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Distribution of fibronectin, laminin and entactin in the environment of migrating neural crest cells in early mouse embryos

J. STERNBERG AND S. J. KIMBER

Experimental Embryology and Teratology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK

SUMMARY

The distribution of the extracellular matrix molecules fibronectin, laminin and entactin was studied in frozen sections of 9½-day mouse embryos in order to relate their presence to neural crest cell migration. It was found that all three components were present in basement membranes, laminin and entactin being mainly restricted to these. Fibronectin was present at high levels in basement membranes and extracellular spaces throughout the embryo, including the regions of neural crest cell migration. Fibronectin is known to affect migration in a variety of cell systems, so its presence in the embryo at the time of migration may indicate that it is influencing cell movement. This influence is likely to be via the cell surface through interactions with other matrix components such as glycosaminoglycans, and possibly entactin and laminin.

INTRODUCTION

Neural crest cells (NCC) form at the dorsal ridge of the neural folds prior to fusion. After fusion of the folds, the cells detach from the neural tube and migrate away to different areas of the embryo where they differentiate into a variety of tissues including melanocytes, bones of the face and nervous system. For details of neural crest derivatives see reviews by Le Douarin (1982) and Weston (1983). Because neural crest cells migrate unusually large distances and differentiate so diversely they provide a unique system for the study of the mechanisms of these developmental events.

The migration pathways of NCCs in avian embryos are now well defined. Much of the evidence was derived from following radioactively labelled crest cells using autoradiography (Johnston, 1966; Noden, 1975) and from the injection of quail NCCs, identifiable by the large amounts of heterochromatin associated with the nucleoli, into chick embryos (Bronner & Cohen, 1979; Bronner-Fraser & Cohen, 1980). Information from scanning and transmission electron microscopy has contributed to our knowledge of the routes taken by these cells (Bancroft & Bellairs, 1976; Tosney, 1978). Although the migration of mammalian neural crest cells is still in the preliminary stages of investigation, recent observations made with the

Key words: neural crest cells, extracellular matrix, cell migration, fibronectin, laminin, entactin, mouse embryo.

scanning electron microscope indicate that the early phase of migration occurs in a similar manner to that in avian embryos (Erickson & Weston, 1983).

It is thought that one of the initial events after neural fold fusion is the breakdown of the basement membrane of the neural tube at its most dorsal aspect (Erickson & Weston, 1983) thus removing a physical barrier to potentially migrating cells. Coincidentally, in avian embryos the ectoderm overlying this area moves away from it and a comparatively large extracellular space appears (Pratt, Larsen & Johnston, 1975). Initially mouse neural crest cells in the trunk emerge through the basement membrane in the dorsal region of the neural tube and migrate laterally over the surface of the tube. Subsequently some cells pass ventrally in the space between neural tube and somites.

Various components of the extracellular matrix have been implicated as influencing cell movement in a number of cell systems (Yamada, 1982; Rollins, Cathcart & Culp, 1982; Hay, 1981). In the chick embryo increased hyaluronic acid in the enlarged extracellular spaces is associated with the region of initial migration of neural crest cells dorsal and lateral to the neural tube (Pratt *et al.* 1975). Glycosaminoglycans (GAGS), mainly hyaluronic acid, are also found in areas where crest cells are migrating in the quail (Pintar, 1978) and the mouse (Derby, 1978). The role of the glycoprotein fibronectin is well established in cell adhesion and motility (Carter, Rauvala & Hakomori, 1981; Nicol & Garrod, 1982; Yamada, 1982; Turner *et al.* 1983). Fibronectin has been demonstrated in the neural crest migration pathway, in all basement membranes and in the mesenchyme in avian embryos (Newgreen & Thiery, 1980; Mayer, Hay & Hynes, 1981; Duband & Thiery, 1982; Thiery, Duband & Delouvee, 1982) and may provide a substrate for the cells to migrate over. Studies *in vitro* confirm the importance of interactions between neural crest cells and fibronectin in avian neural crest cell movement (Maxwell, 1976; Newgreen & Gibbons, 1982; Newgreen *et al.* 1982). One of the earliest extracellular matrix molecules which can be identified in mouse embryogenesis at the 8- to 16-cell stage is laminin (Leivo, Vaheri, Timpl & Wartiovaara, 1980; Wu, Wan, Chung & Damjanov, 1983), a glycoprotein found in basement membranes. *In vitro* laminin has been shown to promote the attachment and spreading of various epithelial cells (Johansson, Kjellen, Höök & Timpl, 1981; Carlsson, Engvall, Freeman & Ruoslahti, 1981; Couchman, Höök, Rees & Timpl, 1983) and it may play a role in morphogenesis (Ekblom *et al.* 1980). In the chick embryo laminin is also present (Duband & Thiery, 1982) but does not support avian neural crest cell migration *in vitro* as effectively as fibronectin (Rovasio *et al.* 1983). The distribution of entactin in the preimplantation and early postimplantation embryo is similar to laminin (Wu *et al.* 1983) and this molecule may also be involved in cell–matrix interactions (Carlin, Yaffe, Bender & Chung, 1981).

