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# Glycosaminoglycans modulate fibronectin matrix assembly and are essential for matrix incorporation of tenascin-C

**Chang Y. Chung* and Harold P. Erickson†**

Department of Cell Biology, Duke University Medical Center, Durham, NC 27710, USA

*Present address: Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093-0634, USA
†Author for correspondence (e-mail: Harold_Erickson@cellbio.duke.edu)

## SUMMARY

**We have investigated the role of glycosaminoglycans in fibronectin matrix assembly and the incorporation of tenascin-C into matrix fibrils. Chinese hamster ovary cell mutants with a total block in heparan and chondroitin sulfate production failed to assemble a fibronectin matrix, and incorporated no tenascin-C. Another mutant with reduced heparan sulfate produced a normal fibronectin matrix but failed to incorporate tenascin-C. Excess soluble glycosaminoglycans inhibited the binding of tenascin-C to purified fibronectin in ELISA, and completely blocked incorporation into matrix fibrils. Treating cultured cells with xyloside, which interferes with glycosaminoglycan attachment to proteoglycans, also completely blocked their ability to incorporate tenascin-C into matrix fibrils. We conclude that proteoglycans bound to fibronectin fibrils play a major role in binding tenascin-C to these fibrils. We examined more closely the large heparan sulfate proteoglycan, perlecan, and found that it co-localizes with tenascin-C and fibronectin in the matrix. The perlecan binding site in tenascin-C was mapped to the fibronectin type III domains 3-5, but this binding was strongly enhanced for the small splice variant, which is the major form incorporated into the matrix. Apparently when the alternative splice segment is inserted after domain 5 it inhibits perlecan binding. Thus heparan sulfate glycosaminoglycans, and perlecan in particular, may play a role in incorporation of the small splice variant of tenascin-C into fibronectin matrix fibrils.**

Key words: Fibronectin, Tenascin, Glycosaminoglycan, Proteoglycan, Perlecan, Extracellular matrix

## INTRODUCTION

Tenascin-C (TN-C), a large glycoprotein of the extracellular matrix (ECM), is expressed in many embryonic and some adult tissues (Erickson and Bourdon, 1989; Erickson, 1993). The distribution of TN-C in tissues is regulated by expression in the cells that secrete it, but probably also by binding to other ECM proteins. Binding to ECM proteins may also affect presentation of TN-C to cellular receptors, modulating biological activities.

TN-C largely co-localizes with fibronectin (FN) fibrils both in cell cultures and in embryos (Chiquet and Fambrough, 1984b; Riou et al., 1988). Direct binding of the small splice variant of TN-C to purified FN and to FN fibrils of cell cultures has been demonstrated (Chiquet-Ehrismann et al., 1991; Chung et al., 1995). The strongest binding of TN-C to FN was obtained in conditioned medium of cultured cells, suggesting the presence of a third molecule that might bridge and augment the binding of TN-C to FN. Since proteoglycans bind to both TN-C and FN, they are likely candidates for this enhancement of binding.

Two heparin-binding sites have been identified in TN-C. A heparin binding site in TNfn5, the fifth fibronectin type III (FN-III) domain, was demonstrated with bacterial expression proteins (Aukhil et al., 1993; Weber et al., 1995). The second heparin-binding site is in TNfbg, the C-terminal fibrinogen-like domain of TN-C (Aukhil et al., 1993), and this site is active in native TN-C (Joshi et al., 1993; Fischer et al., 1995). Aukhil et al. (1990) also demonstrated that rat embryo fibroblasts can adhere to TNfbg, and this adhesion was inhibited by exogenous heparin or by perturbing the cells' glycosaminoglycan (GAG) chain synthesis with sodium chlorate. This result suggests cell surface heparan-sulfate proteoglycan(s) on fibroblasts can bind to TNfbg.

Several types of proteoglycans have already been demonstrated to bind TN-C. When TN-C is immobilized on an antibody affinity column it can bind chondroitin sulfate proteoglycans derived from smooth muscle culture (Chiquet and Fambrough, 1984a) and from cartilage (Vaughan et al., 1987). Binding of TN-C to a brain proteoglycan was demonstrated in vitro (Hoffman and Edelman, 1987). Two cell surface heparan sulfate proteoglycans bind TN-C via their GAG chains: syndecan, from embryonic tooth-mesenchyme (Salmivirta et al., 1991); and glypican, from brain (Vaughan et al., 1994). Neurocan and receptor protein tyrosine phosphatase β, a transmembrane chondroitin sulfate proteoglycan from brain, were shown to bind TN-C in vitro and co-localize with it in vivo (Barnea et al., 1994; Grumet et al., 1994). The binding of these proteoglycans was not mediated by the GAGs (Grumet et al., 1994), but probably by asparagine-linked carbohydrates on the proteoglycans (Milev et al., 1994).

Since several proteoglycans have been shown to bind TN-C, it seems that some matrix-bound proteoglycans might mediate the incorporation of TN-C into the ECM. In the present study we examined matrix assembly by Chinese hamster ovary (CHO) cell lines that are defective in specific aspects of GAG synthesis. We discovered that GAGs modulate the matrix assembly of both FN and TN-C. We also examined directly the large proteoglycan, perlecan, demonstrating that it binds to TNfn3-5, and implicating it for a role in the localization of TN-C to FN matrix fibrils.

