Dual function of tenascin: simultaneous promotion of neurite growth and inhibition of glial migration

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INTRODUCTION

The extracellular matrix (ECM) molecule tenascin has attracted interest due to its restricted and rapidly changing pattern of expression during embryogenesis. Tenascin is found especially at sites of mesenchyme-epithelium interaction in many developing organs (for review see Erickson and Bourdon, 1989). In the developing central and peripheral nervous systems tenasin neurite growth occurred only after a lag phase and at a slower rate. Neurite growth on tenasin was inhibited by antibodies to β1 integrin and by heparin. While tenasin promotes neurite outgrowth of peripheral neurons, we found that it does not allow satellite cell migration when it is present on the substratum, and it inhibits migration of satellite cells on fibronectin when added in soluble form. In contrast, soluble tenasin did not significantly alter the rate of neurite growth on tenasin, fibronectin or laminin substrata, although neurites were straighter and less attached. When isolated satellite cells were added to neurites grown on tenasin, they preferentially adhered to and elongated along neurite surfaces. Using patterned substrata of tenasin versus fibronectin or laminin confirmed that tenasin borders allow neurites to pass but act as barriers to migrating satellite cells. We postulate that tenasin or related molecules with dual functions in cell adhesion are important for peripheral nerve morphogenesis. Tenasin allows axonal growth, but may restrict random satellite cell migration into the fibronectin-rich mesenchyme, thereby inducing the compaction of nerve fascicles.

Key words: tenasin, neurite growth, satellite cells, extracellular matrix

SUMMARY

The extracellular matrix molecule tenascin is expressed within the developing peripheral nervous system, first by migrating neural crest cells and later by satellite (Schwann precursor) cells at the growing tips of peripheral nerves. Here we found that the neurite promoting activity of tenascin for sensory neurons is developmentally regulated: very young sensory ganglia of stage 23 (4 days old) embryos grew neurites on tenascin as fast as on laminin and fibronectin. The growth response of older (day 7 and 9) ganglia on laminin or fibronectin was similar to that of 4-day-old ganglia, while on tenasin neurite growth occurred only after a lag phase and at a slower rate. Neurite growth on tenasin was inhibited by antibodies to β1 integrin and by heparin. While tenasin promotes neurite outgrowth of peripheral neurons, we found that it does not allow satellite cell migration when it is present on the substratum, and it inhibits migration of satellite cells on fibronectin when added in soluble form. In contrast, soluble tenasin did not significantly alter the rate of neurite growth on tenasin, fibronectin or laminin substrata, although neurites were straighter and less attached. When isolated satellite cells were added to neurites grown on tenasin, they preferentially adhered to and elongated along neurite surfaces. Using patterned substrata of tenasin versus fibronectin or laminin confirmed that tenasin borders allow neurites to pass but act as barriers to migrating satellite cells. We postulate that tenasin or related molecules with dual functions in cell adhesion are important for peripheral nerve morphogenesis. Tenasin allows axonal growth, but may restrict random satellite cell migration into the fibronectin-rich mesenchyme, thereby inducing the compaction of nerve fascicles.

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REFERENCES

Wehrle-Haller, B., Chiquet, M., Chiquet, E., 1991. Inhibition experiments done in vivo to try and understand the function of tenasin are difficult to interpret. Injection of a tenasin-specific mAb into embryos blocked migration of neural crest cells prior to the onset, but had
no effect during migration (Bronner-Fraser, 1988). In cultured cerebellar slices, anti-tenascin antibodies could block ganglion cell migration along the surface of Bergmann glia cells (Choung et al., 1987; Husmann et al., 1992). On the other hand, it has been reported that mice can develop normally in the complete absence of tenasin (Saga et al., 1992), suggesting that this protein is either not required or functionally redundant during embryogenesis (see Discussion).

Tenascin substrata promote neurite growth of peripheral and central neurons in vitro (Wehrle and Chiquet, 1990; Lochter et al., 1991; Husmann et al., 1992). On the other hand, Lochter et al. (1991) reported that addition of soluble tenasin to the medium was able to reduce neurite growth of central nervous system neurons grown on any substrate. Although allowing neurite outgrowth, tenasin is a poor substrate for the attachment of peripheral and central neuronal cell bodies (Wehrle and Chiquet, 1990; Faissner and Kruse, 1990). For neural crest cells (Halfter et al., 1989; Tan et al., 1987) and fibroblastic cells (Chiquet-Ehrismann et al., 1988) tenasin is antiadhesive. Recent data suggest the presence of adhesive domains in tenasin, which can be modulated by the presence of anti-adhesive domains (Spring et al., 1989; Lotz et al., 1989; Murphy-Ullrich et al., 1991; Prieto et al., 1992; Aukhil et al., 1993).

