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Abstract
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Nerve-dependent and -independent tenasin expression in the developing chick limb bud

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Summary

The extracellular matrix protein, tenasin, appears in a restricted pattern during organ morphogenesis. Tenasin accumulates along developing peripheral nerves as they leave the spinal cord and enter the limb mesenchyme (Wehrle and Chiquet, Development 110, 401–415, 1990). Here we found that most but not all tenasin deposited along growing nerves is of glial origin. By in situ hybridization with a tenasin cDNA probe, we determined the site of tenasin mRNA accumulation both in normal and nerve-free limbs. In normal wing buds, tenasin mRNA was first detected within the developing limb nerves. Vinculin-positive glial precursor cells, which comigrate with the axons, are the likely source of this tenasin message. In nerveless wing grafts, tenasin was first expressed in tendon primordia in the absence, and thus independently, from innervation. In contrast to normal limbs, grafted wing buds neither contained vinculin-positive glial precursor cells, nor expressed tenasin in regions proximal to tendon primordia. In normal wing buds, tenasin deposited by tendon primordia transiently parallels and surrounds certain developing nerves. After the major nerve pattern is established, tenasin mRNA disappears from nerves in the upper limb, but is expressed in perichondrium and tendons. We propose that glial tenasin facilitates the penetration of axons into the limb bud and is important for nerve fasciculation. In some places, early tendon primordia might help to guide the migration of axons and glial precursor cells towards their target.

Abbreviations: ECM, extracellular matrix; TBS, Tris buffered saline; mAb, monoclonal antibody, IgG, immunoglobulin gamma; SDS, sodium dodecyl sulfate; TGF-β, transforming growth factor-β; FCS, fetal calf serum.

Key words: tenasin, in situ hybridization, peripheral nerve, tendons, chick limb bud.

Introduction

The pattern of peripheral nerve innervation in limb buds of chick embryos has been the subject of many investigations (for references, see Hollyday, 1983). Independently of their origin, the nerves, which consist of motor and sensory axons and satellite cells (glial precursor cells), grow along common pathways into the limb bud. The spatial and temporal pattern of nerves is specified by the limb mesenchyme, while developing bone and cartilage is avoided by growing nerves (Lewis et al. 1983; Tosney and Landmesser, 1984). Neural tube rotation (Lance-Jones and Landmesser, 1981) and rotation of the wing bud (Stirling and Summerbell, 1985) do not alter the proximal nerve pattern. However, distally to these fixed tracks, growth cones are able to reach their displaced target muscles by following new pathways within the limb. Experiments suggest that the migrating growth cones have the ability to guide the nerves to their targets. After removing the motor neurons and their axons, glial precursor cells and sensory fibers avoid migration on motor nerve pathways (Landmesser and Honig, 1986; Swanson and Lewis, 1986). In addition, motor axons devoid of sensory axons and glial precursor cells are not able to penetrate into the limb mesenchyme and remain at the plexus region (Noakes et al. 1988), suggesting an important function for the nerve satellite cells in establishing the nerve pattern (Noakes and Bennett, 1987; for review see Keynes, 1987). The question remains which factors are required for guiding neurons and glial precursor cells through the limb mesenchyme. Such cues are known to be provided locally by the limb connective tissue (Lewis et al. 1983; Tosney and Landmesser, 1984). Since ECM glycoproteins are found along specific migratory pathways in the embryo (Boucaut et al. 1984; Wehrle and
Chiquet, 1990), and are able to promote neurite outgrowth in vitro (Edgar et al. 1984; Humphries et al. 1988; Wehrle and Chiquet, 1990), they appear as candidates for transmitting local signals in the developing limb.

