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A proteoglycan with HNK-1 antigenic determinants is a neuron-associated ligand for cytotactin

(cell–cell adhesion/cell–substratum adhesion/extracellular matrix/glial cells/embryogenesis)

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ABSTRACT **Cytotactin is an extracellular matrix protein that is involved in neuron–glia adhesion and is found in both neural and nonneural sites. It is synthesized by glia but not by neurons. In this study, we have examined the binding of cytotactin to a variety of extracellular matrix components using uniform microscopic beads (Covaspheres) that could be labeled and then linked to purified molecules. Cytotactin-coated beads bound well to neurons, and this binding was strongly inhibited by anti-cytotactin antibodies but not by anti-neural cell adhesion molecule (anti-N-CAM) antibodies. In contrast, the binding of N-CAM-coated beads to neurons was inhibited by anti-N-CAM antibodies and not by anti-cytotactin antibodies. To identify a neuronal ligand for cytotactin, we tested several molecules for their ability to block the binding of cytotactin-coated beads to cells. A proteoglycan-containing fraction that copurified with cytotactin from brain extracts strongly inhibited binding, whereas neither a heparan sulfate proteoglycan from Engelbreth-Holm–Swarm tumor cells nor soluble cytotactin itself had a significant inhibitory effect. The neural proteoglycan also inhibited the binding of cytotactin-coated beads to fibroblasts. Digestion with chondroitinase, heparitinase, and hyaluronidase as well as immunological analyses suggested that the predominant species in the active fraction was a chondroitin sulfate proteoglycan with a M_r 280,000 core protein bearing HNK-1 antigenic determinants and also indicated that hyaluronic acid was present in this fraction. In experiments on *in vitro* synthesis, it was found that the proteoglycan was synthesized in culture by embryonic chicken brain tissue but not by embryonic chicken glial cells. A series of binding experiments was performed on appropriately derivatized beads to confirm that the proteoglycan is a ligand for cytotactin and to check for the possibility that other extracellular matrix proteins might interact with one or the other member of this binding couple. Proteoglycan-coated beads and cytotactin-coated beads coaggregated readily. The aggregation was inhibitable by anti-cytotactin antibodies, soluble cytotactin, or soluble proteoglycan. Addition of laminin inhibited the binding of cytotactin-coated beads to proteoglycan-coated beads or to cells; this is consistent with data indicating that laminin interacts with a component of the proteoglycan-containing fraction. In contrast, fibronectin bound to cytotactin, but it did not bind to proteoglycan or interfere with the binding of cytotactin to proteoglycan. The results of this study are in accord with the idea that the functions of extracellular matrix components during neural and nonneural development may be modulated both by competition for shared cell surface receptors and by a network of molecular interactions among the matrix components themselves.**

Morphogenesis in higher organisms depends upon the properties of cell collectives that are maintained by molecular interactions between apposing cells and between cells and the surrounding extracellular matrix. A number of molecules involved in cell–cell (1) and cell–substratum (2, 3) adhesion have been identified using various adhesion assays *in vitro*. Although the structures and biological distributions of several of these molecules have been characterized to various extents (1–3), the interpretation of their functional roles during development *in vivo* is complicated by the presence of multiple adhesive molecules interacting in various ways at different locales.

A comparison of the extracellular matrix (ECM) proteins fibronectin, laminin, and the recently discovered cytotactin clearly illustrates the need to resolve the differential contributions to morphogenesis of substrate adhesion molecules that are present in overlapping spatial distributions *in vivo*. For example, cytotactin, laminin, and fibronectin are all found together in locations such as neural crest pathways and in a variety of basement membranes (4). In general, laminin and fibronectin are more widely distributed than cytotactin in the embryo (4). However, in the central nervous system, cytotactin is present at high levels in a broad distribution (4, 5) whereas laminin is present only at certain sites (6), and (excluding the vascular component) fibronectin may not be present at all. Both laminin and fibronectin bind to a cell surface protein of M_r 140,000 (7), to heparan sulfate proteoglycan (2, 3), and to various forms of collagen (2, 3). In addition, laminin binds to a surface protein of M_r 67,000 (8). Although cytotactin is known to mediate neuron–glia adhesion and to be synthesized by glia (5), no ligand has heretofore been found for cytotactin.