To test the proposed dual function on neural cell adhesion of tenasin directly, we used a culture system that allowed us to characterize at the same time neurite growth and satellite cell migration on extracellular matrix substrata. Dorsal root ganglia (DRG) of different embryonic ages were cultured on tenasin or other extracellular matrix molecules. On patterned substrata of tenasin versus fibronectin or laminin, or by adding soluble tenasin to ECM substrata, we studied cellular movements of cocultured neurites and satellite cells in response to combinations of tenasin with other ECM molecules.

MATERIALS AND METHODS

Extracellular matrix proteins

Tenasin and fibronectin were isolated from medium conditioned by 11-day chick embryo fibroblasts by affinity chromatography on mAb M1-Sepharose and gelatin-Sepharose, respectively, as described by Chiquet and Fambrough (1984). For the experiments presented here, tenasin preparations consisting of a mixture of 230, 200 and 190 kDa splice variants in a ratio of about 2:1:1 were used. In control experiments with purified (Chiquet et al., 1991a) 230 kDa or 190 kDa tenasin, respectively, we could not detect any significant difference in neurite-promoting activity between variants (not shown). Tenasin was routinely checked for contaminating fibronectin by nonreducing SDS-PAGE and immunoblotting with mAb M6 against chick fibronectin (Pearson et al., 1988; Wehrle and Chiquet, 1990). Laminin-nidogen complex isolated from mouse Engelbreth-Holm-Swarm tumor (Paulsson et al., 1987) was a gift from Dr M. Paulsson (Bern).

Antibodies and immunohistochemistry

The following monoclonal antibodies were used: anti-chick fibronectin mAb M6 (Pearson et al., 1988), mAb ID5 against the C-terminal peptide of detyrosinated mouse α-tubulin (Wehland and Weber, 1987; a gift from Dr J. Wehland, Braunschweig), and the neural crest marker mAb HNK-1 (obtained from Ready systems, Bad Zurzach, Switzerland, and used as recommended by the company).

Polyclonal antibodies against chick fibroblast tenasin, human plasma fibronectin, chick gizzard vinculin and mouse laminin-nidogen complex (obtained from Dr M. Paulsson, Bern, Switzerland) were the same as described by Wehrle and Chiquet (1990) and Wehrle-Haller et al. (1991).

For immunohistochemistry, sensory explant cultures were fixed for 20 minutes at room temperature by adding 100 µl of 37% formaldehyde to 1 ml of culture medium. Cultures were washed with PBS, permeabilized with 0.5% Triton X-100 in PBS, and stained as described by Wehrle and Chiquet (1990) and Wehrle-Haller et al. (1991). Micrographs were taken with Ilford HPS film on an Olympus Microscope. Internal reflection micrographs were taken on an Axiopt with a microscope (Zeiss).

Preparation of culture substrata

Round glass coverslips (12 mm) were washed in 70% ethanol, wiped with cotton gauze and air dried. Coverslips were incubated with one 50-µl drop of tenasin, laminin, fibronectin or heat-denatured (5 minutes at 70°C) BSA (hdBSA), respectively, diluted to 40 µg ml⁻¹ in PBS. After 1-2 hours at room temperature in a closed dish, coverslips were washed twice in PBS and placed in the wells of a 24-well culture dish containing Ham’s F-12 medium (Gibco). To prepare a patterned substratum (Goodman and Newgreen, 1985) or to overlay the first protein with a second one, the PBS-washed coverslips were blocked with 5 mg ml⁻¹ of hdBSA in PBS for 30 minutes and washed again in PBS. With the obliquely cut end of a yellow Eppendorf tip, stripes were scraped free of coated proteins in two perpendicular directions, creating a rectangular pattern. The coverslips were then incubated with a 50 µl drop of the second substratum molecule at the desired concentration for 1 hour, washed, blocked in hdBSA as above and put into the wells of a 24-well plate containing Ham’s F-12 medium. Prior to the addition of neuronal explants the coverslips were immersed in culture medium (see below).

Neural explant and glial cell cultures

Sensory ganglia from 4- to 9-day-old chicken embryos were dissected in L-15 medium (Gibco) by opening decapitated embryos at their dorsal side, removing the neural tube and then the basement membrane of the neural tube. The ganglia were then peeled free from sclerotomal tissue, picked up with fine forceps at their ventral nerve exits, and put in L-15 medium containing 1 mg ml⁻¹ hdBSA. Whole ganglia or pieces of ganglia were aspirated into a capillary tube and transferred into culture medium consisting of Ham’s F-12 medium supplemented with 1 mg ml⁻¹ hdBSA, 0.25 mM reduced glutathione and 30 ng ml⁻¹ 7S-NGF (Sigma).