## MATERIALS AND METHODS

### Cells and cell cultures

A human glioma cell line U-251MG (clone 3, obtained from Dr Darell Bigner, Duke University Medical Center), and the BHK cells transfected with the small and large splice variants of TN-C (Aukhil et al., 1993) were grown in Dulbecco's modified Eagle's medium, high glucose supplemented with 10% heat inactivated fetal calf serum. CHO mutant cell lines (Esko, 1991) were generously provided by Dr Jeffrey Esko, University of Alabama at Birmingham, and grown in Ham's F-12 medium supplemented with 10% fetal bovine serum. We tested the CHO cell lines for expression of FN and perlecan by western blotting cell lysates, and found no differences in the different lines.

### Proteins and antibodies

Splice variants of human TN-C were produced in transfected BHK cells (Aukhil et al., 1993). HxB.L is the large splice variant, and HxB.S is the small variant, missing the segment TnfnA-D (see Aukhil et al., 1993, for the domain structure of TN-C and nomenclature of the segments). The purified tenascin showed no detectable fibronectin or perlecan when tested by western blotting. TN-C was purified from culture supernatant of the BHK or U-251MG cells by gel filtration and mono Q ion exchange chromatography as described by Aukhil et al. (1990). FN was purified from human plasma or horse serum by gelatin-agarose affinity chromatography (Engvall and Ruoslahti, 1977). Bacterial expression proteins were purified as described by Aukhil et al. (1993). A rabbit polyclonal antibody, HxB-9172, was produced by immunizing with recombinant TNfn1-5. Rat monoclonal antibody RCB-1 (Hiraiwa et al., 1993) against human TN-C was obtained from Dr Moriaki Kusakabe, RIKEN, Japan. This antibody binds to the EGF or central knob region of TN-C, and was previously shown to bind both HxB.L and HxB.S in cultures fixed by different protocols (Chung et al., 1995; the antibody was called by its earlier name 8C9 in that study). Anti-perlecan antiserum and purified perlecan were provided by Dr John Hassell, University of Pittsburgh. Purified perlecan was also obtained from Dr Peter Yurchenko, University of Medicine and Dentistry of New Jersey. Heparan sulfate (from bovine kidney) and chondroitin sulfate (from shark cartilage) were purchased from Sigma.

### Immunostaining

For immunocytochemistry, cells were grown in Labtek chamber slides (Nunc) to confluence and incubated with HxB.S for 24-48 hours (CHO cells) or left 24 hours in their own conditioned medium (BHK cells). Cells were washed with phosphate-buffered saline (PBS), and fixed for 5 minutes in ice-cold methanol:acetone (1:1). Primary rabbit antiserum against FN or perlecan was added at a final dilution of 1:100 in PBS containing 0.1% Triton X-100 and 0.01% bovine serum albumin. Anti-TN monoclonal antibody RCB-1 was added at a concentration of 10 μg/ml. The slides were incubated for 1 hour at 4°C and then washed three times with PBS containing 0.05% Tween-20 for 15 minutes and fluorescein-conjugated anti-rat IgG (Sigma) and rhodamine-conjugated anti-rabbit IgG (TAGO, Inc.) were added at a final dilution 1:100. After a one hour incubation, slides were washed three times with PBS-Tween-20, the chambers were detached carefully and slides were mounted for observation and photography.

### Isolation of glycosaminoglycans from BHK cells

GAGs were isolated as described by Bame and Esko (1989) with minor modifications. For most preparations shark cartilage chondroitin sulfate (Sigma) was added as a carrier at a concentration of 1 mg/ml after the initial extraction with NaOH. The final purification was on a mono Q column, where the GAGs eluted at 1.0-1.2 M NaCl. The concentration of GAGs was measured as described by Dische (1947). In preparations with added carrier chondroitin sulfate, the total yield of GAG was 10-20% greater when purified from normal BHK cells than from cells treated with sodium chlorate, allowing us to estimate that about 10-20% of the total GAG was from BHK cells, and ~80% was carrier chondroitin sulfate.

### Solid phase binding assay

A solid-phase binding assay was used to study the interaction between TN-C and FN or perlecan. In this assay, 96-well plates (Falcon 3912) were coated overnight at 4°C or 2 hours at 37°C with FN, 20 μg/ml in D-TBS (Tris buffered saline, with divalent cations; 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$); or with HxB.S or HxB.L (50-70 μg/ml). Plates were washed, blocked for 1-2 hours at 37°C with 5% non-fat dry milk in PBS. FN plates were then incubated with soluble HxB.S (20 μg/ml in D-TBS) containing the indicated GAG. Plates coated with TN-C or fragments were incubated with different concentrations of perlecan in D-TBS containing 100 μg/ml bovine serum albumin for 2 hours at room temperature. After washing the wells, bound proteins were detected by conventional ELISA technique using polyclonal anti-perlecan antibody and horseradish peroxidase-conjugated secondary antibody.

## RESULTS

### Altered FN matrix assembly and incorporation of TN-C by CHO cells with defective GAG assembly

To investigate the possible role of proteoglycans, we tested the matrix assembly and incorporation of HxB.S into the ECM, using several mutant CHO cell lines that are defective in different aspects of GAG synthesis. These CHO cells make very little TN-C (unpublished data), and none detectable with the human-specific RCB-1 antibody. After growing CHO cells to confluence, purified human HxB.S, the small splice variant of TN-C, was added to cells, and incubated a further 24-48 hours to allow incorporation into the matrix.