In the present study, we have evaluated the ability of microscopic beads (Covaspheres) coated with cytotactin to bind to neurons or to other beads coated with putative ligands for cytotactin. Using this assay, we have identified a neuronal proteoglycan that is a ligand for cytotactin. Other matrix proteins were found to have differential effects on the interaction of cytotactin and proteoglycan in accord with the notion that the function of components of the extracellular matrix may be modulated by a network of differential synthesis and binding interactions at different embryonic sites.

MATERIALS AND METHODS

Preparation of Cytotactin and Proteoglycan and Characterization of Proteins. Buffer extracts without detergent were prepared from 14-day embryonic chicken brains and were incubated with monoclonal antibody HNK-1 bound to Sepharose CL-2B as previously described (5). A mixture containing essentially only cytotactin and proteoglycan was eluted from the HNK-1 beads with phosphate-buffered saline/0.375 M NaCl/1 mM EDTA and was further fractionated by immunoaffinity chromatography using monoclonal antibody 1D8 (which is specific for a polypeptide determinant in cytotactin) bound to Sepharose CL-2B. The 1D8 beads were

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Abbreviations: ECM, extracellular matrix; N-CAM, neural cell adhesion molecule.

eluted with 50 mM diethylamine, pH 11.5/1 mM EDTA, and the eluate was neutralized. The unbound fraction (proteoglycan) and eluted fraction (cytotactin) were then dialyzed against H_2O, lyophilized, and desalted by gel filtration in phosphate-buffered saline on Sepharose CL-6B. The concentration of proteoglycan was determined by uronic acid analysis (9) using chondroitin 6-sulfate as the standard.

Fibronectin and laminin were obtained from the New York Blood Center and GIBCO, respectively. Levels of uronic acid (9) in these preparations were below detection; this suggests that the functions attributed to fibronectin and laminin in these studies are not due to proteoglycans present in the preparations.

Preparation and Use of Covaspheres. To couple proteins covalently to Covaspheres, 100 μl aliquots of Covaspheres (Duke Scientific, Palo Alto, CA) were separately incubated for 1 hr at room temperature with 50 μg of cytotactin, 15 μg of cytotactin (low level), 100 μg of ovalbumin, 100 μg of bovine serum albumin, 100 μg of neural cell adhesion molecule (N-CAM), 15 μg of proteoglycan, 100 μg of laminin, or 50 μg of fibronectin. When radioiodinated Covaspheres were needed, 10% of the protein was labeled by using chloramine T (10). To free Covaspheres from unbound protein and to block unused reactive sites, Covaspheres were subjected to two rounds of centrifugation in a microfuge and resuspension in 1 ml of bovine serum albumin (5 mg/ml) in phosphate-buffered saline/10 mM sodium azide. Covaspheres were used in binding experiments as described in the table legends.

Biosynthesis of Cytotactin and Proteoglycan. Glial cell cultures (11), each containing 1×10^7 cells per 100-mm dish, and brain organ cultures (12), each containing 10 embryonic chicken brains (9-day), were radioactively labeled with 1 mCi (1 Ci = 37 GBq) of [^{3}H]leucine or $^{35}SO_4$. Cytotactin and proteoglycan were purified from Nonidet P-40 extracts of these cultures by using protein A-Sepharose coated with a rabbit antibody prepared against the cytotactin–proteoglycan complex.

RESULTS

Binding Properties of Cytotactin. To investigate the ability of cytotactin to bind to cells, the purified molecule was coupled to 0.5-μm beads (Covaspheres), and the ability of these beads to bind to neurons was evaluated. Several observations (Table 1) suggest that cytotactin-coated beads were bound to neurons by a specific molecular mechanism. Cytotactin-coated beads bound to a much greater extent than did beads coated with control proteins such as ovalbumin or bovine serum albumin. The ability of cytotactin-coated beads to bind to neurons was dependent on the cytotactin concentration on the beads and was ablated by boiling or treating the derivatized beads with trypsin. Binding to neurons by means of N-CAM was used as a specific control. The binding of cytotactin-coated beads was strongly inhibited by Fab' fragments of anti-cytotactin antibodies but not by Fab' fragments of anti-N-CAM antibodies. In contrast, the binding of N-CAM-coated beads to neurons was inhibited by anti-N-CAM Fab' fragments but not by anti-cytotactin Fab' fragments.