Tenascin is a large oligomeric extracellular matrix molecule (for reviews see Erickson and Bourdon, 1989; Chiquet, 1989; Chiquet-Ehrismann, 1990) with a highly restricted and regulated pattern of expression during morphogenesis (Chiquet and Fambrough, 1984a; Chiquet-Ehrismann et al. 1986; Crossin et al. 1986). During development of the peripheral nervous system, tenascin is accumulated in defined areas by mesenchymal and glial cells (Crossin et al. 1986; Rieger et al. 1986). It is re-expressed during nerve regeneration (Sanes et al. 1986; Danilloff et al. 1989; Martini et al. 1990). At the time when the limb buds are innervated, tenascin is accumulated along ingrowing peripheral nerves which form a bracket-like structure around the cartilage primordia (Wehrle and Chiquet, 1990; Martini and Schachner, 1991). In addition, an early tenascin expression in the limb bud can be localized to ligament and tendon anlagen (Chiquet and Fambrough, 1984a; Hurle et al. 1990). At later stages, tenascin disappears from nerves, but continues to accumulate in perichondrium and tendons (Chiquet and Fambrough, 1984a; Wehrle and Chiquet, 1990).

In this paper, we asked whether tenascin is produced by the ingrowing limb nerves themselves, or by the limb mesenchyme along nerve pathways. In the latter case, tenascin could have a role in axonal pathfinding, whereas the former possibility would point to a function in morphogenesis and maturation of limb nerves. To answer this question, we determined the sites of tenascin mRNA production in normal limbs as well as in regressed wing buds, which were free of invading nerves and hence only expressed mesenchymal tenascin. Specific immunological markers allowed us to correlate the positions of axons and satellite cells in normal limbs with the location of accumulated tenascin mRNA and protein.

Materials and methods

Antibodies and immunocytochemistry

The following monoclonal antibodies (mAbs) were used: M1 against chick tenascin (Chiquet and Fambrough, 1984a); M6 against chick fibronectin (Pearson et al. 1988); and ID-5 against the C-terminal peptide of detyrosinated mouse alphatubulin (Wehland and Weber, 1987; gift of Dr Juergen Wehland, Braunschweig). In the developing limb buds, this antibody stains exclusively axonal fibers and their growth cones; background staining in other tissues in the limb is extremely low (Wehrle and Chiquet, 1990). All three mAbs are mouse IgGs. A culture supernatant of ID-5 was used; the other mAbs were partially purified from ascites fluid by ammonium sulfate precipitation and dialysis against TBS (Chiquet and Fambrough, 1984a).

The characterization of polyclonal antiserum against chick fibroblast tenascin and human plasma fibronectin has been published (Chiquet and Fambrough, 1984a). A rabbit antiserum against chick gizzard vinculin was obtained as follows. Adult chick gizzard vinculin was purified according to O'Halloran et al. (1986). Purity of the vinculin (Mr=116,000) was judged by SDS–polyacrylamide gel electrophoresis. A rabbit was injected with 300 μg vinculin and boosted twice. The serum was tested by immunoblotting (Chiquet et al. 1988) on purified vinculin (not shown) and on SDS extracts of tissues (Fig. 1). We used this vinculin antiserum to detect Schwann precursor cells associated with the outgrowing peripheral axons (Duband and Thiery, 1990). IgGs were precipitated from antiserum with ammonium sulfate, redissolved in the original volume, and dialyzed against TBS (Chiquet and Fambrough, 1984a).

Embryos were staged according to Hamburger and Hamilton (1951); fixation, cryostat sectioning and immunofluorescence with the appropriate antibodies was done as described in Wehrle and Chiquet (1990). The distribution of tenascin and fibronectin was compared with neuronal and glial markers by double immunofluorescence on tissue sections. The following antibody pairs were used: anti-tenascin mAb M1/anti-fibronectin antiserum; anti-tenascin antiserum/anti-tubulin mAb ID5; anti-vinculin antiserum/anti-fibronectin mAb M6.
Chick tenasin cDNA clones and in situ hybridization
A labeled cDNA probe specific for tenasin mRNA was obtained as follows. Chick tenasin cDNA clones were isolated from λgt11 libraries and subcloned into plasmids (Spring et al. 1989). Five fragments were selected that span the entire coding region of the 190×10^2 M_t tenasin variant (Spring et al. 1989). Gel-purified DNA fragments were mixed in an equimolar ratio, and nick translation was performed with tritiated nucleotides (Hafen et al. 1983; Baumgartner et al. 1987). Parafomaldehyde-fixed cryostat sections of 10 μm were incubated with labeled cDNA probe, washed and autoradiographed as described by Baumgartner et al. (1987). Sections were photographed under dark-field illumination and phase contrast on a Zeiss Photomicroscope III using Ilford Pan F film.

