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# Topology of cell adhesion molecules

**(protein structure/immunoglobulin superfamily/rotary shadowing)**

JOSEPH W. BECKER*, HAROLD P. ERICKSON†, STANLEY HOFFMAN*, BRUCE A. CUNNINGHAM*, AND GERALD M. EDELMAN*

*The Rockefeller University, 1230 York Avenue, New York, NY 10021; and †Department of Cell Biology, Duke University Medical Center, Durham, NC 27710

*Contributed by Gerald M. Edelman, November 18, 1988*

ABSTRACT **The neural cell adhesion molecule (N-CAM) exists in two major forms [ld (large cytoplasmic domain) peptide and sd (small cytoplasmic domain) peptide] that contain transmembrane segments and different cytoplasmic domains and in a third form [ssd (small surface domain) peptide] that lacks transmembrane and cytoplasmic regions. All forms have the same extracellular region of more than 600 amino acid residues, a region also found in a fragment (Fr2) that can be released from cells by proteolysis. The liver cell adhesion molecule (L-CAM) is expressed as a single species that is distinct from N-CAM, but its extracellular region can also be obtained as a proteolytic fragment (Ftl). Examination of the various forms of N-CAM and the Ftl fragment of L-CAM by electron microscopy of rotary shadowed molecules indicated that they all have rod-shaped structures that contain a hinge region which is apparently flexible. Both the ssd chain and the Fr2 fragment of N-CAM are single rods bent into arms approximately 18 and 10 nm long. The ld and sd chains are longer bent rods that form rosettes comprising two to six branches; detergent treatment disrupts these rosettes into single rods. Specific antibodies that block homophilic N-CAM binding labeled the distal ends of the branches of the ld/sd rosettes and the ends of the longer arm of both the ssd chain and the Fr2 fragment. Antibodies that bind to the sialic acid-rich region of N-CAM bound near the hinge. These data indicate that the N-CAM rosettes are formed by interaction between their transmembrane or cytoplasmic domains and not by interactions involving their homophilic binding sites. The L-CAM Ftl fragment is also a bent rod with an apparently flexible hinge; like the ssd chain and the Fr2 fragment of N-CAM, it does not form aggregates. The similarities between L-CAM and N-CAM, despite their differences in amino acid sequence, suggest that their general configuration and the presence of a flexible hinge are important elements in assuring effective and specific cell–cell adhesion.**

The neural cell adhesion molecule (N-CAM) and liver cell adhesion molecule (L-CAM) are membrane-associated glycoproteins that mediate cell–cell interactions through specific intermolecular binding (1, 2). They appear in different developmentally regulated patterns during development, suggesting that they play critical roles in morphogenesis (3). Modulation of the expression, distribution, local concentration, and posttranslational modification of CAMs affects the activities of these molecules and the cellular and developmental processes that they mediate (4).

N-CAM from chicken brain contains three distinct polypeptides that share a common extracellular sequence of 682 residues extending from their $NH_2$ termini to the cell membrane (5). The two larger forms, ld (large cytoplasmic domain) peptide and sd (small cytoplasmic domain) peptide, have identical transmembrane domains but the ld polypeptide contains a unique segment of 261 residues in its cytoplasmic region. The ssd (small surface domain) peptide terminates in a unique hydrophobic sequence (5) and is apparently attached to the membrane by a phosphatidylinositol-containing anchor (6–8). The three forms of N-CAM are derived by alternative RNA splicing of a single gene that contains at least 19 exons spanning 50 kilobases (9). Other forms of N-CAM, containing additional inserts in the extracellular region, have been found in muscle (10, 11) and rat brain (12).

The chemical structure of N-CAM has three striking features: similarity to immunoglobulins, similarity to the cell-binding region of fibronectin, and large amounts of polysialic acid. The $NH_2$-terminal half of the molecule contains five contiguous regions of approximately 100 residues that are similar to one another and to the domains of immunoglobulins (5, 13). A homophilic binding site is apparently contained within the first four of these domains (14). This immunoglobulin-like region is followed by two regions of similarity to the type III repeats of fibronectin (15). Asparagine-linked oligosaccharides are distributed among seven potential attachment sites in the third, fourth, and fifth domains (14, 16). N-CAM contains large amounts of $\alpha$-2,8-linked polysialic acid, which is attached to two or more of the oligosaccharides in the fifth domain. Expression of polysialic acid is developmentally regulated (17–20), and in many tissues its amount decreases with age with a concurrent increase in binding activity (1, 21). Two extracellular fragments of N-CAM can be released by proteolysis: Frl ($M_r$ 65,000) includes the $NH_2$ terminus and the homophilic binding site but lacks the polysialic acid, and Fr2 ($M_r$ 108,000) includes all of Frl plus the polysialic acid-containing region (14).