Identification of a Ligand for Cytotactin. In a previous study (5), material detectable with the dye StainsAll (Eastman) and containing high levels of uronic acid was found to copurify with cytotactin. This putative proteoglycan and cytotactin were separated by immunoaffinity chromatography using a monoclonal antibody specific for cytotactin polypeptides. The bound and subsequently eluted fraction contained essentially all of the cytotactin in the preparation (Fig. 1, lane 1), little StainsAll-positive material (lane 3), and only 5% of the uronic acid as determined in colorimetric assays (9); the unbound fraction contained almost no cytotactin (lane 2), high levels of StainsAll-positive material (lane 4), and 95% of the uronic acid.

Table 1. Binding of protein-coated Covaspheres to neurons

Protein on Covaspheres	Fab' fragments	% input bound	% inhibition
Cytotactin		4.9 ± 0.5	
Ovalbumin		0.5 ± 0.1	
Bovine serum albumin		0.4 ± 0.1	
Cytotactin: low level		1.3 ± 0.2	
Cytotactin: boiled*		1.5 ± 0.3	
Cytotactin: trypsinized†		0.9 ± 0.2	
Cytotactin	Non-immune	4.8 ± 0.4	—
Cytotactin	Anti-cytotactin	0.6 ± 0.1	87
Cytotactin	Anti-N-CAM	4.5 ± 0.5	6
N-CAM	Non-immune	3.8 ± 0.3	—
N-CAM	Anti-cytotactin	3.7 ± 0.4	3
N-CAM	Anti-N-CAM	2.2 ± 0.4	42

Covaspheres bearing radiolabeled proteins were diluted 1:5 in medium and were sonicated for 20 s in a bath sonicator; 100-μl aliquots were incubated at 25°C with 1 mg of Fab' fragments. After 10 min, 1×10^7 neurons (in 200 μl of medium containing 5 mg of bovine serum albumin per ml) prepared by the light trypsin-EDTA method (13) were added to each aliquot of Covaspheres and further incubated 20 min. The cells were then separated from unbound Covaspheres by centrifugation through 3.5% bovine serum albumin in medium. Covaspheres bound to cells were quantitated by gamma spectroscopy (average of duplicate points ± SD).
*Cytotactin-coated Covaspheres were boiled for 1 min prior to use.
†Cytotactin-coated Covaspheres were treated with 1 mg of trypsin per ml for 10 min at 25°C followed by 1.5 mg of soybean trypsin inhibitor per ml.

To investigate the possibility that the cytotactin-associated proteoglycan may have been the ligand for cytotactin on cells, the abilities of the soluble proteoglycan and of several ECM molecules to perturb the binding of cytotactin-coated beads to neurons were compared. Soluble proteoglycan strongly inhibited binding at low doses (Table 2). In contrast, fibronectin and a heparan sulfate proteoglycan had no effect on the binding of cytotactin-coated beads to neurons, and soluble cytotactin itself had little effect (Table 2). Unlike fibronectin, laminin inhibited the binding of cytotactin-coated beads to cells. The same ECM molecules were tested for their ability to perturb the binding of N-CAM-coated

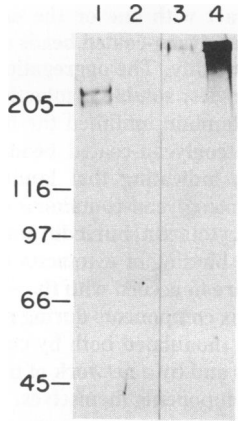

FIG. 1. Electrophoretic comparison of cytotactin and proteoglycan preparations. One-two-hundredth of the total isolated cytotactin (lanes 1 and 3) or proteoglycan (lanes 2 and 4) in a preparation from 2000 embryonic chicken brains (14-day) was resolved in a $NaDodSO_4$/6% polyacrylamide gel (14) and detected using Coomassie blue stain (lanes 1 and 2) or StainsAll stain (15) (lanes 3 and 4). StainsAll stains polyanions such as glycosaminoglycans an intense blue or purple color while it less intensely stains proteins pink. The migration and $M_r \times 10^{-3}$ of standard proteins are indicated.