To test whether the hybridization patterns obtained with the described cDNA probe are specific, a second 330 bp long cDNA probe was prepared from the extra fibronectin type III repeats of tenasin which are present only in the largest, 230×10^3 M_t splicing variant (Spring et al. 1989). This cDNA had no sequence overlap with the probe described above, yet showed an identical hybridization pattern in the developing limb nerves (data not shown).

**Grafting**

Grafting was essentially performed as described in Pardanaud

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**Fig. 2.** Cross sections through a normal stage 26 embryo at the wing bud level. Consecutive sections were either hybridized with tenasin cDNA probe (A) or double labeled with anti-tenasin mAb M1 (B) and anti-fibronectin antiserum (C). Black and white arrow pair in A indicate the plane of the cross sections shown in Fig. 5A–H. Note the high expression of tenasin mRNA (A) overlapping with tenasin positive (B), fibronectin-negative (C) outgrowing limb nerves. In addition, tenasin mRNA can be located to precartilage of the vertebral column and to the aortic wall, whereas the chorda appears negative at this stage and level in the embryo (cf. Fig. 7). TN, tenasin; FN, fibronectin; nt, neural tube; ch, chorda; ao, aorta; p, brachial plexus; bls, brachialis longus superior nerve; bli, brachialis longus inferior nerve; r, radialis nerve; h, humerus primordium. Bar, 500 μm.
et al. (1989). Eggs were incubated with their pointed end down in a humidified chamber at 37°C for 3 to 3.5 days. The egg was opened at the blunt end. The egg membrane and the underlying vitelline membrane were opened, and the embryos were staged according to Hamburger and Hamilton (1951). The amnion of a chosen embryo (stage 18–19) was slit open with watchmaker forceps over the right wing bud. The limb bud was cut away with a scalpel and pushed through a slit in the flap into the coelomic cavity of the same embryo. The egg was sealed with Scotch tape and returned to the incubator. 2 or 3 days later the graft and the contralateral control wing were staged and fixed. Successful grafts were examined by immunocytochemistry and in situ hybridization. The possible innervation of grafts by ectopic nerves was checked routinely by staining for mAb ID-5-positive axons and vinculin-positive nerve satellite cells.

Results

Nerve-dependent tenascin expression in the normal wing bud

During embryonic day 5 of chick development (stages 24–25), the peripheral axons that have accumulated in a plexus at the hip and shoulder region start to invade the limb mesenchyme as large nerve bundles. At stage 26, they have formed bracket-like structures that encircle the femur or humerus primordia at their dorsal and ventral surfaces. These nerves are positive for tenascin and exclude fibronectin (cf. Wehrle and Chiquet, 1990; Fig. 2B,C). In situ hybridization during nerve invasion of the wing bud showed that tenascin mRNA is associated with the brachial plexus, with the proximal wing nerves (brachialis longus superior and inferior) and with the radial nerve, which runs dorsally of the humerus anlage (Fig. 2A; see Fig. 3 for a scheme). Since the hybridization signal is high throughout the nerves and sharply outlines their borders, the likely source of tenascin mRNA in this case is nerve satellite cells, i.e. glial precursors (cf. Tosney and Landmesser, 1985; Fig. 5), rather than mesenchymal cells that surround the nerve.