Wild-type CHO K1 cells deposited FN predominantly in a pericellular matrix, as demonstrated by immunostaining (Fig. 1). HxB.S added to the medium was incorporated into these fibrils and mostly co-localized with the FN. CHO 745 cells, which lack both heparan sulfate and chondroitin sulfate GAGs, failed to deposit a FN matrix, and did not incorporate exogenous HxB.S. CHO 606 cells, which make heparan sulfate chains with 2- to 3-fold lower *N*- and *O*-sulfation, as well as more dispersed *N*-sulfated glucosamine (Zhang and Esko, 1995), produced a FN matrix equal to or stronger than wild type. In Fig. 1 the staining of the wild-type CHO K1 matrix varies from quite strong on the left to much weaker on the right. The staining of the CHO 606 matrix in Fig. 1 is equivalent to the strongest staining of the wild-type matrix. Examination of many fields showed that FN fibrils in CHO 606 cultures were

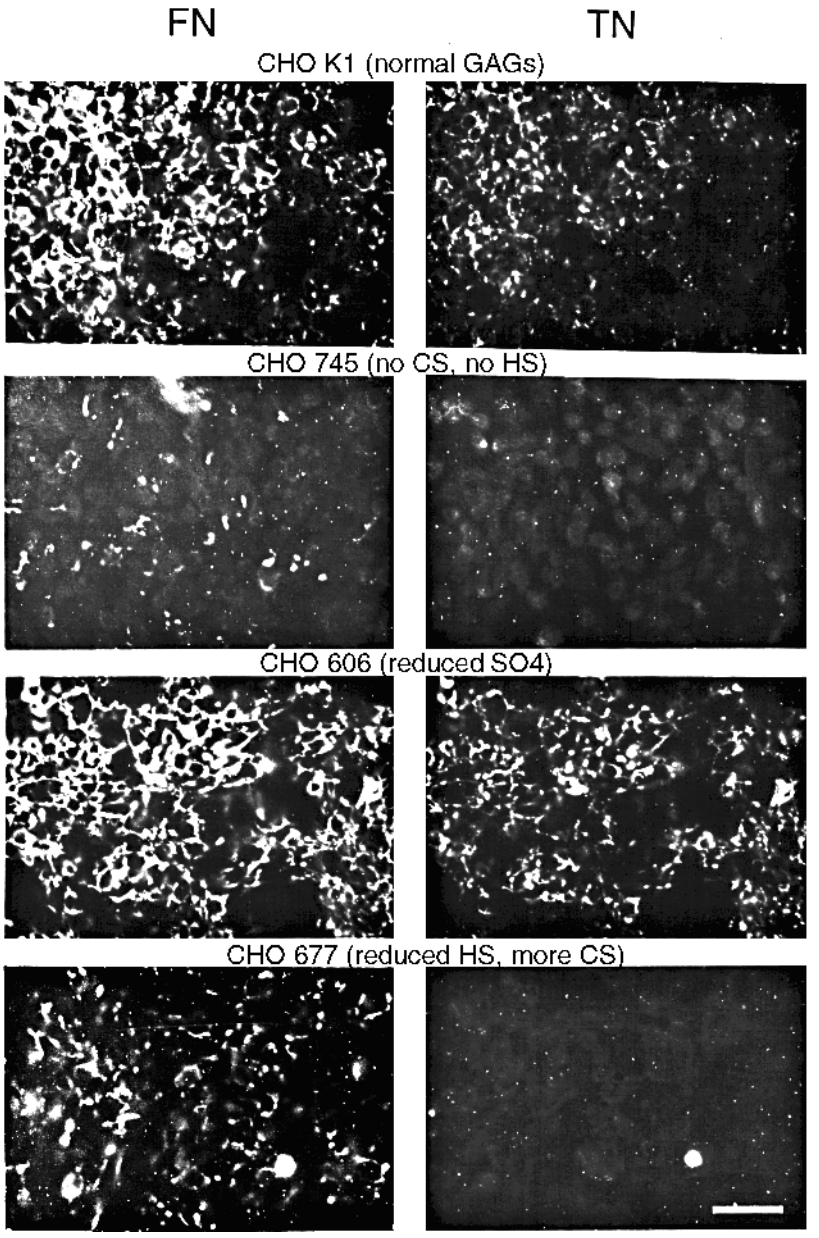


**Fig. 1.** Distribution of FN and TN-C in normal and mutant CHO cell cultures. Purified HxB.S was added to confluent cells at a concentration of 15 μg/ml and incubated for 24 hours. Cells plus matrix were then fixed with cold acetone:methanol (1:1) and stained with affinity purified anti-FN polyclonal antibody and RCB-1 monoclonal antibody against human TN. Bar, 50 μm.

generally longer and thicker than wild-type matrix, with more extensive branching and integration with fibrils from neighboring cells. This matrix also showed enhanced incorporation of HxB.S.

The most specific effect on matrix was seen with CHO 677 cells, which have a block in heparan sulfate synthesis (Lidholt et al., 1992). These cells showed a substantial reduction in FN fibrils, but the most striking effect was an almost total block of incorporation of HxB.S into the ECM by the CHO 677 cells.

### Binding of HxB.S to purified FN and matrix fibrils can be blocked by BHK-GAGs and by xyloside treatment

To examine the role of GAGs in the interaction between TN-C and FN, we tested commercial heparan sulfate and chondroitin sulfate, as well as GAGs purified from BHK cells (BHK-GAG), for their effect on the binding of TN-C to FN in a solid phase binding assay (Fig. 2). Commercial GAGs had no effect, but soluble BHK-GAG inhibited the binding of HxB.S to FN. The BHK-GAG seemed to interact primarily with the soluble HxB.S because pretreatment of the FN substrate with GAG did not affect the binding (not shown).

