gels and ligated into the *Bgl* II site of pKSV-10 (Pharmacia) adjacent to the SV40 early promoter. The orientation of the insert in isolated clones was determined by restriction mapping. We designate pKSV-10 having the pEC1301 insert as pEC1311 and the construct having the pEC1302 insert as pEC1312.

Cell Culture and Transfection. All transfections were performed with a calcium phosphate protocol (15) on mouse L-M(TK$^-$) cells (American Type Culture Collection CCL 1.3) grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Cells (3–5 × 10^6) were transfected with 40 μg of the appropriate CAM plasmid DNA and 5 μg of pSV2neo DNA for times ranging from 4 to 16 hr. After a 2-min glycerol shock, the cells were allowed to recover for 24 hr, replated in five 10-cm tissue culture dishes, and selected with G418 (GIBCO) at 400 μg/ml (200 μg/ml active). Individual colonies, which appeared 2.5–3 weeks after initiation of selection, were isolated with cloning rings and expanded. Cells from colonies that did not have uniform expression of the transfected genes were cloned once by limiting dilution in 96-well tissue culture plates. Some cells growing on a 10-cm tissue culture dish were cultured for an additional 12–16 hr with medium containing 10 mM sodium butyrate (16).

Immunofluorescent Staining. Cells growing in 35-mm tissue culture dishes were rinsed once with phosphate-buffered saline (P_i/NaCl), fixed (15 min) in P_i/NaCl containing 2.5% formaldehyde and 0.02% glutaraldehyde, quenched (15 min) in P_i/NaCl containing 0.1 M glycine, and incubated (15–60 min) in P_i/NaCl containing 5% goat serum. Cells were then treated with first antibody (100 μg/ml) in P_i/NaCl containing 5% goat serum for 1 hr at room temperature, washed five times with P_i/NaCl, incubated with fluorescein-conjugated goat anti-rabbit IgG (ICN) (1:50 to 1:100 dilution) for 0.5 hr at room temperature, washed five times with P_i/NaCl, and viewed and photographed with a Zeiss Universal microscope.

Immunoblotting Analysis. Cells were rinsed once with P_i/NaCl and then scraped off the dish in 0.5 ml of $NaDodSO_4$ sample buffer. Extracts were heated 5 min at 95°C and clarified by centrifugation. Fifty microliters of each extract was resolved by $NaDodSO_4$/PAGE (17). The proteins were electrophoretically transferred to nitrocellulose and visualized by immunoblotting (18).

Aggregation Assays. For the L-CAM aggregation assay, cells were released from the culture dishes by treating for 30 min with P_i/NaCl containing 2% fetal calf serum and 5 mM EDTA on ice. Cells were collected in Eagle's minimal essential medium modified for spinner culture and containing DNase at 10 μg/ml (SMEM-DNase), pelleted by centrifugation, and resuspended in SMEM-DNase. Aliquots of cell suspension were preincubated with Fab′ solutions in medium NCTC 135 on ice for 15 min and assayed for aggregation in shaking culture at 37°C in Eagle's minimal essential medium as described (19).

For N-CAM aggregation assays, transfected cells on culture plates were first rinsed with P_i/NaCl and then released from the dishes by trypsinization (20 μg/ml) at 37°C for 5–10 min in SMEM-DNase containing 1 mM EDTA; cell concentrations were measured with a Coulter Counter.

The binding of chick brain vesicles to transfected cells and the inhibition of binding by anti-N-CAM Fab′ (1 mg/ml) were carried out as described by Grumet and Edelman (20). Cell–cell binding was assessed by measuring the disappearance of single cells (21).

RESULTS

Synthesis and Expression of CAMs. Immunoblotting experiments with specific antibodies to L-CAM and N-CAM (Fig. 1) showed that the permanently transfected cell lines expressed L-CAM and N-CAM polypeptides corresponding to the appropriate cDNA in each construct. Anti-L-CAM antibodies revealed that the cells transfected by chimeric L-CAM constructions made with a small 5′ portion of the cDNA for