Expression of tenascin mRNA and protein in nerveless wing grafts

To distinguish between nerve-dependent versus -independent tenascin expression in the developing wing bud, we wanted to compare the pattern of tenascin mRNA and protein in normal limbs with the one in nerve-free limbs. To obtain nerveless wings, we grafted stage 18–19 wing buds into the coelomic cavity of the same embryos. We operated on 22 embryos of which 13 have grown successful grafts. Typically the grafts were smaller than the contralateral control wing and were also developmentally delayed by about half a day. Of the 13 grafts, two were innervated by ectopic peripheral nerves. These nerves could be detected by using two antibodies as markers: mAb ID5 against detyrosinated α-tubulin (Wehland and Weber, 1987) specifically labels peripheral axons (Wehrle and Chiquet, 1990), whereas an anti-tenascin antiserum (Fig. 1) stains neural-crest-derived nerve satellite cells strongly compared to mesenchymal cells (Duband and Thiery, 1990). The innervation pattern of these two grafts was basically the same as in ungrafted controls (not shown). The remaining grafts were judged nerveless because neither axons nor satellite cells could be detected with the respective marker antibodies (not shown). Of these grafts, three at stage 24–25 and three at stage 26 were sectioned and stained for tenascin and fibronectin. Three grafts at stage 27 were sectioned and in situ hybridization for tenascin mRNA as well as immunofluorescence staining for tenascin and fibronectin were performed.

In wing grafts at stage 24–25, no staining with anti-tenascin could be detected, except where the graft was attached to the host tissue. Fibronectin-positive precartilage condensations could be observed (data not shown). Wing grafts at stage 26 exhibited tenascin-positive stripes on the surface of the fibronectin-positive humerus primordium (Figs 4A,B). Two such structures, of which the dorsal is seen in Fig. 4A, were oriented in a proximal to distal direction. They are the first tendon primordia. However, no tenascin was detected in regions proximal to the humerus primordium, where the tenascin-positive plexus and the upper brachial nerves are found in normal limbs (Fig. 2B; Wehrle and Chiquet, 1990).

At stage 27, the anti-tenascin staining of the tendon primordia in nerveless wing grafts had increased in intensity and diameter (Figs 4C,D). Ventrally, the tenascin-positive material lay close to the main blood vessel (Fig. 4C) and ended at the mid forewing level (not shown). Dorsally, the staining extended along the humerus (Fig. 4C) to the proximal end of the ulna, disappeared and started again a bit more distally in between radius and ulna (not shown). In situ hybridiz-
Fig. 4. Protein distribution of tenasin (A,C) and fibronectin (B,D) and localization of tenasin mRNA (E) in cryosections of chick coelomic wing grafts. A longitudinal section of a stage 26 grafted wing bud was double stained with anti-tenasin mAb M1 (A) and anti-fibronectin antiserum (B). A cross section through a stage 27 wing graft at the mid-humerus level was double labeled with anti-tenasin mAb M1 (C) and anti-fibronectin antiserum (D). A longitudinal section of a stage 27 wing graft was hybridized with tenasin cDNA probe (E). Dorsal is up; in the cross section, anterior is oriented to the right, and in longitudinal sections, distal is to the right. TN, tenascin; FN, fibronectin; dt, dorsal tendon primordium; vt, ventral tendon primordium; h, humerus primordium; bv, blood vessel; c, coracoid primordium. Bar, 400 μm (A,B) and 500 μm (C-E).

ation (Fig. 4E) revealed the same location of tenasin mRNA in tendon primordia as detected for the protein by staining with anti-tenasin.

In the grafts, anti-tenasin staining was restricted to perichondrium, ligament and tendon anlagen at this stage. Mesenchyme proximal to the tendon primordia, which normally accumulates tenasin protein along the developing nerve, was devoid of tenasin in nerveless wings. Wing grafts lacked both vinculin-positive satellite cells (not shown) and tenasin mRNA expression (Fig. 4E) in the region where the plexus and brachial nerves would form. This supports the notion that tenasin mRNA accumulated throughout proximal nerves in normal wing buds (Fig. 2A) is synthesized by these satellite cells.