A single-cell suspension of gli from embryonic peripheral nerve was obtained as follows. Brachial and sciatic peripheral nerves were dissected from 6-day-old chick embryos and cultured for 20 hours on glass coverslips coated with fibronectin in Ham’s F-12 medium with 1 mg ml⁻¹ hdBSA. During this time fibroblasts migrated onto the fibronectin away from the explants. The remaining axon and glial cell aggregates were flushed from the substratum and treated for 5 minutes with 0.25% trypsin/EDTA in Puck’s saline (Gibco) at 37°C. Digestion was stopped by addition of 5 mg ml⁻¹ hdBSA in F-12 and a single-cell suspension was obtained by trituration with a cell culture pipet. Cells were centrifuged at 150 g for 5 minutes, resuspended in culture medium, and added to the explants.

Cultures were viewed under phase contrast on an inverted
Kinetics of neurite outgrowth
To determine the rate and the morphology of neurite outgrowth and satellite cell migration obtained on the different substratum molecules, at least three and maximally five ganglia were plated per coverslip. The average length of neurites for a given data point (e.g. tenascin substratum, 10 hours of culture) was determined from photographs as follows. Concentric circles were drawn around ganglia with radii increasing at 100 µm intervals. The number of growth cones within a certain distance interval (e.g. between 100 and 200 µm) from the surface of the ganglion was scored and multiplied with the medial radius of this interval (e.g. 150 µm). For each data point, the sum of the length of all measured neurites was divided by the total number of growth cones to obtain the mean single neurite length, and the standard deviation of the mean was calculated.

Growth and migration inhibition experiments
A stock solution of heparin (sodium salt) from porcine intestine (140 units mg⁻¹; Fluka) was prepared at 10 mg ml⁻¹ in sterile PBS. The mAb JG22 against chick β₁ integrin (Greve and Gottlieb, 1982) was obtained from the Developmental Studies Hybridoma Bank (maintained by the Department of Biology, University of Iowa, under contract NO1-HD-2-3144 from the NICHD). Antibody JG22 was used as a 0-40% ammonium sulfate fraction of ascites fluid, redissolved in the original volume and dialyzed against PBS. The same procedure was followed for polyclonal antisera against ECM proteins. For experiments where soluble tenascin was to be added to the culture medium, a mixture of 100 µg ml⁻¹ tenascin and 1 mg ml⁻¹ hdBSA was dialyzed against Ham’s F12/PBS (1:1). Stock solutions were added to the medium of neural explant cultures to yield the concentrations indicated in Results.

Characterization of non-neuronal cells from sensory ganglia
Isolated 6-day-old DRGs consist of differentiated sensory neurons, neuronal precursors, satellite cells and fibroblasts. All neural crest-derived cells are known to express the HNK-1 epitope at this developmental stage (Bronner-Fraser, 1988). In addition, Rohrer (1985) has shown that fibroblasts, but not satellite cells, from sensory ganglia secrete and secrete fibronectin in culture. Moreover, satellite cells of developing peripheral nerves and sensory ganglia have been shown to express much higher levels of the cytoskeletal protein, vinculin, in comparison to mesenchymal cells (Duband and Thiery, 1990; Wehrle-Haller et al., 1991). In order to distinguish between satellite cells and fibroblasts in explants of 6-day DRGs, we stained neuronal explants with antibodies to the HNK-1 epitope, to the ECM components tenascin and fibronectin, and to cytoplasmic vinculin. In addition we observed the explants with interference reflection microscopy to detect focal adhesion plaques.

In cultured DRGs two different non-neuronal cell populations could easily be distinguished morphologically. The first population consisted of elongated, rapidly migrating cells (up to 1 mm per 24 hours on laminin and fibronectin), which were in contact with neuritic surfaces and bore one or multiple lamellipodia and a long distinct tail. These cells expressed the HNK-1 epitope (not shown), were stained with anti-tenascin antibodies (arrow in Fig. 1B) and were rich in cytoplasmic vinculin (Fig. 1E,F). However, these cells were not labeled by anti-fibronectin (arrow in Fig. 1A) and did not develop focal contacts during migration (Fig. 1H,I). The second type of non-neuronal cells was restricted to the vicinility of the explant and had a multipolar and well-spread appearance. The latter cells were not labeled by the HNK-1 antibody but expressed fibronectin within intracellular vesicles as shown by mAb M6 staining (Fig. 1A,C). By internal reflection microscopy, dark focal adhesion contacts were observed underneath these cells (Fig. 1G).

By means of the morphological and molecular differences, the multipolar flat cells were identified as fibroblasts, and the more bipolar cells, which have lamellipodia on one end and a distinct tail on the other, as satellite cells.