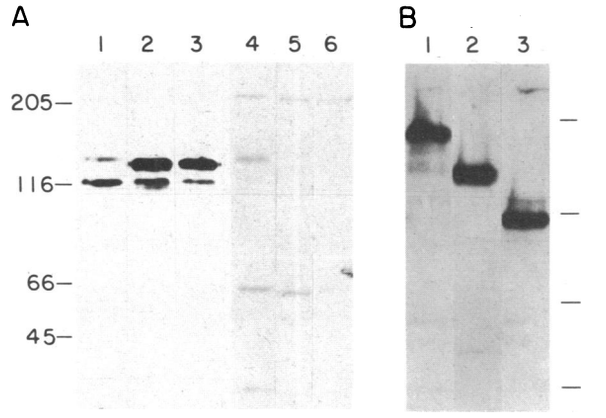

FIG. 1. Immunoblots of proteins produced by transfected cell lines. (*A*) Extracts of L-CAM transfectant clones NE2-5 (lanes 1 and 4), NA8-4 (lanes 2 and 5), and NA8-6 (lanes 3 and 6) resolved by $NaDodSO_4$/PAGE and visualized with antibodies to chicken L-CAM (lanes 1–3) or chicken N-CAM (lanes 4–6). Lines NE2-5 were obtained by transfection with pEC1312, whereas lines NA8-4 and NA8-6 were obtained by transfection with pEC1311. Cells were induced with sodium butyrate for 14 hr before extraction. The band at M_r 65,000 seen in lanes 4–6 was also present in lanes 1–3 but is not apparent because of different exposure times; it possibly represents a proteolytic fragment or a contaminant. (*B*) Extracts of clones 1LB4 (lane 1), 1LA4 (lane 2), and 2LA5 (lane 3) encoding ld, sd, and ssd chains of N-CAM, respectively, resolved by $NaDodSO_4$/PAGE and visualized with antibody to chicken N-CAM. The positions of standards (M_r × 10^{-3}) are shown at the left for *A* and at the right for *B*.

N-CAM linked to the cDNA for L-CAM expressed two kinds of molecules, one corresponding in size to the L-CAM polypeptide and the other to higher molecular weight chimeric polypeptides containing both L-CAM and segments of N-CAM (in Fig. 1*A*, compare lanes 1–3 with lanes 4–6). Only the clone (NE2-5) with the larger N-CAM insert reacted with anti-N-CAM antibodies, however. Antibodies to N-CAM showed that each of the lines transfected with N-CAM constructions expressed the corresponding polypeptide of N-CAM (Fig. 1*B*). Cells induced by butyrate treatment (16) expressed larger amounts of each CAM form than did the cells not so treated (data not shown).

An immunohistological comparison of untransfected cells with NE2-5 cells transfected with an L-CAM chimeric construct is shown in Fig. 2 *A–D*. Individual transfected cells 12 hr after butyrate induction showed no surface immunofluorescence with anti-N-CAM antibodies but exhibited strong uniform surface fluorescence with anti-L-CAM (compare *B* and *D* in Fig. 2). Correlation of these findings with those shown in Fig. 1 indicates that some of the protein in these cells, although made from a chimeric construct, is cleaved at the appropriate site to be expressed at the cell surface without N-CAM determinants as mature L-CAM. After their release from the dishes by EDTA treatment, these cells aggregated rapidly and effectively in an L-CAM-specific manner (Fig. 2 *E–J*). Univalent fragments of antibodies to L-CAM strongly inhibited this aggregation, whereas corresponding fragments of nonimmune Ig and of anti-N-CAM had no effect.

