

Cell Adhesion and Migration in the Early Vertebrate Embryo: Location and Possible Role of the Putative Fibronectin Receptor Complex

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Abstract. Using a combined *in vivo* and *in vitro* approach, we have analyzed the immunofluorescent localization and function of a 140,000-mol-wt glycoprotein complex implicated in cell adhesion to fibronectin (FN), with particular emphasis on neural crest cell adhesion and migration. This putative fibronectin receptor complex (FN-receptor) was detectable in almost all tissues derived from each of the three primary germ layers. It was present in both mesenchymal and epithelial cells, and was particularly enriched at sites close to concentrations of FN, e.g., at the basal surfaces of epithelial cells. It was also present on neural crest cells.

The distribution and function of this putative receptor was then analyzed on individual cells *in vitro*. It was diffusely organized on highly locomotory neural crest cells and somitic fibroblasts. Both motile cell types also displayed relatively low numbers of focal contacts and microfilament bundles and limited

amounts of localized vinculin, α -actinin, and endogenous FN. In contrast, the FN-receptor in stationary embryonic cells, i.e., somitic cells after long-term culture or ectodermal cells, existed in characteristic linear patterns generally co-distributed with α -actinin and fibers of endogenous FN. Anti-FN-receptor antibodies inhibited the adhesion to FN of motile embryonic cells, but not of stationary fibroblasts. However, these same antibodies adsorbed to substrata readily mediated adhesion and spreading of cells, but were much less effective for cell migration.

Our results demonstrate a widespread occurrence *in vivo* of the putative FN-receptor, with high concentrations near FN. Embryonic cell migration was associated with a diffuse organization of this putative receptor on the cell surface in presumably labile adhesions, whereas stationary cells were anchored to the substratum at specific sites linked to the cytoskeleton near local concentrations of FN-receptor.

DURING embryonic development, interactions of cells with extracellular matrix are important in regulating cell behavior (27, 59, 65). The extracellular molecule fibronectin (FN)¹ promotes the adhesion, spreading, and formation of specialized adhesion sites in a variety of cells (25, 31, 40, 49). Besides this role, FN stimulates the *in vitro* locomotion of several embryonic cell types, including chick heart fibroblasts and avian neural crest cells (17, 42, 43, 48). *In vivo*, the presence of FN has often been correlated with the migration of cells, e.g., gastrulating cells, primordial germ cells, and neural crest cells (5, 18, 21, 22, 28, 39, 50, 58, 64). In addition, the interaction of FN with the cell surface is a prerequisite for cell movement, since blocking the FN cell-

binding domain inhibits the migration of cells (6, 7, 48). These observations imply that the same molecule is involved in both the transient adhesions involved in cell movement and in the firm anchorage of a cell to a substratum. These two functions may reside in distinctly different interactions of FN with receptor molecules in a motile compared to a nonmotile cell, rather than in differences in the molecular structure of FN itself.

Because they cannot be observed *in vivo*, cell-to-substratum interactions have been studied in cells cultured on two-dimensional substrates. Chick embryo fibroblasts or fibroblastic cell lines adhere strongly to and spread extensively on FN-coated substrates. They develop microfilament bundles, which are often aligned with FN fibers deposited on the substrate by the cells (3, 29, 30, 52, 63). At the sites of closest contact with the substrate, termed focal contact sites, the microfilament

¹ Abbreviations used in this paper: DME, Dulbecco's modified Eagle's medium; FN, fibronectin; FN-receptor, putative fibronectin receptor complex; NCS, newborn calf serum.

bundles interact with vinculin, α -actinin, and the plasma membrane in association with FN fibers outside of the cell (12, 13, 53). In contrast to stationary fibroblasts, motile cells tend not to form focal contact sites (16, 34a) and lack the ability to synthesize FN; during migration, they use exogenous FN as a substrate (17, 38, 42, 51). However, it is still not known how these cells use FN to move, e.g., by means of FN receptors.