The purpose of this study was to describe the distribution of the extracellular matrix components fibronectin, laminin and entactin in the mouse embryo during initial stages of neural crest cell migration dorsally and laterally around the neural tube. The 9½-day embryo was chosen for this study because at this age neural crest

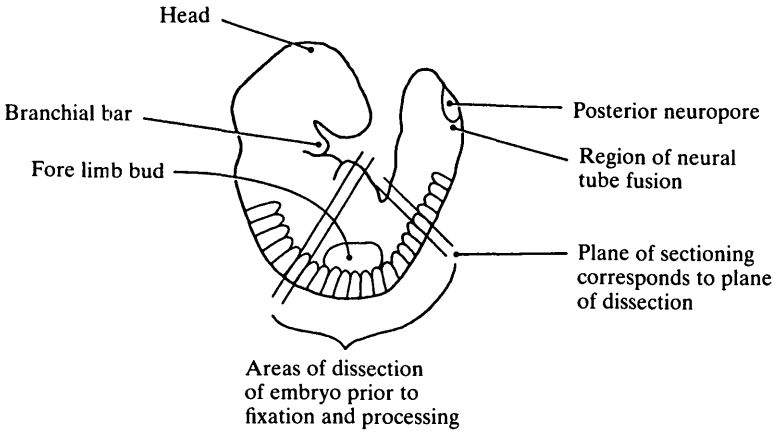


Fig. 1. Diagram of 9½-day (22-somite) embryo showing regions of dissection.

cells at various stages of migration are present along the length of the trunk. The distribution of these extracellular matrix molecules was investigated using a double layer antibody technique with a fluorescent second antibody. The pattern of fluorescence was related to the position of early migrating neural crest cells in stained sections taken at different levels along the length of the trunk and in embryos prepared for scanning electron microscopy. Neural crest cells could be positively identified both in sections and in scanning electron micrographs.

MATERIALS AND METHODS

Preparation of sections for immunofluorescence

(a) *Isolation of embryos*

Random-bred MFI female mice were naturally mated with F_1 C57BL/6×CBA males. Fertilization was assumed to be around the midpoint of the light–dark cycle at 1 a.m. The presence of a vaginal plug the following morning indicated pregnancy, and the day of the vaginal plug was designated day 1 of pregnancy. On day 10 (actual embryonic age approx. 9½ days) the mice were sacrificed between 10 and 11 a.m. and the decidua removed from the uteri and placed in phosphate-buffered saline (PBS) pH 7·4 at 37°C (Dulbecco 'A', Oxoid Ltd, U.K.). The embryos, which had 20 to 25 somite pairs, were dissected free of decidua, yolk sac and amnion and the somite number of individual embryos noted. Each embryo was dissected into three or four pieces, using watchmaker's forceps to cut perpendicularly to the anteroposterior axis at specific somitic levels. For example, a particular embryo was dissected at somites 6 and 16 to give anterior, central and posterior portions (see Fig. 1).

(b) *Preparation of embryos for immunocytochemistry*

The embryonic pieces were fixed in 4 % paraformaldehyde (EMScope) in PBS for 1 h at room temperature. The fixed tissue was incubated in 5 % sucrose in PBS for 1 h followed by 15 % sucrose in PBS for 1–2 h. 7 % gelatin (Swine skin Type I, 300 bloom, Sigma) in 15 % sucrose in PBS was warmed, dissolved, and maintained in a liquid state at 37°C until required. The embryos were impregnated in the gelatin for 1 h at 37°C and then transferred to fresh gelatin in plastic moulds and left to solidify.

The gelatin was cut into blocks, each containing one piece of embryonic material which was oriented so that the long axis of the embryo was perpendicular to the cutting face of the block.

Each block was mounted on a cork base with O.C.T. (Tissue-Tek) and quick frozen by plunging into liquid nitrogen for approx. 1 min. 8 μm transverse sections of the neural tube were cut at −20 to −30°C on a Reichert-Jung Cryocut E cryostat and mounted on microscope slides previously coated with 1 % gelatin.

(c) *Antibody staining procedure*

Antibodies against extracellular matrix components were gifts kindly given to us by R. O. Hynes, Massachusetts Institute of Technology (rabbit anti-hamster fibronectin); D. Stott, St George's Hospital Medical School, London (rabbit anti-mouse entactin). Rabbit anti-mouse laminin was acquired from Bethesda Research, Bethesda, U.S.A. Serial dilutions of antibody were tested to determine the most suitable concentration. For all three antibodies this was found to be a dilution of 1 in 50 in 1 % BSA in PBS. For the non-immunoreactive control normal rabbit serum was used at the same concentration. The optimum concentration of second layer antibody, goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) (Miles-Yeda, Israel), was also found to be 1 in 50 in 1 % BSA in PBS. Sections were initially washed for 3×10 min in PBS with 1 mM-$CaCl_2$, followed by incubation for 10 min in a humidified petri dish with 50 μl of 2 % glycine in PBS to reduce non-specific binding of antibody to free aldehyde groups. After further washing (3×10 min) in PBS, 50 μl of 5 % BSA (fraction V) in PBS, to block non-specific protein binding sites, was placed on the sections for 10 min. The blocking buffer was removed and replaced by 50 μl of appropriate 1st antibody, for 30 min at room temperature. The slides were then washed (3×15 min) in PBS and then in 5 % BSA in PBS for 10 min before addition of 50 μl aliquots of the FITC-conjugated 2nd antibody. After incubation for 30 min at room temperature the sections were washed finally in PBS (3×15 min), dipped in distilled water and mounted with 'Univert' mountant (Gurr) under No. 1 glass coverslips. The sections were viewed with a Leitz Ortholux microscope, using excitation filter BP 450-490 (1/2) and suppression filter LP515, and photographed using the Leitz Vario-Orthomat on Ilford HP5 film.

Preparation of embryos for histochemistry

Intact decidua were fixed in 4 % paraformaldehyde in PBS at room temperature for 4 h. The paraformaldehyde solution contained 0·25 % polyvinylpyrrolidone and 0·5 % cetylpyridinium chloride to retain GAGs. After fixation, decidua were washed twice in buffer and placed in 70 % ethanol. The material was then dehydrated and subjected to wax infiltration in an automatic histokinette (Hendry) and vacuum embedded with paraffin wax. The decidua were placed individually in plastic moulds filled with liquid paraffin wax and accurately orientated. The solidified blocks were mounted to give transverse sections of the neural tube and sectioned at 8 μm on a rotary microtome.

