

Neuronal Cell Adhesion Molecule Contactin/F11 Binds To Tenascin Via Its Immunoglobulin-like Domains

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Abstract. Adhesive interactions between neurons and extracellular matrix (ECM) play a key role in neuronal pattern formation. The prominent role played by the extracellular matrix protein tenascin/cytotactin in the development of the nervous system, tied to its abundance, led us to speculate that brain may contain yet unidentified tenascin receptors. Here we show that the neuronal cell adhesion molecule contactin/F11, a member of the immunoglobulin(Ig)-superfamily, is a cell surface ligand for tenascin in the nervous system.

Through affinity chromatography of membrane glycoproteins from chick brain on tenascin-Sepharose, we isolated a major cell surface ligand of 135 kD which we identified as contactin/F11 by NH₂-terminal sequencing. The binding specificity between contactin/F11 and tenascin was demonstrated in solid-phase assays. Binding of immunopurified ¹²⁵I-labeled contactin/F11 to immobilized tenascin is completely inhibited by the addition of soluble tenascin or contactin/F11, but not by fibronectin. When the fractionated isoforms of tenascin were used as substrates, contactin/F11 bound preferentially to the 190-kD isoform. This isoform differs in having no alternatively spliced fibronectin type III domains. Our results imply that the introduction of these additional domains in some way disrupts the contactin/F11 binding site on tenascin.

To localize the binding site on contactin/F11, proteolytic fragments were generated and characterized by NH₂-terminal sequencing. The smallest contactin/F11 fragment which binds tenascin is 45 kD and also begins with the contactin/F11 NH₂-terminal sequence. This implies that contactin/F11 binds to tenascin through a site within the first three Ig-domains.

Through affinity chromatography of membrane glycoproteins from chick brain on tenascin-Sepharose, we isolated a major cell surface ligand of 135 kD which we identified as contactin/F11 by NH₂-terminal sequencing. The binding specificity between contactin/F11 and tenascin was demonstrated in solid-phase assays. Binding of immunopurified ¹²⁵I-labeled contactin/F11 to immobilized tenascin is completely inhibited by the addition of soluble tenascin or contactin/F11, but not by fibronectin. When the fractionated isoforms of tenascin were used as substrates, contactin/F11 bound preferentially to the 190-kD isoform. This isoform differs in having no alternatively spliced fibronectin type III domains. Our results imply that the introduction of these additional domains in some way disrupts the contactin/F11 binding site on tenascin.

MOLECULES of the extracellular matrix have been implicated to play a pivotal role in tissue morphogenesis. Tenascin (also known as cytotactin, hexabrachion, J1-220) is a large multidomain extracellular matrix (ECM)¹ glycoprotein that shows a highly restricted expression pattern in developing embryos (reviewed by Erickson and Bourdon, 1989). In rotary-shadowed electron micrographs, tenascin appears as a hexabrachion consisting of six individual polypeptide chains joined via their amino termini (Erickson and Iglesias, 1984; Vaughan et al., 1987). Each polypeptide chain is composed of tandemly connected domains, either homologous to EGF or to the type III repeats of fibronectin or to fibrinogen (see Fig. 4 A) (Jones et al., 1989; Spring et al., 1989). Structural variation is provided by alternative splicing within the fibronectin type III homology region (see Fig. 4 A). In the chick, up to three additional

domains are incorporated, giving rise to isoforms which range in size from 190 kD, with no additional domains, to 220 kD with three extra domains (Spring et al., 1989; Jones et al., 1989). The site for alternative splicing, between domains five and nine, is conserved between species, but the number of additional domains differs, with mouse tenascin having up to five (Weller et al., 1991) and human tenascin up to seven extra type III repeats (Gulcher et al., 1989). This variation appears to be under tight cellular control as only homohexamers have been isolated from either cell cultures (Kaplony et al., 1991; Chiquet et al., 1991) or tissue extracts (Kaplony et al., 1991). The spatial and temporal expression of tenascin isoforms during development is also tightly regulated (Aufderheide et al., 1988; Matsuoka et al., 1990; Kaplony et al., 1991).

The functional role(s) of the alternatively spliced forms may be established, in part, through the identification of isoform-specific ligands. Tenascin has been reported to mediate contrary functions: a tenascin substratum can be either repulsive or adhesive for cells, with the EGF-repeats mediating the former and the type III region, including the alternatively spliced domains, the latter effect (Spring et al., 1989). Little is known, however, about the cellular receptors interacting with these domains. Until now a β_1 -integrin,

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1. *Abbreviations used in this paper:* β OG, β -octylglucopyranoside; CAM, cell adhesion molecule; CEF-Tn, chicken embryo fibroblast tenascin; CNS, central nervous system; ECM, extracellular matrix; hd, heat-denatured; PNS, peripheral nervous system; PVDF, polyvinylidene difluoride; WGA, wheat germ agglutinin; tn 190 and tn 220, 190- and 220-kD tenascin isoforms.

isolated from a human glioma cell line (Bourdon and Ruoslahti, 1989), and two different proteoglycans, the membrane bound syndecan (Salmivirta et al., 1991) and an extracellular matrix chondroitin sulfate proteoglycan, the cytotactin binding proteoglycan (Hoffman and Edelman, 1987), have been proposed as tenascin ligands, yet their binding sites on tenascin remain obscure.

In the nervous system, the distribution of tenascin is correlated with key developmental processes such as cell migration, axonal outgrowth, and tissue boundary formation (reviewed by Chiquet, 1989; see also Edelman and Crossin, 1991; Reichardt and Tomaselli, 1991). Tenascin is synthesized by glial cells of the central and peripheral nervous system (CNS and PNS, respectively), and in the PNS by mesenchymal cells that are in contact with developing neurons (Wehrle and Chiquet, 1990; Crossin et al., 1986; Chuong et al., 1987; Prieto et al., 1990). For instance, during granule cell migration in the cerebral cortex, the expression of tenascin on the Bergman glia coincides with the migration of the granule neurons along the glial processes (Chuong et al., 1987). Direct evidence for a role of tenascin in neuronal migration was obtained by blocking granule cell migration within the molecular layer in cerebellar explant cultures (Chuong et al., 1987; Husmann et al., 1992). A role for tenascin in migration of the neural crest is implicated from its distribution along the paths of cranial neural crest cells (Bronner-Fraser, 1988; Kaplony et al., 1991) and its expression in the rostral sclerotome of the trunk (Tan et al., 1987; Mackie et al., 1988). As neural crest cells migrate exclusively through the rostral portion of the sclerotome (presumptive vertebrae) and avoid the caudal half, this pattern correlates with their metameric migration pattern giving rise to the segmental organization of the PNS (Keynes and Stern, 1988). It is debated, however, if tenascin is present in the rostral sclerotome before the entry of the neural crest (Tan et al., 1987; Mackie et al., 1988) or accumulates in this region in response to neural crest immigration (Stern et al., 1989). Certainly, tenascin is available as a substrate for motor axons that emerge from the ventral neural tube several hours after neural crest cells and similarly project exclusively across the rostral sclerotome region (Keynes and Stern, 1984; Wehrle and Chiquet, 1990). In vitro studies using spinal cord explants showed that motor axons efficiently grow on plain tenascin substrata (Wehrle and Chiquet, 1990). Studies in different laboratories have shown that tenascin promotes the growth of neurites from both CNS and PNS neurons. Provided the cell bodies are attached through polyamines, the growth cones of the extending neurites efficiently attach and are highly motile on a tenascin substratum, although their morphology differs from growth cones extending on highly permissive substrata such as laminin (Wehrle and Chiquet, 1990; Lochter et al., 1991). Together with the observation that neurites avoid tenascin substrata if given a choice between tenascin and other ECM substrata such as fibronectin (Faissner and Kruse, 1990), it appears that tenascin is able to modulate adhesive interactions between developing axons and their surroundings and thus contribute to the establishment of neuronal circuitry.