L-CAM has no amino acid sequence similarity to N-CAM, contains no polysialic acid, and, unlike N-CAM, its binding activity is calcium dependent. Recent studies indicate that the single genes for L-CAM and N-CAM are very different in structure (9, 22). At its $NH_2$ terminus, L-CAM has three contiguous regions of 113 amino acid residues that are homologous to one another. The remaining sequence includes an additional extracellular region, a transmembrane segment, and an intracellular domain of 151 amino acid residues (23). Trypsin digestion of cell membranes in the presence of calcium releases the soluble fragment Ftl, which contains the $NH_2$-terminal two-thirds of the molecule and the homophilic binding site (24, 25). Recent studies indicate that a number of other $Ca^{2+}$-dependent CAMs closely resemble L-CAM in amino acid sequence (26).

Electron microscopy of rotary shadowed mixtures of the ld and sd polypeptides of N-CAM (27, 28) has revealed rod-shaped molecules of uniform thickness. The rods are bent at

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Abbreviations: N-CAM, neural cell adhesion molecule; L-CAM, liver cell adhesion molecule; ld, large cytoplasmic domain; sd, small cytoplasmic domain; ssd, small surface domain; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate.

a single location and associate with one another to form rosettes with two to six branches. Because N-CAM molecules contain both a homophilic binding site and a hydrophobic membrane anchor, such rosettes might be formed either through specific interactions at their $NH_2$ termini or through hydrophobic association involving their membrane-spanning or cytoplasmic domains. Preliminary electron microscopic studies (27) and experiments on the effects of the detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) on the structure of N-CAM (see below) suggested that these rosettes formed through hydrophobic interactions. It was subsequently proposed (28) that rosettes form through their $NH_2$-terminal binding domains. Comparable data have not been obtained for L-CAM. We therefore pursued more detailed structural characterization of these molecules.

The results described here indicate that the N-CAM ld and sd forms that have been sprayed from dilute solution form rosettes through their cytoplasmic or transmembrane regions as originally proposed (27). In contrast, N-CAM molecules on cell surfaces bind to similar molecules on apposing cells through the immunoglobulin-like domains at their $NH_2$ termini (14). The $NH_2$-terminal region of N-CAM has dimensions consistent with a tandem array of unpaired immunoglobulin-like domains. L-CAM was also seen as a rod-shaped molecule with a flexible hinge; its binding domain also does not appear to form aggregates in solution. The presence of common structural traits, despite the chemical and functional differences between these two CAMs, suggests that the flexible hinge and rod-like shape have important roles in specific cell–cell adhesion.

## MATERIALS AND METHODS

Antibodies, N-CAM, its Fr2 proteolytic fragment, and the Ft1 fragment of L-CAM were prepared (14, 24, 29), and the identity of the Fr2 fragment was confirmed by $NH_2$-terminal amino acid sequence determination. The ssd polypeptide was prepared from adult chicken brains (7) by using a phosphatidylinositol-specific phospholipase C from *Bacillus thuringiensis* (provided by Martin G. Low of the College of Physicians and Surgeons, New York) that removes the lipid anchor from the glycosyl-phosphatidylinositol moiety (30). For electron microscopy, samples were further purified by density gradient sedimentation. Typically, 300 $\mu$l of concentrated eluate was layered onto a 12-ml gradient of 15–40% (vol/vol) glycerol in 0.20 M ammonium formate/1.0 mM Pipes, pH 7.2. Some gradients also contained 2.0% (vol/vol) CHAPS. For the purification of L-CAM Ft1, gradients contained 1 mM $CaCl_2$. Gradients were centrifuged at 20°C, either for 18 hr at 288,000 × *g* or for 22 hr at 218,000 × *g*. Fractions of 0.4–0.6 ml were collected and analyzed by $NaDodSO_4$/PAGE (31). CAM samples were incubated with purified antibodies for either 1 hr at room temperature or 24 hr at 4°C. Samples were sprayed onto mica and rotary shadowed with platinum (32). Micrographs were taken at a magnification of ×50,000. The locations of specific epitopes were assigned when between 30 and 100 examples of apparently specific binding were observed with no more than two exceptions. Each antibody labeled between 1 and 10% of the N-CAM molecules photographed, except for anti-N-CAM 12 which bound to 10–50% of the molecules. Molecular dimensions are averages of 30–100 equivalent measurements from ×250,000 prints of micrographs.

