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The distribution of tenascin coincides with pathways of neural crest cell migration

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Summary

The distribution of the extracellular matrix (ECM) glycoprotein, tenascin, has been compared with that of fibronectin in neural crest migration pathways of *Xenopus laevis*, quail and rat embryos. In all species studied, the distribution of tenascin, examined by immunohistochemistry, was more closely correlated with pathways of migration than that of fibronectin, which is known to be important for neural crest migration. In *Xenopus laevis* embryos, anti-tenascin stained the dorsal fin matrix and ECM along the ventral route of migration, but not the ECM found laterally between the ectoderm and somites where neural crest cells do not migrate. In quail embryos, the appearance of tenascin in neural crest pathways was well correlated with the anterior-to-posterior wave of migration. The distribution of tenascin within somites was compared with that of the neural crest marker, HNK-1, in quail embryos. In the dorsal halves of quail somites which contained migrating neural crest cells, the predominant tenascin staining was in the anterior halves of the somites, codistributed with the migrating cells. In rat embryos, tenascin was detectable in the somites only in the anterior halves. Tenascin was not detectable in the matrix of cultured quail neural crest cells, but was in the matrix surrounding somite and notochord cells *in vitro*. Neural crest cells cultured on a substratum of tenascin did not spread and were rounded. We propose that tenascin is an important factor controlling neural crest morphogenesis, perhaps by modifying the interaction of neural crest cells with fibronectin.

Key words: tenascin, neural crest, cell migration, extracellular matrix, fibronectin.

Introduction

The neural crest is a transient embryonic tissue that migrates as individual cells or streams of cells in an anterior-to-posterior wave from the dorsal neural tube to numerous locations in the embryo. Neural crest cells differentiate into a variety of cell types, including pigment cells, neurosecretory cells, and the neurones and accessory cells of the peripheral nervous system (for reviews see Noden, 1980; Le Douarin, 1982; Erickson, 1986). The pathways of trunk neural crest cell migration are well defined (Weston, 1963; Le Douarin & Teillet, 1974; Thiery *et al.* 1982; Vincent & Thiery, 1984; Bronner-Fraser, 1986*a*; Tucker, 1986; Teillet *et al.* 1987; Loring & Erickson, 1987), and the extracellular matrix (ECM) that lines these pathways has been implicated in directing the early phases of neural crest cell migration. For example, it has been suggested that collagen (Löfberg *et al.* 1980; Tucker & Erickson, 1984), the glycosaminoglycans chondroitin sulphate and hyaluronate (Derby, 1978; Pintar, 1978; Newgreen *et al.* 1982, 1986; Erickson & Turley, 1983; Tucker & Erickson, 1984) and the glycoproteins laminin (Newgreen, 1984; Krotoski *et al.* 1986; Loring & Erickson, 1987) and fibronectin (Newgreen & Thiery, 1980; Duband & Thiery, 1982; Thiery *et al.* 1982; Krotoski *et al.* 1986; see also Löfberg *et al.* 1985)

are important for neural crest cell migration. Most of the evidence collected so far concerning the roles of these molecules has resulted both from correlations between the presence of the molecule in the pathway and the behaviour and morphology of neural crest cells confronted with isolated ECM *in vitro*. Recently, however, it has been possible to manipulate the ECM *in vivo* and to present more direct evidence for the role of one of these matrix components, fibronectin, in neural crest cell migration (Boucaut *et al.* 1984; Bronner-Fraser, 1985, 1986*b*). Each study reports that neural crest cell interactions with fibronectin are necessary for normal migration. Although fibronectin is a preferred substratum for neural crest cells, its ubiquitous distribution (e.g. see Krotoski *et al.* 1986; Tucker & Erickson, 1986) in the embryonic ECM precludes the possibility that fibronectin alone is guiding the more restricted migration of the neural crest.

Recently, the ECM glycoprotein, tenascin, has been proposed as a factor that could affect neural crest migration. Tenascin, originally described as myotendinous antigen, is a disulphide-linked oligomer consisting of subunits of about 220, 200 and $190\times10^3 M_r$ (Chiquet & Fambrough, 1984*b*). It has been implicated in oncogenesis (Chiquet-Ehrismann *et al.* 1986; Mackie *et al.* 1987*a*) and in a variety of embryonic events, including tendon and muscle development (Chiquet & Fambrough, 1984*a*), bone and cartilage formation (Mackie *et al.* 1987*b*; Vaughan *et al.* 1987) and tooth (Thesleff *et al.* 1987), kidney (Aufderheide *et al.* 1987) and mammary gland (Chiquet-Ehrismann *et al.* 1986) development.

GMEM (Bourdon *et al.* 1985), J1 (Kruse *et al.* 1985), and cytotactin (Grumet *et al.* 1985) have similar structures and tissue distributions to tenascin and are probably identical. Antibodies to cytotactin have been used by Crossin *et al.* (1986) to investigate the distribution of this protein in the trunk of the avian embryo. Cytotactin was found lining the neural crest migration pathways in a spatial and temporal pattern more closely correlated with the presence of neural crest cells than fibronectin. We have undertaken a more detailed study of the distribution of tenascin in the trunk of avian embryos, examining the precise localization of tenascin within somites. We have carried out a survey of *Xenopus laevis* and rat embryos to see whether the correlation between tenascin and neural crest pathways holds true in other classes of vertebrates. We have also investigated the effect of tenascin-coated substrata on the morphology of cultured neural crest cells and whether neural crest cells themselves are able to synthesize tenascin and organize it into a matrix *in vitro*. Our results indicate that tenascin is a likely candidate for a major factor controlling the pathways of neural crest migration.

Materials and methods

Experimental animals

Xenopus laevis embryos were obtained from hormone-induced matings of animals maintained in a colony in Basel. Embryos were dejellied in 1 % cysteine (pH 8·0) and kept at room temperature in tap water until appropriate stages (stages 25/26 and 32, Nieuwkoop & Faber, 1975) for sectioning were reached. Japanese quail embryos (*Coturnix coturnix*) were kept in a humidified incubator at 38°C for 54 h (16–20 somites) before processing for primary cultures or immunostaining (see below). Rat embryos (embryonic day 10 and 11) were dissected from outbred Sprague-Dawley rats supplied by the Ciba-Geigy (Basel) animal care facility.

Antibodies

The rabbit antibodies to fibronectin or tenascin used in this study have been described and characterized elsewhere (Ehrismann *et al.* 1981; Chiquet-Ehrismann *et al.* 1986). A mouse monoclonal antibody to HNK-1 (anti-Leu-7; Becton Dickinson, Mountain View, CA) was used as a marker for neural crest cells (Vincent *et al.* 1983; Tucker *et al.* 1984).