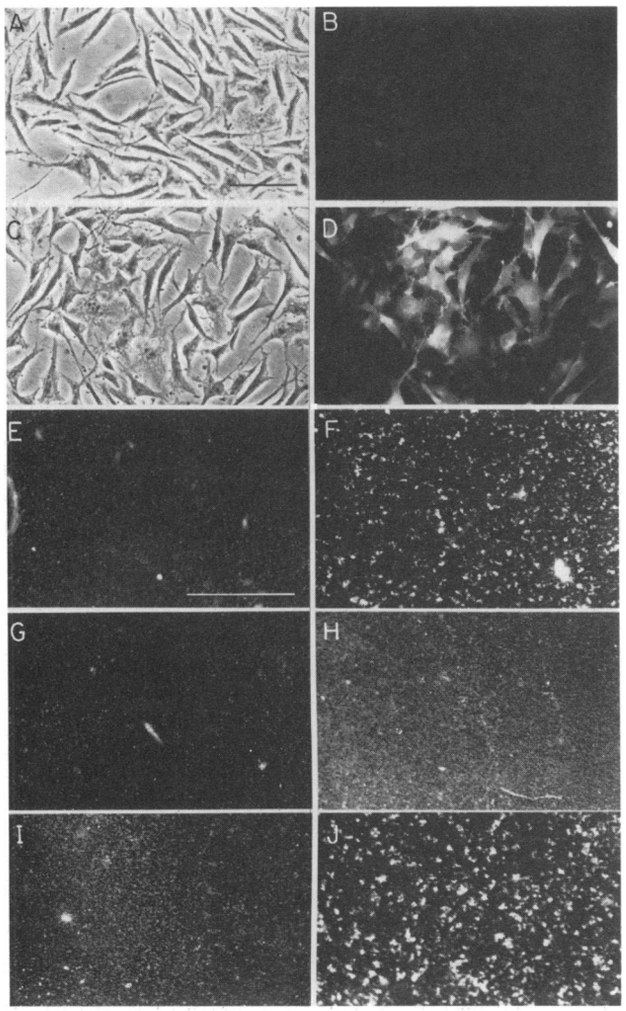

FIG. 2. Matched phase-contrast (*A* and *C*) and fluorescence (*B* and *D*) photographs of NE2-5 cells induced with 10 mM butyrate for 12 hr; the cells yielded no immunofluorescent staining for N-CAM (*B*) but gave bright uniform surface staining for L-CAM (*D*). (*E–I*) The cultured cells were released from the culture dish, incubated in a standard aggregation assay (19) for 45 min (see Table 1), and then photographed at low magnification under dark-field illumination to score aggregation visually. Untransfected L cells did not aggregate in the presence of nonimmune rabbit Fab′ (*E*), anti-L-CAM Fab′ (*G*), or anti-N-CAM Fab′ (*I*) after 65 min. In contrast, transfected line NE2-5 formed visible aggregates in the presence of nonimmune Fab′ after 45 min (*F*). This aggregation was totally inhibited by anti-L-CAM Fab′ (*H*) and was unaffected by anti-N-CAM Fab′ (*J*). (Bar in *A* = 50 μm for *A–D*; bar in *E* = 2 mm for *E–J*.)

Cells separately transfected with vectors containing cDNA sequences corresponding to the sd and ld chains of N-CAM expressed the respective chains at their cell surfaces; cells expressing the ld chain are shown in Fig. 3 *C* and *D*. In most cases, it was observed that cells permanently transfected to express these molecules became more rounded than did untransfected L cells and showed evidence of blebbing. Cells transfected with vectors containing cDNA sequences for the ssd chain of N-CAM (Fig. 3 *A* and *B*) released the polypeptide into the medium, showed no surface staining with fluorescently labeled anti-N-CAM, and underwent no shape change. As indicated below, the transfected cells expressing ld and sd chains showed homophilic binding either among themselves or to N-CAM-containing vesicles from brain membranes.

Quantitation of Binding by Transfected Cells. Aggregation and binding behavior of the transfected cells were tested by quantitative assays measuring disappearance of single cells and its inhibition by appropriately specific antibodies. In Table 1, results are presented for various permanent lines expressing L-CAM and for a line expressing high levels of the sd chain of N-CAM. In both cases, there was specific aggregation inhibitable only by the appropriate specific anti-CAM antibody. The two kinds of cells did not bind to each other or to untransfected L cells (data not shown). Fab′ fragments of anti-L-CAM antibodies were more efficient than fragments of anti-N-CAM antibodies in inhibiting aggregation of their corresponding cells; nonetheless, the anti-N-CAM