Despite this evidence for a role in nervous system organization, so far only one neuronal ligand for tenascin, the cytotactin-binding proteoglycan, has been reported (Hoffman and Edelman, 1987). A number of cell adhesion mole-

cules (CAMs) with restricted neuronal expression have been identified (Jessell, 1988; Rutishauser and Jessell, 1988), and evidence is gathering for them having prominent roles in the establishment of neuronal networks. Sequence analysis of these proteins showed that many neural cell adhesion molecules (N-CAM) are members of larger gene families (for reviews see Edelman and Crossin, 1991; Rathjen and Jessell, 1991). A prominent family of CAMs is the immunoglobulin superfamily that includes the N-CAM (Cunningham et al., 1987), neuron-glia cell adhesion molecule (Ng-CAM) (Burgoon et al., 1991), L1 (Moos et al., 1988), TAG-1 (Furley et al., 1990), axonin-1 (Stoeckli et al., 1991), chick contactin/F11 (Ranscht, 1988; Brummendorf et al., 1989) and its mouse homologue F3 (Gennarini et al., 1989). These molecules have been shown to bind to other members within this family by homophilic (Lemmon et al., 1989) or heterophilic interactions (Kuhn et al., 1991). The chick extracellular matrix protein restriction (Rathjen et al., 1991) has been copurified with contactin/F11, indicating that extracellular matrix components could act as additional heterophilic ligands for this class of CAMs.

In the present study we describe the identification of the neuronal glycoprotein contactin/F11 as a tenascin receptor. We present evidence for the binding between the fibronectin type III homology region of tenascin and a site within the three distal immunoglobulin-like repeats of contactin/F11. The interaction between these domains may not only be relevant for neuron-glia interactions, but constitute a general mechanism to regulate cell-cell and cell-matrix interactions.

Materials and Methods

Antibodies

The mouse monoclonal IgG antibodies T16 and T17 against chicken tenascin have been described (Kaplony et al., 1991). mAb 4D1 is specific for contactin/F11 (Ranscht et al., 1984). Antibodies T16, T17, and 4D1 were purified from ascites fluid by affinity chromatography on protein A-Sepharose CL 4B (Pharmacia, Uppsala, Sweden). mAb affinity columns were prepared by coupling of isolated IgG to CNBr-activated Sepharose 4B (Pharmacia) to a final concentration of 2 mg IgG per ml resin.

ECM Molecules

Chicken embryonic fibroblast tenascin (CEF-Tn) was purified from conditioned culture media of primary chicken embryo fibroblasts, grown in bulk cytodex microcarrier cultures (Pharmacia) by affinity chromatography on a T16-Sepharose column (Kaplony et al., 1991). The purity of the isolated tenascin was judged by SDS-PAGE. For the preparation of the tenascin-Sepharose affinity column, CEF-Tn was coupled to CNBr-activated Sepharose 4B (Pharmacia) to a final concentration of 5 mg/ml resin. Critical for the preparation of such an affinity column was the isolation of tenascin in a concentrated, soluble form from fibroblast conditioned medium. Through the use of microcarrier cultures, a high cell/volume ratio is obtained in the culture medium, of which batches of 10–20 liters were then concentrated 10-fold by ultrafiltration, using a 100-kD cut-off, tangential flow filter. This medium concentrate was then used for the affinity purification of tenascin on a mAb T16-column. 15–20 mg CEF-Tn were isolated per batch. Four batches were used for the setup of a high capacity CEF-Tn-column. Coupling of CEF-Tn to CNBr-activated Sepharose 4B was performed following the manufacturer's instructions with CEF-Tn in 0.1 M NaHCO₃, 0.5 M NaCl, pH 8.3, as coupling solution. Coupling was performed on a stirring wheel for 2 h and the efficiency of the coupling step (between 70 and 80% of tenascin were coupled) was determined by photometrical measurement of the concentration of uncoupled tenascin at 280 nm.

Chick brain tenascin was purified from 17-d-old embryonic chick brain by immunoaffinity chromatography. In a typical experiment, 100 g chick

brain was homogenized in 400-ml ice cold buffer containing 1 M NaCl in buffer A (20 mM Tris, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM PMSF, 25 mM 6-aminocaproic acid, 4 μg/ml leupeptin, 4 μg/ml pepstatin, 0.02% NaN₃) and extracted overnight at 4°C. The extract was centrifuged for 2 h at 15,000 g. The supernatant was diluted with 4 vols buffer A and used as source of tenascin, while the pellet was used for the isolation of putative tenascin receptors. The supernatant was passed sequentially over a gelatine-Sepharose 4B column (to remove fibronectin) and a T16-Sepharose column. To remove unspecifically bound material, the T16 affinity column was washed with 0.1% Triton X-100, 0.5 M NaCl in buffer A and extensively washed with 0.15 M NaCl in buffer A in a subsequent step. Bound material was eluted with 0.1 M triethylamine buffer, pH 11.5, containing 0.15 M NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM PMSF, and 0.02% NaN₃. Fractions were immediately neutralized with 1 M Tris, pH 5.8, and analyzed on SDS-PAGE. The peak fractions were frozen at -18°C. A typical yield was 4–5 mg tenascin per 100 g chick brain.

Isoforms of chick brain tenascin were isolated using serial immunoaffinity chromatography (Kaplon et al., 1991). The supernatant, prepared as described in the above isolation procedure, was passed sequentially over a gelatine-Sepharose 4B column, followed by T17-Sepharose (to retain tn 220) and T16-Sepharose (to retain the remaining isoforms). After loading, the antibody columns were washed and eluted separately, as described above.

Fibronectin was isolated from chicken blood according to Ruoslahti et al. (1982). For the preparation of the affinity column, fibronectin was coupled to CNBr-activated Sepharose 4B to a final concentration of 2 mg/ml resin.

Mouse laminin was purchased from Collaborative Research, Inc. (Lexington, MA).

Affinity Chromatography

E17 chick brains were homogenized in 1 M NaCl in buffer B (20 mM Tris, 2 mM CaCl₂, 2 mM MgCl₂, 1 mM MnCl₂, 2 mM PMSF, 25 mM 6-aminocaproic acid, 4 μg/ml leupeptin, 4 μg/ml pepstatin, 0.02% NaN₃, pH 7.5) and extracted overnight at 4°C. The extract was centrifuged for 2 h at 15,000 g. The pellet was again homogenized, but now in a 5–10-fold vol of buffer B containing 150 mM β-octylglucoside (βOG) (Sigma Chemical Co., Deisenhofen, BRD) and 150 mM NaCl and extracted overnight at 4°C. The extract was centrifuged at 100,000 g for 1 h at 4°C; the supernatant was passed over a wheat germ agglutinin (WGA)-Sepharose column (Pharmacia) and a membrane-glycoprotein fraction was eluted with 10% (wt/vol) *N*-acetyl-D-glucosamine, 30 mM βOG, and 50 mM NaCl in buffer B. The WGA-fraction was first passed over a Sepharose 4B precolumn (Pharmacia) and then over tenascin-Sepharose 4B. The columns were washed extensively with 30 mM βOG, 50 mM NaCl in buffer B, and eluted separately with a NaCl-gradient (0.05–0.4 M NaCl, 30 mM βOG in buffer B). Aliquots of the eluate from the tenascin-column were precipitated with 30% trichloroacetic acid, run in a SDS-PAGE 5–10% gradient-gel under nonreducing conditions, and either fixed and silverstained or blotted onto a polyvinylidene difluoride (PVDF)-membrane (Immobilon; Millipore Corp., Bedford, MA).

Affinity chromatography with fragments of contactin/F11 was performed in the following manner: ¹²⁵I-labeled fragments of contactin/F11 were added to binding buffer G, and applied to a Sepharose-4B precolumn followed by a tenascin-Sepharose column in series. After loading, the columns were disconnected and extensively washed with binding buffer; the columns were subsequently eluted with 0.5 M NaCl, 2 mM CaCl₂, 2 mM MgCl₂ in buffer C (20 mM Tris, 0.02% Triton X-100, 1 mM PMSF, pH 7.5), then with 10 mM EDTA in buffer C, and finally with 1.5 M NaCl in buffer C. Fractions were collected and radioactivity measured. As a control, the same procedure was performed on a fibronectin-Sepharose 4B column. Aliquots of each fraction were analyzed by autoradiography after 8–15% SDS-PAGE.