Immunohistochemistry

Freshly excised embryos or tadpoles were fixed in 4 % paraformaldehyde in 0·1 M-potassium phosphate buffer (pH 7·4) at 4°C for 1–4 h or overnight. The embryos were then rinsed in phosphate-buffered saline (PBS) and cryoprotected in 25–30 % sucrose in PBS overnight or until the embryos sank into the solution. Specimens were then embedded in OCT (Miles, Naperville, IL), quickly frozen and sectioned at 20 or 14 μm (*Xenopus* and quail), or 10 μm (rat). Serial frozen sections were collected on chrome alum/gelatin-coated glass slides and air dried for 1–3 h. Sections were then rinsed in PBS, blocked with 0·5 % bovine serum albumin (BSA) in PBS, and incubated in pre-immune, anti-tenascin or anti-fibronectin serum (diluted 1:100) or anti-Leu-7 (undiluted) for 1 h to overnight at room temperature. Sections were then rinsed in PBS and incubated in an appropriate TRITC- or FITC-labelled secondary antibody for 2–4 h. Sections which were double stained with anti-tenascin and anti-Leu-7 were first incubated with anti-Leu-7 then with FITC-labelled goat anti-mouse IgG. They were then incubated with anti-tenascin followed by TRITC-labelled goat anti-rabbit IgG.

Immunocytochemistry

Quail neural tubes, posterior somites (the second and third somites from the segmental plate), and notochords were isolated from 54 h embryos and plated on 8-well plastic slides (Miles) coated with laminin (see below). Primary cultures were grown in Ham's F-12 medium with antibiotics for 48 h and then processed for immunocytochemistry. Cells were rinsed gently with PBS and then fixed in 4 % paraformaldehyde in PBS for 5 min at room temperature. Cells were then rinsed with PBS, treated with −20°C methanol for 5 min, rinsed again in PBS and blocked in 0·5 % BSA in PBS for 15 min. Cultures were then incubated in primary antibody (preimmune, anti-tenascin or anti-fibronectin serum) diluted 1:100 in PBS for 2 h. Cultures were rinsed in

PBS and stained with TRITC-labelled goat anti-rabbit secondary antibody for 1 h. After thorough rinsing in PBS, the cultures were mounted using 50% glycerol (with 1% azide) and viewed with a Zeiss photomicroscope.

Neural crest cells in vitro *on ECM-coated substrata*

Neural crest cultures were made using slight modifications of previously described methods (Loring *et al.* 1981). In brief, 54 h quail embryos were cut free from extraembryonic membranes and yolk in PBS, and the trunks from the segmental plate to the fifteenth somite were dissected free and digested briefly in collagenase/dispase (Boehringer, Mannheim, FRG). After placing the digested tissues in cold Dulbecco's medium with 10% fetal calf serum, the neural tube, somites and notochord were gently dissociated using tungsten needles. Neural tubes were rinsed in three changes of unsupplemented Ham's F-12 and cultured in Ham's F-12 with antibiotics on untreated Corning (Corning, NY) tissue culture dishes or on tissue culture dishes coated with laminin (BRL, Bethesda, MD; 50 $\mu g\,ml^{-1}$ in PBS applied for 20 min, aspirated, dried and thoroughly rinsed with PBS), chick fibroblast tenascin (~50 $\mu g\,ml^{-1}$, purified according to methods described by Chiquet & Fambrough, 1984*b*), or fibronectin (~50 $\mu g\,ml^{-1}$, Chiquet *et al.* 1979). After 18 h at 37°C, the cultures were photographed using a Zeiss inverted microscope.

Results

The distribution of tenascin and fibronectin in situ

At the onset of neural crest cell migration in the midtrunk of *Xenopus laevis* embryos (stage 25/26), anti-tenascin stained the ECM surrounding the neural crest along the dorsal surface of the neural tube, as well as the ECM in the intersomitic furrows (Fig. 1A). Anti-fibronectin, in contrast, stained ECM throughout the embryo (Fig. 1B). In slightly older embryos, when the neural crest cells are actually spread along the ventral pathway (stage 32), staining with anti-tenascin was found in the dorsal fin matrix, around the neural tube, notochord, and ventrally between the endoderm and somites (Fig. 1C). Anti-tenascin did not stain the matrix underlying the ectoderm. Anti-fibronectin staining at stage 32 was intense and essentially unchanged from the earlier period (Fig. 1D).

In the 54 h-old quail embryo, both anti-tenascin and anti-fibronectin stained ECM in cross-sections through the trunk at an axial level where the neural crest cells are migrating (Fig. 2A,B). Tenascin was found around and within the somites, as well as surrounding the neural tube, notochord and underlying the ectoderm. Tenascin staining was weak or absent in extraembryonic tissues. The staining with anti-fibronectin was identical to staining reported by others (e.g. see Newgreen & Thiery, 1980; Krotoski *et al.* 1986); fibronectin was found in the ECM surrounding all of the blocks of embryonic tissues, as well as in extraembryonic membranes. At a more posterior axial level (2–4 somites from the segmental plate), where the neural crest cells are just beginning to emigrate from the dorsal neural tube, staining with anti-tenascin in the vicinity of the neural tube was almost undetectable (Fig. 2C), in contrast to the intense, widespread staining seen with anti-fibronectin (Fig. 2D). Anti-tenascin staining was encountered again at more posterior axial levels, at the level of Hensen's node and the primitive streak (Fig. 2E), where it was found associated with the primitive ridge. Anti-fibronectin staining was ubiquitous and intense at this axial level (Fig. 2F).

Four distinct pathways for neural crest cell migration in the trunk of avian embryos have been described. At a particular axial level, neural crest cells first move ventrally in the intersomitic spaces and then later through the anterior half of the somite as well as between the somite and neural tube (Loring & Erickson, 1987). They also migrate between the ectoderm and somite (Weston, 1963). Cross-sections demonstrated abundant tenascin staining in the former two pathways. Serial frontal and sagittal sections were examined to determine the distribution of tenascin in the latter two pathways. In addition, double staining of tenascin-stained sections with an antibody to the neural crest cell marker HNK-1 was used to compare the distribution of tenascin with that of migrating neural crest cells. Strong staining was seen with anti-tenascin in the spaces between somites at all dorsoventral levels (Fig. 3A–I). In the dorsalmost part of the somites through which neural crest cells were migrating both tenascin and HNK-1 (not shown) were present in a continuous line between the neural tube and dermamyotome (Fig. 3A,B). More ventrally, but still within the dorsal halves of the somites, HNK-1 staining was only present in the anterior halves (Fig. 3L). At this level, tenascin was predominantly, but not exclusively, found in the anterior halves (Fig. 3A–F,J–M). In the ventralmost part of these somites, where HNK-1 staining was not detectable (not shown), tenascin was present in both anterior and posterior halves (Fig. 3E–I). In contrast to tenascin, fibronectin was present homogeneously within the somite (Fig. 3M,N). Posterior to the level of somitic HNK-1 staining, where somites had not yet formed distinct dermamyotome and sclerotome, some tenascin staining was seen in the central part of the somite, as well as surrounding the somites and neural tube (not shown). As demonstrated in the cross-section in Fig. 2C, tenascin was virtually absent at the level of the last-formed somites.