Table 2. Competition by soluble molecules of Covasphere to neuron binding

Protein on Covaspheres	Soluble protein*	% input bound	% inhibition
Cytotactin	None	4.7 ± 0.2	—
Cytotactin	Cytotactin	3.9 ± 0.3	17
Cytotactin	Proteoglycan	1.2 ± 0.2	74
Cytotactin	HS proteoglycan†	4.4 ± 0.3	6
Cytotactin	Fibronectin	5.3 ± 0.3	(13)‡
Cytotactin	Laminin	2.1 ± 0.2	55
N-CAM	None	3.6 ± 0.3	—
N-CAM	Cytotactin	3.4 ± 0.3	6
N-CAM	Proteoglycan	3.4 ± 0.1	6
N-CAM	HS proteoglycan†	3.5 ± 0.2	3
N-CAM	Fibronectin	3.6 ± 0.1	0
N-CAM	Laminin	3.8 ± 0.2	(6)‡
N-CAM	N-CAM	1.7 ± 0.1	53

Covasphere to neuron binding was performed as described in Table 1.
*Covaspheres were preincubated with 20 μg of N-CAM, fibronectin, or laminin or 10 μg of cytotactin or proteoglycan.
†HS proteoglycan, saline-extracted heparan sulfate proteoglycan from Engelbreth-Holm–Swarm tumor cells (16).
‡Promotion.

beads to cells (Table 2), but none had any effect. Consistent with the idea that N-CAM-mediated adhesion is homophilic (N-CAM to N-CAM), only soluble N-CAM inhibited the binding of N-CAM-coated beads to cells.

Direct Demonstration of Cytotactin–Proteoglycan Binding. To assay directly for adhesion between cytotactin and its associated proteoglycan, the purified molecules were individually bound to separate batches of beads. When the two types of beads were coincubated, aggregates rapidly formed (Table 3). In contrast, little or no aggregation occurred among pure populations of cytotactin-coated beads or proteoglycan-coated beads. Anti-cytotactin antibodies, cytotactin itself, and proteoglycan all strongly inhibited the binding between cytotactin-coated beads and proteoglycan-coated beads (Table 3).

Covasphere aggregation was also used to evaluate further the possibilities that laminin and fibronectin may bind to cytotactin, to proteoglycan, or to both molecules. Soluble laminin inhibited the binding of cytotactin-coated beads to proteoglycan-coated beads (Table 3) whereas fibronectin had little effect. These results are in accord with the effects of laminin and fibronectin on the binding of cytotactin-coated beads to cells (Table 2). The effect of laminin on cytotactin–proteoglycan binding appears to be due to an interaction between laminin and a component of the proteoglycan fraction rather than to an interaction with cytotactin. Soluble laminin was found to aggregate proteoglycan-coated beads while soluble fibronectin had no effect; conversely, soluble proteoglycan aggregated laminin-coated beads while soluble fibronectin or cytotactin had little effect. The specificity of this interaction was further suggested by the observation that soluble laminin had little effect on cytotactin-coated beads or bovine serum albumin-coated beads. When laminin-coated beads or fibronectin-coated beads were incubated with cytotactin-coated beads or proteoglycan-coated beads, significant binding was observed only in the fibronectin–cytotactin pairing (Table 3). This indicates that, although fibronectin does not block cytotactin–proteoglycan binding, it nevertheless binds to cytotactin. The failure of laminin and proteoglycan to bind when both molecules are immobilized on beads may be due to steric effects that do not come into play when only one of the molecules is immobilized.