Relationship between the growing radialis nerve and the dorsal tendon primordium

It seems clear from the last paragraph that tendon primordia are the only tenasin-positive structures in the developing limb which appear at the same time and about the same place as two of the peripheral limb nerves, namely the radialis and the interosseus (see Fig. 3). This tenasin expression is mesenchymal and is
not triggered by innervation. Since tendon primordia might provide guidance to these two developing nerves, we wanted to look at their spatial and temporal relationship more closely. Growing axons of the radialis nerve were detected with anti-tubulin mAb ID5 (Wehland and Weber, 1987; Wehrle and Chiquet, 1990). Comigrating nerve satellite cells were specifically labeled by anti-vinculin antisem (Fig. 1; Duband and Thiery, 1990). By double immunofluorescence labeling, it was possible to correlate the positions of the dorsal tendon primordium with the developing radialis nerve.

At stage 26 in the wing, the upper brachial nerves have a large diameter, whereas the radialis nerve is long and tall (Fig. 2). Examination of cross sections at the same stage (26) by immunofluorescence revealed tenascin staining between the nerve fascicles of the brachialis longus inferior (Fig. 5A). ID-5-positive axons (Fig. 5B) were encircled by vinculin-positive nerve satellite cells, i.e. glial precursor cells (Fig. 5C). Fibronectin was excluded from nerve fascicles but stained the precartilage condensation of the humerus (Fig. 5D). On the same section, tenascin staining was distributed in the mesenchyme around the radialis nerve (Figs 5A,E). As for tenasin, staining for fibronectin was prominent adjacent to the nerve (Figs 5D,H). The enhanced staining for tenasin and fibronectin around the nerve is due to the dorsal tendon primordium which could be detected in the grafted nerve-free limbs (Fig. 4) and which in normal limbs partially overlaps with the radialis nerve at this stage. One stage later in development (stage 27), the now strongly tenasin- and fibronectin-positive tendon primordium, which parallels the radialis nerve, has increased in diameter, and the axons with their glial sheet are detached from it (Figs 5I–L; Tosney and Landmesser, 1985).

At the tip of the deep branch of the radialis nerve (Figs 5M–P), the most-distal tenasin-positive ECM fibers (Fig. 5M) were detected within 20 μm from the tip of the nerve as determined by ID5 (Fig. 5N) and vinculin (Fig. 5O) staining. The vinculin-positive satellite cells were always found in close association with axonal fibers and growth cones (Figs 5N,O). Fibronectin staining is homogeneous throughout the limb mesenchyme at this distal level (Fig. 5P).

It should be stressed that the association of growing nerves with tendon primordia cannot be generalized. Of the ventral wing nerves, for example, the unlaris penetrates into tenasin-free mesenchyme but itself expresses tenasin. Interestingly, however, this nerve advances more slowly than the interosseus nerve which runs parallel to the ventral tendon primordium (data not shown). A summary of findings concerning the stage 26 wing bud is shown in a schematic drawing (Fig. 3).

**Cessation of tenasin mRNA accumulation within nerves at later stages**

At stages 28 and later, detectable tenasin mRNA has disappeared completely from the plexus region (not shown) and within the nerves at the upper wing level (Figs 6A–D). However, the tenasin protein that has accumulated around the nerves is still visible (Fig. 6E).

Expression of tenasin mRNA in perichondrium (Figs 6A,B) and tendon primordia (Figs 6A–D) is now clearly, although weakly, detectable. This seems to contrast with the strong labeling of tendon primordia by anti-tenasin (Fig. 6E). Apparently, low but constant levels of tenasin mRNA synthesis lead to a high protein accumulation in tendons. In contrast, a short but transient burst of tenasin mRNA is found in growing nerves.