In the rat embryo (embryonic day 10·5), there was relatively little staining with anti-tenascin relative to *Xenopus* or the quail in the dorsal portion of the embryo. Ventrally, intense staining was limited to the

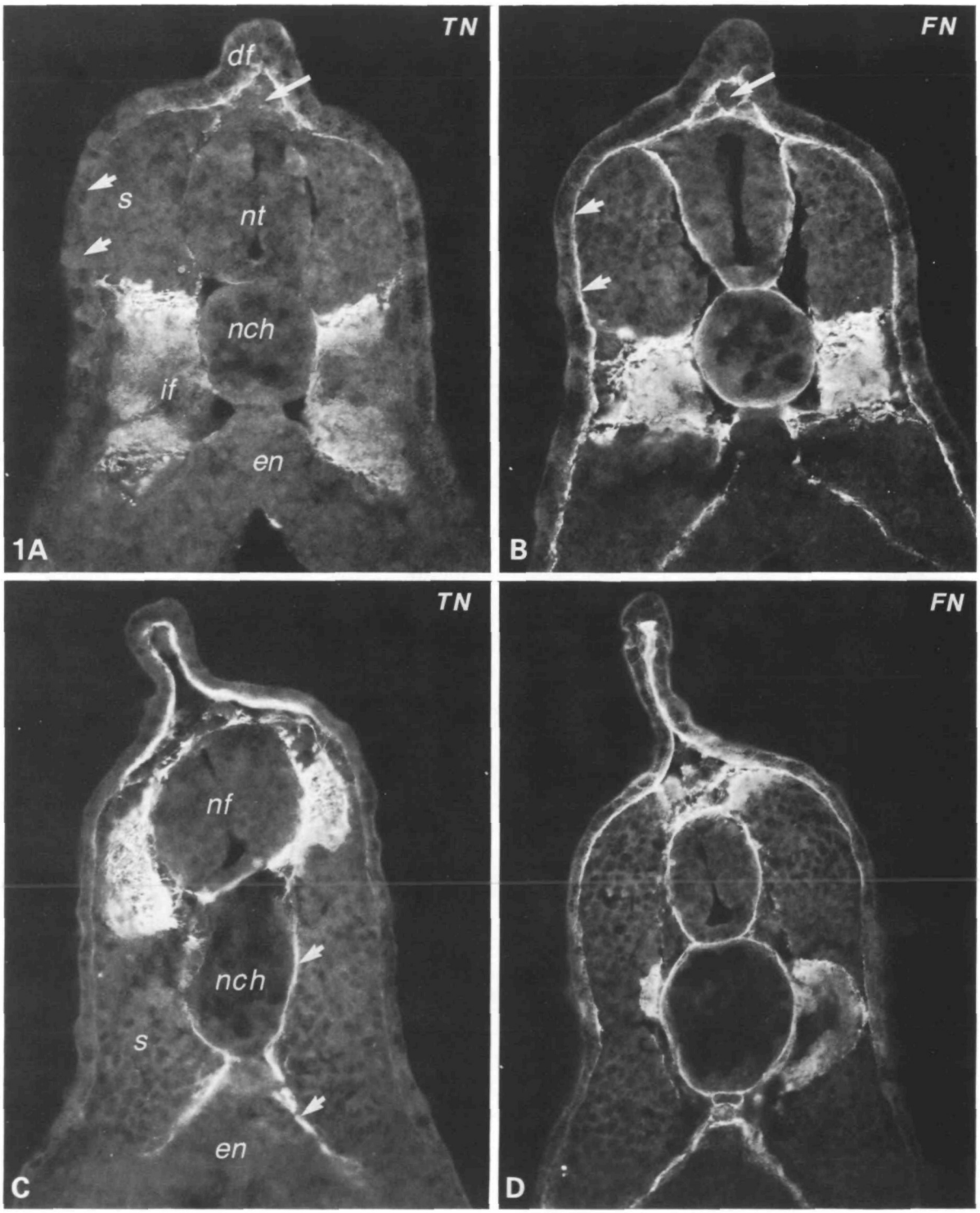

Fig. 1. Immunohistochemical staining of cross-sections through the anterior trunk of *Xenopus laevis* embryos with antibodies to tenascin (*TN*) and fibronectin (*FN*). (A) Anti-tenascin staining is limited to the matrix of the dorsal fin (*df*) and the intersomitic furrows (*if*) in stage 25/26 embryos. The neural crest (large arrow) is still found along the dorsal surface of the neural tube (*nt*) at this stage. No anti-tenascin staining is seen between the ectoderm and the somites (*s*, small arrows). *nch*, notochord; *en*, endoderm. (B) Anti-fibronectin stains the ECM throughout the stage 25/26 embryo. (C) At stage 32, when neural crest cells are found in the dorsal fin and along the ventral pathway (along the medial surface of the somites, arrows), anti-tenascin stains the ECM of the dorsal fin, intersomitic furrows and ventral pathway. (D) Anti-fibronectin stains the ECM throughout the embryo, including the matrix underlying the ectoderm where the neural crest cells are not found.

mesenchyme surrounding the gut and heart (results not shown). There was, however, staining with anti-tenascin in the somites during the time of neural crest migration. In cross-sections, this staining was found in the lateral portion of the sclerotome (Fig. 4A), or associated with the basement membrane underlying the dermamyotome (Fig. 4C). As in quail embryos, tenascin was absent from the somites in the posterior part of the embryo, where neural crest migration has not yet begun (results not shown).