Biosynthesis of Proteoglycan. Cytotactin is synthesized in the brain primarily by glial cells (5). If proteoglycan is the ligand for cytotactin in neuron–glia adhesion, one would expect it to be synthesized by neurons. To evaluate this possibility, the abilities of cultured glial cells (>95% glia) and cultured brain fragments (primarily neurons) to synthesize these molecules were compared. When cultures were incubated with [^{3}H]leucine, a glial culture containing 1×10^7 cells synthesized a higher level of cytotactin than did an organ culture containing ten 9-day embryonic brains (Fig. 2, compare lanes 1 and 2). In contrast, in sister cultures labeled with $^{35}SO_4$, high levels of incorporation into proteoglycan were obtained in the brain organ culture, but incorporation into proteoglycan was below detection in the glial cell culture (compare lanes 3 and 4). While these experiments do not completely rule out the possibility that glial cells synthesize proteoglycan in the presence of neurons or that glial cells synthesize an unsulfated form of the proteoglycan, it appears that the type of proteoglycan examined here is predominantly synthesized by neurons.

Table 3. Quantitative analysis of Covasphere–Covasphere aggregation

Protein(s) on Covaspheres	Soluble protein	Superthreshold particles*
Cytotactin		1,660 ± 140
Proteoglycan		420 ± 60
Cytotactin and proteoglycan		49,500 ± 2,100
Cytotactin and proteoglycan	Anti-cytotactin	2,480 ± 310
Cytotactin and proteoglycan	Cytotactin	11,800 ± 570
Cytotactin and proteoglycan	Proteoglycan	14,700 ± 1,100
Cytotactin and proteoglycan	Laminin	1,690 ± 250
Cytotactin and proteoglycan	Fibronectin	44,800 ± 2,300
Proteoglycan	Laminin	17,800 ± 800
Proteoglycan	Fibronectin	450 ± 40
Laminin		550 ± 80
Laminin	Proteoglycan	5,900 ± 600
Laminin	Fibronectin	800 ± 110
Laminin	Cytotactin	980 ± 70
Cytotactin	Laminin	1,480 ± 130
Bovine serum albumin		570 ± 60
Bovine serum albumin	Laminin	920 ± 80
Fibronectin		250 ± 60
Cytotactin and fibronectin		17,300 ± 2,200
Cytotactin and laminin		650 ± 40
Proteoglycan and fibronectin		320 ± 20
Proteoglycan and laminin		610 ± 30

Covaspheres (either 80 μl coated with a single protein or a mixture of two 40-μl aliquots each coated with an individual protein) were sonicated for 20 s in a bath sonicator, then they were incubated for 3 hr at 25°C with 20 μl of phosphate-buffered saline or the indicated soluble proteins (anti-cytotactin Fab', 200 μg; cytotactin, 2 μg; proteoglycan, 2 μg; fibronectin, 20 μg; laminin, 20 μg) in 20 μl phosphate-buffered saline. Finally, the samples were diluted to 20 ml, and 0.5-ml aliquots were analyzed in a Coulter Counter model ZBI with a 100-μm aperture set at amplification = 1/4, aperture current = 1/8, threshold = 10 to 100 (these settings should count aggregates larger than 3.84 μm^3, which is equivalent to an aggregate of 60 Covaspheres).
*Average ± SD of duplicate points.

Partial Characterization of the Proteoglycan. In the above studies, the uronic acid-containing macromolecular fraction that copurifies with cytotactin was tentatively identified as a proteoglycan in nature because it contains about 1.5 μg of glycosaminoglycans per μg of protein. To characterize this material further, the cytotactin–proteoglycan complex was treated with chondroitinase, hyaluronidase, or heparitinase. In immunoblotting experiments, rabbit antibodies prepared against the cytotactin–proteoglycan complex detected only cytotactin in control, heparitinase-treated, and hyaluronidase-treated samples (Fig. 3, lanes 1–3). However, in a chondroitinase-treated sample, an additional M_r 280,000

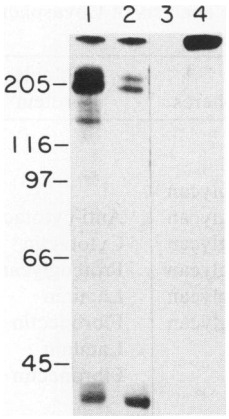

FIG. 2. Biosynthesis of cytotactin and proteoglycan in glial cell cultures and brain organ cultures. Glial cell monolayers (lanes 1 and 3) or fragments of brain tissue (lanes 2 and 4) were cultured (11, 12) in the presence of [^{3}H]leucine (lanes 1 and 2) or $^{35}SO_4$ (lanes 3 and 4). A rabbit antibody prepared against the cytotactin–proteoglycan complex was used to immunoaffinity-purify cytotactin and proteoglycan from Nonidet P-40 extracts of these cultures, and equivalent aliquots were resolved in a $NaDodSO_4$/7.5% polyacrylamide gel (14) and detected by fluorography. The migration and $M_r \times 10^{-3}$ of standard proteins are indicated.