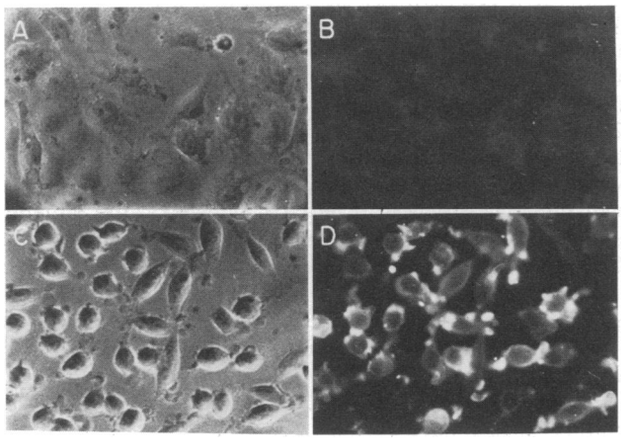

FIG. 3. Cell surface expression of N-CAM in transfected cells: matched phase-contrast (*A* and *C*) and fluorescence micrographs (*B* and *D*) of clone 2LA5 expressing the ssd chain of N-CAM (*A* and *B*) and of clone 1LB4 expressing the ld chain of N-CAM (*C* and *D*) stained with rabbit antibodies to chicken N-CAM. Clone 2LA5 had no detectable cell surface immunoreactive material, although it secreted N-CAM into the medium as detected by electrophoresis and immunoblotting. Clone 1LB4 was brightly stained at the cell surface and also in blebs; this clone, like others that express cell surface N-CAM, had a rounded morphology with cell surface membrane blebs and adhered less tightly to the culture dish than did untransfected cells.

Table 1. Quantitation and specificity of aggregation of transfected cells

	Cell line*	Fab'	% aggregation†	% inhibition
Controls	L	NR	6	
		Anti-L-CAM	0	
		Anti-N-CAM	0	
L-CAM transfectants	NE2-5	NR	59	—
		Anti-L-CAM	0	100
		Anti-N-CAM	61	0
	NA8-6	NR	34	—
		Anti-L-CAM	0	100
		Anti-N-CAM	33	3
N-CAM (sd chain) transfectants	Retina	NR	80	—
		Anti-N-CAM	46	42
	8B2	NR	31	—
		Anti-N-CAM	19	41
	8C2	NR	29	—
		Anti-N-CAM	18	38

NR, normal rabbit Ig.

*Cells were untransfected L cells or L cells transfected with (*i*) pEC1312 for NE2-5, (*ii*) pEC1311 for NA8-6, or (*iii*) pEC1402 for lines 8B2 and 8C2. Retinal cells were prepared from day 10 chicken embryos (26).

†Aggregation was determined as in refs. 19 and 20; results are averages of two separate determinations. In separate experiments under similar conditions, liver cells aggregated 48–50%, and this aggregation was almost completely inhibited by the anti-L-CAM antibodies used in these experiments.

Fab' fragments led to considerable inhibition that was clearly specific.

Confirmation of this specificity was obtained by using vesicle binding assays (Table 2). Brain plasma membrane vesicles bound to a permanently transfected line expressing N-CAM ld chains, and these vesicles did not bind to cells transfected with a control vector. Anti-N-CAM Fab' fragments, but not those of normal rabbit Ig, inhibited the binding. Similar results were obtained with cells expressing the N-CAM sd chain (data not shown).

Qualitative Observations of Phenotypic Changes Following Transfection. Untransfected L cells and transfected cells expressing N-CAM and L-CAM had identical growth rates. Cells permanently expressing L-CAM after transfection spread more rapidly than did untransfected cells when plated into culture dishes and tended to be found in small aggregates that formed colonies. Although these cells were initially flattened, after 36 hr in culture they began to assume morphologies similar to that of their parent L cells (see Fig. 2*C*).

In contrast, most permanent lines of L cells expressing sd and ld chains of N-CAM took on a rounded morphology with blebbing (see Fig. 3). These cells plated less efficiently and were more loosely adherent to the tissue culture substratum than were the untransfected L cells. Cells expressing ssd chains that were released into the medium resembled untransfected L cells in these properties, suggesting that the phenotypic changes seen with sd and ld chains resulted from interactions with the cell surface or cortical regions rather than from the transfection itself.

Table 2. Binding of brain vesicles to transfected cells expressing the ld chain of N-CAM

Cell*	Fab'	% binding†	% inhibition
1LB4	NR	23	—
1LB4	anti-N-CAM	2	90
1LA2	NR	5	—
1LA2	anti-N-CAM	5	0

NR, normal rabbit Ig.

*1LB4 is an L cell line transfected with pEC1401 and expressing the ld chain of N-CAM at the cell surface. 1LA2 is a control L cell line transfected with pCH110, which encodes β-galactosidase but contained no cDNA specifying a CAM.

†Performed as in ref 20; values are averages of duplicate determinations.