RESULTS
Developmentally regulated sensory neurite growth on pure tenascin substrata
In earlier studies (Wehrle and Chiquet, 1990) we have shown that motor axons from 3-day chick embryo spinal cord grow in distinct patterns when exposed to different ECM substrata, such as tenascin, fibronectin and laminin coated directly on glass. Like 3-day embryonic spinal cord explants, cultured sensory ganglia grow neurites on pure tenascin. Since tenascin expression is prominent in the anterior sclerotome when sensory axons start to grow (Wehrle and Chiquet, 1990), we first asked whether newly formed sensory neurons respond differently to tenascin than more mature ones.

DRGs were isolated from 4-, 7- and 9-day-old embryos, dissected to fragments of similar diameter (0.2-0.3 mm) and plated onto ECM substrata. Sensory ganglia from stage 22-23 (day 4) embryos formed neurites on tenascin, laminin and fibronectin within 4 hours after plating. Although the time course of neurite extension was similar on all three substrata, several differences in the morphology of neurites and the explants could be found (Fig. 2). On tenascin (Fig. 2K), the explants remained round in appearance, whereas on laminin and fibronectin, explants flattened already after 4 hours of culture, a process that continued up to an explant diameter of 0.6-0.8 mm after 10 hours. On fibronectin the ganglia dissociated completely within 24 hours, and smaller clusters of neurons formed instead (Fig. 2M). Neurites were relatively straight on tenascin and laminin, whereas on fibronectin they were well attached and curved in appearance (compare Fig. 2K and L with M). On BSA, a few short neurites only were visible (Fig. 2D,H,I). To quantitate these data, the mean length of single neurites was plotted against the time of incubation (Fig. 3A). The mean velocity of neurite growth on tenascin was estimated to be about 40 µm per hour or 1 mm per day, which was similar to the neurite growth rate on laminin and fibronectin (Fig. 3A).

Neurite outgrowth from explants of older sensory ganglia was slower on tenascin (Fig. 3B). Moreover 7-day DRG explants were characteristic for their long lag phase of over 10 hours before neurites were established on tenascin. After 24 hours in culture the morphology of the formed neurites was very similar to neurites from 4-day-old DRGs (not shown). Explants of 9-day-old DRGs established processes after an apparent lag phase of 5 to 10 hours on tenascin (Fig. 3B); however, at the tips of the neurites more branches were formed than in younger explants (not shown). In contrast to the results obtained with tenascin, on
laminin or fibronectin neurites were formed with similar kinetics by explants from all developmental stages (Fig. 3C, D). Generally, neurites on laminin (Fig. 3C) were longer than on fibronectin (Fig. 3D), and sensory neurons from older stages grew even somewhat faster on this substratum (Fig. 3C). For further experiments DRGs isolated within the 6th embryonic day were used because they were easier to dissect than ganglia from 4-day-old embryos.

**Neurite growth on tenascin is inhibited by anti-tenascin, anti-β1 integrin and heparin**

The specificity of neurite growth on the various substrata was tested as follows: antibodies to the original substratum were added after the ganglia had been cultured for 24 hours. On tenascin, neurites could be detached within 15 minutes of incubation with anti-tenascin antiserum (1:100), whereas addition of anti-laminin or anti-fibronectin antiserum had no effect (not shown). Similar results were obtained with anti-laminin and anti-fibronectin antiserum (1:100) on the respective substratum (cf. Wehrle and Chiquet, 1990). These results indicate that the interaction of neurites with the substratum still depended on the original coat of protein after 24 hours in culture.

An antiserum raised against and specific for a C-terminal, heparin-binding 60 kDa fragment of tenascin subunits (Chiquet et al., 1991) completely inhibited neurite growth on tenascin at a 1:100 dilution (not shown). This indicates that the neurite-promoting activity of tenascin subunits maps to this domain, which consists of three fibronectin type III repeats and a fibrinogen homologue (Spring et al., 1989).

The mAb JG22 against chick β1 integrin (Greve and Gottlieb, 1982) proved to be a potent inhibitor of neurite growth on tenascin (Fig. 4). Not only did this antibody completely block primary neurite outgrowth from explants (Fig. 4B), but it also rapidly detached already formed neurites from a tenascin substratum (Fig. 4D). Similarly, heparin (100 µg ml⁻¹) inhibited primary outgrowth of neurites (Fig. 4C); it did not, however, cause preformed neurites to collapse within 2 hours after addition (Fig. 4E). These results point to an involvement of β1 integrin receptors and perhaps cell surface proteoglycans in the response of sensory neurites to tenascin.