Alcian blue 8GX (Sigma) was used to stain GAGs at two pHs. The stain was used either at a concentration of 1 % in 0·1 N-hydrochloric acid (pH 1·0), or 1 % in 3 % acetic acid (pH 2·5). Two consecutive sections were taken at 32 μm intervals and placed on separate slides for staining at the two pHs. Staining of hydrated sections was for 10 min at room temperature. The staining reaction depends on precipitation of GAGs by the binding of the cationic dye to multiple negatively charged sites (Scott & Dorling, 1965). At pH 1·0 phosphate and carboxyl groups are undissociated, and only sulphate groups react while at the higher pH, both sulphate and carboxyl groups stain (Ashurst & Costin, 1971). After Alcian blue staining the sections were dehydrated through graded ethanols, cleared in xylene and mounted in D.P.X. (R. A. Lamb) under No. I coverslips, viewed with the same microscope as for fluorescence, and photographed on Ilford Pan F film.

Scanning electron microscopy

9½-day embryos were dissected out of decidua in PBS or Hank's balanced salt solution. Embryos were prefixed in 0·25 % glutaraldehyde in 0·1 M-sodium cacodylate buffer for 30 min at room temperature. They were then washed in 0·1 M-sodium cacodylate, and the ectoderm overlying the neural tube was removed using sharpened tungsten needles. In some cases, embryos were transversely sectioned at different axial levels of the neural tube. The embryos were then fixed in 6 % glutaraldehyde in 0·1 M-sodium cacodylate buffer with 2 mM-$CaCl_2$ (pH 7·4)

for 2 h at room temperature, washed four times in the buffer and left overnight at 4 °C. The material was treated with 1 % osmium tetroxide in 0·1 M-sodium cacodylate for 1 h at room temperature, washed well with buffer, then dehydrated through an ethanol series. After critical-point drying, embryos were mounted and orientated on stubs with conducting paint, and coated with gold/palladium using a Polaron E5000 sputter coater with a high resolution head. Specimens were viewed in an ISI DS130 scanning electron microscope at 10 kV.

RESULTS

Identification of neural crest cells

SEM

It was possible to identify the axial level of the neural tube being viewed by counting the adjacent somites, which were clearly visible. NCCs on the dorsal surface of the neural tube were clearly identifiable by their position above the basement membrane of the neuroepithelium and their morphology, which was characteristic of migrating cells. The cells could only be identified during the early stages of migration, between the stage of dissociation from the neuroepithelium and the stage at which they began to mingle with the somitic mesenchyme. It is therefore possible to make a direct correlation between the stage of early migration of the cells and the somite (axial) level of the neural tube in embryos of given age.

In a typical 22-somite embryo, the dorsal neural tube in the postsegmented region shows little evidence of NCCs (Fig. 2A). Anterior to this, at somite 16 of a 22-somite embryo, cells can be seen on the surface of the neural tube (Fig. 2B). The cells have a migratory morphology, which can be seen at higher magnification (Fig. 2G). The cells appear to be spreading over the neural tube and each other, using cell processes (lamellipodia and filopodia) and many have an obvious leading and trailing edge. Also seen in the region of early migration (Fig. 2D) is a fibrous matrix on the surface of the neural tube, with which cells appear to be in association. At progressively anterior regions of the neural tube of a 9½-day embryo, NCCs can be seen migrating away from the dorsal midline of the tube towards the somites on either side. The dorsal surface of the neural tube is gradually revealed as the NCCs vacate it. At somites 5–8 of a 23-somite embryo, most of the NCCs have migrated off the neural tube and are grouped in the somite–neural tube space underlying the ectoderm. In the region of the anterior-most somites of a 22-somite embryo, the neural tube surface is devoid of cells and has a smooth, continuous surface (Fig. 2C). If cross sections of the embryo are compared at different levels, it can be seen that in the early stages of migration the NCCs are layered along the dorsal midline and there is no clear delineation from the neuroepithelial cells. At anterior regions, the neuroepithelium is compact, with an apparently continuous basement membrane, with no cells visible on top of it (Fig. 2E,F).

Histological sections

NCCs could be identified in the stained sections by their appearance and position. In the region of early migration (at the level of the last somites) the