BHK-GAG was also tested for its effect on the incorporation of HxB.S into FN fibrils of cultured CHO and BHK cells. As shown in Fig. 3, cultures of BHK cells secreting HxB.S formed normal FN fibrils and incorporated the HxB.S into these fibrils when grown without additive. An identical matrix containing both FN and HxB.S was formed in the presence of 100 μg/ml commercial chondroitin sulfate or heparan sulfate (not shown). (The failure of commercial heparan sulfate to mimic the BHK-GAG is probably due to the very different pattern of sulfation in GAGs from different

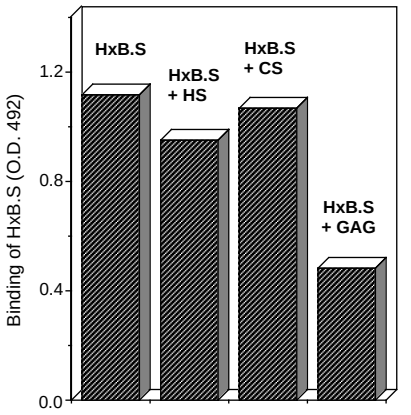


**Fig. 2.** Binding of HxB.S to FN-coated plastic, and the effect of soluble GAGs. FN was coated on plastic at 20 μg/ml in D-TBS. Wells were then incubated with HxB.S (15 μg/ml), or HxB.S plus commercial heparan sulfate (100 μg/ml); chondroitin sulfate (100 μg/ml); or BHK-GAG (estimated to be 10-20 μg/ml, plus 80 μg/ml carrier chondroitin sulfate). The amount of bound HxB.S was determined by ELISA. The values are the mean of duplicates in one experiment (similar results were obtained from two more independent experiments).

sources.) However, when cells were cultured in the presence of BHK-GAG (estimated to be 10-20 μg/ml, plus 80-90 μg/ml carrier chondroitin sulfate), deposition of the FN matrix was substantially inhibited. The thin FN branches were absent and thicker FN fibrils were reduced (Fig. 3c). Incorporation of HxB.S into the FN fibrils was almost completely blocked; TN-C staining was reduced to a few patches that did not co-localize with FN (Fig. 3d). Thus, the presence of BHK-GAG noticeably inhibited the deposition of the FN matrix, and blocked even more strongly the incorporation of HxB.S into the matrix.

The involvement of GAGs in TN-FN binding was further examined by treating BHK cells with *p*-nitrophenyl-β-D-xyloside which acts as an artificial acceptor and blocks the addition of GAG chain to core proteins. Cells treated with xyloside showed normal deposition of FN fibrils, but incorporation of HxB.S into the FN fibrils was completely blocked (Fig. 4). The residual staining of HxB.S was confined to patches on or inside cells, i.e. it did not co-localize with FN.

### Co-distribution of perlecan with FN and TN

Perlecan, a large heparan sulfate proteoglycan, co-localizes with FN in the ECM (Yamagata et al., 1993). Since we have demonstrated that TN-C largely co-localizes with FN fibrils (Figs 1, 3, and 4) perlecan should also be localized in these fibrils. Double label immunofluorescence confirmed this expectation for BHK-HxB.S cells (Fig. 5). The co-localization of perlecan with both FN and TN-C was also observed in CHO 606 cells (not shown). This result suggests that perlecan might be a proteoglycan that mediates or strengthens the binding of TN-C to FN matrix fibrils.

### Binding of perlecan to TN-C in a solid-phase binding assay

We used an ELISA to explore the binding of perlecan to HxB.S and HxB.L coated on plastic and different concentrations of perlecan in solution (Fig. 6). In this experiment about five times more perlecan bound to the HxB.S substrate than to HxB.L. In Figs 7 and 8 the ratio was greater than ten (note that the binding to BSA has been subtracted from the values plotted in Figs 6 and 7, while Fig. 8 shows binding without subtracting this background).

To map the perlecan binding site, we initially tested expression proteins containing the FN-III domains. As shown in Fig. 7, perlecan bound TNfn1-8, which contains all the FN-III domains of HxB.S, much better than TNfnALL, which contains also the alternatively spliced segment like HxB.L. We then used smaller fragments to map the binding site more precisely. Fig. 8 shows prominent binding to TNfn1-5 and TNfn3-5, and a weaker binding to TNfn3. We conclude that the binding site for perlecan is primarily in TNfn3-5, with partial contributions from both TNfn3 and TNfn4-5. Note that the binding to TNfn1-5 and TNfn3-5 (Fig. 8), and TNfn1-8 (Fig. 7) are large and comparable to binding to HxB.S. This implies that the binding site is completely in TNfn3-5, with little contribution from TNfn6-8.

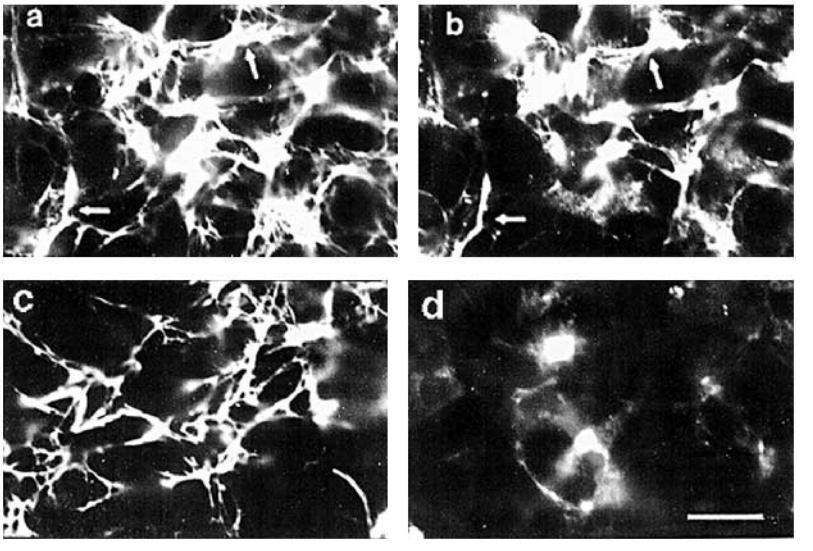


**Fig. 3.** BHK-GAG inhibits incorporation of TN-C into FN fibrils in cell culture. BHK-HxB.S secreting cells were grown to confluence, GAG was added, and growth continued for two more days. Cultures were then stained for FN (a,c) and TN-C (b,d). A control culture without added GAG is shown in a and b. A culture with BHK-GAG (10-20 μg/ml plus 80 μg/ml carrier chondroitin sulfate) is shown in c and d. Cultures with 100 μg/ml commercial chondroitin or heparan sulfate appeared identical to the control cultures. Arrows indicate co-localization of FN and TN-C. The automatic exposure was longer in d to show the weak staining of cellular TN-C. Bar, 20 μm.