Immunoaffinity Purification of Contactin/F11

Contactin/F11 was isolated from either detergent-containing or detergent-free E 17 chick brain extracts by lectin- and immunoaffinity chromatography. Detergent-free chick brain extract was prepared as described above for the isolation of tenascin. Chick brains were homogenized with 1 M NaCl in ice-cold buffer A and extracted overnight at 4°C. The extract was centrifuged for 2 h at 15,000 g. Both the supernatant and the pellet were used as source of contactin/F11. For the isolation of detergent-free contactin/F11, the supernatant was diluted with 4 vol of buffer A and passed over a WGA-column. A glycoprotein fraction was eluted with 10% *N*-acetyl-D-glucosamine in buffer containing 20 mM Tris, 0.2 M NaCl, 1 mM CaCl₂, 1 mM

PMSF, pH 7.5. The eluate was subsequently loaded onto the mAb 4D1-column. Bound material was eluted with 0.1 M triethylamine buffer, pH 11.5, with 0.15 M NaCl, 1 mM PMSF. Fractions were neutralized immediately by addition of 1 M Tris buffer, pH 5.8.

Detergent extracts of contactin/F11 were obtained by overnight extraction of the pellet with 150 mM βOG in buffer A containing 0.2 M NaCl at 4°C. The extract was centrifuged at 100,000 g for 1 h at 4°C; the supernatant was passed over a WGA-Sepharose column and a membrane-glycoprotein fraction was eluted with 10% (wt/vol) *N*-acetyl-D-glucosamine in buffer D (30 mM βOG, 20 mM Tris, 0.2 M NaCl, 1 mM CaCl₂, 1 mM PMSF, pH 7.5). The eluate was applied onto the mAb 4D1 column. After extensive washing with buffer D, bound material was eluted with 0.1 M triethylamine buffer, pH 11.5, with 30 mM βOG, 0.15 M NaCl, 1 mM PMSF. Fractions were neutralized immediately by addition of 1 M Tris buffer, pH 5.8.

Radiolabeling

Proteins were radiolabeled with 125-iodine (Amersham International, Amersham, UK) by use of the Enzymobead method (BioRad Laboratories, Richmond, CA), following the manufacturer's instructions.

Fragmentation of Contactin/F11

Collagenase (EC 3.4.24.3, SERVA) was added to a final concentration of 0.6 U/ml to 300 μg immunopurified soluble contactin/F11 in 20 mM Tris, 150 mM NaCl, 10 mM CaCl₂, 1 mM PMSF, pH 7.5, incubated for 4 h at 37°C, and stopped by addition of EDTA. The contactin/F11 fragments were immediately freed of collagenase by binding to a WGA-column. Fragments were eluted from the WGA-column with 10% *N*-acetylglucosamine in 20 mM Tris, 150 mM NaCl, 0.02% Triton X-100, 1 mM PMSF, pH 7.5. Aliquots of the eluate were analyzed by silverstaining or by autoradiography after labeling with 125-iodine by the Enzymobead method on SDS-PAGE.

Solid Phase Assay

Removable 96-well microtiter plates (Maxi Sorp; Nunc, Roskilde, Denmark) were coated with 20 μg protein/ml in buffer E (20 mM Tris, 50 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, 0.02% NaN₃, pH 7.5) in a 100 μl vol per well and incubated overnight at 24°C. Negative controls received the same treatment with 0.1% heat-denatured (hd) BSA in buffer E. After incubation, the wells were washed three times with washing buffer F (0.02% Tween 20, 20 mM Tris, 50 mM NaCl, 0.02% NaN₃, pH 7.5) and unused binding sites blocked by addition of 0.2 ml of 0.1% hd BSA in buffer E for 2 h at 24°C. After blocking, wells were washed three times with buffer F. To allow complete saturation with Ca²⁺/Mg²⁺, wells were incubated with buffer E for 30 min at 24°C. Then constant amounts (100,000 cpm) of ¹²⁵I-labeled contactin/F11 (see Fig. 1, lane 4) in binding-buffer G (0.02% Triton X-100, 20 mM Tris, 50 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, 0.02% NaN₃, pH 7.5) were added to the wells and incubated for 2 h at 37°C. In our standard assay conditions, the salt concentration of the binding buffer was 50 mM NaCl, however similar results were obtained using a salt concentration of 150 mM NaCl. For the inhibition studies, ¹²⁵I-labeled contactin/F11 was incubated in a 100 μl vol of different dilutions of tenascin, fibronectin, contactin/F11, and BSA in buffer G. After incubation, the wells were washed four times with 0.2 ml of washing buffer F, and counted for bound activity. Specific binding was determined as total minus nonspecific binding. Nonspecific binding was determined on BSA. Binding of ¹²⁵I-labeled contactin/F11 to immobilized tenascin in the absence of competing soluble proteins was taken as 100%. In some cases bound radiolabel was extracted with sample buffer for SDS-PAGE, run on a 5–10% SDS-PAGE gradient gel, and autoradiographed.

Gel Electrophoresis, Immunoblotting, NH₂-terminal Sequencing

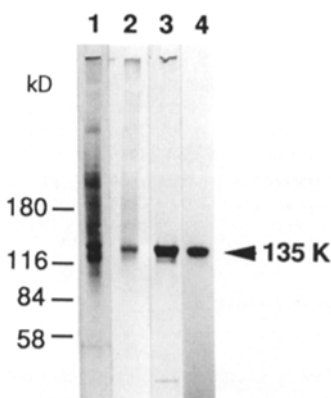
SDS-PAGE was carried out as described by Laemmli (1970) and gels were stained with silver as described by Nielsen and Brown (1984). Immunoblotting was performed in the following way: proteins electrophoresed in SDS-PAGE were transferred to nitrocellulose (BA 85; Schleicher & Schull, Dassel, Denmark) using a semidry blotting chamber system (Sartorius Corp., Göttingen, Denmark) according to the instructions of the manufacturer. The nitrocellulose was incubated with mAb, followed by alkaline phosphatase-conjugated goat anti-mouse IgG (Tago, Inc., Burlingame, CA), and stained with 5-bromo-4-chloro-3-indonyl-phosphate and nitroblue tetrazolium (Sigma Chemical Co., St. Louis). For NH₂-terminal sequencing, pro-

teins were separated by SDS-PAGE and blotted onto a PVDF-membrane (Immobilon, Millipore Corp.). Ponceau S (Serva Biochemicals, Paramus, NJ) stained bands were cut out and sequenced through their NH₂-termini with an Applied Biosystems 470 A sequencer and a 120 A phenylthiohydantoin-derivative analyzer.