A number of possible candidates for the FN receptor have been proposed. Besides nonprotein components such as heparan sulfate (35) and gangliosides (32, 55), proteins are the leading candidates for the major endogenous binding mechanism for FN. Photoaffinity labeling experiments have suggested that a 47-kD glycoprotein comes into close contact with FN (4). Protease treatments abolish the ability of cells to attach to FN (56). Recently, two different approaches, one using monoclonal antibodies that interfere with cell attachment (9, 11, 14, 15, 20, 24, 26, 33, 34, 41) and the other based on affinity between the cell-binding sequence of FN and solubilized membrane proteins (47), have led to the identification of a complex of three glycoproteins of approximately 120, 140, and 160 kD involved in the interaction of cells with FN. Immunofluorescent localization of the 140-kD glycoprotein on fibroblasts and myoblasts shows that it co-distributes with extracellular FN fibrils and intracellular α -actinin at cell-to-substratum contact sites (14, 15, 19). In addition, the 140-kD glycoprotein complex and FN are excluded from vinculin-rich focal adhesion sites and are enriched at the periphery of these structures (14, 15, 19). Taken together, these and other biochemical, functional, and immunocytological studies strongly suggest that the 140-kD complex functions as an FN receptor. For conciseness, we shall refer hereafter to this 140-kD glycoprotein complex implicated in FN receptor function as FN-receptor.

In the present study, we have examined the distribution and redistribution of this putative FN-receptor *in vivo* in a variety of embryonic tissues, particularly at the time of neural crest cell migration and differentiation. We then focused on its organization on cultured neural crest cells, which are known to be actively migrating (for reviews, see references 36 and 57), compared to other motile or nonmotile embryonic cells such as somitic and ectodermal cells. Finally, *in vitro* perturbation experiments were performed to determine the possible role of this molecule in cell motility. The results are discussed in terms of possible roles of the FN-receptor in migratory processes.

Materials and Methods

Embryos

Japanese quail (*Coturnix coturnix Japonica*) embryos were used throughout the study. Eggs were incubated at $38 \pm 1^\circ\text{C}$ in a humidified air chamber and staged according to the number of somite pairs and to the duration of incubation.

Cell Cultures

Cultures were generated as described previously (48). Briefly, the caudal regions of embryos incubated for 60 h were excised with a scalpel. The trunk fragments were incubated for 30–60 min at room temperature with 750 U/ml Dispase (Godo Shusei, Tokyo, Japan) in Dulbecco's modified Eagle's medium (DME). Somites, notochords, ectoderms, and neural tubes were teased apart with tungsten needles until free of contaminating tissues. Under these conditions, the neural tubes, somites, and ectoderms were devoid of any contaminating mesenchymal cells. After dissociation, tissues were allowed to recover from enzyme treatment by an incubation in DME for 30 min. Somites and ectoderms

were dissociated with 0.1% crude trypsin (1/250, Gibco Europe, Scotland) for 10 min at 37°C ; the enzyme was inactivated by serum, and the isolated cells were harvested by centrifugation. Somitic and ectodermal cells and neural tubes were explanted onto appropriate substrata (see below) and cultured at 37°C in a humidified 7% $\text{CO}_2/93\%$ air incubator.

Antibodies

Polyclonal antibodies to the 140,000-D putative FN-receptor complex were produced in rabbits and their specificity established as described previously (15). Fab' fragments of anti-FN-receptor antibodies were prepared according to Brackenbury et al. (8).

In Vivo Localization of the FN-Receptor

The FN-receptor complex distribution was studied by immunofluorescent staining of cryostat sections. After fixation in 3.7% formaldehyde in phosphate-buffered saline (PBS consisting of 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4 , and 1.5 mM KH_2PO_4 , pH 7.4) for 1–4 h, and extensive washes in PBS, embryos were embedded in a graduated series of sucrose solutions in PBS (12–18% wt/vol) and frozen in Tissue Tek (Lab-Tek Products) in liquid nitrogen. Sections were cut at 10 μm on a cryostat (Bright Instrument Co. Ltd., Huntington, England) and mounted on slides coated with gelatin according to the procedure of Lohmann et al. (37). Immunofluorescent staining of the slides was similar to that of cultures (see below). Simultaneous staining for FN-receptor, FN, and crest cells and their derivatives was performed on successive sections.

Immunofluorescent Staining of Cell Cultures and Interference Reflection Microscopy