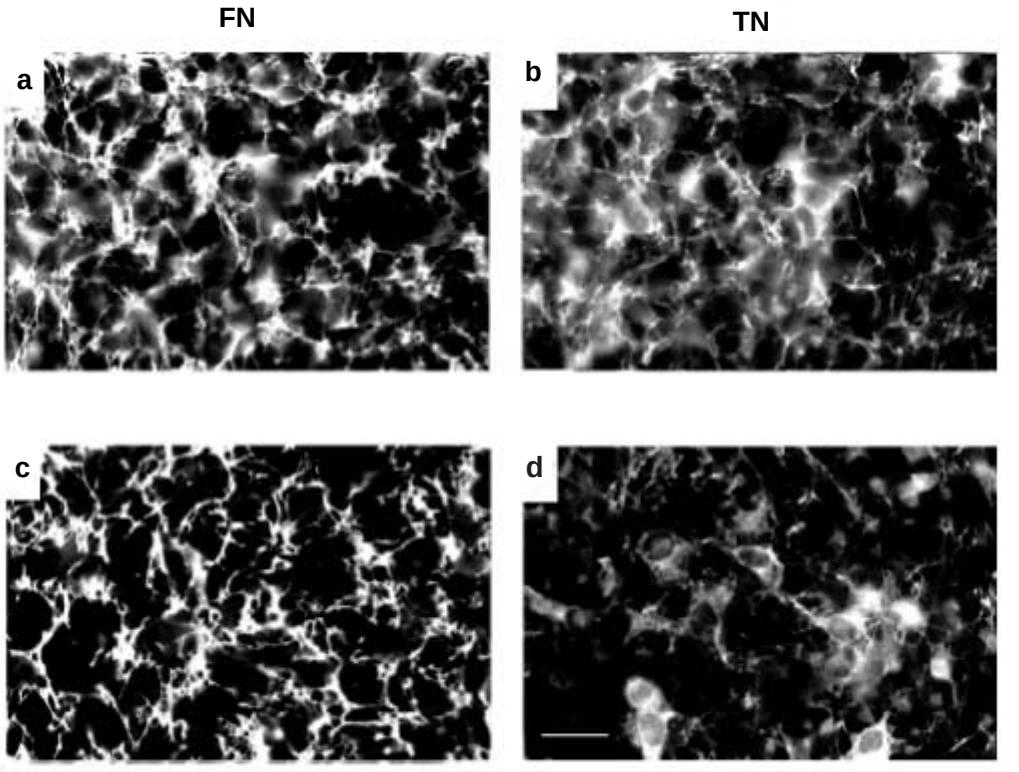


**Fig. 4.** Inhibition of the TN-C incorporation into the matrix of BHK cells by treatment with *p*-nitrophenyl-βD-xyloside. BHK cells secreting HxB.S were plated ($5\times10^5$ cells/ml) and grown to confluence in the absence (a and b) or presence (c and d) of 200 μM xyloside and stained with antibodies against FN and TN-C. The automatic exposure was longer in d to show the weak staining of cellular TN-C.

## DISCUSSION

We found two prominent defects in matrix assembly by CHO cells mutant in proteoglycan synthesis. First and most surprising was the effect of proteoglycans on assembly of the FN matrix. The FN matrix was blocked in the CHO-745 cell line, which is completely deficient in GAGs. FN matrix assembly was also partially blocked by addition of soluble BHK-GAGs to BHK cell cultures. However, an apparently normal FN matrix was assembled by the CHO cells with reduced heparan sulfate, and by BHK cells treated with xyloside. The block of FN matrix assembly may be modulated differently depending on the specific defects in GAGs. Until now studies of FN matrix assembly have focused on interactions between FN molecules and between FN and cell receptors. Our observations now suggest that proteoglycans play an essential role in assembly of FN matrix fibrils.

The second observation confirmed our initial hypothesis that proteoglycans may have an important role in attachment of TN-

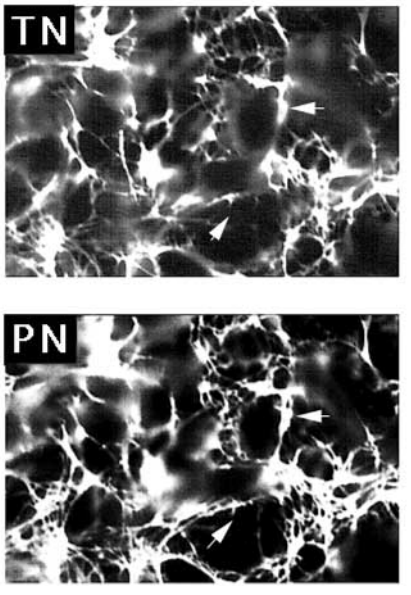


**Fig. 5.** Co-distribution of perlecan with TN-C in BHK-HxB.S cell cultures. The distribution of perlecan and TN-C was determined by staining with anti-perlecan polyclonal antibody and rat monoclonal anti-TN antibody. PN, perlecan, TN, tenascin. The arrows mark some sites of co-distribution. Bar, 20 μm.