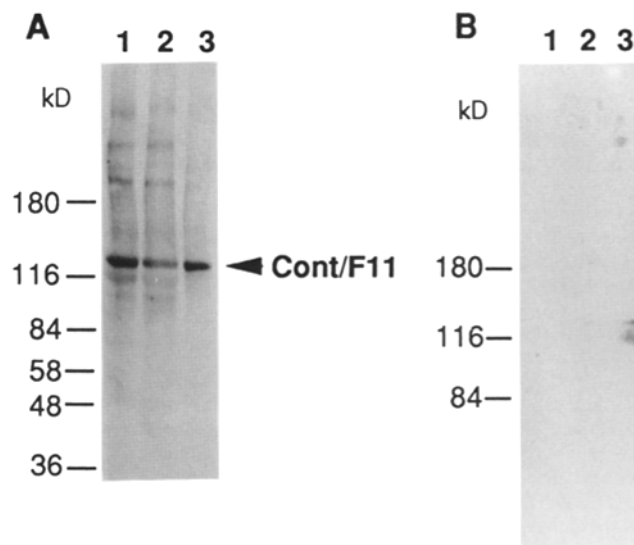
Results

Contactin/F11 Binds to the Tenascin-Affinity Column

Tenascin is expressed at high levels in the developing brain and is implicated to play an important role in establishing neural patterns (Steindler et al., 1989; Crossin et al., 1989; Bartsch et al., 1992). In brains of 17-d-old chick embryos, two of the major tenascin isoforms, the 220-kD isoform (tn 220) and the 190-kD isoform (tn 190) can be detected in high amounts (Kaplon et al., 1991). For these reasons, we prepared a membrane glycoprotein fraction from chick brain of this embryonic stage to search for putative tenascin receptors. To exclude extracellular matrix (ECM)- and cytoplasmic proteins from our receptor preparations, brains were homogenized and extracted in detergent-free buffer, containing 1 M NaCl. With this approach most, if not all, tenascin could be removed from the tissue, indicating that this extraction is highly efficient for ECM components. Insoluble proteins were collected by centrifugation and the membrane-rich pellet extracted in detergent- (150 mM β OG) containing buffer. Detergent-soluble proteins were used as a crude membrane fraction and enriched for membrane glycoproteins through lectin-affinity chromatography on WGA-Sepharose (Fig. 1, lane 1). To isolate putative tenascin receptors in this fraction, the WGA-binding glycoproteins were passed over a tenascin-Sepharose affinity column. As interactions between ECM molecules and their cellular receptors are generally of low affinity, a tenascin-affinity column of extra high capacity was used. In total, >50 mg chick embryo fibroblast (CEF)-tenascin were coupled to 10 ml CNBr-activated Sepharose 4B. The possibility of non-specific binding was eliminated by passing the WGA-binding glycoproteins through a Sepharose 4B precolumn before the tenascin column. No proteins were detected binding to this precol-



umn. The major ligand in chick brain detergent extracts retained on the tenascin column was eluted with a NaCl gradient and migrated as a protein of 135 kD on SDS-PAGE (Fig. 1, lane 2). It was identified as contactin/F11 (Ranscht et al., 1988; Brümmendorf et al., 1989) by NH₂-terminal sequencing which gave the following sequence: XHFSEE-GNKGYPVFEEQ (single letter code, X stands for a non-identified amino acid). None of the known tenascin-binding proteins in brain such as the cytotactin-binding proteoglycan (Hoffman and Edelman, 1987) or restrictin (Rathjen et al., 1991) was detected in this fraction. As both the cytotactin-binding proteoglycan and restrictin are extracellular matrix proteins and can be extracted without detergents (Hoffman and Edelman, 1987; Rathjen et al., 1991), it seems likely that they were solubilized along with tenascin in the 1.0 M NaCl extract and were therefore not present in the membrane preparation applied to the tenascin-affinity column.



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A further indication of the specificity of the tenascin-contactin/F11 interaction comes from experiments showing that contactin/F11 is depleted in the flow-throughs of the tenascin-Sepharose column. Dominant in silver-stained gels of the membrane glycoprotein fraction (Fig. 2 A, lane 1) applied to the tenascin column is a broad band of 135 kD. Although the staining intensity of this band is diminished in the flow-through of the tenascin column (Fig. 2 A, lane 2), con-

Figure 2. Affinity chromatography on tenascin-Sepharose. (A) Chick brain (E17) membrane glycoprotein eluate from WGA-Sepharose, prior loading onto the tenascin-column (lane 1), and in the flow-through after the tenascin column (lane 2), run on SDS-PAGE and detected by silver staining. The prominent band in the eluate of the tenascin column (lane 3), which was identified as contactin/F11, contributes to the broad band at 135 kD. (B) Binding of contactin/F11 to the tenascin column was efficient. Flow-throughs of three independent experiments were subjected to SDS-PAGE, and electrotransferred to nitrocellulose. For immunodetection of contactin/F11, the nitrocellulose was incubated with mAb 4D1. For visualization, an alkaline phosphatase-conjugated secondary antibody was used. Immunostaining shows a complete depletion of contactin/F11 in the first two experiments (lanes 1 and 2). In the third, where double the amount of membrane glycoprotein extract was applied to the tenascin column, the flow-through now contained some contactin/F11 (lane 3).

tactin/F11 can no longer be detected immunologically (Fig. 2 B), suggesting that other proteins of identical molecular mass contribute to this band. This can be seen in Fig. 2 B, where aliquots from the flow-throughs of three independent experiments were tested with the contactin/F11 specific mAb 4D1 (Ranscht et al., 1984) after immunoblotting and separation on SDS-PAGE (Fig. 2 B). In each of the first two experiments, the complete eluate from a 20-ml WGA-Sepharose column was applied to the tenascin-Sepharose. In the third, two such eluates were combined before loading onto the tenascin column. Contactin/F11 was removed to completion from each of the first two WGA eluates, however, not all contactin/F11 was removed when the amount loaded onto the tenascin column was doubled, as can be seen in Fig. 2 B, lane 3. These results show that providing the capacity of the tenascin column is not exceeded, contactin/F11 can be efficiently and selectively depleted from extracts containing a complex mixture of membrane glycoproteins.

Binding of Contactin/F11 to Immobilized Tenascin

To determine directly the binding specificity between contactin/F11 and tenascin, we used a solid-phase assay on different substratum-bound molecules, including immunopurified contactin/F11. Microtiter wells were coated with either chick brain tenascin or chick fibronectin or chick contactin/F11, or mouse laminin. Contactin/F11 (Fig. 1, lane 3), used for the coating of wells, was isolated from detergent-free extracts of embryonic chick brain through lectin-affinity chromatography on WGA-Sepharose and immunoaffinity chromatography on the contactin/F11 specific mAb 4D1-column. Wells, coated with the mAb 4D1 served as positive controls for maximal binding and wells coated with 0.1% hd BSA as negative controls in this assay. Binding studies were carried out using ^{125}I -labeled immunopurified contactin/F11 (Fig. 1, lane 4), isolated from either detergent-free or detergent-containing extracts of chick brain as described in Materials and Methods. No difference in the electrophoretic mobility in SDS-PAGE could be detected between contactin/F11 from these two sources. Wells were incubated with ^{125}I -labeled contactin/F11 for 2 h at 37°C, washed, and counted for bound radioactivity. The absolute amount of bound radioactivity varied between assays, but the ratio of binding between contactin/F11 and various substrates remained constant. As a consequence, the binding values for contactin/F11 are expressed as 100% relative to tenascin with the binding to the negative control, BSA, set as 0%. In comparison, five times more contactin/F11 binds to wells coated with the contactin/F11 specific mAb 4D1 than to tenascin (data not shown), reflecting the value for a high affinity binding and the maximum possible in this assay. Assuming tenascin and IgG (4D1) coat with similar efficiencies, and that each possesses one binding site per monomer of 190 and 75 kD, respectively, then the binding of contactin/F11 per mole of coated substratum compares very favorably. All values were means of at least triplicate determinations and their standard deviations were <10%, apart for that of binding to laminin which was 14%. The differences of the means were compared statistically using *t* test. Binding of ^{125}I -labeled contactin/F11 to chick brain tenascin was 4–5 times higher than to the negative control, BSA, alone ($p < 0.001$) (Fig. 3). The binding between contactin/F11 and tenascin was also significantly higher than the binding to all the other

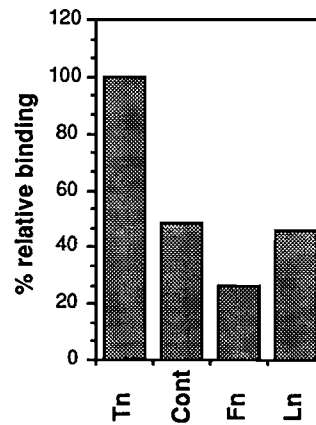


Figure 3. Binding of ^{125}I -labeled contactin/F11 to various substrates was tested in a solid phase assay (see Materials and Methods). Binding of ^{125}I -labeled contactin/F11 to tenascin (Tn, chick brain tenascin, containing all isoforms) was significantly higher compared with binding to contactin/F11 (Cont) or chick fibronectin (Fn) or mouse laminin (Ln). Nonspecific binding was determined on BSA. Specific binding was determined as total minus nonspecific binding. Binding activity is plotted

relative to the activity obtained on the tenascin substrate, which was 4–5 times higher than binding to the negative control, BSA alone. All values are means of at least triplicate determinations. Standard deviations were <10%, other than for laminin where it was 14%.

substrates tested ($p < 0.01$). The homophilic binding to itself was significantly higher compared with heterophilic binding to laminin or fibronectin ($p < 0.01$), and all were significant when compared with binding to BSA ($p < 0.01$).