## RESULTS

Both the Fr2 fragment and the ssd polypeptide of N-CAM migrated in density gradients as homogeneous species at a slower velocity than the ld and sd polypeptides (Fig. 1). The

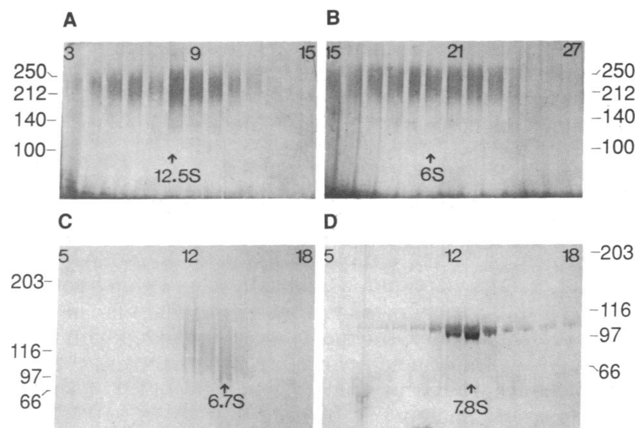


FIG. 1. $NaDodSO_4$/PAGE of fractions from density gradient centrifugation of CAMs. Molecular weight standards ($\times 10^{-3}$), gradient fraction numbers, and approximate S values are indicated. Lower-numbered fractions contain faster sedimenting components. (*A*) N-CAM ld/sd mixtures. Fractions 3–15 of 20 fractions. (*B*) N-CAM ld/sd mixture in 2% CHAPS. Fractions 15–27 of 30. (*C*) N-CAM Fr2. Fractions 5–18 of 20. (*D*) N-CAM ssd. Fractions 5–18 of 20.

ssd polypeptide, which was isolated from adult tissue by removing its lipid anchor, produced relatively sharp bands in $NaDodSO_4$/PAGE whereas the ld and sd chains gave the diffuse bands that are characteristic of the embryonic forms of N-CAM with their higher levels of polysialic acid (14). In the electron microscope, both the ssd chain and the Fr2 fragment appeared as discrete rod-shaped molecules that did not form aggregates (Fig. 2 *A* and *B*). Fr2 rods are 27.8 ± 0.4 nm (mean ± SEM) long and have a uniform thickness of 4–5 nm; the ssd chain is also 27.8 ± 0.4 nm long with a similar thickness. Approximately one-half of the Fr2 molecules are bent, producing a longer arm of 18.7 ± 0.5 nm and a shorter arm of 9.2 ± 0.3 nm. Virtually all ssd molecules are bent into similar arms, 17.6 ± 0.4 nm and 10.1 ± 0.2 nm long. The hinge between these arms is apparently flexible: angles between them ranged from 50° to 140°, with an average value of 98° (standard deviation, 17°).

The L-CAM Ft1 fragment, like the N-CAM ssd chain and Fr2 fragment, was seen as a bent rod-shaped molecule that did not form aggregates (Fig. 2*C*). The molecules are 28.4 ± 0.4 nm long, with arms that are 17.9 ± 0.2 and 10.5 ± 0.2 nm long. The hinge in this molecule is also apparently flexible

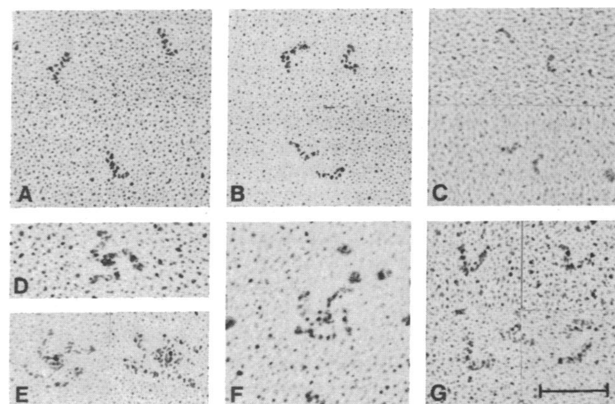


FIG. 2. Electron micrographs of CAMs. (*A*) N-CAM Fr2. (*B*) N-CAM ssd. (*C*) L-CAM Ft1. (*D*) N-CAM ld/sd. (*E*) N-CAM ld/sd showing prominent globular region at center of rosette. (*F*) N-CAM ld/sd with nearby Fab′ fragments. (*G*) N-CAM ld/sd mixture in 2% CHAPS. (Bar = 50 nm.)