**In contrast to sensory neurites, satellite cells of sensory ganglia are not able to migrate on a tenascin substratum**

Whole 6-day-old chick embryonic DRGs were isolated and plated onto glass coverslips coated with the appropriate ECM protein. On tenasin, neurites formed after a lag phase of 5 to 10 hours. The processes were often bundled into fascicles, or grew as single straight neurites with distinct growth cones (Fig. 5E,F). The growth cones had rapidly moving lamellipodia and filopodia (Fig. 5F). Only a few
satellite cells were found tightly associated with neurites (arrows in Fig. 5F).

In contrast to what was observed on tenascin, on laminin and fibronectin the DRGs explants extended a neurite network that was populated by a large number of satellite cells migrating on the substratum away from the explant (Fig. 5A-D). On laminin, the satellite cells established large lamellipodia and were in close contact with the neurites (Fig. 5B). On fibronectin, satellite cells had smaller lamellipodia and were less associated with neurites (Fig. 5D).

**On a tenascin substratum, isolated satellite cells**
Fig. 3. Kinetics of neurite outgrowth by sensory ganglion explants from embryos of different stages on glass coated with 40 μg ml$^{-1}$ tenascin (TN), laminin (LN), fibronectin (FN), or BSA, respectively. In (A) the mean neurite length of DRGs from 4-day embryos was compared on different substrates as a function of time in culture. To test the influence of developmental age, 4-, 7- or 9-day embryonic DRGs were cultured on tenascin (B), laminin (C) or fibronectin (D). Error bars indicate the s.d. of the mean neurite length, which was determined as described in Materials and Methods. Data are from one representative large experiment; each data point represents the mean length of 50 to 200 measured neurites originating from 3 to 4 different ganglion explants per developmental age and per substratum. The growth response did not differ qualitatively between independent experiments.

Fig. 4. Inhibition of neurite growth on tenascin by anti-β$_1$ integrin antibody and by heparin. Sensory ganglion explants from 6-day embryos were cultured for 17 hours on coverslips coated with 40 μg ml$^{-1}$ tenascin (A) in normal medium, (B) in medium supplemented with anti-β$_1$ integrin mAb JG22 (ascites fluid diluted 1:100), and (C) with heparin (100 μg ml$^{-1}$). In (D) and (E), explants were grown in normal medium on tenascin for 16 hours. Then, mAb JG22 (1:100) was added for another 75 minutes (D), or heparin (100 mg ml$^{-1}$) for 120 minutes (E), respectively, before photographing the cultures. Bar, 320 μm.
Satellite cells do not migrate from DRGs plated on tenascin. To observe their behavior on a tenascin substratum directly, we obtained satellite cells from 6-day embryonic peripheral nerve explants and added them to neurites from 6-day-old DRGs that had been grown on tenascin for 20 hours as single cells. The interaction of single satellite cells with the tenascin substratum and with neurites was followed over a period of 4 hours.

Most of the satellite cells of the peripheral nerve remained round when they attached to tenascin. They were able to form filopodia and even small active lamellipodia.

**Fig. 5.** Sensory ganglion explants from 6-day embryos cultured for 17 hours on glass coverslips coated with 40 µg ml⁻¹ laminin (A, B), fibronectin (C, D) or tenascin (E, F), respectively. Note that the satellite cells are migrating on the substratum on laminin (A) and fibronectin (C) but not on tenascin (E). Enlargements of A, C, E are shown in B, D, F, respectively. The arrows in F point to elongated satellite cells associated with the neurite network on tenascin. Bar: A, C, E, 320 µm; B, D, F, 100 µm.
Figure 6. (A-E) Time sequence of a satellite cell interacting with a growing neurite on a tenascin substratum. The neurite is part of a DRG explant from a 6-day embryo cultured for 20 hours on a glass coverslip coated with 40 µg ml\(^{-1}\) tenascin. After 18 hours in culture a suspension of peripheral nerve satellite cells was added and the first picture was taken 2 h later (A). Note how the satellite cell already in contact with the neurite in (A) elongates along its shaft with time (B-E). The arrow in (A) points to another, substratum-attached satellite cell, which develops small lamellipodia even before it comes into contact with the neurite. Bar, 100 µm. Time is given on the right in minutes.

(arrow Fig. 6A) but did not move. When satellite cells established contact with growing neurites, several different reactions were observed. In some cases growth cones made initial contacts with satellite cells, but later changed direction and passed the satellite cell (not shown). More frequently, satellite cells that contacted neurites elongated to a spindle-like shape along the neurite surface. In the example shown in Fig. 6, a satellite cell attached to the surface of a single growing neurite just behind its migrating growth cone (Fig. 6A). With time the growth cone advanced and the satellite cell elongated along the neurite surface, increasing its area of contact (Fig. 6B-E). Interestingly, in this case the satellite cell body did not move actively along the neurite surface but remained stationary in respect of the advancing growth cone. In other cases, due to extensive neurite growth the satellite cells associated with the axonal network could undergo passive movements (not shown).