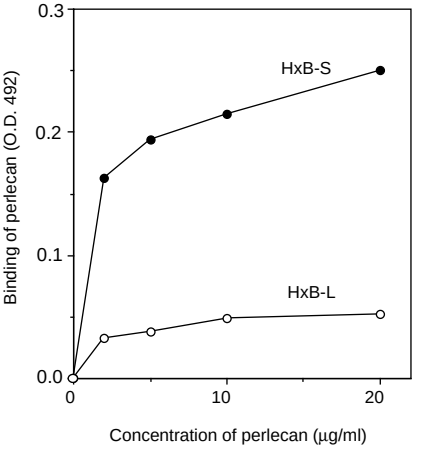


**Fig. 6.** Binding of perlecan to native TN-C splice variants. HxB.L (20 μg/ml) and HxB.S (15 μg/ml) were coated on 96-well plates overnight at 4°C, and the remaining binding sites were blocked by incubation with 5% non-fat dry milk for 1 hour at 37°C. Increasing concentrations of perlecan in D-TBS plus 0.01% bovine serum albumin were incubated for 2 hours at room temperature, and the amount of bound perlecan was measured by ELISA with a polyclonal antibody against perlecan. The values represent the mean of three determinations.

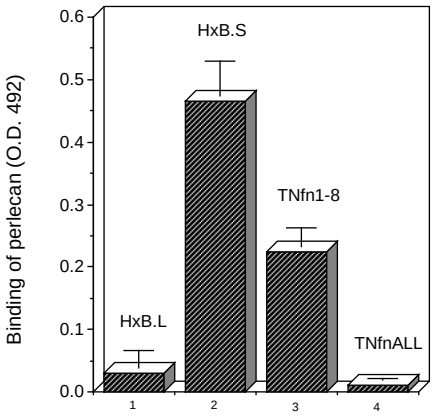


**Fig. 7.** Binding of perlecan to native TN-C and to expression proteins containing only FN-III domains. HxB.S (50 μg/ml), HxB.L (70 μg/ml), TNfn1-8 (50 μg/ml), or TNfnALL (70 μg/ml) were coated on plastic wells and incubated with perlecan (20 μg/ml). The amount of bound perlecan was determined by ELISA. The monomeric TNfn1-8 and TNfnALL always gave a smaller binding response than the hexameric native tenascin when coated on the basis of equal molar subunits. In the present case the monomeric fragments are coated at equal protein weight, but 2-3 fold higher molar concentration relative to native tenascin.

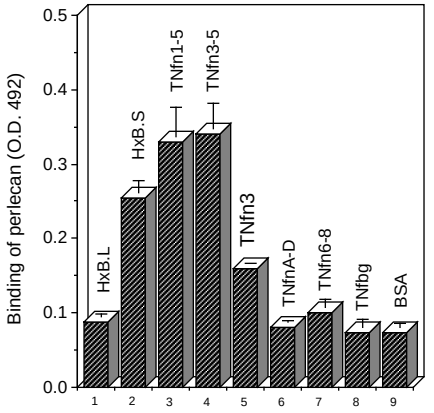


**Fig. 8.** Binding of perlecan to small segments of TN-C. Plastic wells were coated with HxB.S (50 μg/ml), HxB.L (70 μg/ml), or recombinant TN-C fragments (50 μg/ml) as in Fig. 3. After blocking and washing, perlecan (20 μg/ml) was incubated at room temperature for 2 hours, and bound perlecan was determined by ELISA. The values represent the mean of three determinations.

C to the matrix. Incorporation of TN-C into FN matrix fibrils was blocked completely by a genetic deficiency in heparan sulfate in CHO cells, by adding excess soluble BHK-GAGs to the medium, or by treatment of BHK cells with xyloside. Canfield and Shor (1995) also found that bovine aortic endothelial cells cultured in the presence of xyloside contained a reduced level of tenascin while the level of fibronectin was not markedly affected. They further demonstrated that the level of tenascin was reduced by β-D-xyloside more than by its non-active isomer α-D-xyloside, suggesting that the decrease was related to GAG assembly rather than a non-specific inhibition of protein synthesis. There are two possibilities for the mechanism of xyloside inhibition. It could be due to reduction of GAGs on the matrix proteoglycans, or to the release of free GAGs into the medium that could bind TN and inhibit its attachment like the added BHK GAGs.

Previous studies have shown that TN-C can bind directly to purified FN, but binds much more strongly to FN matrix fibrils. The present results suggest that the direct binding of TN-C to FN is not sufficient to incorporate it into the ECM. Heparan sulfate proteoglycans seem to be essential for efficient incorporation.

In order for proteoglycans to mediate TN-C matrix incorporation, they must have binding sites for both TN-C and the matrix. Since soluble GAGs purified from BHK cells and removed from the core protein can block incorporation when added with TN-C, but not when added to the matrix before TN-C, this implies that TN-C binds to GAG chains. Binding of proteoglycans to the FN matrix may involve both the core protein and the GAG chains. A previous study showed that binding of perlecan to the ECM of fibroblast cultures was mediated primarily by the core protein (Heremans et al., 1988). Our observation that alterations in GAG synthesis can affect assembly of the FN matrix suggest that the GAG chains may also participate in this binding. A consistent picture is that proteoglycans are first attached to the matrix, and their heparan sulfate GAGs can subsequently bind TN-C.