The binding between contactin/F11 and tenascin was directly demonstrated in competitive binding studies, again using ^{125}I -labeled contactin/F11 in the solid phase assay. Microtiter wells, coated with chick brain tenascin, were incubated with ^{125}I -labeled contactin/F11 in the presence of different concentrations of competing soluble proteins. The specificity of the binding between chick brain tenascin and contactin/F11 is demonstrated in Fig. 4. Binding of ^{125}I -labeled contactin/F11 to immobilized tenascin was inhibited to completion (i.e., to background values) in a dose-dependent manner by tenascin in solution (Fig. 4 A), while the addition of soluble chicken fibronectin (Fig. 4 C) or BSA (Fig. 4 D) had only marginal effects. Moreover, the binding of ^{125}I -labeled contactin/F11 to immobilized tenascin was also quantitatively blocked by the addition of unlabeled soluble contactin/F11 (Fig. 4 B). These results demonstrate a specific interaction between contactin/F11 and tenascin.

Contactin/F11 Binds Preferentially to the tn 190 Isoform of Tenascin

A demanding test in the work with a protein-like tenascin, which exists in several isoforms, is to establish whether the property being examined is isoform specific. The homo-hexameric (or homotrimeric) chick tenascin exists in at least three different variants, generated by alternative splicing of fibronectin type III homology domains (Fig. 5 A). Relative to tn 190, tn 200 contains one and tn 220 three additional type III domains (Spring et al., 1989; Jones et al., 1989). The major isoforms in 17-d chick embryo brain are tn 190 and 220, while tn 200 is less abundant (Fig. 5 B, lane 2). Serial affinity chromatography (Kaplon et al., 1991) on T17-Sepharose (to isolate tn 220) and T16-Sepharose (to retain the remaining tn 190) permitted separation of tn 220 from tn 190, the latter containing minute amounts of tn 200 (Fig. 5 B). Binding tests of ^{125}I -labeled contactin/F11 to the immobilized isoforms indicated that contactin/F11 clearly fa-

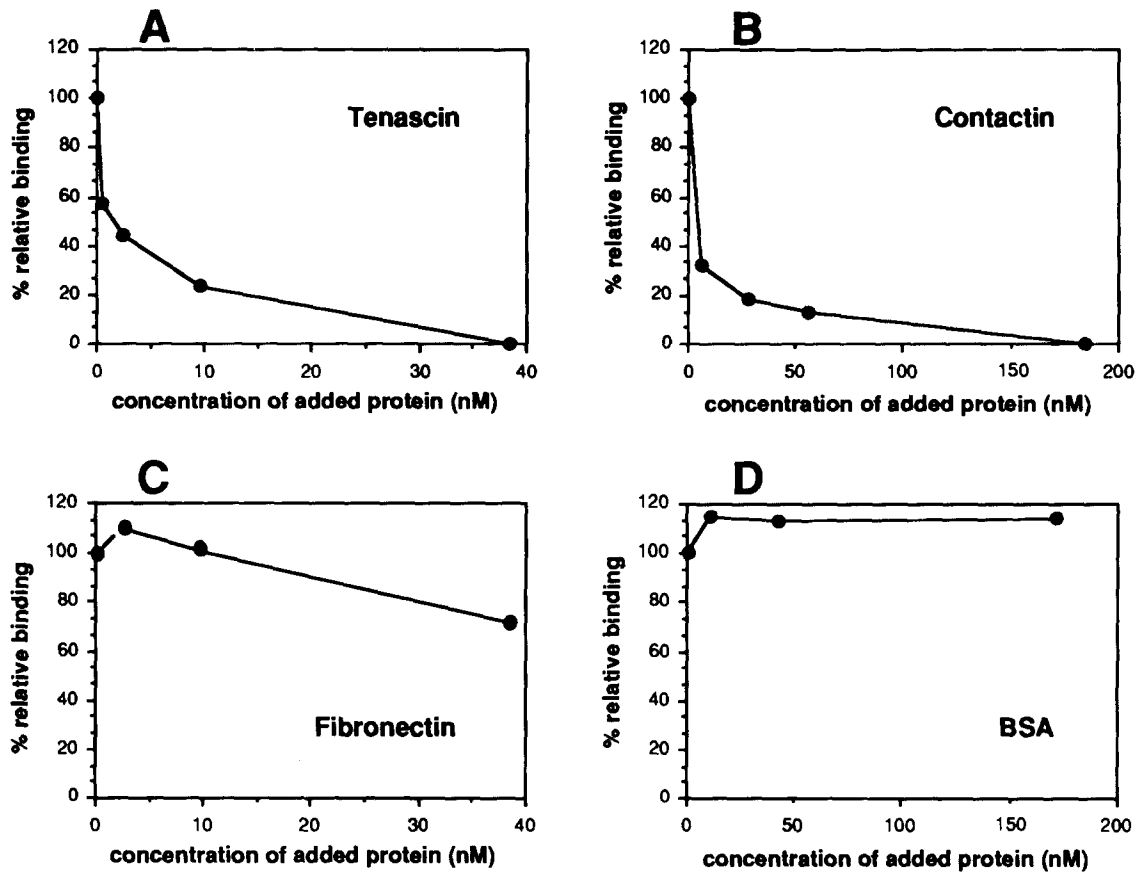


Figure 4. Competition-assays: binding of ^{125}I -labeled contactin/F11 to immobilized chick brain tenascin was completely inhibited through addition of unlabeled soluble tenascin (A) or contactin/F11 (B) in solid-phase assays (described in Materials and Methods). In comparison, little or no inhibition through addition of fibronectin (C) or BSA (D) was observed.

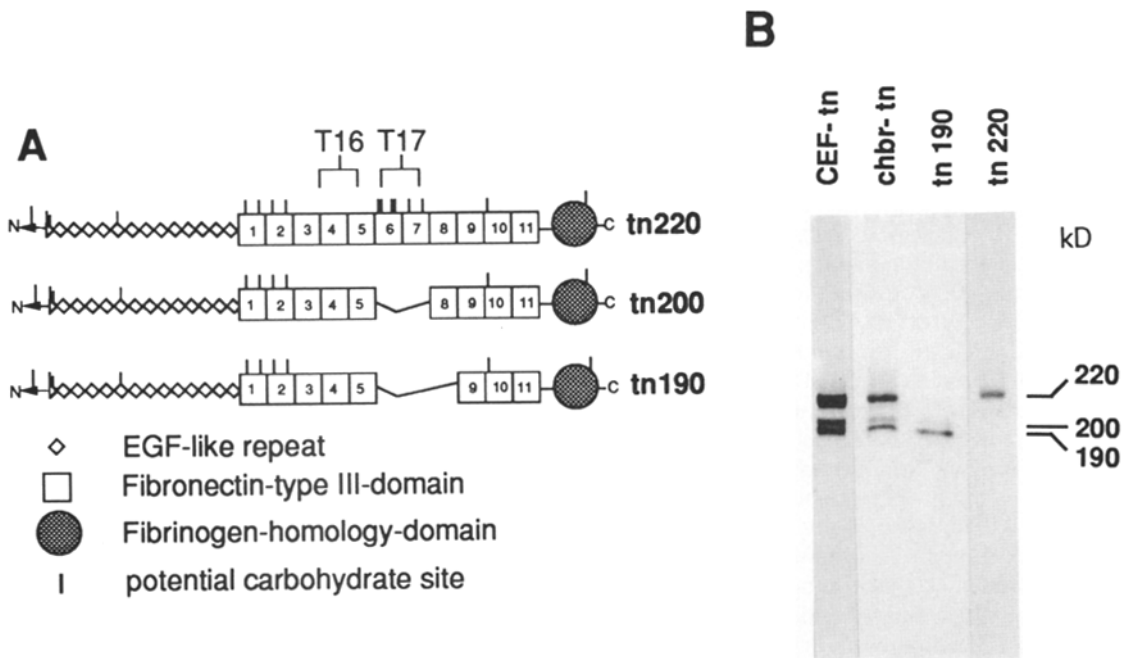


Figure 5. Chick tenascin exists in at least three different isoforms generated by alternative splicing. (A) Structural model of these isoforms. (B) Tenascin and its isoforms detected by silver staining after running on SDS-PAGE under reducing conditions. *CEF-tn* chicken embryo fibroblast tenascin (lane 1); *chbr-tn*, chicken brain tenascin, containing all three isoforms (lane 2); *tn 190*, 190-kD isoform of tenascin, containing minute amounts of the 200-kD isoform (lane 3); and *tn 220*, 220-kD isoform (lane 4).