**Expression of tenasin in the hindlimb bud**

In the hindlimb bud at stages 24–25, the nerves have not yet left the plexus region; however, tenasin mRNA can be detected in ventral as well as in dorsal regions of the femoral precartilage cell condensation (Fig. 7A). This expression is obviously independent of the presence of nerves, as is the case in nerveless wing grafts of stage 26. Thus, tenasin expression in tendon primordia starts earlier in the leg than in the wing. At stage 26, the dorsal and ventral branches of the sciatic nerve have reached these tenasin mRNA positive pretendinous structures (Figs 7B,C). The dorsal tendon primordium parallels the dorsal sciatic nerve branch anteriorly. In the hindlimb bud, tenasin expression by peripheral nerves (Figs 7B,C) is similar to that observed in the wing bud, however, the tendon primordia are formed prior to nerve innervation.

In conclusion, when the peripheral nerves start to innervate the limb bud, satellite cells, which are likely to be glial precursor cells, accumulate high amounts of tenasin mRNA. Newly synthesized tenasin is deposited at the interface between the glial cell sheet and the mesenchyme, which in most places does not express tenasin mRNA. However, specialized mesenchyme,
Tenascin expression in limb buds

like the tendon primordia, produce and accumulate tenascin basically at the same time when some limb nerves (radialis and interosseus in the wing; sciatic in the leg) are formed. These nerves are first tightly associated with tendon primordia, but later separate from them and finally run parallel to the tendons. After
During invasion of embryonic chick limbs by peripheral nerves, tenasin mRNA accumulation by nerve satellite cells ceases, but continues in mesenchymal structures.

**Discussion**

During invasion of embryonic chick limbs by peripheral nerves, tenasin is deposited between axons at their growing tips, and more proximally around developing axon fascicles (Wehrle and Chiquet, 1990). In contrast, the mesenchyme not in contact with nerves is mostly free of tenasin at this stage. The specific accumulation of tenasin along growing peripheral nerves peaks during the main growth period, whereas later the molecule is found only in the perineurium (Chiquet and Fambrough, 1984a) and at the nodes of Ranvier (Rieger et al. 1986). Looking for a function of tenasin in nerve morphogenesis, it is important to know which cells synthesize the protein. We could think of the following possibilities: Either, tenasin is synthesized by mesenchyme along prospective nerve pathways independently from the growing nerves. In this case, tenasin might be involved in axonal pathfinding. Alternatively, the ingrowing nerves could induce tenasin production in the neighboring mesenchyme, or the protein could be made by nerve satellite cells (i.e., prospective Schwann cells; Noakes et al. 1988), which accompany the growing axons. In the latter two cases, tenasin could have a function in nerve morphogenesis and maturation rather than in establishing the nerve pattern. To distinguish between these possibilities, we determined the sites of tenasin mRNA accumulation in normal as well as in grafted nerve-free limbs. Since in the absence of nerves only the tendon primordia express tenasin mRNA at the relevant stages, we can conclude that all other tenasin associated with nerves...
is deposited as a consequence of nerve invasion in normal limbs. For the following reasons, we strongly believe that neural-crest-derived nerve satellite cells are the main source of tenascin that is associated with growing nerves. These glial precursor cells are positive for neural crest markers (Vincent and Thiery, 1984) and have been shown to express high levels of cytoskeletal vinculin (Duband and Thiery, 1990), which we used as a marker in this study. In most cases, we found an exact overlap between the vinculin-positive, fibronectin-negative area of growing nerves and the in situ hybridization pattern obtained with tenascin cDNA. The border of the labeling between nerves and mesenchyme was very sharp; except in the few cases where nerves follow tendon primordia, no tenascin mRNA could be detected in the adjacent tissue at this stage. Thus, nerves are not likely to induce tenascin synthesis in the surrounding mesenchyme, but themselves produce most of the protein. Since axons contain little if any mRNA (Davis et al., 1987), vinculin-positive satellite cells are the only candidates to accumulate tenascin mRNA within the nerve proper. In accordance with our conclusion, Martini and Schachner (1991) have found intracellular tenascin immunoreactivity in Schwann precursor cells by electron microscopy. Moreover, primary glial cells (Grunet et al., 1985) and glial cell lines (Bourdon et al., 1985) are known to produce the protein.