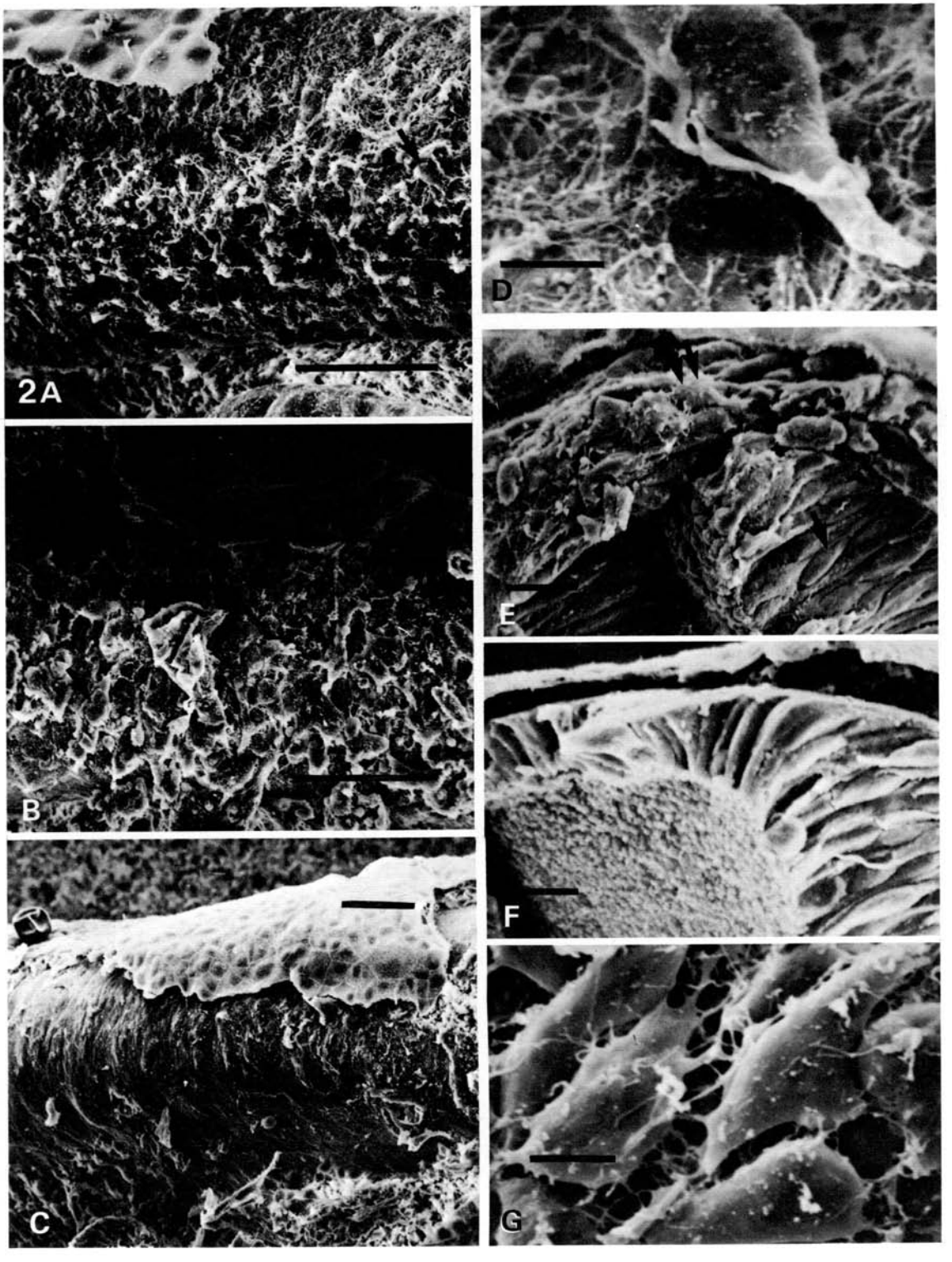
NCCs were seen in a space between the dorsal surface of the neural tube and the ectoderm, extending laterally to the neural tube/somite cleft (Fig. 3A, arrows). Somite and surface ectoderm are closely apposed at this level whereas further anterior (somites 16–18) a space is apparent between the two structures. In contrast the space overlying the neural tube is diminished at the level of somites 16–18. At approximately somite 12, cells are seen accumulating between the lateral neural tube and the somite, while few cells remain dorsally (Fig. 3C). At the most anterior level of the trunk, there is no space between neuroepithelial and ectodermal basement membranes, and NCCs have migrated laterally and ventrally to become indistinguishable from surrounding mesenchyme (Fig. 3D).

Observations obtained using SEM and light microscopy correlate well with regard to the position of NCCs in relation to somite level. At a given somite level of a 9½-day embryo the stage of initial migration can therefore be accurately predicted. Because of the lack of a specific marker for mammalian NCCs their subsequent migration into the ventral areas of the mouse embryo cannot be accurately followed, because the cells mingle with mesenchyme of similar morphology.

Glycosaminoglycans

Sections stained with Alcian blue revealed the presence of GAGs in the regions of NCC migration. Consecutive sections of 9½-day embryos were stained at two pHs, to demonstrate the presence of sulphated and unsulphated GAGs. Both types of GAGs were present in association with NCCs and their early migration pathways. The heaviest staining was seen in basement membrane structures