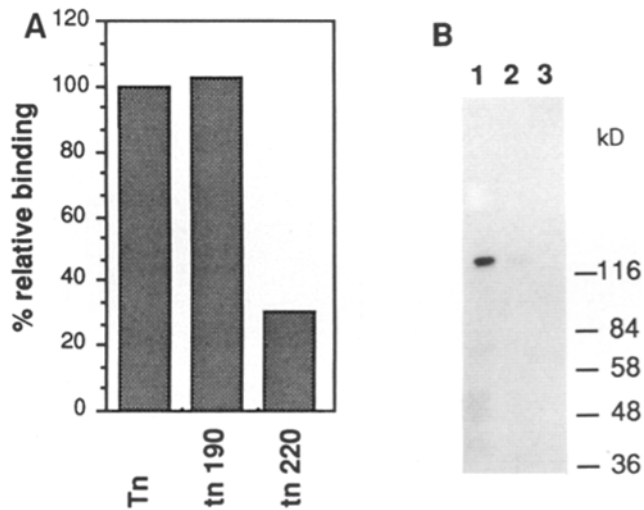


Figure 6. (A) Comparative binding of contactin/F11 to immobilized tn 190 or tn 220 or to the unfractionated isoforms, Tn. Extraction of bound radiolabel (B) from wells coated with tn 190 (lane 1) in comparison to tn 220 (lane 2). No binding to BSA was observed (lane 3). Tenascin isoforms were coated at a concentration of 20 $\mu\text{g}/\text{ml}$ in a pH 7.5 buffer containing 20 mM Tris, 50 mM NaCl, 2 mM CaCl_2 , 2 mM MgCl_2 , 0.02% NaN_3 , and control wells were coated with 0.1% heat denatured BSA. After washing, blocking, and saturation with $\text{Ca}^{2+}/\text{Mg}^{2+}$, wells were incubated with equal amounts of ^{125}I -labeled contactin (100,000 cpm/well) for 2 h at 37°C. After extensive washing, wells were extracted with sample buffer for SDS-PAGE, run on a 5–10% gradient gel, and autoradiographed.

vored binding to the tn 190 fraction. The binding values to tn 190 were identical to values of binding between contactin/F11 and unfractionated chick brain tenascin (Tn), and were significantly higher than the binding to tn 220 ($p < 0.001$) (Fig. 6 A). Binding to tn 220, although low, was still significant when compared with binding to BSA ($p = 0.01$). The differences between tn 190 and 220 were not artefacts due to differences in coating efficiency as both isoforms bound equally well to the plastic wells (results not shown). Extraction of bound radiolabel and visualization by autoradiography after SDS-PAGE shows the striking binding preference of contactin/F11 for tn 190 (Fig. 6 B).

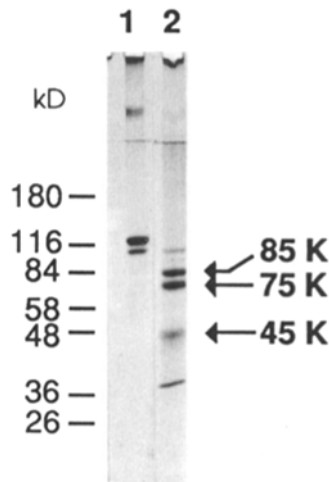


Figure 7. Fragmentation of contactin/F11 by collagenase digestion, followed by SDS-PAGE and silverstaining. Contactin/F11, before collagenase digestion (lane 1) and after digestion (lane 2). Peptides were freed of contaminating collagenase by use of a WGA column (see Materials and Methods).

Contactin F11 Binds to Tenascin Via Its Ig-Domains

To localize the tenascin-binding region within contactin/F11, the six Ig-like domains were separated from the fibronectin type III repeats by digestion with collagenase at a 7-amino acid collagen-like sequence (-Gly-Pro-Pro-Gly-Pro-Pro-Gly-) which separates the two domains (Ranscht et al., 1988; Brümmendorf et al., 1989) (see Fig. 9 C). The digestion led to a highly reproducible pattern of peptide fragments, although from their sizes it was evident that cleavage also occurred at additional sites. Contactin/F11-fragments were separated from the enzyme by affinity chromatography on WGA-Sepharose as described in Materials and Methods and analyzed by SDS-PAGE (Fig. 7, lane 2). These collagenase-freed fragments were labeled with 125-iodine (see Fig. 9 A, lane 1) and tested for binding to tenascin by affinity chromatography on tenascin-Sepharose. As a control, the ^{125}I -labeled fragments were also applied to a Sepharose 4B column and to a fibronectin-Sepharose column. Fragments bound exclusively to the tenascin-Sepharose column (Fig. 8 A) and were completely eluted with 0.5 M NaCl. No further radioactivity was recovered by subsequent elution with 10 mM EDTA and 1.5 M NaCl. Similar elution of the two control columns, Sepharose alone (data not shown) and the fibronectin-Sepharose column (Fig. 8 B), revealed no bound activity. Although a wide range of contactin/F11 fragments were labeled with 125-iodine (Fig. 9 A, lane 1), only fragments of 85, 75, and 45 kD specificity bound to the tenascin column (Fig. 9 A, lane 2). Bound fragments were identified by NH_2 -terminal sequencing of the corresponding peptides of the unlabeled fragment pool. Fragments were subjected to SDS-PAGE, electrotransferred to a PVDF membrane, and visualized by Ponceau S-staining. As can be seen in Fig. 9 B, lanes 2 and 3, the 85-, 75-, and 45-kD fragments were cleanly separated after SDS-PAGE. These three bands were cut out, and sequenced, revealing that the NH_2 -terminal sequence of each fragment was that of contactin/F11 itself. Some of the lower molecular mass fragments, which were labeled with 125-iodine but which did not bind to the tenascin column are presumably derived from the fibronectin type III homology region of contactin/F11. However, it was difficult to cleanly separate these on SDS-PAGE and only mixed NH_2 -terminal sequences were obtained after blotting onto PVDF membranes and sequencing, precluding a clear identification.

By correlation of size, potential glycosylation, and sequence of the bound fragments, it is estimated that the smallest 45-kD peptide comprises the first three Ig-domains, while the 75- and 85-kD peptides represent most or all the Ig-region (Fig. 9 C). This result shows that the three NH_2 -terminal Ig-like domains of contactin/F11 most likely contain the binding region for tenascin.

Discussion

We have used an affinity chromatography approach to select for cell surface receptors of the extracellular matrix glycoprotein tenascin in chick brain. With this approach, we have identified contactin/F11, a CAM on the surface of neurons, as a major tenascin receptor.