It is an open question how the highly restricted expression pattern of tenascin is controlled. It seems from our results that tendon cells produce relatively low amounts of tenascin mRNA over prolonged periods of time, which results in the accumulation of a lot of the protein as the tendon matures. This process might be regulated by factors such as TGF-β, which is known to stimulate tenascin mRNA transcription in cultured fibroblasts (Pearson et al., 1988) and which is expressed in embryonic tendons (Pelton et al., 1989). In contrast, expression of tenascin mRNA in nerve satellite cells seems to be high but lasts for only about one day. This leads to a delayed, transient accumulation of the protein around axon fascicles. It is known that direct contact with growing axons induces Schwann cells to deposit laminin (Bunge et al., 1989), and such cell contacts might also be important for the expression of tenascin mRNA in these cells. On the other hand, the short burst of tenascin expression, which is also found in some growing epithelia (Prieto et al., 1990), might be coupled to an endogenous developmental program of glial precursor cells.

Could any of the tenascin that is expressed in developing limbs be involved in axonal guidance? We found that most of the tenascin at the tips of growing peripheral nerves is produced by nerve satellite cells and not by mesenchymal cells along prospective axonal pathways. Therefore, other molecules or structures at the interface between the central cartilage condensation and the peripheral limb mesenchyme must be responsible for guiding the nerves. The early tendon primordia, for which tenascin happens to be an early
mesenchymal marker, are candidates for guiding two of the wing nerves, the radialis and the interosseus. For a short period of time, the developing radialis nerve is embedded into the tendon primordium, which is consistent with a guiding function of this structure. The molecules involved have yet to be identified. Since tenascin is accumulated here by tendon mesenchyme as well as by nerve satellite cells, it is not likely to act as a vectorial signal between the two tissues. Certain monoclonal antibodies (Tanaka et al. 1989) might recognize putative candidates for a guiding function which are present in prospective nerve pathways.

Tenascin is produced by nerve satellite cells during the main growth phase of peripheral axons and is therefore likely to be essential for the morphogenesis of limb nerves. For example, tenascin is much more protease resistant than fibronectin (Chiquet and Fambrough, 1984b). Since growing axons are known to secrete proteases (McGuire and Seeds, 1990), the growing nerve might digest mesenchymal fibronectin, which is then replaced by tenascin secreted by the satellite cells. In vitro, tenascin inhibits the spreading of cells of mesenchymal origin (Chiquet-Ehrismann et al. 1988). It is a non-adhesive substratum for most cell types except for some of glial origin (Lotz et al. 1989). Thus, tenascin might be used to demarcate the boundaries of developing axon fascicles. Such a boundary function has been proposed for tenascin during development of the somatosensory barrel fields in the cortex (Steindler et al. 1989). However, we do not believe that tenascin exerts such a function by being a general inhibitor of axonal attachment and growth (Caroni and Schwab, 1988; Faissner and Kruse, 1990). From our observations, it does not seem that growing axons are deflected away from tenascin-containing tendon primordia, as it has been suggested (Martini and Schachner, 1991). We have shown that tenascin does allow growth cone attachment and movement, although it is a poor adhesive substratum for the neurites of developing motor neurons (Wehrle and Chiquet, 1990). Therefore, tenascin-containing matrix around axons might facilitate the formation of fascicles, but still allow the sorting of axons between them during final nerve morphogenesis. Tenascin could thus allow the plasticity that is necessary for a refinement of neuronal projections during development of the nervous system. More experiments both in vitro and in vivo are needed to support this hypothesis.

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