with an average angle between arms of 101° (standard deviation, 25°).

Electron microscopy of mixtures of the ld and sd chains in the absence of detergent (Fig. 2*D*) revealed rosettes of rod-shaped molecules like those that have been observed (27, 28). Some of these rosettes had globular regions at their centers (Fig. 2*E*) and, in some gradients, fractions corresponding to higher sedimentation velocities had more and larger globular regions than did slower sedimenting fractions. In contrast to other structural features of N-CAM, the globular regions were quite variable in size: within fraction 4 (Fig. 1*A*) these regions vary from 100 to 400 $nm^2$ in area. When replicas of Fab′ fragments were compared with those of N-CAM within single fields (Fig. 2*F*), the average thickness of N-CAM replicas (including shadowed Pt), $4.4 \pm 0.6$ nm was approximately one-half that of Fab′, $9.5 \pm 1.2$ nm, suggesting that the N-CAM molecules contain unpaired domains.

Rosettes were disrupted by detergent treatment. When the ld and sd polypeptides were purified on density gradients containing 2.0% CHAPS, they sedimented more slowly than in detergent-free gradients (Fig. 1 *A* and *B*). This treatment also produced a significant change in the appearance of these molecules: they were single rods $42.6 \pm 10.9$ nm long and virtually no aggregates were apparent (Fig. 2*G*). Treatment with 0.1% Triton X-100, a much milder detergent, also disrupted rosettes completely, as demonstrated by sedimentation (data not shown).

Specific points of reference within the structures of the various forms of N-CAM were located by observing their complexes with specific monoclonal antibodies. It was difficult to identify unambiguously antibodies bound at or near the center of ld/sd rosettes because this region is extremely constricted and frequently contains globular regions that can be confused with immunoglobulin molecules (Fig. 2*E*). To avoid misinterpretation, we selected an antibody that clearly marks the distal ends of the branches of rosettes and characterized its epitope. We then located additional specific landmarks within the ssd form and the Fr2 fragment because these molecules have no globular or constricted regions and thus allow clear observation of bound antibodies.

To mark the $NH_2$-terminal region of N-CAM, we utilized monoclonal antibody anti-N-CAM 12, which blocks specific N-CAM to N-CAM binding (5). To localize its epitope, N-CAM was allowed to autolyze (29), and the resulting mixture was passed over an anti-N-CAM 1 (see below) affinity column to remove large fragments; material that did not bind to this column was then purified on an anti-N-CAM 12 affinity column. A 13-kDa peptide from this second eluate was purified by gel filtration. Its $NH_2$-terminal amino acid sequence was identical to the $NH_2$ terminus of N-CAM. The antibody thus binds within the $NH_2$-terminal 13 kDa of N-CAM. In electron micrographs, anti-N-CAM 12 bound at the distal ends of the branches of the ld/sd rosettes and the divalent antibody frequently cross-linked the ends of adjacent branches (Fig. 3*A*). This antibody also bound at the ends of the longer arms of the ssd rods (Fig. 3 *B* and *C*) and at the ends of the Fr2 rods (Fig. 3*D*).

Within the ssd polypeptide, the monoclonal antibody anti-N-CAM 1, which also blocks specific N-CAM to N-CAM binding and whose epitope appears to be in the third domain of all forms of N-CAM (5), bound in the longer arm (Fig. 3*E*). Monoclonal antibody anti-N-CAM 2, which is specific for the polypeptide near the point of polysialic acid attachment (16), bound near the bend in the ssd polypeptide (Fig. 3*F*).