Contactin/F11 is a member of the immunoglobulin-like family of CAMs that was originally reported to be a trans-

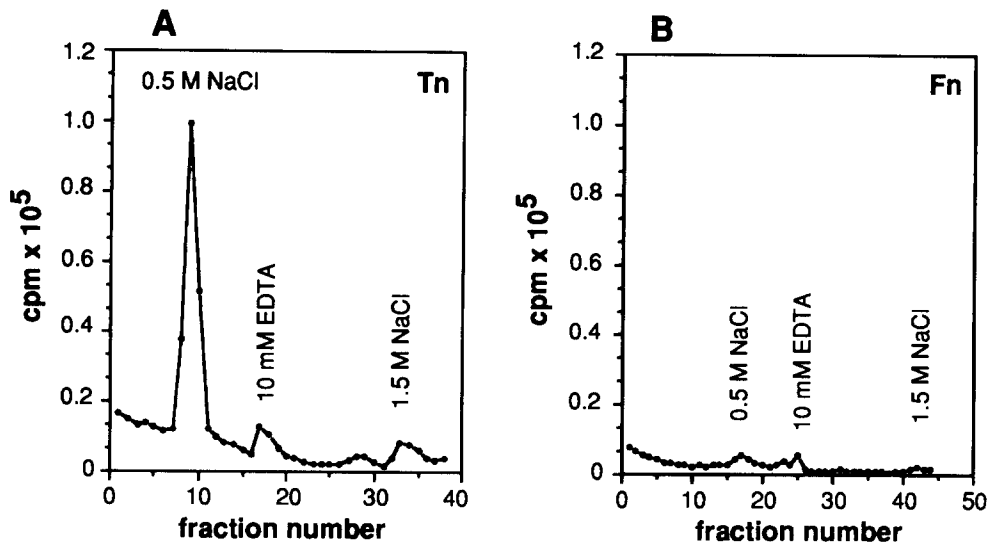


Figure 8. Affinity chromatography of contactin/F11 peptides on tenascin and fibronectin columns (*Tn* and *Fn*). ¹²⁵I-labeled fragments of contactin/F11 bound to the Tn column (*A*) and were eluted with 0.5 M NaCl. No binding was observed to the control Fn column (*B*) and subsequent washes with 10 mM EDTA or 1.5 M NaCl eluted no additional radiolabeled material from either column.

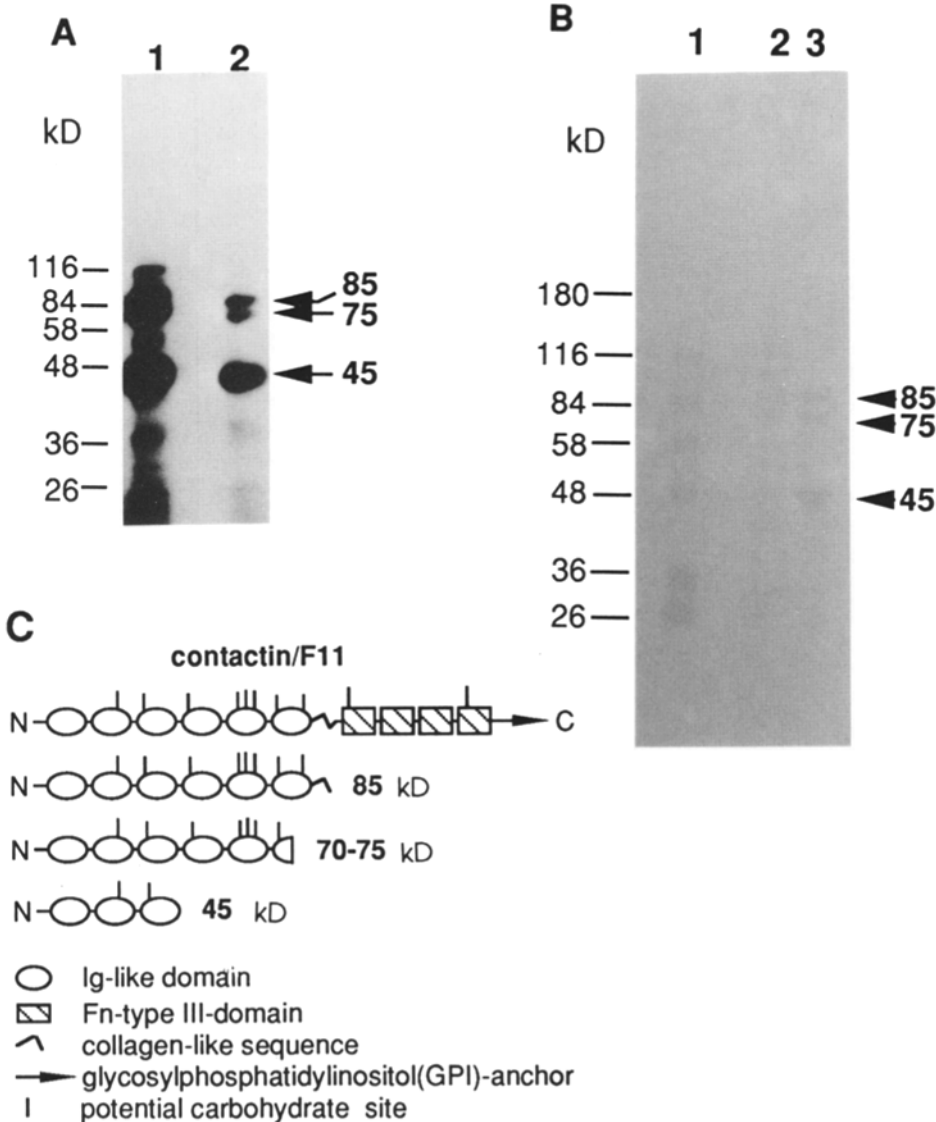


Figure 9. Characterization of tenascin binding contactin/F11 fragments. (*A*) Shows the selective binding to the tenascin column of 85-, 75-, and 45-kD fragments (lane 2) from a ¹²⁵I-labeled pool of contactin/F11 fragments (lane 1), detected by autoradiography after SDS-PAGE. (*B*) NH₂-terminal sequencing of tenascin binding contactin/F11-fragments. Contactin fragments (lanes 2 and 3) were separated by 5–15% gradient SDS-PAGE, electrotransferred to PVDF membrane, and stained with Ponceau S. Lane 1, high molecular mass standard (SDS 7B, Sigma). The 85-, 75-, and 45-kD fragments were cut out and sequenced through their NH₂ termini. The 85-kD fragment began with amino acid residues 3–9, the 75-kD fragment with residues 3–15, and the 45-kD fragment with residues 3–16 of the contactin/F11 amino-terminal. (*C*) A model of contactin/F11 and tenascin-binding fragments shows that the binding site of contactin/F11 for tenascin lies within the first three Ig-like domains.

membrane protein (contactin) (Ranscht, 1988). Molecular cloning of a molecule with identical extracellular domains in chick (F11) (Brümmendorf et al., 1989) and mouse (F3) (Gennarini et al., 1989) suggested the membrane attachment of these latter molecules is through a GPI moiety and raised the possibility that contactin and F11/F3 are isoforms of the same molecule containing a different membrane anchor. For several reasons this appears not to be the case. Firstly, the majority of contactin, like F11, is released from cultured neurons by phosphatidyl inositol specific phospholipase C (PI-PLC) (B. Ranscht, unpublished data). Secondly, contactin is expressed from the corresponding cDNA in CHO and COS cells as a membrane protein susceptible to treatment with PI-PLC, although contactin is never completely removed from the cell surface by enzyme treatment (D. Vestal and B. Ranscht, manuscript in preparation). Lastly, reassessment of the contactin cDNA sequence revealed agreement with the data published by Brümmendorf et al. (1989) (E. O. Berglund and B. Ranscht, manuscript in preparation). These data indicate that contactin and F11 are identical proteins that contain a GPI anchor.

Solid-phase assays using immunopurified contactin/F11 demonstrated the binding specificity between contactin/F11 and tenascin and revealed the preferential binding of contactin/F11 to the tn 190 isoform of tenascin. This implies that the binding site on tenascin is either disrupted or masked by inclusion of the alternatively spliced type III domains. The masking alternative requires that an extra domain(s) in the 220 isoform somehow folds to block the actual contactin/F11 binding site, located in another region of tenascin. As the binding to tn 220 alone is almost reduced to background values, this would require that nearly each arm of the tn 220 hexamer is folded into this inhibitory conformation. We examined rotary shadowed electron micrographs of such preparations and could find no evidence to support such a proposal. A selective denaturation of the tn 220 isoform is also unlikely, as tn 220 is subjected to identical purification conditions as tn 190, with the only difference being that it is bound via the T17 rather than the T16 mAb, both of which recognize epitopes within the fibronectin type III region (Kaplon et al., 1991). In addition, the alternatively spliced region retained its functional activity when human tenascin was also purified with a similar protocol to that of our own (see below; Murphy-Ullrich et al., 1991). For these reasons, we conclude that the contactin/F11 binding site is disrupted by the splicing-in of the additional type III domains. This proposed mechanism for regulating functional activity of tenascin is an intriguing one and may also apply to interactions with other ligands. Fibronectin, for example, was also shown to bind preferentially to tn 190 (Chiquet-Ehrismann et al., 1991).