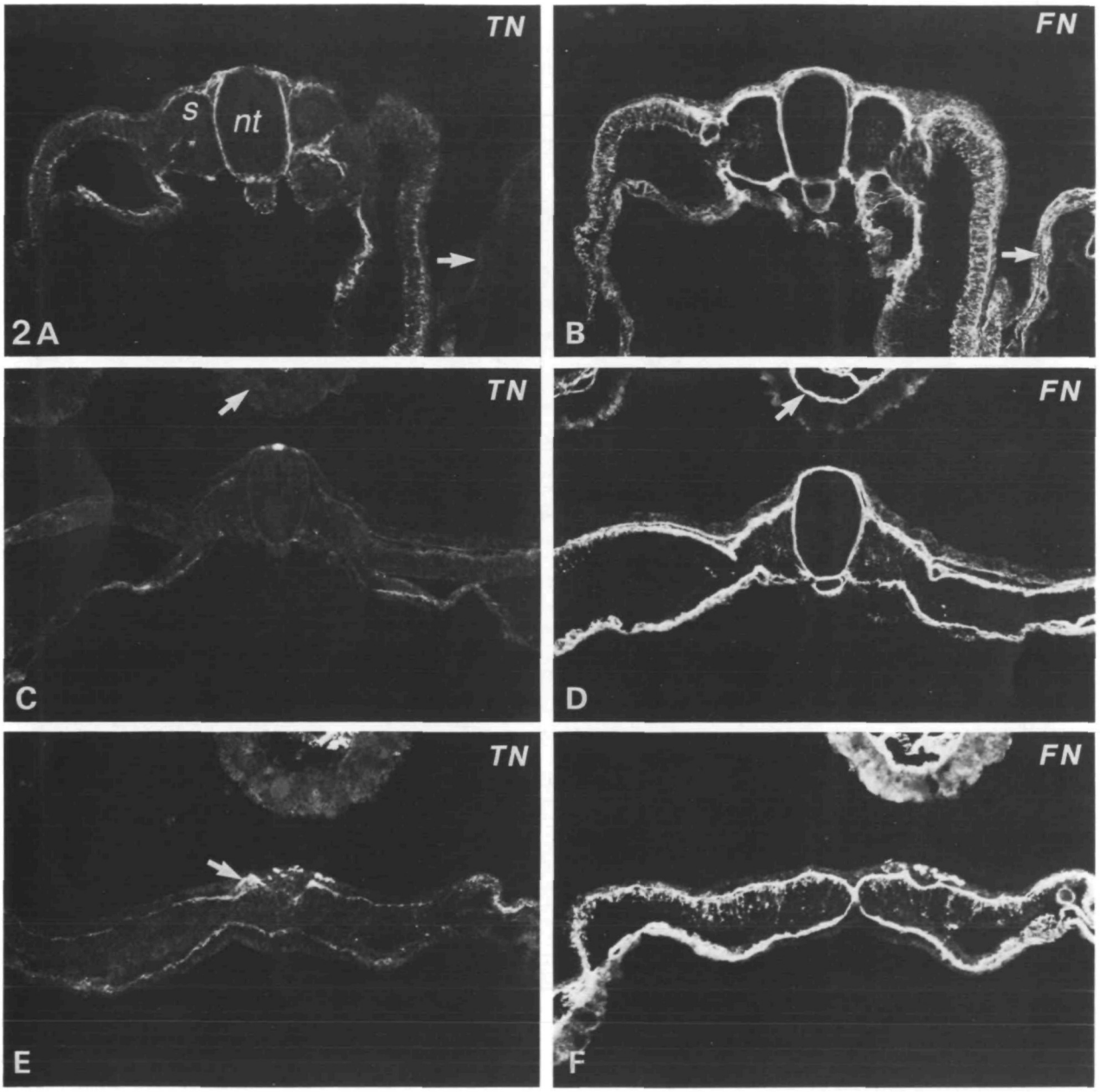

Fig. 2. Anti-tenascin (*TN*) and anti-fibronectin (*FN*) staining of cross-sections through different axial levels of a 54 h-old quail embryo. (A) In the anterior trunk, where neural crest cells have begun to migrate, anti-tenascin stains the ECM surrounding the neural tube (*nt*), notochord and somites (*s*). Anti-tenascin staining is seen only in the embryo and not in extraembryonic membranes (arrow). (B) In an adjacent section, anti-fibronectin stains the ECM throughout the embryo and the extraembryonic membranes (arrow). (C) At an axial level posterior to (i.e. preceding) neural crest cell migration, anti-tenascin staining is very faint in the embryo and is not seen in extraembryonic membranes (arrow). (D) In contrast to the staining with anti-tenascin, anti-fibronectin stains the ECM throughout the embryo and extraembryonic membranes (arrow) in a section adjacent to that shown in C. (E) At an axial level posterior to that shown in C and D, anti-tenascin stains cells at the ingressing margin of Hensen's node (arrow), but elsewhere in the embryo anti-tenascin staining is very faint. (F) Anti-fibronectin intensely stains ECM in a section (adjacent to that shown in E) through Hensen's node.

The precise distribution of tenascin in the rat somites was determined in sagittal and frontal sections. Anti-tenascin staining was relatively strong at the dorsalmost portion of the intersomitic furrow (Fig. 5A) and, more ventrally, the tenascin was found in the lateral sclerotome in only the anterior half of

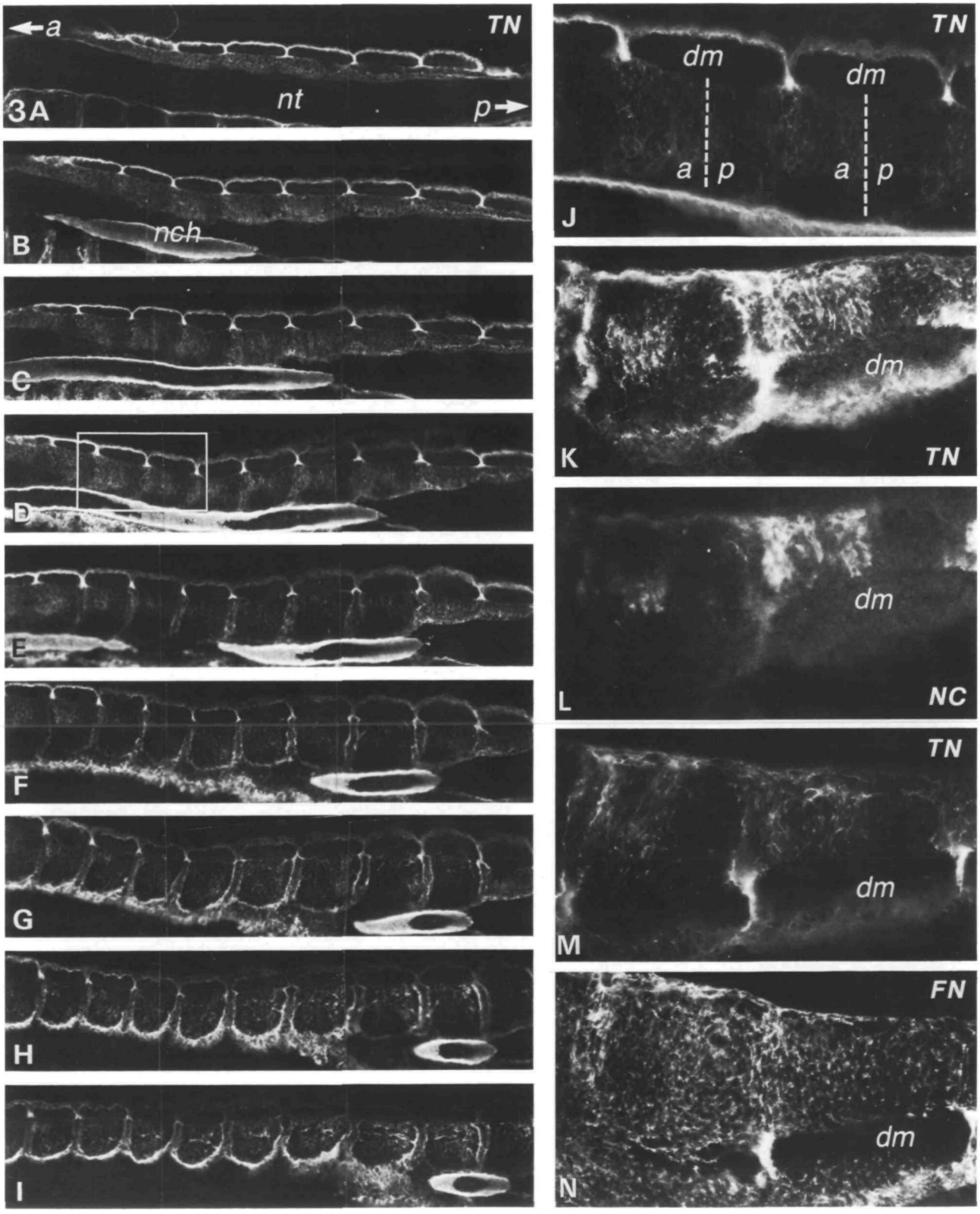

Fig. 3. For legend see p. 244

each somite (Fig. 5C,E). In contrast to the very limited distribution of tenascin in the dorsal portion of the rat embryo, fibronectin was distributed throughout the ECM being found between blocks of tissues and throughout the somites (Figs 4B,D 5B,D,F).