Fig. 2. Scanning electron micrographs of 9½-day embryos after ectodermal removal. (A) Dorsal view of neural tube of a 22-somite embryo in the postsegmented region. The embryonic axis runs anterior to posterior (right to left) across the micrograph. A few cell outlines (arrow) are visible on the surface of the neural tube, but it is mostly at a stage prior to NCC migration. A piece of remaining ectoderm can be seen in the top left. ×435. Bar equals 50 μm. (B) Dorsal view of neural tube in a 22-somite embryo, at somites 16–18, anterior to posterior (right to left). Cells are clearly visible on the surface, apparently migrating over each other and the neural tube (arrows). ×437. Bar equals 50 μm. (C) Anterior region of neural tube (anterior to posterior is right to left). The neural tube surface is virtually free of neural crest cells. It is covered by a smooth, continuous basement membrane. Undissected ectoderm covers part of the dorsal surface. ×220. Bar equals 50 μm. (D) A single cell is seen migrating over the neural tube in the last somite region. It is in association with an extensive fibrous matrix covering the dorsal neural tube. ×3300. Bar equals 5 μm. (E) Part of a cross section of a neural tube of a 9½-day embryo. The neural tube lies in the lower half of the micrograph, and its closely packed cells are seen (arrows). Dorsally neural crest cells lie on top of the tube and are hard to distinguish from neural tube cells. This is an early stage in migration, with the cells still multilayered and tightly packed (double arrows). ×1070. Bar equals 10 μm. (F) A cross section of neural tube at a more anterior region than Fig. 6E. No neural crest cells can be seen lying between dorsal neural tube and ectoderm. The dorsal cells of the neural tube are tightly apposed and highly organized. ×1060. Bar equals 10 μm. (G) Neural crest cells actively migrating show many cells' processes (lamellipodia and fine filopodia) extending between each other. Cells are apparently continually in contact and migrating over each other. ×2500. Bar equals 5 μm.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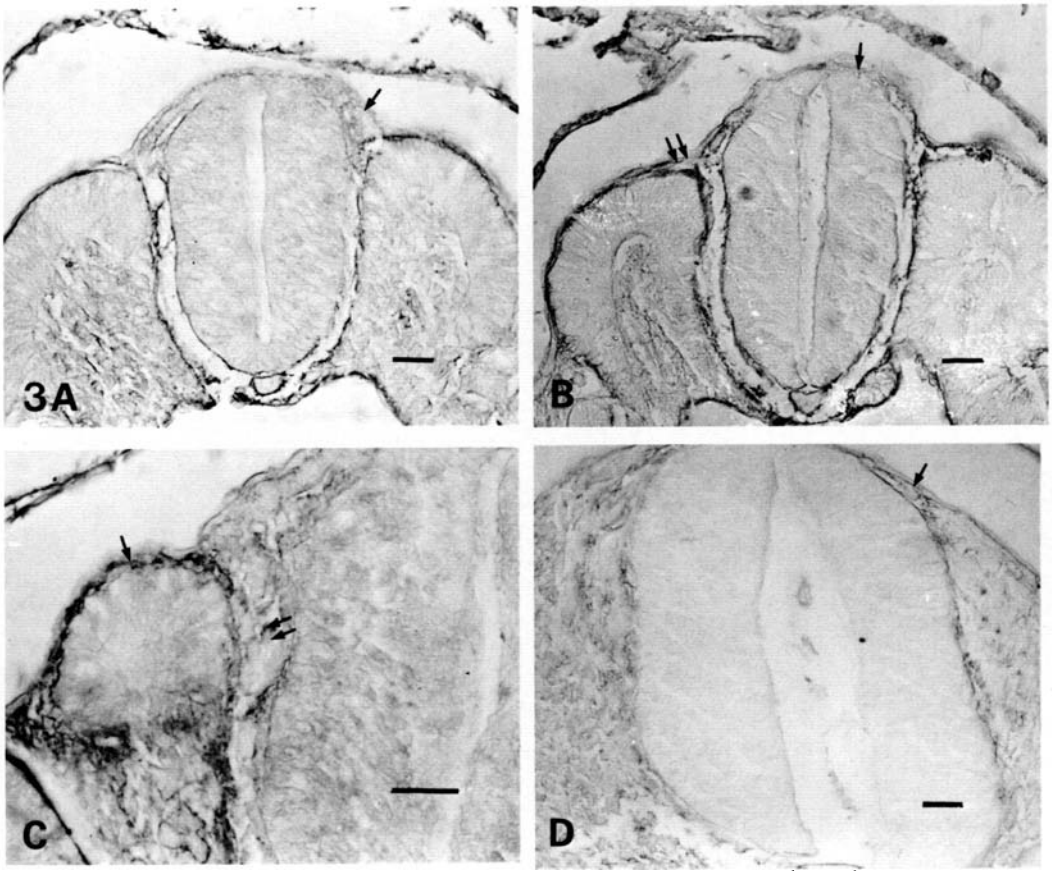

Fig. 3. 8 μm sections of 9½-day embryos, embedded in paraffin and stained with Alcian blue at pH 1·0 or 2·5. (A) Section through one of the last somites of a 9½-day embryo, in which the neural crest cells can be seen between dorsal neural tube and overlying ectoderm (arrow). Staining at pH 2·5. ×315. Bar equals 20 μm. (B) One or two somites anterior to section A, the space between neural tube and ectoderm is obliterated (arrow), but a space is beginning to form between somites and ectoderm (double arrow). Staining at pH 1·0. ×315. Bar equals 20 μm. (C) A section approximately 5 somites anterior to B, shows the glycosaminoglycan-rich area above the somite (arrow), and cells presumably of neural crest origin between somite and neural tube (double arrow). Staining at pH 2·5. ×440. Bar equals 20 μm. (D) At this anterior level of trunk neural tube the basement membranes of neural tube and ectoderm are refused (arrow) and neural crest cells have migrated away to become indistinguishable from surrounding mesenchyme. Staining at pH 1·0. ×250. Bar equals 20 μm.

(Fig. 3A–D) but some intercellular stain was seen, particularly associated with the NCCs. The lowest levels of staining were within the neural tube and within the developing somites. There was no obvious difference in distribution of stain at the two pHs, suggesting colocalization of sulphated and unsulphated GAGs.