component was detected (lane 4), which was also strongly recognized by monoclonal antibody HNK-1 (lane 5). Similar results were obtained with the purified proteoglycan fraction free of cytotactin used in the binding experiments described above; i.e., although no immunoreactive material was detected in the untreated sample, chondroitinase treatment revealed a M_r 280,000 molecule (data not shown). These results suggest that the proteoglycan that binds to cytotactin is a chondroitin sulfate proteoglycan with a core protein of M_r 280,000. They also indicate that the intact form of this proteoglycan, which contains HNK-1 antigenic determinants, does not transfer well to nitrocellulose probably because of its large size or charge.

StainsAll staining of $NaDodSO_4$ gels of the enzymatic

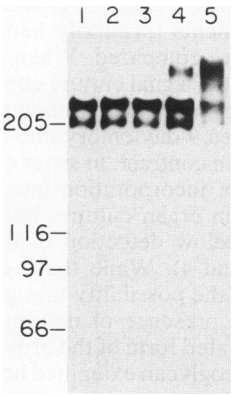

FIG. 3. Immunological detection of cytotactin and chondroitin sulfate proteoglycan. Equal aliquots of the cytotactin–proteoglycan complex (see *Methods*) containing 0.5 μg of cytotactin were incubated with no enzyme (lane 1), 0.5 unit of heparitinase (lane 2), 1 unit of hyaluronidase (lane 3), or 0.05 unit of chondroitin ABC lyase (lanes 4 and 5) for 2 hr at 37°C. Samples were then resolved in $NaDodSO_4$/6% polyacrylamide gels (14) and detected by immunoblotting (17) using a rabbit antibody prepared against the cytotactin–proteoglycan complex (lanes 1–4) or the monoclonal antibody HNK-1 (lane 5). All enzymes were obtained from Miles Laboratories (Naperville, IL), and the units of enzyme activity are as defined in their catalog. The migration and $M_r \times 10^{-3}$ of standard proteins are indicated.

digests described above showed that untreated and heparitinase-treated samples were similar in staining pattern to the proteoglycan sample shown in Fig. 1, lane 4. As expected from the immunoblot analysis described above, chondroitinase treatment removed much of the StainsAll-positive material. Nonetheless, StainsAll-positive material, which was not destroyed by heparitinase treatment or further chondroitinase treatment, remained; this residual material was destroyed by hyaluronidase treatment. These observations suggest that hyaluronic acid may be a part of the cytotactin–proteoglycan complex, which raises the possibility that it may participate in the binding to one or more of the ECM proteins examined here.

DISCUSSION

The cellular and molecular properties of cytotactin described in this study and the differential binding of various ECM proteins to the cytotactin–proteoglycan couple are pertinent to an understanding of the properties of cytotactin at both neural and nonneural sites (4). Cytotactin, which is synthesized by glial cells, binds to a proteoglycan that is synthesized by neurons and thus provides a potential mechanism for the previously observed neuron–glia adhesion mediated by cytotactin (5). Recent immunoblotting and immunofluorescent localization experiments (unpublished) have further confirmed the conclusion that this proteoglycan is primarily associated with neurons in the brain.

The predominant species in the purified fraction that binds to cytotactin is a chondroitin sulfate proteoglycan with a M_r 280,000 core protein that carries HNK-1 antigenic determinants. As indicated by the effects of hyaluronidase, the fraction containing this proteoglycan also appears to contain hyaluronic acid. The proteoglycan isolated from brain extracts inhibited binding of cytotactin-coated beads to fibroblasts by about 75% and also affected binding to gizzard cells (unpublished experiments). These results raise the possibility that proteoglycan or related molecules may be general cell-associated ligands for cytotactin. In accord with the observation that cytotactin is a general ECM component, we have found that fibronectin binds to cytotactin but does not inhibit cytotactin–proteoglycan binding and that laminin inhibits the binding of cytotactin to proteoglycan by binding to a component of the proteoglycan fraction. The presence of hyaluronidase-sensitive material in this fraction raises the possibility that the laminin binding observed here may be affected by hyaluronic acid. Further quantitative biological and structural analyses of the proteoglycan and cytotactin as well as examination of fibronectin and laminin fragments will be required to establish the physiological significance and the detailed properties of these interactions.