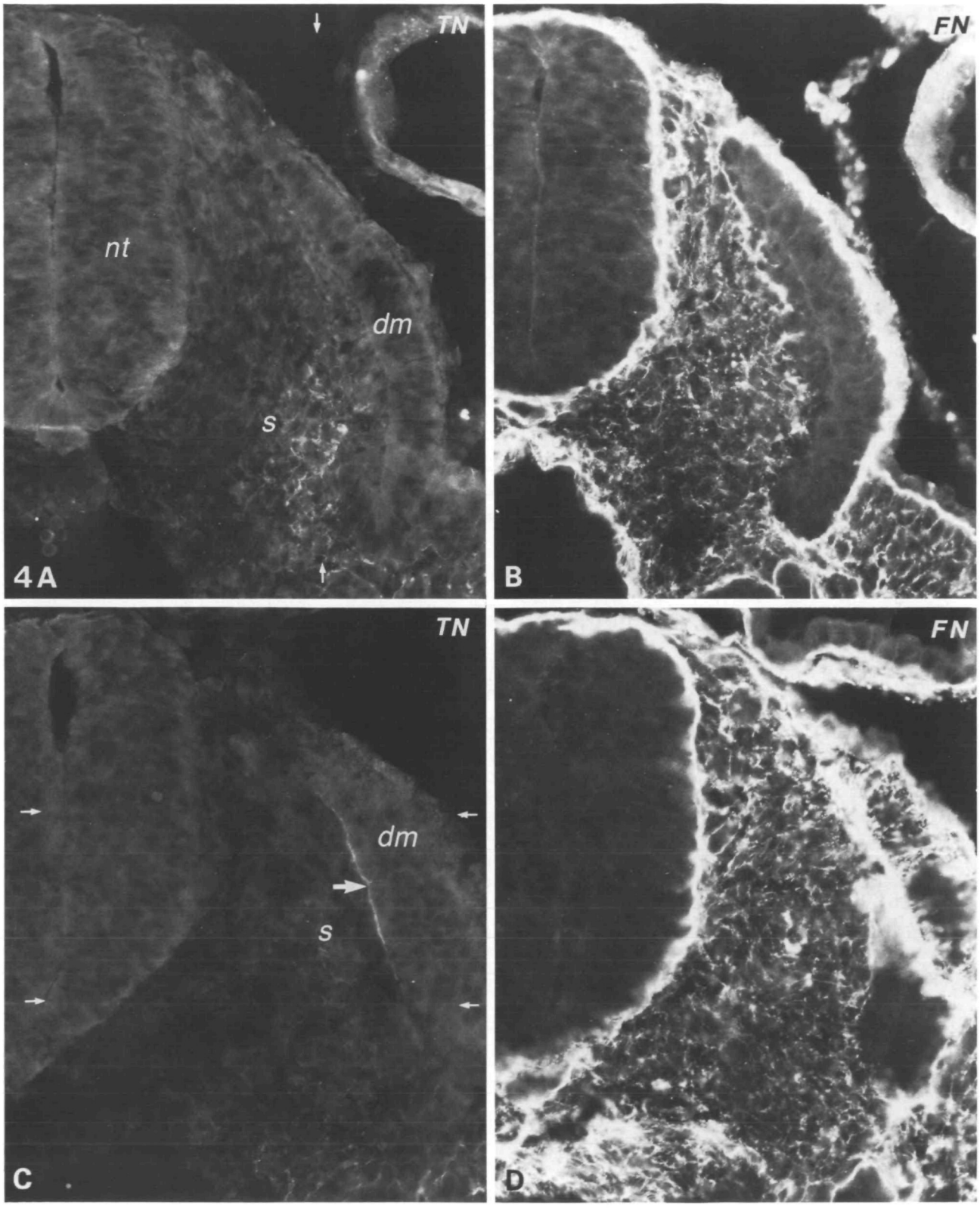

Fig. 4. For legend see p. 244

Tenascin and fibronectin in vitro

When 48 h-old primary cultures of neural crest cells were stained with either anti-tenascin or anti-fibronectin, no fluorescence was observed (Fig. 6A,B). In contrast, fibrillar extracellular fluorescence was observed in cultures of somite cells (Fig. 6C,D) and notochord cells (Fig. 6E,F) stained with anti-tenascin or anti-fibronectin. This suggests that the tenascin in the neural crest pathways is produced by the cells surrounding the pathways and not by the neural crest cells themselves.

The morphology of neural crest cells on ECM in vitro

Quail neural crest cells cultured for 24 h on tissue culture plastic coated with tenascin were rounded, indicating relatively weak adhesion to the substratum (Fig. 7A). Flattened processes were rarely seen in these cultures. Neural tubes were usually coiled when plated onto tenascin, and frequently detached from the substratum if the dish was disturbed. In spite of the nonadhesive character of tenascin, neural crest cells were able to migrate from the neural tube during the first 24 h *in vitro* to distances comparable to those found when the cells were cultured on fibronectin or tissue culture plastic alone. The morphology of neural crest cells on fibronectin-coated substrata was similar to the morphology of these cells on fibronectin reported by others (Newgreen *et al.* 1982; Rovasio *et al.* 1983; Erickson & Turley, 1983): the cells were flattened, with large lamellipodia, suggesting strong adhesion to the substratum (Fig. 7B). When neural crest cells were cultured on uncoated plastic, the morphology was intermediate between that on tenascin and fibronectin (Fig. 7C). The cells had flattened processes, but these lamellipodia were smaller than those encountered in cells on fibronectin. Neural crest cells cultured on laminin-coated substrata also had flattened processes, but these processes tended to be relatively small and found around the entire circumference of the cell, giving the cells a saucer-like morphology that was quite distinct from the cell shapes seen on other substrata (Fig. 7D).

Fig. 3. Frontal sections through the somites in the midtrunk region of quail embryos, stained with anti-tenascin (*TN*), anti-fibronectin (*FN*) and antibody to the neural crest marker, HNK-1 (*NC*). (A–I) Serial 14 μm sections through somites 9–18 of a quail embryo of about 30 somites. The section in A is dorsal to that in I. The sections are not exactly frontal, but rather slightly diagonal from side to side so that the section in A shows the most ventral part of the somites at the bottom of the field and the most dorsal part of the somites at the top of the field. The embryo is also slightly curved dorsoventrally, so that the somites in the middle of the field are cut through a more ventral part than the somites at the two ends in any particular section. In all sections, tenascin is present in the intersomitic furrows and outlining the neural tube (*nt*), as well as around the notochord (*nch*). In the dorsal halves of the somites, staining with anti-tenascin is much more intense in the anterior (*a*) halves than in the posterior (*p*) halves. Moving more ventrally within a particular somite the staining becomes more even anteroposteriorly. (J) A higher magnification of the somites outlined in D. *dm*, dermamyotome; *a*, anterior; *p*, posterior. (K,L) A single section of somites 14 and 15 from a quail embryo of about 30 somites, stained with anti-tenascin (K) and anti-HNK-1 (L) shows codistribution of tenascin and neural crest cells in the anterior (left) half of each somite. (M,N) In two sections adjacent to that in K and L, fibronectin (N) is evenly distributed within the somites, whereas tenascin (M) is almost entirely restricted to the anterior (left) half.