Fibronectin

As shown by the fluorescent staining FN (fibronectin) was present at all axial levels along the neural tube. At the level of the posterior neuropore (not shown)

of a 23-somite embryo fluorescence was visible around the basement membrane of the neural tube and that of the ectoderm, forming a fork between the two basement membranes. Fibronectin was also widely distributed in the extracellular matrix of the surrounding mesenchyme. No anti-fibronectin staining was present between the cells of the neural tube or along the apposed surfaces of the closing neural folds. Anterior to the posterior neuropore, but still in the region posterior to

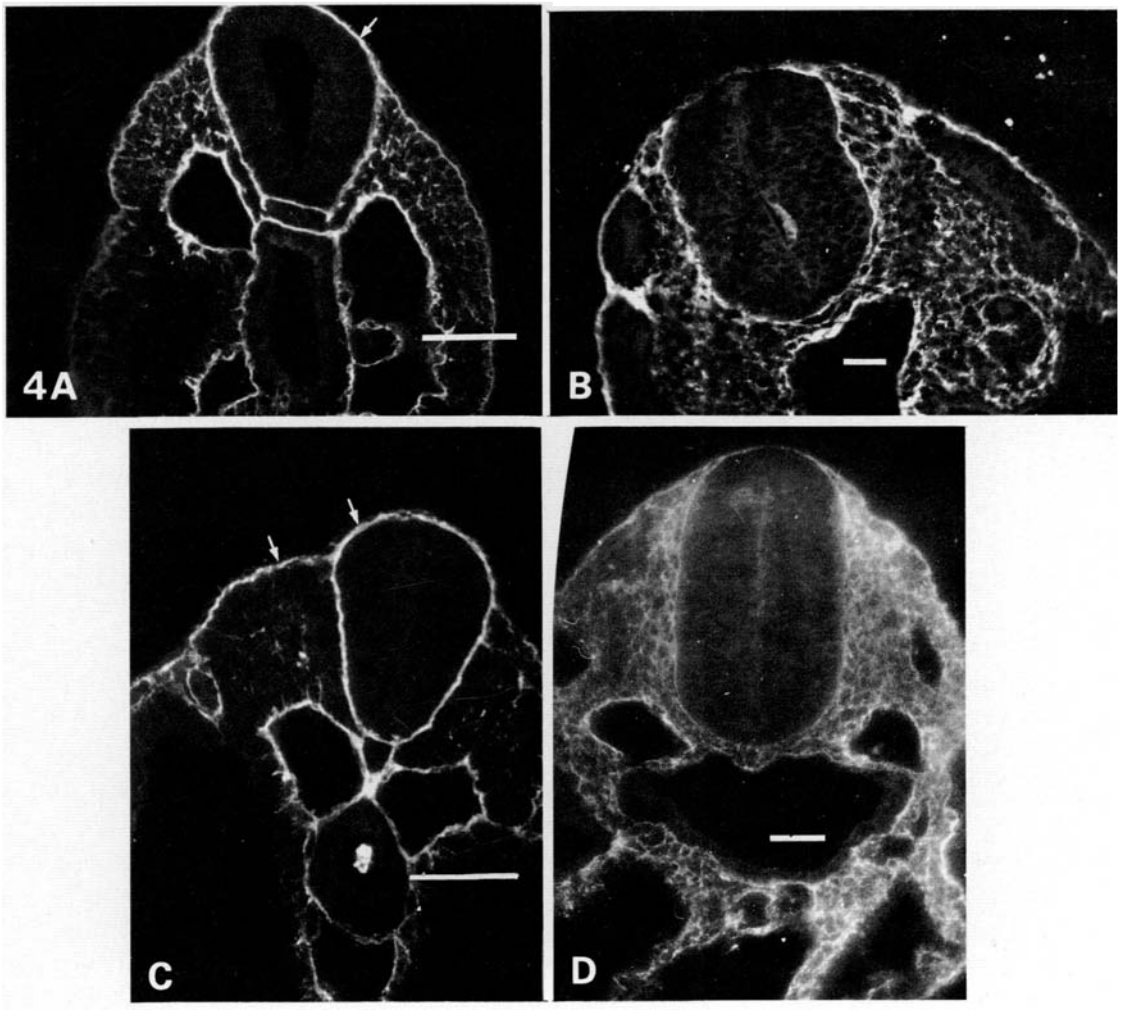

Fig. 4. 8 μm sections of 9½-day embryos stained by indirect immunofluorescence for fibronectin. (A) Postsomite area of the neural tube, showing anti-fibronectin staining of basement membranes (arrow) and extracellular regions. ×120. Bar equals 100 μm. (B) Last somite region of a 21-somite embryo. Basement membranes are still strongly stained (arrows). ×140. Bar equals 100 μm. (C) 14–15 somite region of a 23-somite embryo. Staining of the basement membranes is reduced compared to more posterior areas. ×100. Bar equals 50 μm. (D) Anterior region of trunk neural tube (approx. somite 2). Staining no longer restricted to basement membranes. ×130. Bar equals 50 μm.

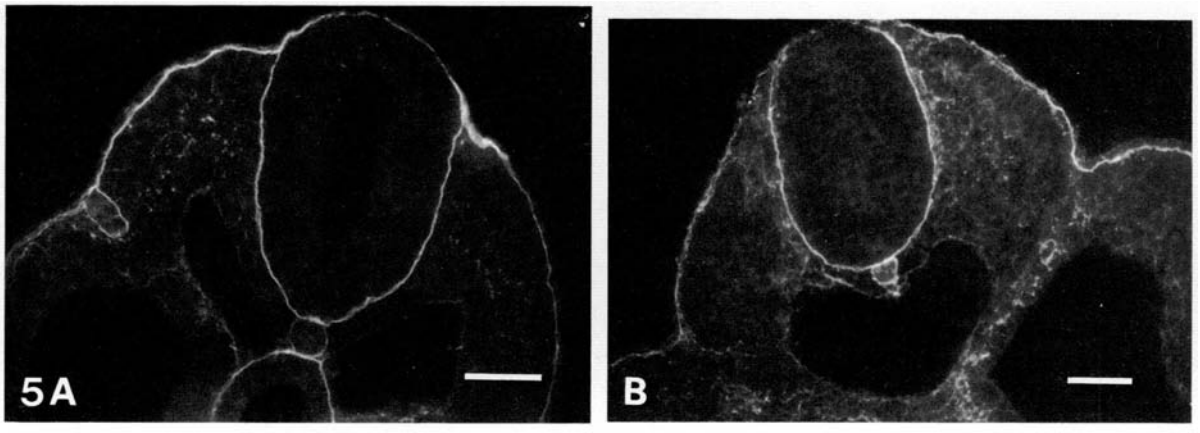

Fig. 5. 8 μm sections of 9½-day embryos stained by indirect immunofluorescence for laminin. (A) Last somite region of a 21-somite embryo, showing intense staining for laminin in basement membranes. ×200. Bar equals 50 μm. (B) Somite 6 of a 21-somite embryo. Some laminin is stained for in extracellular areas as well as basement membranes. ×150. Bar equals 50 μm.