Recently, three molecules with various degrees of relatedness to cytotactin have been described: myotendinous antigen (18), hexabrachion (19), and J1 antigen (20). Although no function has previously been assigned to the myotendinous antigen, it appears to be similar or identical to cytotactin in its biochemistry, immunological properties, and distribution (K. L. Crossin and G.M.E., unpublished observations). Hexabrachion is the name given to the 6-armed image (19) that is observed by electron microscopic examination of a molecule having similar biochemical properties to cytotactin or myotendinous antigen. This molecule was isolated as a nonfibronectin component of cellular fibronectin preparations. The presence of this cytotactin-like material in fibronectin preparations may be due to the interaction described here between fibronectin and cytotactin. It is as yet unclear whether the hexabrachion morphology represents only cytotactin or a complex of cytotactin and proteoglycan, but given the observations reviewed above it is a reasonable hypothesis that cytotactin, myotendinous antigen, and

hexabrachion represent the same ECM component. In contrast to these molecules, J1 antigen (20) was defined by a rabbit antiserum prepared against a M_r 160,000 glycoprotein from adult mouse brains. The J1 antiserum crossreacted with an embryonic protein of M_r 220,000 (presumably cytotactin), but the M_r 160,000 adult protein and the M_r 220,000 embryonic protein appear to be unrelated in polypeptide structure (5). While antibodies to J1 inhibit neuron–glia adhesion (20), it is not clear whether this effect is due to their binding to cytotactin or to some other molecule.

The partial characterization performed here indicates that the chondroitin sulfate proteoglycan that binds to cytotactin is distinct from the heparan sulfate proteoglycan that is known to bind to fibronectin and laminin through its glycosaminoglycan chains (3). Moreover, we have shown that not all proteoglycans are ligands for cytotactin. One of the most intriguing aspects of the results presented here is the observation that a proteoglycan recognized by monoclonal antibody HNK-1 serves as a ligand in cell adhesion. Although at least three glycoproteins involved in cell adhesion [cytotactin (5), N-CAM (21), and Ng-CAM (21)] and a glycolipid (22) are recognized by HNK-1 or the closely related monoclonal antibody anti-N-CAM clone 5, to our knowledge a proteoglycan containing HNK-1 antigenic determinants has not been previously detected. Whether other distinct proteoglycans are also HNK-1 antigens remains to be determined.

The findings described here open the possibility that the molecular interactions between cytotactin and proteoglycan may be analogous to the molecular interactions that mediate the formation of proteoglycan aggregates that are a component of cartilage. In both systems, a chondroitin sulfate proteoglycan with a M_r 200,000–300,000 core protein, hyaluronic acid, and proteins not bearing glycosaminoglycan chains are present. In cartilage, the chondroitin sulfate proteoglycans bind to hyaluronic acid; this interaction is stabilized by link proteins, which interact with both hyaluronic acid and the proteoglycan (23). Further experiments will be needed to determine whether, in some of its interactions, cytotactin is functionally analogous to the link proteins.

Given the complexity of the intermolecular associations involving ECM components, it is not surprising that the functional role of the extracellular matrix in pattern formation cannot be readily described in terms of the distribution of individual matrix components. Rather, it seems more likely that a network of interactions among multiple matrix components selectively influences the ability of various molecules to bind to cell-associated receptors. Cellular behavior and particularly cell surface modulation (24, 25) could be differentially affected depending on the various amounts of interactive matrix molecules present; the particular composition of such a mixture could depend upon the epigenetic and synthetic history of the cells. The idea of an interactive complex of substrate molecules is not new, but the notion that the dynamics of morphogenesis might be specifically altered by site-regulated synthesis and cell surface modulation of multiple interactive ECM components in a network adds additional versatility to previously proposed (24, 25) mechanisms of modulation.

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