This example shows that on a pure tenascin substratum, satellite cells attach but do not locomote actively. However, they are able to adhere to and spread on neurites formed on tenascin. So far, we have not found any means of making glial cells migrate on a plain tenascin substratum.

**Soluble tenascin inhibits satellite cell migration on fibronectin by binding to the substratum**

In vivo, tenascin is synthesized and secreted by migrating neural crest cells and satellite cells of the peripheral nerve in vivo and in vitro (Wehrle-Haller et al., 1991; Tan et al., 1991; Tucker and McKay, 1991). In order to mimic the high concentrations of tenascin observed around satellite cells of the growing peripheral nerves, soluble tenascin was added at 15 µg/ml to explants grown on fibronectin, laminin and tenascin. ECM-coated glass coverslips were extensively blocked with heat-denatured BSA prior to addition of tenascin to prevent non-specific binding of tenascin to the substratum.

For ganglia plated on laminin (Fig. 7A), soluble tenascin did not significantly reduce neurite length compared to the control (0.83±0.22 mm versus 1.03±0.18 mm; 18 hours in culture), and satellite cell migration was similar although cellular morphology was slightly different (cf. Fig. 7A with Fig. 5A,B). On a tenascin substratum in the presence of soluble tenascin (Fig. 7C), neurite length was again comparable to the control (0.55±0.18 mm versus 0.49±0.26 mm; 18 hours in culture), but neurites were less fasciculated (compare Fig. 7C with Fig. 5E,F). On fibronectin substrata after addition of soluble tenascin (Fig. 7B), neurites were straighter and less attached while their length was not significantly reduced (0.53±0.17 mm to 0.74±0.16 mm; 18 hours in culture), and their growth cones appeared normal in size (cf. Fig. 7B with Fig. 5C,D). However, a concentration of 15 µg ml\(^{-1}\) soluble tenascin almost completely blocked satellite cell migration on fibronectin (Fig. 7B). The ganglia treated with soluble tenascin remained as compact as on a tenascin substratum (not shown).

To test whether the inhibitory effect of tenascin on satellite cell migration was in part mediated by its interaction with coated fibronectin, we incubated pure fibronectin substratum (after blocking with BSA) with soluble tenascin. After washing, such substrata were exposed to 6-day-old DRGs. While neurite growth was similar to that on pure fibronectin, the ability of satellite cells to migrate on fibronectin was significantly reduced. Subsequent immunofluorescence staining of the substratum revealed a concentration-dependent deposition of tenascin on the fibronectin
substratum. A similar interaction of tenascin with laminin or BSA was not observed by this technique (data not shown). These results indicate that the inhibition of satellite cell migration on fibronectin substrata by soluble tenascin is in part due to its binding to the coated fibronectin.

### Substratum borders between tenascin versus laminin or fibronectin restrict satellite cell migration but not neurite growth

To test the effect of discontinuities in ECM substratum composition, 6-day-old DRGs were cultured on patterned substrata of tenascin versus fibronectin and tenascin versus laminin, respectively. The general growth and migration behavior of neurites and satellite cells on areas not divided by a border were the same as on the respective homogeneous substrata. At substratum borders between fibronectin and pure tenascin, neurites were able to cross in both directions. Since tenascin did not allow the migration of satellite cells, they were stopped at the border between fibronectin and tenascin and aligned to form a cellular sheet (Fig. 8A). As a control for tenascin-induced neurite growth, a pattern of fibronectin versus BSA is shown (Fig. 8B). Only a few neurites were found to continue on BSA for a short distance (Fig. 8B). In Fig. 9 patterns of laminin versus tenascin are shown. Also in these experiments neurites were able to cross from laminin to tenascin and vice versa. On laminin, satellite cells were rapidly migrating, whereas they did not migrate over onto the tenascin-coated substratum (Fig. 9A,C). The specificity of the substratum borders was visualized by immunofluorescence staining for coated laminin (Fig. 9B) and found to be correlated to morphological boundaries. Inverting the sequence of coating to create patterns between laminin and tenascin did not change the behavior of neurites and migrating satellite cells (Fig. 9C). In control experiments where BSA was used as a second coat instead of tenascin, both neurite growth and satellite cell migration were completely blocked at the border from laminin to BSA, indicating that tenascin selectively induced the growth of neurites, but restricted satellite cell migration (Fig. 9D).