DISCUSSION

The present experiments indicate that permanently cloned tissue culture lines can be obtained that express CAMs of different specificities as tested by immunoblotting, surface immunofluorescence, and appropriately specific quantitative aggregation assays. Mouse L cells were successfully transfected with different vectors containing cDNAs encoding L-CAM or each of the three major polypeptides of N-CAM. The qualitative and quantitative findings on aggregation unequivocally demonstrate the function of CAMs in ligating the cells that synthesize them (2). Inasmuch as untransfected L cells do not express genes for N- or L-CAM and are generally nonadhesive in the assays, the data confirm previous observations (4) that N-CAM binding is homophilic and provide evidence that the binding of the Ca^{2+}-dependent L-CAM is also homophilic.

Transfected cells expressing L-CAM showed high levels of uniform surface immunofluorescence, and when plated, they aggregated and spread rapidly to form small colonies of cells lacking the spindle-shaped morphology of untransfected L cells. After growth for 36 hr, however, these permanently transfected cells continued their L-CAM expression but assumed a more spindle-shaped morphology in dense culture. Such cells spread more rapidly than L cells did after plating and, when removed from culture dishes by P_i/NaCl containing EDTA, they aggregated specifically and rapidly. Anti-L-CAM Fab' fragments inhibited this aggregation almost completely.

Most cell lines expressing sd and ld polypeptides of N-CAM after transfection showed permanent morphological changes from a spindle shape to various rounded shapes with blebs. In a few cases (for example, sd lines 8B2 and 8C2; Table 1) this effect was not seen. In contrast to transfected lines expressing L-CAM, the lines expressing sd and ld chains of N-CAM plated less efficiently and adhered only weakly to the substrate. Those cells transfected with cDNAs

corresponding to the ssd chain showed none of these morphological changes, however, and the expressed ssd chain was detected intracellularly and in the medium but not on the cell surface. This suggests that the L cells either did not synthesize phosphatidylinositol intermediates or did not link them to the carboxyl terminus of the ssd chains (13) or that the polypeptides were spontaneously released from the lipid anchor after it was attached. That this is not attributable to the type of construct used is indicated by the fact that monkey kidney cells [COS cells (22)] transiently transfected with similar constructs under the control of the SV40 late promoter showed ssd chains attached to their cell surfaces that could be released with phosphatidylinositol-specific phospholipase C (J. J. Hemperly, B.A.M., and G.M.E., unpublished data).

The quantitative assays used here left no doubt that the L-CAM and the chains of N-CAM expressed at the cell surface were capable of mediating cell–cell adhesion specific for each CAM. Possibly because of their shape changes, however, the cells expressing the ld and sd chains aggregated less efficiently than those expressing L-CAM, and their aggregation, while specific, was also somewhat less efficiently inhibited by the anti-N-CAM antibody fragments (see Table 1).

Several phenomena of biochemical interest have emerged from the present experiments and require further study. At present, the sialic acid content of the expressed N-CAM chains is not known, but the gel patterns (see Fig. 1) are consistent with the conclusion that polysialic acid is either absent or is not present in the amounts or chain lengths found in the so-called embryonic or E form of the molecule, which shows diffuse electrophoretic behavior (23). The effects induced by the synthesis of such forms may possibly be revealed by further biochemical analyses after transfecting cells that have the appropriate sialyl transferase. Additional biochemical analysis of the cleavage of the mixed chain containing N-CAM and L-CAM sequences specified by the chimeric construct used here may also shed light on the origin of L-CAM from a larger precursor (9) in embryonic cells that naturally express L-CAM.

Transfection experiments could clarify a number of important problems related to cell adhesion. For example, the different phenotypic changes seen here for the same L-cell line transfected with different CAMs are not in keeping with the notion that CAMs function merely to link cells to form boundaries reflecting their different binding specificities. Instead, it appears that specific CAM expression is also correlated with cell changes related to CAM type, to linkage to the cell surface, and possibly to interactions with the cytoskeleton. It is not yet clear, however, whether the observed changes in plating efficiencies and morphology result directly from such interactions (24) or from homophilic CAM binding, or both. Nonetheless, the phenotypic differences are in accord with embryological observations relating CAM specificities and expression to different regions at different times. For example, L-CAM has only one polypeptide (9) that is seen on epithelial tissues in early embryos or in nonneural sites after formation of the neural tube (3, 25). The sd polypeptide of N-CAM is found in both neural and nonneural tissues at a variety of sites in contrast to the ld chain, which appears to be nervous system-specific (12) and is generally expressed in neurite-rich regions.