Fig. 4. Cross-sections through the anterior trunk of a 10·5-day-old rat embryo stained with anti-tenascin (*TN*) and anti-fibronectin (*FN*). (A) In a section through the anterior part of the somite, cells in the lateral portion of the sclerotome (*s*) are stained with anti-tenascin. Small arrows at the top and bottom of the figure indicate the plane of the sections shown in Fig. 5E and F. *dm*, dermamyotome; *nt*, neural tube. (B) In an adjacent section anti-fibronectin stains the ECM surrounding the neural tube and dermamyotome, as well as the entire sclerotome. (C) Sections near the intersomitic furrows show anti-tenascin staining along the basal lamina adjacent to the dermamyotome (large arrow). Small arrows at the right and left sides of the figure indicate the approximate plane and level of the sections shown in Fig. 5A,B (upper arrows) and Fig. 5C,D (lower arrows). (D) As seen in B, anti-fibronectin stains the ECM throughout the somite in a section adjacent to that shown in C. Fluorescence within the neural tube and dermamyotome in A and C is due to background, as demonstrated by sections stained with preimmune serum (not shown).

Discussion

The staining pattern of anti-tenascin in amphibian, avian and mammalian embryos is correlated with the known pathways of neural crest cell migration. In *Xenopus laevis*, neural crest cells migrate almost exclusively along the ventral pathway (between the medial surface of the somites and the neural tube and notochord) and into the dorsal fin matrix; pigment cells reach the dermis by migrating through the intersomitic furrows and the somites themselves from the ventral pathway (MacMillan, 1976; Tucker, 1986). The absence of anti-tenascin staining laterally between the ectoderm and somites is correlated with the absence of neural crest cells in this region. The pattern of anti-tenascin staining in *X. laevis* not only corresponds spatially with the neural crest pathways, but also temporally: before the neural crest cells leave the vicinity of the neural tube, anti-tenascin staining

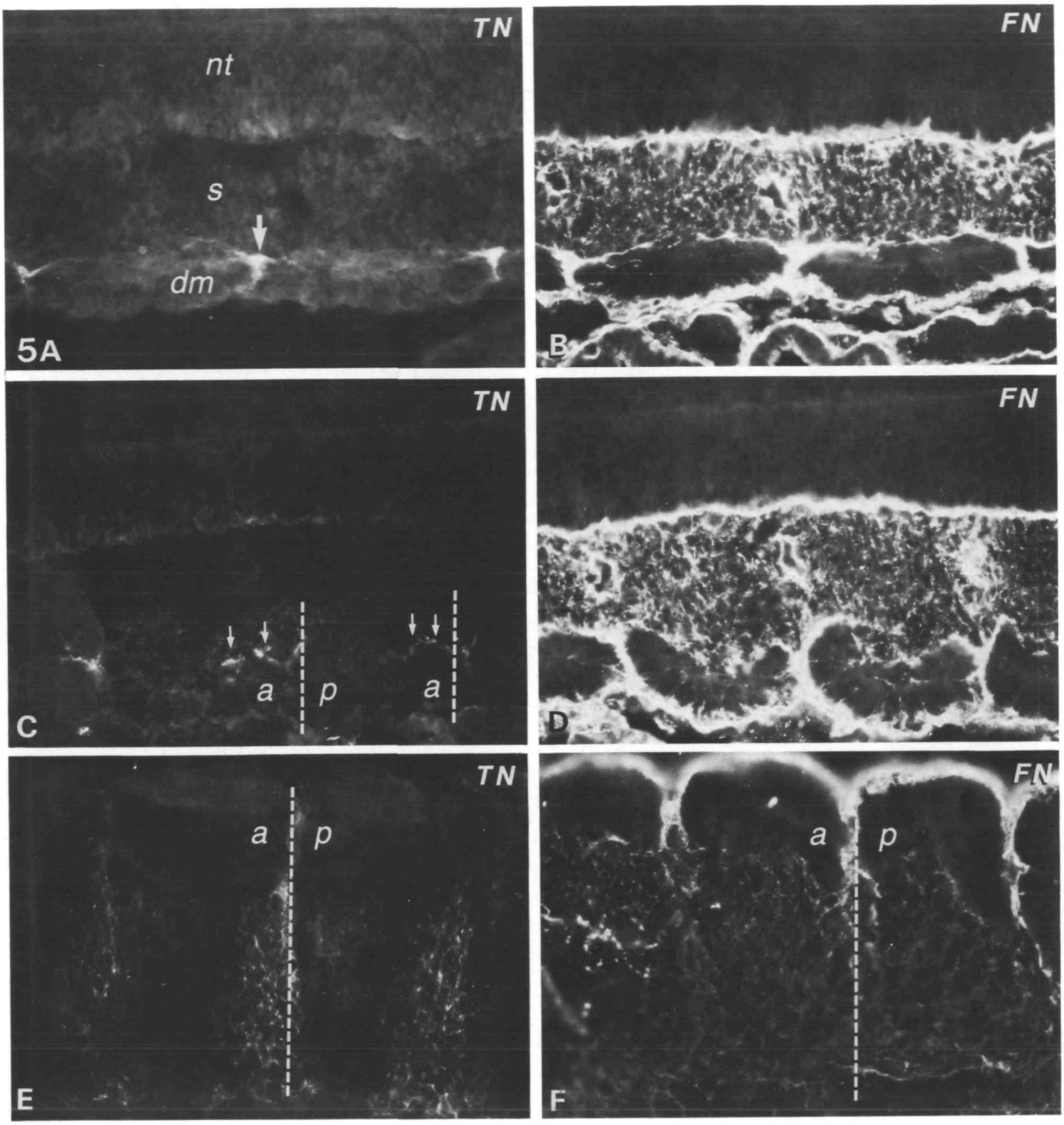

Fig. 5. Frontal and sagittal sections through the somites of a 10·5-day-old rat embryo stained with antibodies to tenascin (*TN*) and fibronectin (*FN*). See small arrows in Fig. 4 for orientation. (A) In frontal sections through the dorsal part of the somites, anti-tenascin stains the intersomitic furrows adjacent to the dermamyotome (arrow) intensely. *dm*, dermamyotome; *nt*, neural tube; *s*, sclerotome. (B) Anti-fibronectin staining of a section adjacent to the section shown in A shows intense labelling of ECM surrounding the dermamyotome and throughout the sclerotome. (C) In frontal sections through the midlevel of the somite, anti-tenascin staining is most intense in the anterior half of each somite, between the dermamyotome and the sclerotome (arrows). The intersomitic furrows are indicated by a dashed line. *a*, anterior; *p*, posterior. (D) Again, in a section adjacent to that shown in C, anti-fibronectin stains both the anterior and posterior halves of the somite with equal intensity, as well as the ECM surrounding the neural tube and underlying the ectoderm. (E) In sagittal sections through the somites, anti-tenascin staining in the anterior half of each somite but not in the posterior half is clear. An intersomitic furrow is indicated by the dashed line. *a*, anterior; *p*, posterior. (F) In contrast to the anti-tenascin staining, anti-fibronectin stains both the anterior (*a*) and posterior (*p*) halves of the somites in a section adjacent to that shown in E. Fluorescence within the neural tube and dermamyotome in A and C is due to background, as demonstrated by sections stained with preimmune serum (not shown).