The large heparan sulfate proteoglycan, perlecan, is a major component of the matrix and it co-localizes with FN fibrils and TN-C. The integration of perlecan into the basement membrane matrix can involve binding of the core protein and glycosaminoglycans to different molecules. Nidogen binds to the core protein of perlecan and laminin binds to the GAGs, resulting in a ternary complex (Battaglia et al., 1992). Perlecan can bind to the second FN-III domain of FN (Heremans et al., 1990), and this may be the primary interaction localizing it to FN matrix fibrils. The binding of perlecan to a FN matrix was also shown to be mediated by the core protein (Heremans et al., 1988). Thus perlecan is a likely candidate for binding simultaneously to FN and TN-C, and thereby mediating the incorporation of TN-C into the matrix.

Remarkably, both perlecan and FN bind preferentially the small splice variant of TN-C, which is missing the alternatively spliced segment inserted between TNfn5 and TNfn6. The cell surface molecule contactin/F11 also binds preferentially to the small splice variant, and a simple mechanism has been elucidated for this case. The binding of contactin/F11 requires both domains TNfn5 and TNfn6, and the binding site is interrupted by insertion of the alternatively spliced segment between these two (Weber et al., 1996). However, this mechanism does not seem to explain the preference of perlecan and FN for the small tenascin splice variant, since both molecules appear to bind as well to TNfn1-5 and TNfn3-5 as to TNfn1-8 (Figs 7 and 8; see also Chung et al., 1995).

An alternative mechanism for preferential binding to the small splice variant would invoke a conformational change in TNfn5, depending on whether TNfn6 or TNfnA were interfaced as the adjacent domain. This is a somewhat ugly hypothesis, since most FN-III domains seem to fold as independent units. However, there is a substantial interface between most FN-III domains (Leahy et al., 1996), so it is possible that contacts across this interface could modify the structure of the adjacent domains. New expression proteins will be needed to test this hypothesis.

A preferential binding of the small splice variant was observed for FN, but it was much more pronounced for perlecan and matrix fibrils. This suggests that perlecan or another proteoglycan is largely responsible for the binding of small TN-C to the matrix. Since perlecan (Gauer et al., 1996) and TN-C (Chiquet-Ehrismann et al., 1988; Lightner and Erickson, 1990; Joshi et al., 1993) can both modulate cell adhesion to fibronectin, the matrix containing these molecules may have substantially altered adhesion properties, in particular enhancing the migration of cells through it.

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## REFERENCES

**Aukhil, I., Slemp, C. A, Lightner, V. A., Nishimura, K., Briscoe, G. and Erickson, H. P.** (1990). Purification of hexabrachion (tenascin) from cell culture conditioned medium, and separation from a cell adhesion factor. *Matrix* **10**, 98-111.

**Aukhil, I., Joshi, P., Yan, Y. and Erickson, H. P.** (1993). Cell- and heparin-binding domains of the hexabrachion arm identified by tenascin expression proteins. *J. Biol. Chem.* **268**, 2542-2553.

**Bame, K. J. and Esko, J. D.** (1989). Undersulfated heparan sulfate in a Chinese hamster ovary cell mutant defective in heparan sulfate N-sulfotransferase. *J. Biol. Chem.* **264**, 8059-8065.

**Barnea, G., Grumet, M., Milev, P., Silvennoinen, O., Levy, J. B., Sap, J. and Schlessinger, J.** (1994). Receptor tyrosine phosphatase beta is expressed in the form of proteoglycan and binds to the extracellular matrix protein tenascin. *J. Biol. Chem.* **269**, 14349-14352.

**Battaglia, C., Mayer, U., Aumailley, M. and Timpl, R.** (1992). Basement-membrane heparan sulfate proteoglycan binds to laminin by its heparan sulfate chains and to nidogen by sites in the protein core. *Eur. J. Biochem.* **208**, 359-366.

**Canfield, A. E. and Schor, A. M.** (1995). Evidence that tenascin and thrombospondin-1 modulate sprouting of endothelial cells. *J. Cell Sci.* **108**, 797-809.

**Chiquet, M. and Fambrough, D. M.** (1984a). Chick myotendinous antigen. II. A novel extracellular glycoprotein complex consisting of large disulfide-linked subunits. *J. Cell Biol.* **98**, 1937-1946.

**Chiquet, M. and Fambrough, D. M.** (1984b). Chick myotendinous antigen. I. A monoclonal antibody as a marker for tendon and muscle morphogenesis. *J. Cell Biol.* **98**, 1926-1936.

**Chiquet-Ehrismann, R., Kalla, P., Pearson, C. A., Beck, K. and Chiquet, M.** (1988). Tenascin interferes with fibronectin action. *Cell* **53**, 383-390.

**Chiquet-Ehrismann, R., Matsuoka, Y., Hofer, U., Spring, J., Bernasconi, C. and Chiquet, M.** (1991). Tenascin variants: Differential binding to fibronectin and distinct distribution in cell cultures and tissues. *Cell Regul.* **2**, 927-938.

**Chung, C. Y., Zardi, L. and Erickson, H. P.** (1995). Binding of tenascin-C to soluble fibronectin and matrix fibrils. *J. Biol. Chem.* **270**, 29012-29017.

**Dische, Z.** (1947). A new specific color reaction of hexuronic acids. *J. Biol. Chem.* **167**, 189-198.

**Engvall, E. and Ruoslahti, E.** (1977). Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. *Int. J. Cancer* **20**, 1-5.

**Erickson, H. P. and Bourdon, M. A.** (1989). Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. *Annu. Rev. Cell Biol.* **5**, 71-92.

**Erickson, H. P.** (1993). Tenascin-C, tenascin-R and tenascin-X: A family of talented proteins in search of functions. *Curr. Opin. Cell Biol.* **5**, 869-876.