In each of these cases, the cytoplasmic domains of these CAMs have characteristic and different structures (8, 9). Thus, the opportunity is provided to test the relationship between the specific function of CAM binding (carried out by the extracellular domains) and the functions of CAM cytoplasmic domains that interact with the cell surface and cortex. Hybrid constructs can be made containing the cytoplasmic domain of a given CAM and the extracellular homophilic binding domain of another CAM. Moreover, cotransfection with CAMs of different specificities showing different degrees of expression or coculturing of cells containing one, the other, or both CAMs should shed further light on the role (2, 6) of these molecules in linking cell collectives and in forming boundaries between them.

Note Added in Proof. After this manuscript was submitted, Nagafuchi *et al.* (27) reported transfection of L cells with a cDNA clone of E-cadherin, the mouse homolog of L-CAM, with results comparable to those presented here for L-CAM.

We thank M. Grumet, J. Hemperly, and F. Matsuzaki for their help and advice. This work was supported by National Institutes of Health Grants HD-09635; HD-16550, and DK-04256 and by a Senator Jacob Javits Center Grant for Excellence in Neuroscience, NS-22789. R.M.M. received support from the Institut National de la Santé et de la Recherche Médicale and the Fondation Singer–Polignac; W.J.G. was an R. J. Reynolds Fellow.

1. Edelman, G. M. (1983) *Science* **219,** 450–457.
2. Edelman, G. M. (1985) *Annu. Rev. Cell Biol.* **2,** 82–116.
3. Crossin, K. L., Chuong, C.-M. & Edelman, G. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6942–6946.
4. Hoffman, S. & Edelman, G. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5762–5766.
5. D'Eustachio, P., Owens, G. C., Edelman, G. M. & Cunningham, B. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7631–7635.
6. Gallin, W. J., Chuong, C.-M., Finkel, L. H. & Edelman, G. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8235–8239.
7. Hemperly, J. J., Murray, B. A., Edelman, G. M. & Cunningham, B. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3037–3041.
8. Cunningham, B. A., Hemperly, J. J., Murray, B. A., Prediger, E. A., Brackenbury, R. & Edelman, G. M. (1987) *Science* **236,** 799–806.
9. Gallin, W. J., Sorkin, B. C., Edelman, G. M. & Cunningham, B. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2808–2812.
10. Owens, G. C., Edelman, G. M. & Cunningham, B. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 294–298.
11. Edelman, G. M. (1987) *Immunol. Rev.* **100,** 9–43.
12. Murray, B. A., Owens, G. C., Prediger, E. A., Crossin, K. L., Cunningham, B. A. & Edelman, G. M. (1986) *J. Cell Biol.* **103,** 1431–1439.
13. Hemperly, J. J., Edelman, G. M. & Cunningham, B. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9822–9826.
14. Hall, C. T., Jacob, P. E., Ringold, G. M. & Lee, F. (1983) *J. Mol. Appl. Genet.* **2,** 101–109.
15. Davis, L. G., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York).
16. Gorman, C. M., Howard, B. H. & Reeves, R. (1983) *Nucleic Acids Res.* **11,** 7631–7648.
17. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
18. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
19. Bertolotti, R., Rutishauser, U. & Edelman, G. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4831–4835.
20. Grumet, M. & Edelman, G. M. (1984) *J. Cell Biol.* **98,** 1746–1756.
21. Brackenbury, R., Thiery, J.-P., Rutishauser, U. & Edelman, G. M. (1977) *J. Biol. Chem.* **252,** 6835–6840.
22. Gluzman, Y. (1981) *Cell* **23,** 175–182.
23. Rothbard, J. B., Brackenbury, R., Cunningham, B. A. & Edelman, G. M. (1982) *J. Biol. Chem.* **257,** 11064–11069.
24. Edelman, G. M. (1976) *Science* **192,** 218–226.
25. Levi, G., Crossin, K. L. & Edelman, G. M. (1987) *J. Cell Biol.*, in press.
26. Brackenbury, R., Rutishauser, U. & Edelman, G.M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 387–391.
27. Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda, K. & Takeichi, M. (1987) *Nature (London)* **329,** 341–343.