the last somites, the neural folds have fused and there was a continuous basement membrane overlying the neural tube which stained with anti-fibronectin. The basement membrane of the surface ectoderm also contained fibronectin, as did the basement membranes of all the structures ventral to the neural tube, including the notochord (Fig. 4A, arrows). The fluorescence produced by anti-fibronectin staining between the cells lying lateroventral to the neural tube (Fig. 4A) was very intense, indicating high levels of fibronectin in this region.

At the level of the last somites of a 21-somite embryo the neural crest cells were emerging from the neural tube and migrating across its dorsal surface. In this region (compare with Fig. 2D), there was intense fluorescence after anti-fibronectin staining, particularly in the basement membrane surrounding the neural tube (arrow, Fig. 4B) and those of ventral structures such as gut, dorsal aortae and notochord. The extracellular region above the neural tube, which contained neural crest cells at this stage was also rich in fibronectin.

Approximately four somites anterior to the level shown in Fig. 4B, at somite 14–15 of a 23-somite embryo, fluorescent staining was still seen. Fibronectin fibrils in the extracellular spaces surrounding the mesenchymal tissue were more in evidence (Fig. 2C, arrow) but basement membranes were less strongly delineated and that of the notochord was barely visible (Fig. 4C). Within the neural tube itself and the dermomyotome no fibronectin was present. However, extracellular fibrils of fibronectin were visible in the region of the sclerotome where NCCs were present by extrapolation from paraffin sections as well as between many other cells in this region of the embryo.

At anterior levels of the neural tube, such as somites 2–5, FN appeared as a fairly evenly distributed network throughout the matrix and most basement membranes, but the basement membrane of the notochord no longer stained at all.

There was an apparent increase in the intensity of the overall fluorescence at more ventral levels, particularly around the dorsal aortae (Fig. 4D). Anti-fibronectin stained the basement membranes of neural tube and ectoderm as a continuous layer at this level.

Laminin

Laminin was found throughout the embryo by immunofluorescence. Its distribution resembled more closely that of entactin than that of fibronectin. Intense staining for laminin was seen in basement membranes, particularly in posterior regions of the embryo (Fig. 5A), but basement membrane staining remained at high levels at all trunk axial levels. The staining pattern at somite 6 (approx.) is illustrated in Fig. 5B, where it is seen to be strongest in the basement membranes of neural tube and surface ectoderm, with some extracellular staining. As with fibronectin and entactin, epithelial structures such as the neural tube showed no staining at all between the cells.

Entactin

Entactin, like fibronectin and laminin, was present throughout the embryo in all regions examined. Particularly in the posterior of the embryo entactin-associated fluorescence was exclusively in basement membranes, i.e. those of ectoderm, neural tube, notochord, aortae etc. (Fig. 6A). At more anterior levels entactin

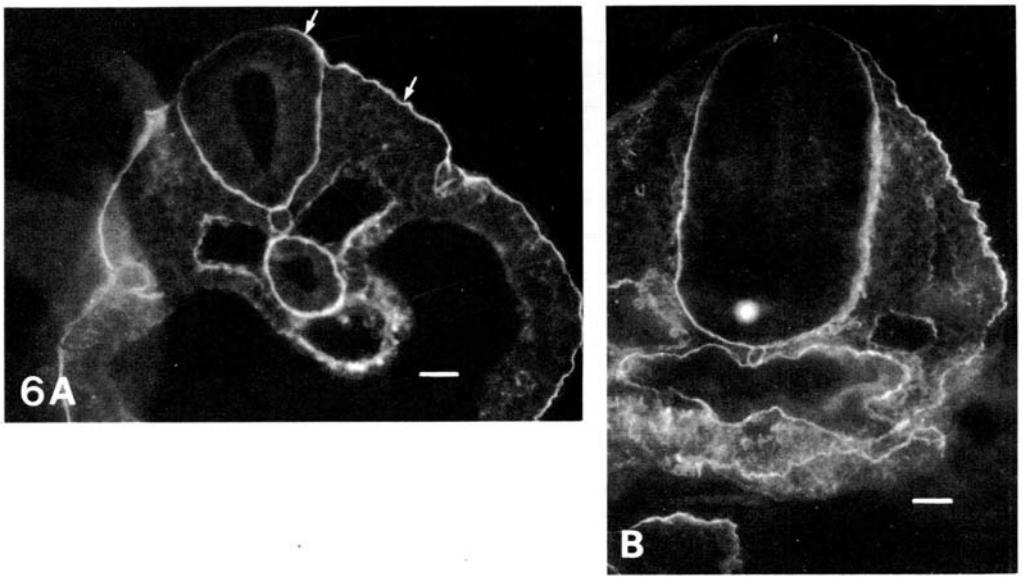

Fig. 6. 8 μm sections of 9½-day embryos stained by direct immunofluorescence for entactin. (A) Posterior (postsomite) level of neural tube. Entactin is mainly restricted to basement membranes (arrows). ×140. Bar equals 100 μm. (B) Anterior (approx. somite 2) level of neural tube. There is still entactin in basement membranes, but it is also more apparent in extracellular spaces. ×170. Bar equals 50 μm.

was also mainly associated with basement membranes (Fig. 6B) but the fluorescence was visible between cells lateral to the neural tube as well. Like fibronectin no staining for entactin was seen within neural tube or dermamyotome. Generally, entactin distribution was similar to that of fibronectin, but less appeared outside basement membranes.