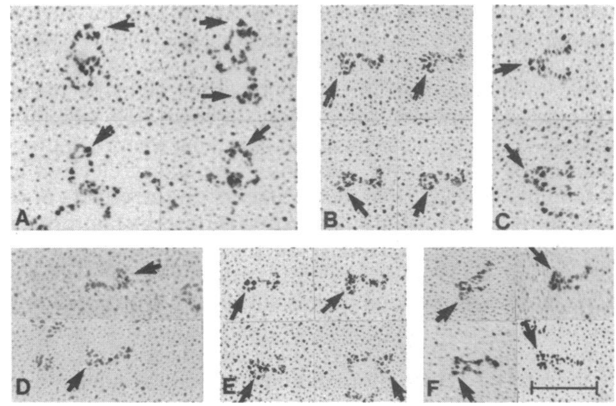


FIG. 3. Electron micrographs of complexes between N-CAM and antibodies. Arrows indicate bound antibody molecules. (*A*) N-CAM ld/sd rosettes and monoclonal antibody anti-N-CAM 12. (*B*) N-CAM ssd and anti-N-CAM 12. (*C*) Two N-CAM ssd molecules bound by a single anti-N-CAM 12 molecule. (*D*) N-CAM Fr2 and anti-N-CAM 12. (*E*) N-CAM ssd and monoclonal antibody anti-N-CAM 1. (*F*) N-CAM ssd and monoclonal antibody anti-N-CAM 2. (Bar = 50 nm.)

## DISCUSSION

The results presented here provide a view of two cell surface glycoproteins, N-CAM and L-CAM, that are exemplars of two distinct families of CAMs. They also clarify earlier views of the structure of N-CAM by demonstrating that the N-CAM rosettes seen in the electron microscope are formed through their cytoplasmic or transmembrane domains, as originally proposed (27), and not through association of their $NH_2$ termini (28). We have also developed a working model of the molecular structures of the various forms of N-CAM (Fig. 4*A*). This model is consistent with the idea (5, 13) that N-CAM resembles other members of the immunoglobulin superfamily (33) in three-dimensional structure as well as in amino acid sequence, and with the suggestion that a gene for an N-CAM precursor was the evolutionary origin of the entire immunoglobulin superfamily (13). The results also suggest how the structural features of N-CAM are utilized in the modulation of cell–cell binding.

The combined antibody-labeling, structural, and detergent data indicate that the rosettes formed in solution by the ld and sd forms of N-CAM have their COOH termini at the centers of the assemblies and that the homophilic binding regions are at the distal ends of the molecular branches (Fig. 4*B*). The structure proposed by Hall and Rutishauser (28), in which the rosettes form by association of the $NH_2$ termini (Fig. 4*C*) is inconsistent with all three lines of evidence. Anti-N-CAM 12 binds at the distal ends of the branches of ld/sd rosettes. Moreover, this divalent antibody frequently cross-links the ends of adjacent branches within a rosette (Fig. 3*A*) demonstrating that this binding is to a specific epitope that is present in each molecular branch. Because this antibody marks the $NH_2$-terminal 13 kDa of N-CAM and blocks homophilic binding, these observations clearly localize that site in the distal ends of the rosette branches.

This conclusion is also supported by comparing the structures of the ld and sd polypeptides of N-CAM with those of the ssd chain and the Fr2 fragment. All four molecules have the same amino acid sequence from their $NH_2$ termini to residue 682, a span that includes the homophilic binding site. If N-CAM rosettes were formed by association of the $NH_2$-terminal regions, all four species of N-CAM would make such assemblies, albeit of different sizes. Rosettes are formed, however, only by ld and sd, the two polypeptides that contain transmembrane and cytoplasmic domains. The lipid anchor of the ssd chain was removed in these experiments, eliminating possible rosette formation involving this lipid. These results indicate that the formation of rosettes is dependent on the presence of transmembrane or cytoplasmic domains.