### DISCUSSION

Previous studies have suggested a role for tenascin in the development of the central and peripheral nervous system, as a neurite-promoting substratum molecule (Wehrle and Chiquet, 1990; Lochter et al., 1991; Husmann et al., 1992) or as an antiadhesive molecule involved in the formation of tissue barriers (for review see Steindler, 1993). However, mice lacking a functional tenascin gene seem to develop normally (Saga et al., 1992). Erickson (1993) has therefore argued that expression of tenascin in development might be superfluous, either because it has no function at all, or because it is functionally redundant. Since tenascin has potent effects on cell adhesion in vitro (Chiquet-Ehrismann et al., 1988) and in vivo (Riou et al., 1990), it is hard to imagine that it does nothing at all in normal development. On the other hand, redundancy, which is widespread in complex biological systems (Rudnicki et al., 1992), is prob-

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**Fig. 7.** Sensory ganglia explants from 6-day embryos cultured for 18 hours on glass coverslips coated with 40 μg ml⁻¹ laminin (A), fibronectin (B) or tenascin (C), respectively, in the presence of 15 μg ml⁻¹ soluble tenascin. Note that soluble tenascin inhibits satellite cell migration on fibronectin (B) but not on laminin substratum (A). Bar, 200 μm.
ably not ‘superfluous and wasteful’ (Erickson, 1993): if two or more proteins can exert the same function at the same place, it is impossible to say which one is superfluous. The biological activity of tenascin could be shared by other molecules of the tenasin gene family such as restrictin (Nörenberg et al., 1992; Fuss et al., 1993) or TN-MHC (Matsumoto et al., 1992), or by non-related molecules with similar activities and expression patterns, like the thrombospondins (Neugebauer et al., 1991; for review see Chiquet-Ehrismann, 1991). Knocking out one of these molecules could be overcome by the overexpression of others.

Recently a series of reports have demonstrated a neurite-promoting activity of tenascin towards different types of neurons within the central and peripheral nervous systems (Wehrle and Chiquet, 1990; Lochter et al., 1991; Husmann et al., 1992). The main problem in these studies was that isolated neurons interacted only weakly with a tenascin substratum, so that they had to be plated onto mixed polylysine/tenascin substrata. Here we used embryonic ganglia explants; omitting polylysine did not diminish the neurite growth response on tenascin. Moreover, different extracellular matrix proteins induced characteristic, morphologically distinct neurite growth patterns, which indicates that a different set of cellular adhesion receptors is used for neurite growth on each substratum.

Our previous work showed different kinetics of neurite growth by two types of neurons on tenascin (Wehrle and Chiquet, 1990). Motoneurons from 3-day embryos responded immediately when plated on tenascin whereas sympathetic neurons from 11-day embryos sprouted after an apparent lag phase of 5 to 10 hours. The more-systematic investigation of the growth response by sensory neurons presented here shows that it is developmentally regulated. Similar results have been described for neurite growth of retinal neurons on laminin. The failure of retinal ganglion neurons to form neurites on laminin could be correlated with a downregulation of the number of receptors on these neurons (Cohen et al., 1989; De Curtis et al., 1991). However, the decrease in neurite growth by other types of retinal neurons on laminin was due to inactivation of integrins on the neuronal surface, which could be reversed by the addition of an activating mAb against β1 integrin (Neugebauer and Reichardt, 1991). This result shows that neurons, like other cell types (for review see Hynes, 1992), can endogenously regulate their integrin expression and activity according to their developmental program. Because neurite growth on tenascin is blocked by antibodies to β1-integrin, the apparent lag phase of older neurons before they grow on tenascin could represent either the time needed for reactivation of integrins or their upregulation in culture. In contrast, very young sensory neurons (of the stage where they form axons in vivo) could provide the necessary set of activated receptors on the surface of their growth cones, leading to immediate neurite formation on tenascin. Exactly which cellular receptor(s) are involved in neurite growth on tenascin is not yet known; besides β1 integrins, members of the Ig superfamily of cell adhesion molecules are possible candidates (Zisch et al., 1992; Pesheva et al., 1993).

The inhibition of satellite cell migration on tenascin is probably correlated with the known antiadhesive properties of tenascin (Chiquet-Ehrismann et al., 1988; Lotz et al., 1989). Neural crest cells and neural crest-derived glial cells are not able to migrate over a pure tenascin substratum (Tan et al., 1987), but they attach and produce very active filopodia and lamellipodia (Halfter et al., 1989). Glial and fibroblastic cells are able to attach to certain recombinant fragments of tenascin (Spring et al., 1989; Prieto et al., 1992; Aukhil et al., 1993), but are not able to spread. In contrast, other domains of tenascin do not allow attachment of cells.
and they inhibit spreading on otherwise adhesive substrata (Spring et al., 1989; Murphy-Ullrich et al., 1991; Prieto et al., 1992). Therefore, specific cellular behavior is apparently achieved by combinations of antiadhesive and adhesive domains in tenascin (Prieto et al., 1992), functioning via distinct receptors. It is unlikely that satellite cells do not