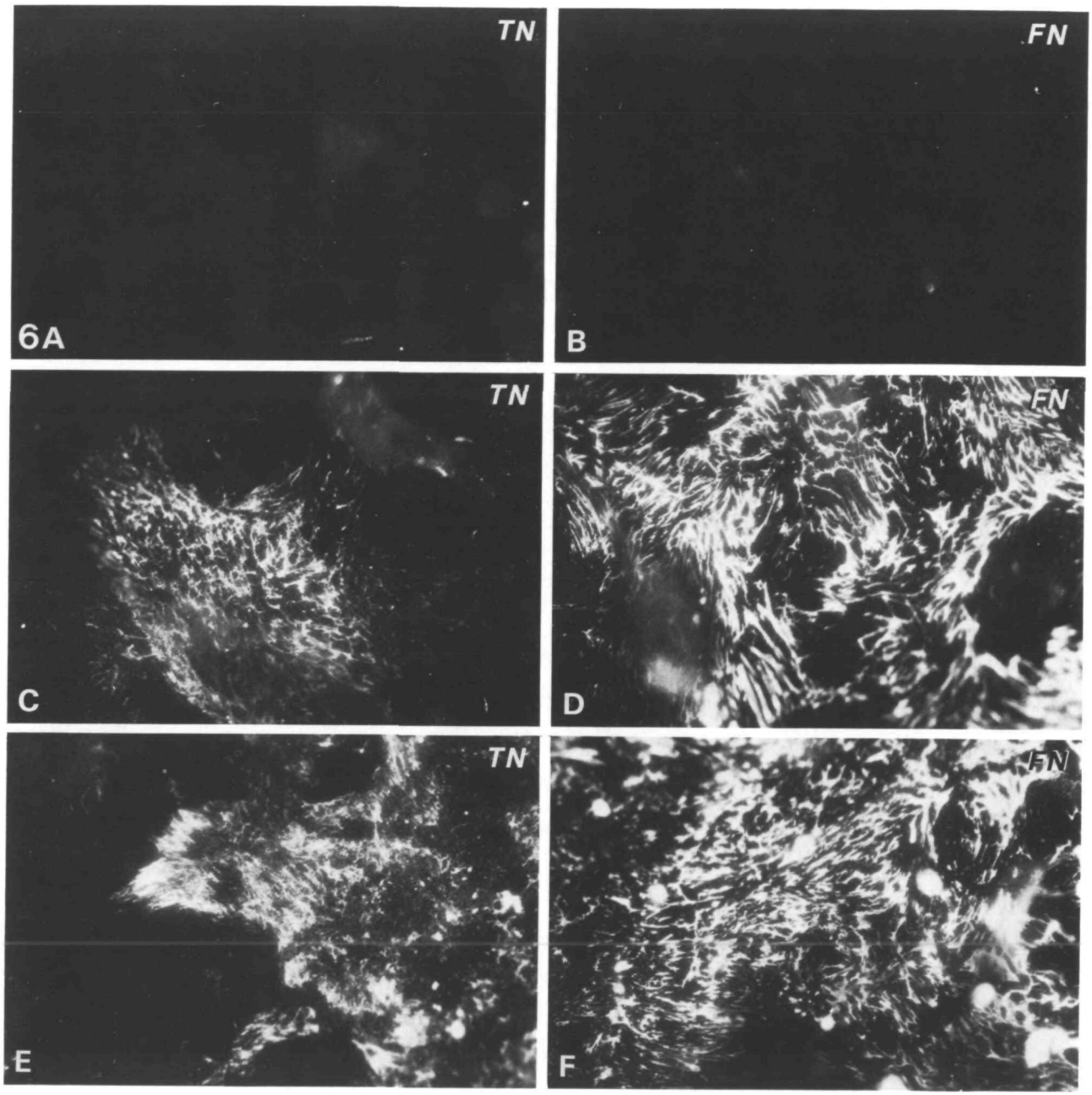

Fig. 6. Immunocytochemical staining of primary cultures of quail neural crest cells, somite cells and notochord cells with antibodies to tenascin (*TN*) and fibronectin (*FN*) 48 h after explantation. No staining with either anti-tenascin (A) or anti-fibronectin (B) is seen in neural crest cell cultures. Anti-tenascin stains matrix deposited by somite cells (C) and notochord cells (E). Similar fibrillar matrix staining is seen when antibodies to fibronectin are used to stain somite cells (D) and notochord cells (F).

is found predominantly in this region; when neural crest cells are spreading ventrally between the somites and endoderm, staining is found more ventrally as well.

In the avian and mammalian embryo, the distribution of tenascin also correlates with the anterior-to-posterior wave of neural crest cell migration: staining is found in the anterior part of the embryo where neural crest cells are actively migrating and is absent at more posterior axial levels where neural crest cells still reside within the neural tube. In the quail embryo, tenascin is abundant in the neural crest pathways in the intersomitic spaces, between neural tube and somites, and between ectoderm and somites. This is largely in agreement with the distribution of cytotactin reported by Crossin *et al.* (1986) in the chick. In addition, in the dorsal halves of the somites intense tenascin staining codistributes with