**Esko, J. D.** (1991). Genetic analysis of proteoglycan structure, function, and metabolism. *Curr. Opin. Cell Biol.* **3**, 805-816.

**Fischer, D., Chiquet-Ehrismann, R., Bernasconi, C. and Chiquet, M.** (1995). A single heparin binding region within the fibrinogen-like domain is functional in chick tenascin-C. *J. Biol. Chem.* **270**, 3378-3384.

**Gauer, S., Schulzelohoff, E., Schleicher, E. and Sterzel, R. B.** (1996). Glomerular basement membrane-derived perlecan inhibits mesangial cell adhesion to fibronectin. *Eur. J. Cell Biol.* **70**, 233-242.

**Grumet, M., Milev, P., Sakurai, T., Karthikeyan, L., Bourdon, M., Margolis, R. K. and Margolis, R. U.** (1994). Interaction with tenascin and differential effects on cell adhesion of neurocan and phosphacan, two major chondroitin sulfate proteoglycans of nervous tissue. *J. Biol. Chem.* **269**, 12142-12146.

**Heremans, A., Cassiman, J.-J., Van den Berghe, H. and David, G.** (1988). Heparan sulfate proteoglycan from the extracellular matrix of human lung fibroblasts: Isolation, purification, and core protein characterization. *J. Biol. Chem.* **263**, 4731-4739.

**Heremans, A., De Cock, B., Cassiman, J.-J., Van den Berghe, H. and David, G.** (1990). The core protein of the matrix-associated heparan sulfate proteoglycan binds to fibronectin. *J. Biol. Chem.* **265**, 8716-8724.

**Hiraiwa, N., Kida, H., Sakakura, T. and Kusakabe, M.** (1993). Induction of tenascin in cancer cells by interactions with embryonic mesenchyme mediated by a diffusible factor. *J. Cell Sci.* **104**, 289-296.

**Hoffman, S. and Edelman, G. M.** (1987). A proteoglycan with HNK-1 antigenic determinants is a neuron-associated ligand for cytotactin. *Proc. Nat. Acad. Sci. USA* **84**, 2523-2527.

**Joshi, P., Chung, C.-Y., Aukhil, I. and Erickson, H. P.** (1993). Endothelial cells adhere to the RGD domain and the fibrinogen-like terminal knob of tenascin. *J. Cell Sci.* **106**, 389-400.

**Leahy, D. J., Aukhil, I. and Erickson, H. P.** (1996). 2.0 Å crystal structure of a four-domain segment of human fibronectin encompassing the RGD loop and synergy region. *Cell* **84**, 155-164.

**Lidholt, K., Weinke, J. L., Kiser, C. S., Lugemwa, F. N., Bame, K. J., Cheifetz, S., Massagué, J., Lindahl, U. and Esko, J. D.** (1992). A single mutation affects both *N*-acetylglucosaminyltransferase and glucuronosyltransferase activities in a Chinese hamster ovary cell mutant defective in heparan sulfate biosynthesis. *Proc. Nat. Acad. Sci. USA* **89**, 2267-2271.

**Lightner, V. A. and Erickson, H. P.** (1990). Binding of hexabrachion (tenascin) to the extracellular matrix and substratum and its effect on cell adhesion. *J. Cell Sci.* **95**, 263-277.

**Milev, P., Friedlander, D. R., Sakurai, T., Karthikeyan, L., Flad, M., Margolis, R. K., Grumet, M. and Margolis, R. U.** (1994). Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia, and neural cell adhesion molecules. *J. Cell Biol.* **127**, 1703-1715.

**Riou, J.-F., Shi, D.-L., Chiquet, M. and Boucaut, J.-C.** (1988). Expression of tenascin in response to neural induction in amphibian embryos. *Development* **104**, 511-524.

**Salmivirta, M., Elenius, K., Vainio, S., Hofer, U., Chiquet-Ehrismann, R., Thesleff, I. and Jalkanen, M.** (1991). Syndecan from embryonic tooth mesenchyme binds tenascin. *J. Biol. Chem.* **266**, 7733-7739.

**Vaughan, L., Huber, S., Chiquet, M. and Winterhalter, K. H.** (1987). A major, six-armed glycoprotein from embryonic cartilage. *EMBO J.* **6**, 349-353.

**Vaughan, L., Weber, P., Dalessandri, L., Zisch, A. H. and Winterhalter, K. H.** (1994). Tenascin-contactin/F11 interactions - a clue for a developmental role. *Persp. Dev. Neurobiol.* **2**, 43-52.

**Weber, P., Zimmermann, D. R., Winterhalter, K. H. and Vaughan, L.** (1995). Tenascin-C binds heparin by its fibronectin type III domain five. *J. Biol. Chem.* **270**, 4619-4623.

**Weber, P., Ferber, P., Fischer, R., Winterhalter, K. H. and Vaughan, L.** (1996). Binding of contactin/F11 to the fibronectin type III domains 5 and 6 of tenascin is inhibited by heparin. *FEBS Lett.* **389**, 304-308.

**Yamagata, M., Saga, S., Kato, M., Bernfield, M. and Kimita, K.** (1993). Selective distributions of proteoglycans and their ligands in pericellular matrix of cultured fibroblasts. Implications for their roles in cell-substratum adhesion. *J. Cell Sci.* **106**, 55-65.

**Zhang, L. and Esko, J. D.** (1995). Accumulation of a pentasaccharide terminating in alpha-N-acetylglucosamine in an animal cell mutant defective in heparan sulfate biosynthesis. *J. Biol. Chem.* **270**, 12557-12562.

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