Fig. 9. Sensory ganglia explants from 6-day embryos cultured for 20 hours on glass coverslips coated with a patterned substratum of laminin versus tenascin (A–C) or laminin versus heat-treated BSA (D). In (A) and (B), tenascin (40 µg ml⁻¹) was coated first, blocked, scraped and strips free of protein were incubated with 40 µg ml⁻¹ laminin. Immunofluorescence with anti-laminin antiserum revealed the striped appearance of the laminin substratum (B). In C, D laminin (40 µg ml⁻¹) was coated first, blocked, scraped and incubated with 40 µg ml⁻¹ tenascin (C) or 5 mg ml⁻¹ heat-treated BSA (D). LN, laminin; TN, tenascin. Bar, 200 µm.
migrate on tenascin substrata, simply because they have no receptors for the molecule: we show here that these cells attach to tenasin, and that soluble tenasin inhibits their migration on fibronectin. For certain CNS neurons that behave in a similar way (but differently from peripheral neurons), it has been suggested that the cell adhesion molecule F3/F11 (contactin) is an ‘inhibitory’ receptor for the tenasin-related protein, J1-160/180 (restrictin) (Pesheva et al., 1993).

Satellite cells that adhere to a supporting neurite surface can change their cellular shape even if they are in contact with a tenasin substratum. Obviously, these cells can establish contacts with other cells and modulate their cytoskeleton in the presence of tenasin. This indicates that the inhibitory activity of tenasin is functional at cell-substratum contacts and does not interfere with intercellular adhesion.

Soluble tenasin was able to inhibit the migration of satellite cells on a fibronectin but not on a laminin substratum. It has been shown that tenasin specifically binds to fibronectin but not to laminin (Chiquet-Ehrismann et al., 1988, 1991; Hoffman et al., 1988). Blocking of satellite cell migration by soluble tenasin might be cooperatively enhanced by its binding to the fibronectin substratum. While we did not observe a significant change in neurite growth, Lochter et al. (1991) have found that soluble tenasin was able to suppress neurite growth of hippocampal neurons by 20–40% independently of the culture substratum. However, suppression started only after 12 hours in tenasin-containing medium. This could be due to a cell type–specific modulation of neurite growth, which is not apparent in sensory neurons. A modulation of cellular activity and second messenger pathways by soluble tenasin has been demonstrated by its inhibition of lymphocyte activation (Rüegg et al., 1989) and of growth factor-induced cytoplasmic alkalization (Crossin, 1991). Further studies will be needed to investigate the tenasin domains, the responsible receptors and the second messengers leading to modulation of cell-substratum interaction.

The use of patterned substrata gives insight into the decisions that a migrating cell in general and a growth cone in particular have to make during migration through the connective tissue. Growth cones attach firmly to adhesive substrata (Letourneau, 1975) and can be guided along an adhesive substratum bordering a nonadhesive substratum in vitro (Hammarback et al., 1988). In vivo, growing nerves avoid proteoglycan-rich barrier tissues during migration (Oakley and Tosney, 1991). Alternatively, heterogeneity in the substratum pattern can change the morphology, direction and branching pattern of neurites (Grumbacher-Reinert, 1989). We show here that growth cones migrating from laminin or fibronectin onto tenasin remain attached and continue to grow in their initial direction. In contrast to neurites, satellite cells and fibroblasts do not cross the substratum border. For these cells a tenasin substratum acts in a repulsive way, like certain proteoglycans act on neural crest cells (Tan et al., 1987). In our experiments the tenasin substratum border constitutes a selective filter that restrains the satellite cells but allows neuronal processes to pass. Tenasin is so far the only ECM protein for which a distinct action on the locomotion of two cell types has been shown directly in cocultures, but it is likely that the structurally related molecule J1-160/180 (restrictin) has a similar function (Pesheva et al., 1989; Nörenberg et al., 1992).

In the context of peripheral nerve morphogenesis, we postulate that tenasin expressed by nerve satellite cells interacts with fibronectin surrounding the nerve. The polarized deposition of tenasin forms transient boundaries between nerve fascicles and the mesenchyme around the nerve (Wehrle-Haller et al., 1991), which are subsequently replaced by stable basement membranes (Bunge et al., 1989). The transient tenasin boundaries can probably still be bridged by growth cones and their axons, allowing sorting of axons along the nerve track and within the nerve plexus. However, tenasin’s ability to block adhesive interactions of satellite cells with the mesenchyme might restrict their range of movements on the surface of growing axons. Although the dual action of tenasin on cell adhesion may be duplicated by other related molecules, this function per se is likely to be important for pattern formation in the peripheral nervous system.

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