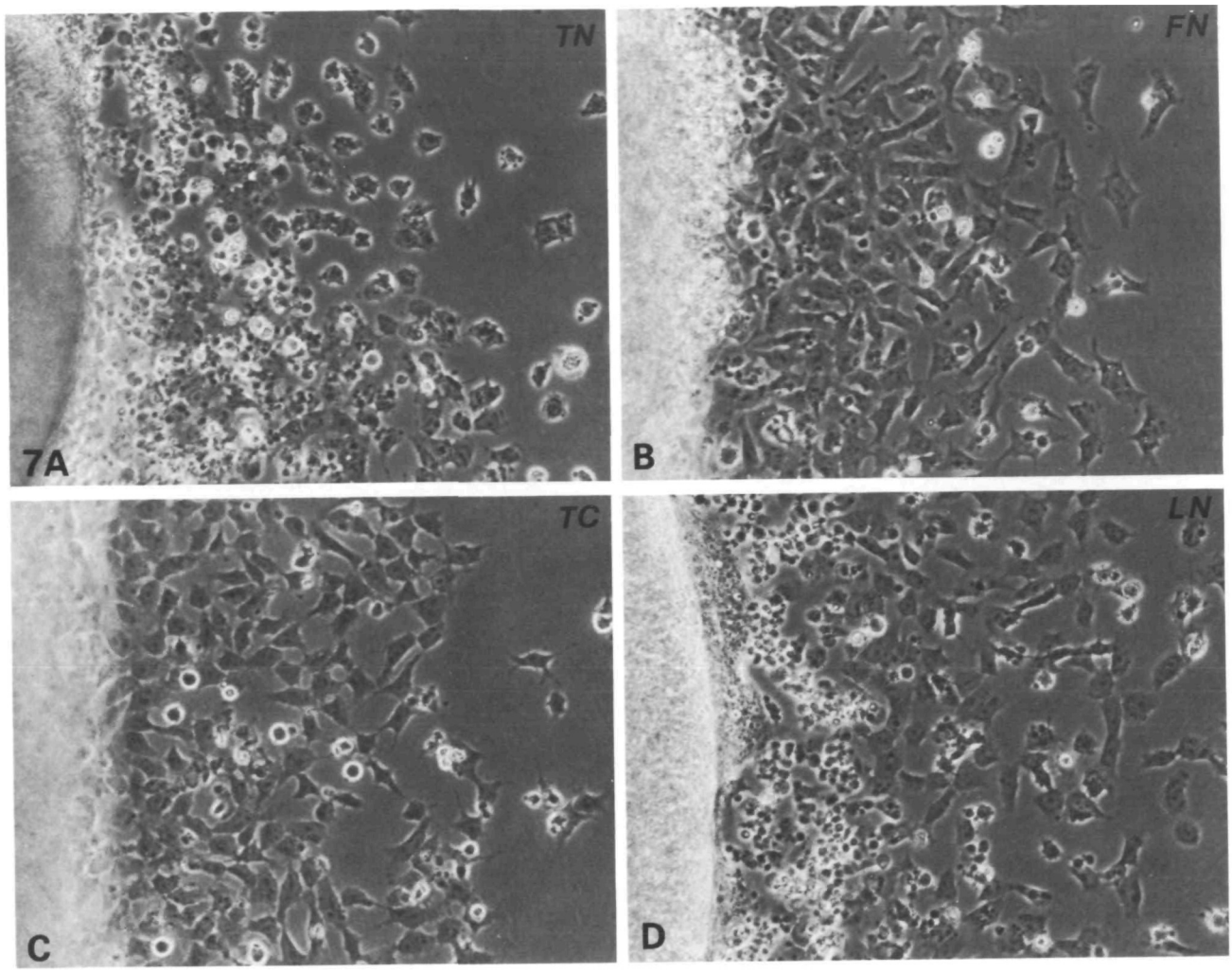

Fig. 7. 24 h-old primary cultures of quail neural crest cells on tissue culture plastic uncoated (TC) or coated with tenascin (TN), fibronectin (FN) or laminin (LN). All cultures were grown in defined medium without serum. (A) Neural crest cells on tenascin-coated plastic spread from the neural tube (at the left). The cells are rounded, and blebs are commonly seen. (B) Neural crest cells cultured on fibronectin-coated substrata are typically flattened, with large lamellipodia. (C) Neural crest cells cultured on uncoated tissue culture plastic are not as flattened as those on fibronectin, but they appear to be more strongly attached to the substratum than cells on tenascin. (D) Neural crest cells cultured on laminin-coated substrata have a saucer-like morphology, distinct from the morphologies of cells cultured on the other substrata.

HNK-1 staining. Thus, where neural crest cells selectively enter the anterior half of the somite, as previously described (Bronner-Fraser, 1986*a*; Teillet *et al.* 1987; Loring & Erickson, 1987), tenascin is selectively distributed in the anterior half. More posteriorly, tenascin staining is present in the centre of the somite, but completely surrounded by the epithelium of the young somite. Thus at this level, where neural crest cells are still uniformly aligned along the neural tube, or just beginning to move between somites, they do not come in contact with intrasomitic tenascin. In the rat, where tenascin staining in the dorsal part of the embryo is more limited than in the quail, it is also present in the intersomitic furrows and selectively within the anterior half of the somite.

In each class of vertebrate studied, the distribution of fibronectin is more widespread than that of tenascin: although present along the pathways of neural crest cell migration, fibronectin is also present in regions where neural crest cells are not found. For example, fibronectin is found laterally beneath the ectoderm of *X. laevis*, whereas the neural crest cells are found in the ventral pathway; in the quail and rat fibronectin is found homogeneously throughout the somite, whereas the neural crest cells are found only in the anterior half. The distribution of fibronectin reported here in the quail and rat is in agreement with previous studies (Newgreen & Thiery, 1980; Duband

& Thiery, 1982; Thiery *et al.* 1982; Krotoski *et al.* 1986).

One possible explanation for the temporal and spatial correlation between the distribution of tenascin and the neural crest is that the neural crest cells themselves are making the tenascin. Our *in vitro* results, however, suggest that cells lining the neural crest pathways and not the neural crest cells make tenascin *in situ*: quail neural crest cells do not organize a tenascin-rich matrix around themselves, whereas somite and notochord cells do. Tenascin is present within somites before neural crest migration but not yet in a differential distribution with respect to anterior and posterior halves. The predominance of tenascin in the anterior half is first seen at the same time as HNK-1 staining is visible within the anterior half. It cannot be excluded, therefore, that neural crest cells induce the production of tenascin in the anterior half of each somite. In this case, tenascin would probably not be responsible for directing migration within the somite but could still influence the rate of migration. On the other hand, the movement of neural crest cells into the somite does not occur until a certain phase of somite maturation, that is, with its dissociation into dermamyotome and sclerotome (Teillet *et al.* 1987). Thus it seems likely that the heterogeneity of tenascin's distribution within the somite is due to intrinsic properties of the somite and results from the stage of somite differentiation with which it coincides. In this case, tenascin could be the factor determining entry of neural crest cells into the anterior but not the posterior half of the somite.

Although the presence of tenascin is clearly correlated with the pathways of neural crest cell migration, we still must speculate as to whether, in fact, tenascin influences migration. Tenascin alone is not a good substratum for neural crest cell adhesion, as was shown by culturing these cells on tenascin-coated substrata. *In vivo* studies have shown that fibronectin is necessary for neural crest cell migration (Boucaut *et al.* 1984; Bronner-Fraser, 1985, 1986*b*), but the distribution of fibronectin is too widespread for fibronectin alone to be able to direct the pathways of neural crest cell translocation. Perhaps tenascin directs neural crest cell migration by means of an interaction with fibronectin, either by making a fibronectin-rich matrix less adhesive for the neural crest, or by loosening the grip of fibronectin that is glueing tissues (e.g. the anterolateral sclerotome) together, making it easier for the neural crest to move through the tissue. In support of these speculations are the observations that tenascin binds to cellular fibronectin (Chiquet-Ehrismann *et al.* 1986), and the rate of neural crest cell migration through three-dimensional collagen matrices *in vitro* decreases with increasing concentrations of collagen (Tucker & Erickson, 1984). Future *in vitro* studies including combinations of matrix materials and *in vivo* studies similar to those done to determine the role of fibronectin are needed.

In conclusion, the distribution of tenascin in the embryos of three classes of vertebrates is much better correlated with pathways of neural crest cell migration than other ECM molecules that have been implicated in neural crest morphogenesis. Neural crest cells themselves do not construct anti-tenascin-stained matrices *in vitro*, and tenascin alone is not an adhesive substratum for neural crest cells. We propose that tenascin plays an important role in determining the pathways of neural crest cell migration, perhaps by altering the interactions of these cells with fibronectin, or by generating pathways of least resistance through fibronectin-rich matrices.

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