The importance of alternative splicing in the function of tenascin was further illustrated in another study (Murphy-Ullrich et al., 1991), where human tenascin, when added to cultures of tightly adherent endothelial cells, changed cellular morphology by reducing the size of focal adhesions. This effect was reversed by monoclonal antibodies (mAb 81C6 and mAb 127) which are specific for different domains within the alternatively spliced region of human tenascin (Bourdon et al., 1985; Murphy-Ullrich et al., 1991). This modulation of endothelial cell adhesion was also inhibited by chondroitin sulfate, implying that a cell-surface chondroitin

sulfate proteoglycan or chondroitin sulfate-binding protein is involved in the destabilizing effect of tenascin on these cells. A neuronal chondroitin sulfate proteoglycan (CTB-proteoglycan) (Hoffman and Edelman, 1987) is reported to bind to tenascin and thereby, at least partly, mediate neuron-glia adhesion. Could the neuronal contactin/F11 and the ECM, cytotactin binding (CTB) proteoglycan (or indeed a cell membrane anchored chondroitin sulfate proteoglycan) be alternative receptors for tenascin? It was recently proposed that an inverse gradient of chondroitin sulfate may guide axons during the early stages of growth along the embryonic retina (Brittis et al., 1992). As contactin/F11 and tenascin are also present in the retina at the time of axonal outgrowth, it is possible that these different molecules may combine to promote axon guidance.

We have previously established that the neurite outgrowth promoting property of tenascin can be inhibited by a monoclonal antibody (J1/tn2) which binds close to the boundary of the alternatively spliced region (Lochter et al., 1991). It therefore seems likely that this site is the same or very close to the binding site on tenascin for contactin/F11. The nature of the neuronal receptor responsible for neurite growth on tenascin was neither identified by Wehrle and Chiquet (1990), nor ourselves. Curiously, the behavior of neurons on a tenascin substratum is density dependent. For neurons plated at high densities, fasciculation is encouraged (Kruse and Faissner, 1990), while neurite extension is favored at low cell culture densities (Lochter et al., 1991). Under the latter conditions the binding of the neurons, however, is restricted to the neurite and its growing tip, as the cell bodies do not adhere to tenascin but require an additional substratum such as polyamines. This implies that the receptor(s) should be concentrated in the neurites, a distribution which matches the expression of contactin/F11 both in vitro and in vivo. Mouse contactin/F11 (F3) is concentrated on neurites in mixed fetal brain cultures (Gennarini et al., 1989) and, like tenascin, can support either neurite fasciculation (Rathjen et al., 1987) or neurite extension (Chang et al., 1987; Gennarini et al., 1991). It will be interesting to determine whether the distribution of contactin/F11 differs under conditions that favor neurite outgrowth or fasciculation on a tenascin substratum, and to examine the role of contactin/F11-tenascin interactions in this process.

It is somewhat surprising that only a few examples of the highly versatile immunoglobulin superfamily have been reported to recognize extracellular matrix glycoproteins. This is especially so for the neuronal members of this family, with their chimeric structures of 5-6 immunoglobulin-like domains and 2-5 fibronectin type III homology domains (for review see Edelman and Crossin, 1991) which could potentially interact with different types of receptors. Neuronal receptors of this family participate in homophilic binding, i.e., N-CAM and N-CAM (Cunningham et al., 1987) and L1-L1 (Lemmon et al., 1989) or in heterophilic interactions such as L1-Axonin-1 (Kuhn et al., 1991) and between N-CAM and heparan sulfate (Cole et al., 1986). Although no information is available about other neural receptors, it has been shown that the endothelial intercellular adhesion molecule ICAM-1, which is composed of five tandemly arranged immunoglobulin-like repeats, binds via the most distal repeat to its leukocyte integrin ligand LFA-1 (Staunton et al., 1990). Our finding that contactin/F11 binds via a site

within its three NH₂-terminal immunoglobulin-like repeats to tenascin rests on the selective binding of only the NH₂-terminal fragments to the tenascin column under the same conditions in which intact contactin/F11 is retained. That this is not an artefact of column chromatography is evident from the same fragments not binding to the control columns of Sepharose alone or fibronectin-Sepharose. Although these results are unequivocal in pointing to the Ig-like region of contactin/F11 containing the binding site, identification of which domain carries this site must await further experiments, currently in progress. Nevertheless, our results are in agreement with published evidence for the importance of the distal domains of the immunoglobulin family of CAMs in ligand binding.

How might contactin/F11-tenascin binding affect neuronal patterning *in vivo*? There is an overlapping distribution of the two proteins in the retina (Crossin et al., 1986; Ranscht and Dours, 1988; Rathjen et al., 1991), in the cerebellum (Chuong et al., 1987; Rathjen et al., 1991; Faivre-Sarrailh et al., 1992; Husmann et al., 1992), and in peripheral nerves (Rieger et al., 1986; Wehrle and Chiquet, 1990; Ranscht, B., 1988, unpublished data). The correlation between contactin/F11 and tenascin expression suggests that the interaction between contactin/F11 and tenascin may affect axonal outgrowth or fasciculation, rather than neuronal migration. Consistent with a spatial separation of migration and axonal outgrowth promoting sites on tenascin, anti-tenascin mAbs blocking the migration of granule neurons in cerebellar explant cultures, map to a site on FN type III homology domains 3-5 (Husmann et al., 1992). In contrast, the J1/tn2 mAb, which inhibits neurite extension on tenascin *in vitro* maps close to the FN type III domains 8,9 on chicken tenascin (Lochter et al., 1991) and has no inhibitory effect on granule cell migration (Husmann et al., 1992). This implies that tenascin interacts with distinct neuronal receptors during neuronal migration and axon growth. During later cerebellar development, contactin/F11 (F3) is specifically localized to the axonal extensions, the parallel fibers, of the granule neurons, while the granule cell bodies and dendrites connecting the mossy fibers are negative (Faivre-Sarrailh et al., 1992). As this expression pattern may indicate a role for contactin/F11 in establishing specific axonal connections, it is important to examine whether the tn 190 isoform is the ligand for this interaction.

Both tenascin and contactin/F11 interact with multiple ligands. In addition to tenascin, contactin/F11 binds weakly to itself (Fig. 3 A), to laminin (Fig. 3 A), and restrictin (Rathjen et al., 1991). Tenascin interacts with a neuronal chondroitin sulfate proteoglycan (Hoffman and Edelman, 1987), syndecan (Salmivirta et al., 1991), collagens (Faissner et al., 1990), fibronectin (Chiquet-Ehrismann et al., 1991), and a β_1 -integrin (Bourdon and Ruoslahti, 1989). Cell adhesion or repulsion is the net effect of an interplay between different cell-surface molecules and so it is likely that different domains within each molecule will control different cellular interactions. It would therefore be surprising if contactin/F11 and tenascin alone controlled neuron-glia adhesion; even though contactin/F11 is an exclusive component of neuronal cell surfaces (Ranscht and Dours, 1988), neural tenascin is synthesized by glial cells (Grumet et al., 1985; Kruse et al., 1985; Prieto et al., 1990), and both molecules have been implicated in this interaction. The majority of contactin/F11 is

released from the cell surface of cultured neurons by phosphatidylinositol-specific phospholipase C, so that the availability of contactin/F11 on the cell surface may be enzymatically regulated. This would provide an additional mechanism for the modulation of adhesive interactions between these cells. Having established contactin/F11-tenascin as a ligand pair, it is important to turn to cellular studies to examine under which conditions this interaction is decisive and to explore the interplay between these and other cell surface receptors.

This demonstration of tenascin-contactin/F11 binding reported here presents the first indication of an interaction between immunoglobulin-like domains and fibronectin-type III repeats. Both of these structural elements are widely spread among various CAMs and ECM-components. It will now be a challenge to see whether interactions between Ig-like domains and FN-type III elements form a basic mechanism through which a wider range of cell-cell and cell-matrix interactions are transmitted.

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