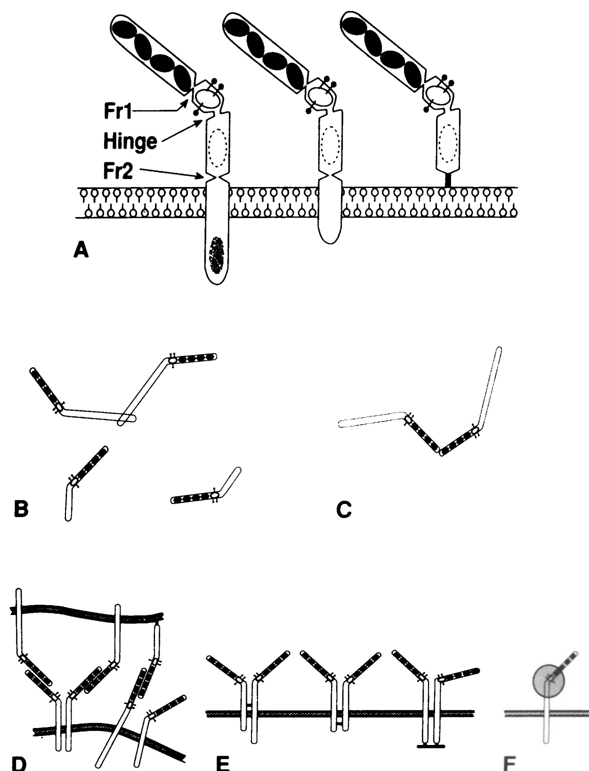


FIG. 4. Model of N-CAM and N-CAM-mediated cell adhesion. All forms of N-CAM contain regions of sequence homology to immunoglobulin domains (ovals) and a homophilic binding domain (dark color). (*A*) N-CAM on a cell membrane. The ld (to the left) and sd (in the center) forms are integral membrane proteins, while the ssd form (to the right) is attached to the membrane by a phosphatidylinositol linkage (■). ld contains a unique insert (stippled) in its cytoplasmic domain. All forms contain a flexible hinge 10 nm from the membrane, three attachment sites for polysialic acid (♀) near the hinge, and regions of homology to fibronectin (dashed ovals). Sites of proteolytic cleavages that make the Fr1 and Fr2 fragments are indicated. (*B*) In solution, N-CAM ld and sd form rosettes through their cytoplasmic and/or transmembrane regions, while ssd and Fr2 do not form aggregates. The specific point of intermolecular contact may be in the transmembrane region, farther up the molecule, rather than in the cytoplasmic region as shown. (*C*) A model of N-CAM rosettes that places their binding domains at the rosette centers (28) is inconsistent with the data presented here. (*D*) Arrays of N-CAM molecules on one cell interact with similar arrays on apposing cells. The flexible hinge in N-CAM allows specific intermolecular contacts to be made even when cell surfaces are in motion or are not complementary in shape. (*E*) N-CAM arrays may be formed by interactions between molecules on the same cell. Sites for such interaction could be extracellular (to the left), intracellular (in the center), or through cytoskeletal structures (to the right). (*F*) The presence of large amounts of polysialic acid (shaded circle) in some forms of the molecule may hinder intercellular adhesion by preventing the formation of arrays and by restricting the flexibility of the hinge region.

The observation that detergent treatment converts ld/sd rosettes into single molecules also suggests that these structures are held together by nonspecific hydrophobic interactions. If N-CAM rosettes were formed through the homophilic binding sites, they might be disrupted by detergent treatment because CHAPS can disrupt specific interactions (34). However, one of us (H.P.E.) participated in a study that showed (35) that, in the absence of detergent, the platelet integral membrane glycoprotein complex IIb–IIIa forms rosettes with membrane anchors inserted into central globular regions of variable size, but appears as single molecules in the presence of detergent. Similar rosettes are formed by other membrane proteins, including the neuraminidase and the hemagglutinin from influenza virus, and these assemblies were also disrupted by detergent (36).

Our results are consistent with a close relationship between N-CAM and other molecules in the immunoglobulin superfamily in terms of three-dimensional structure. All forms of N-CAM contain, at their $NH_2$ termini, five domains that are homologous in sequence both to one another and to the domains of immunoglobulins (5). The three-dimensional structures of such domains have been determined by x-ray crystallography in antibodies (37, 38), $\beta_2$-microglobulin (39), and a class I histocompatibility antigen (40). In every case, the polypeptide chains are folded into the $\beta$-barrel configuration, forming compact ellipsoidal structures approximately $4.0 \times 2.5 \times 2.5$ nm in size. If the immunoglobulin-like domains of N-CAM are folded into such units, each N-CAM chain could contain a tandem array of five domains making up a cylindrical molecule of approximately $20 \times 2.5 \times 2.5$ nm. Such a structure fits well within the longer arms of the ssd chain and Fr2 fragment and the distal arms of ld and sd polypeptides (Fig. 4*A*). In known immunoglobulin structures, domains are paired with extensive areas of interdomain contact (37, 38, 40). However, N-CAM appears to be half as thick as an Fab′ fragment (Fig. 2*F*), suggesting that the immunoglobulin-like regions of N-CAM are not paired. Domain pairing between apposing molecules may thus play a role in N-CAM-mediated cell–cell binding (13).