DISCUSSION

SEM together with light microscopy was used to identify NCCs in the initial stages of their migration from the neural tube. Only the early migration pathway has been examined here; later, more ventral pathways in the mouse embryo remain to be elucidated. NCCs were identified by their position and migratory morphology. The anteroposterior gradient of maturity of the pattern of migration correlated well with that previously described in the mouse (Erickson & Weston, 1983) and with the general pattern observed in other species (Tosney, 1978; Spieth & Keller, 1984). The cells were associated with matrix fibrils which may correspond to the glycoproteins identified with immunofluorescence, and also with the neural tube basement membrane which was heavily stained with fibronectin, laminin and entactin.

We have shown in this study that laminin, entactin and fibronectin are present in basement membranes of the neural tube and other structures along the early NCC migratory pathway in the mouse embryo. Sulphated and non-sulphated GAGs were also seen, especially in basement membrane. FN and low levels of entactin are also closely associated with the cells in the spaces through which they migrate. Although FN is present throughout the embryo and its distribution cannot be specifically correlated with the position of migrating NCCs, it was found to be abundant in regions where early migrating crest cells would be present according to our SEM and histological observations.

Attempts to correlate NCC migration with FN in avian embryos have previously been made. FN is certainly present in a similar distribution to that demonstrated here (Newgreen & Thiery, 1980; Mayer *et al.* 1981) and in the cephalic region fibrils are associated with the leading NCCs (Duband & Thiery, 1982; Meier & Drake, 1984). *In vitro*, FN-coated substrates promote avian NCC migration more effectively than other matrix components (Maxwell, 1976; Greenberg, Seppä, Seppä & Hewitt, 1981; Newgreen & Gibbons, 1982; Newgreen *et al.* 1982; Rovasio *et al.* 1983). Rovasio *et al.* (1983) have shown that this effect is very specific. Although some NCCs appear to synthesize FN in culture (Newgreen & Thiery, 1980), most do not, and this characteristic may account for their response to exogenous FN, as is the case with transformed fibroblasts (Yamada, Kennedy, Kimata & Pratt, 1980*a*; Yamada, Olden & Hahn, 1980*b*). In the rat embryo, migration of cephalic NCCs *in vivo* is correlated with loss of FN staining in adjacent basement membranes (Morriss-Kay & Tuckett, personal communication).

Although the matrix components examined were not found solely in the region in which NCCs were migrating, the presence of FN, laminin and entactin along the

pathways indicates that they do have a role in this process. The close contact between mouse NCCs and basement membranes (Vermeij-Keers & Poelmann, 1980; Erickson & Weston, 1983) suggests extensive contact between cells and matrix.

In this study, the presence of sulphated and non-sulphated GAGs in NCC pathways is also reported. GAGs are particularly abundant in basement membranes adjacent to NCC migration pathways. Hyaluronic acid has been found in this region in avian (Pintar, 1978; Pratt *et al.* 1975; Bolender, Seliger & Markwald, 1980) and mouse embryos (Derby, 1978; Weston, Derby & Pintar, 1978; Weston, 1983). Termination of NCC migration can be correlated with decreased GAG (Weston, 1983) and the cells themselves appear to synthesize GAGs (Greenberg & Pratt, 1977; Pintar, 1978; Manasek & Cohen, 1977). The possible importance of the hydration properties of hyaluronic acid in causing expansion of extracellular space has been stressed (Toole, Jackson & Gross, 1972; Pratt *et al.* 1975; Toole, 1981; Weston, 1983; Le Douarin, 1984). GAGs or other matrix components may also have a role to play in altering the molecular conformation of FN to make it an effective substrate for cell migration (Colvin & Kradin, 1983).

NCCs are inherently invasive (Erickson, Tosney & Weston, 1980; Le Douarin, 1982, 1984) but also follow very specific pathways which are known in avian species. Possible explanations for the unidirectionality of movement include contact guidance, haptotaxis and chemotaxis (see Le Douarin, 1982, 1984; Weston, 1983), but these mechanisms are insufficient to explain the directional migration of NCCs. It is therefore highly likely that ECM components such as fibronectin, laminin, entactin and GAGs influence directional migration. Their effectiveness has been demonstrated in a number of mesenchymal cell systems (fibronectin: Yamada *et al.* 1980*a,b*; Carter *et al.* 1981; Hynes & Yamada, 1982; Turner *et al.* 1983; laminin: Johansson *et al.* 1981; Carlsson *et al.* 1981; Couchman *et al.* 1983; entactin: Carlin *et al.* 1981). Fibrillar fibronectin has been demonstrated to be effective in stimulating unidirectionality of avian NCC migration when cells are cultured at high density (Rovasio *et al.* 1983). The results from the present study show that there is no sudden increase in any of the glycoproteins or GAGs investigated at the time of NCC migration. Changing amounts of these ECM components are obviously not responsible for triggering initial migration, but we propose that once migration has begun, they play a supportive role in the process, besides having other functions in the embryo at this time. The different components probably interact to provide a suitable substrate with which the cells can make contact.

We hope in the future to be able to demonstrate the relationship between mouse NCCs and ECM components more precisely using immunocytochemistry visualized by SEM, and examining the behaviour of mouse NCCs in culture.

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