The images of N-CAM described in this study are of molecules that were sprayed onto surfaces from dilute solution. Such an environment is much different from the cell surfaces where N-CAM is normally expressed. Viewed from the outside, a cell presents a two-dimensional array of apparently similar N-CAM molecules: 30-nm bent rods with homophilic binding domains at their distal ends, a flexible molecular hinge where polysialic acid is attached, and a region resembling the cell-binding domain of fibronectin between the hinge and the cell surface. The site of the cleavage that produces Fr2 is 9 nm from the hinge, while the Fr1 cleavage site is assumed to be within the distal arm, based on the molecular weight of this fragment. The ld and sd forms penetrate the membrane, while the ssd polypeptide is attached to the cell surface by a phosphatidylinositol linkage. On the cytoplasmic side of the membrane, there is a significantly different molecular array: ld molecules have cytoplasmic domains over three times the size of those of sd molecules, whereas the ssd polypeptide has no intracellular domain (Fig. 4*A*). Cell–cell binding presumably occurs when an extracellular array of CAMs interacts with a complementary array on an apposing cell.

In the images seen in these studies, isolated N-CAM and L-CAM molecules do not appear to interact strongly through their homophilic binding regions. This suggests that multivalence is a necessary factor in N-CAM- and L-CAM-mediated adhesion. Thus, our results indicate that the binding of isolated CAMs in dilute solution is weak, but the affinity of cellular arrays of CAMs can be strong. In support of this proposal, both the amount and the chemical nature of the N-CAM on cells and in vesicles have profound and nonlinear effects on cell adhesion: the rate of adhesion of reconstituted vesicles shows a fifth-order dependence on the concentration of N-CAM, and removal of polysialic acid induces a 4-fold increase in aggregation (29).

Three prominent features of the molecular structure of N-CAM, as it appears on cells, can modulate cell–cell binding. (*i*) The flexible hinge in the extracellular part of the molecule may permit and maintain specific interactions between cells whose surfaces are in constant motion and may not have complementary shapes (Fig. 4*D*). (*ii*) The distinct intracellular domains of the ld and sd polypeptides may form specific interactions with the cytoskeleton or other cytoplasmic structures, which could modulate the local concentration

or arrangement of CAM molecules to make local binding domains on apposing cells multivalent and complementary (Fig. 4*E*). This type of modulation may be particularly significant in understanding why the binding domains of N-CAM and L-CAM do not aggregate when sprayed from dilute solution, even though they do bind to one another when they are attached to cell membranes (1, 2). (*iii*) The polysialic acid at the molecular hinge can add a further level of control on the effects of both of these features. The large amounts of polysialic acid seen in embryonic N-CAM might render the hinge less flexible, with a consequent hindrance of intercellular adhesion. In addition, high local concentrations of this large, highly charged moiety near the membrane attachment site may interfere with the formation of ordered CAM arrays (Fig. 4*F*). Consideration of these details should be useful in comparing the activities of other cell surface proteins [e.g., myelin-associated glycoprotein (41), L1 (42), and intracellular (I)-CAM (43)] that are homologous to N-CAM but have different numbers of immunoglobulin-like domains, different cytoplasmic regions, and lack polysialic acid.

In more general terms, the observation that the size and shape of N-CAM is echoed in the Ftl fragment of L-CAM suggests that certain structural characteristics are required for homophilic binding. Despite the fact that L-CAM has no similarity in amino acid sequence or binding specificity to N-CAM and has no unusual carbohydrate groups, images of its binding fragment suggest that it is a rod-shaped molecule of the same general size and shape as N-CAM and that it contains a flexible hinge. A number of $Ca^{2+}$-dependent CAMs resemble L-CAM in amino acid sequence (26, 44), suggesting that the structural features described here may be present in all CAMs of this family. Rod-like structures with extracellular domains 30 nm or longer and containing flexible hinges may thus be characteristic of a broad class of CAMs and may represent a general evolutionary solution to the problem of specific cell–cell adhesion.

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