For immunofluorescent staining and interference reflection microscopy, neural tubes and somitic or ectodermal cells were explanted onto glass coverslips (Corning Glass Works, Corning, NY) in petri dishes (Nunc, Denmark). Coverslips were incubated for 30 min at 37°C with 10–15 $\mu\text{g}/\text{ml}$ human plasma FN before culture. Cultures were grown for 24 h, 48 h, or 4 d in the presence of 10% heat-inactivated newborn calf serum (NCS; Gibco Europe). After washes with serum-free DME, cultures were fixed in 3.7% formaldehyde in PBS for 5 min or 1 h at room temperature, rinsed three times in PBS, extracted with 0.4% Triton X-100 for 3 min, and washed twice in PBS. Antibodies (3–30 $\mu\text{g}/\text{ml}$) were applied for 1 h at 20°C to the cultures in PBS containing 1 mg/ml bovine serum albumin (BSA). After intervening washes, the coverslips were incubated for 30 min with a secondary antibody coupled to rhodamine or fluorescein (Nordic, Tilburg, The Netherlands). For control experiments, non-immune rabbit IgG was substituted for each primary antibody. In all cases, the controls showed negligible degrees of labeling. In some experiments, cultures were immunolabeled without fixation and permeabilization; the staining procedure was completely identical to that for fixed cells except that the antibodies were applied for 10 min at 20°C in the presence of DME buffered with Hepes (50 mM) at pH 7.4. To stain actin-microfilament bundles, fixed and permeabilized cultures were incubated with nitrobenzoxadiazole phalloidin (10 U/ml, Molecular Probes, Junction City, OR) for 35 min, rinsed twice with PBS, and examined with a Leitz epifluorescence microscope (E. Leitz, Inc., Rockleigh, NJ) using the standard fluorescein filters. For interference reflection microscopy, fixed and labeled cells were examined on a Zeiss photomicroscope (Carl Zeiss, Inc., Thornwood, NY) equipped for both interference reflection microscopy and epifluorescence. Cells were observed and photographed for interference reflection, then the same or different fields exposed to fluorescein excitation epiillumination and photographed.

Isolation of Focal Contacts from Cultured Cells

Isolated focal contacts were obtained from cells cultured on glass slides by treating cells with a solution of 0.2% saponin (E. Merck, Darmstadt, FRG) in PBS for 10 min and subsequent pipetting essentially as described by Neyfakh and Svitkina (44). The preparations were then fixed and treated for immunofluorescent labeling as for intact fixed cells.

Assays for Cellular Adhesion and Spreading on Substrata

Cellular adhesion assays were performed on substrata coated with various proteins in Terasaki plates (Nunc, Denmark). Each well of the plates was incubated with 20 μl of human plasma or chick cellular FN, rat tail type I collagen, anti-FN-receptor antibodies, or control IgG at concentrations of 1–1,000 $\mu\text{g}/\text{ml}$ in PBS for 90 min, followed by incubation with heat-treated BSA (3 min at 80°C) in PBS (3 mg/ml) for 60 min and extensive washes with PBS. Routinely, crest cells from 50 explants or somitic cells cultured for 24 h on FN-coated dishes were harvested using treatment for 10 min at 20°C with

0.001% trypsin (type XI, crystallized, Sigma Chemical Co., St. Louis, MO) and 1 mM EDTA in PBS. The protease reaction was stopped by adding DME containing 10% NCS. Cells were collected in conical microcentrifuge tubes, centrifuged at 1,000 rpm for 5 min, and incubated for 3 h at 37°C in DME with 10% FN-free NCS (42) to allow recovery from proteolytic damage. Each well of the Terasaki plates was filled with 20 μ l of cell suspension containing $\sim 10^3$ cells. The plates were then incubated at 37°C in a humidified 7% CO₂/93% air incubator. At the indicated times, the attached cells were fixed with a 3.7% formaldehyde solution in PBS with care to avoid loss of non-attached cells, and counted with a Leitz inverted phase contrast microscope.

Inhibition Assays of Cellular Adhesion and Migration

Each well of Terasaki plates previously coated with human plasma FN (10 μ g/ml in PBS) was filled with 20 μ l of cell suspension (10^3 cells/well) in DME with 10% FN-free NCS in the presence of Fab' fragments of anti-FN-receptor antibodies or of control antibodies (0.1–2.5 mg/ml). The subsequent treatment

of the plates was identical to that described for the adhesion assay. For assaying inhibition of cell migration, somitic cells and neural tubes were cultured in 1-cm-diam wells consisting of a section of polyethylene tubing mounted on FN-coated petri dishes. After 24 h of culture in DME with 10% FN-free NCS (0.1 ml/well), an additional 0.1 ml of medium containing Fab' fragments of anti-FN-receptor antibodies or of control antibodies (0.2–5 mg/ml) was added. Cultures were then incubated at 37°C for varying periods of time, fixed in formaldehyde, and observed with an inverted phase contrast microscope.

Results

In Situ Immunofluorescent Distribution of FN-Receptor in the Early Avian Embryo

We have characterized the distribution of FN-receptor during morphogenesis using immunofluorescent labeling with anti-

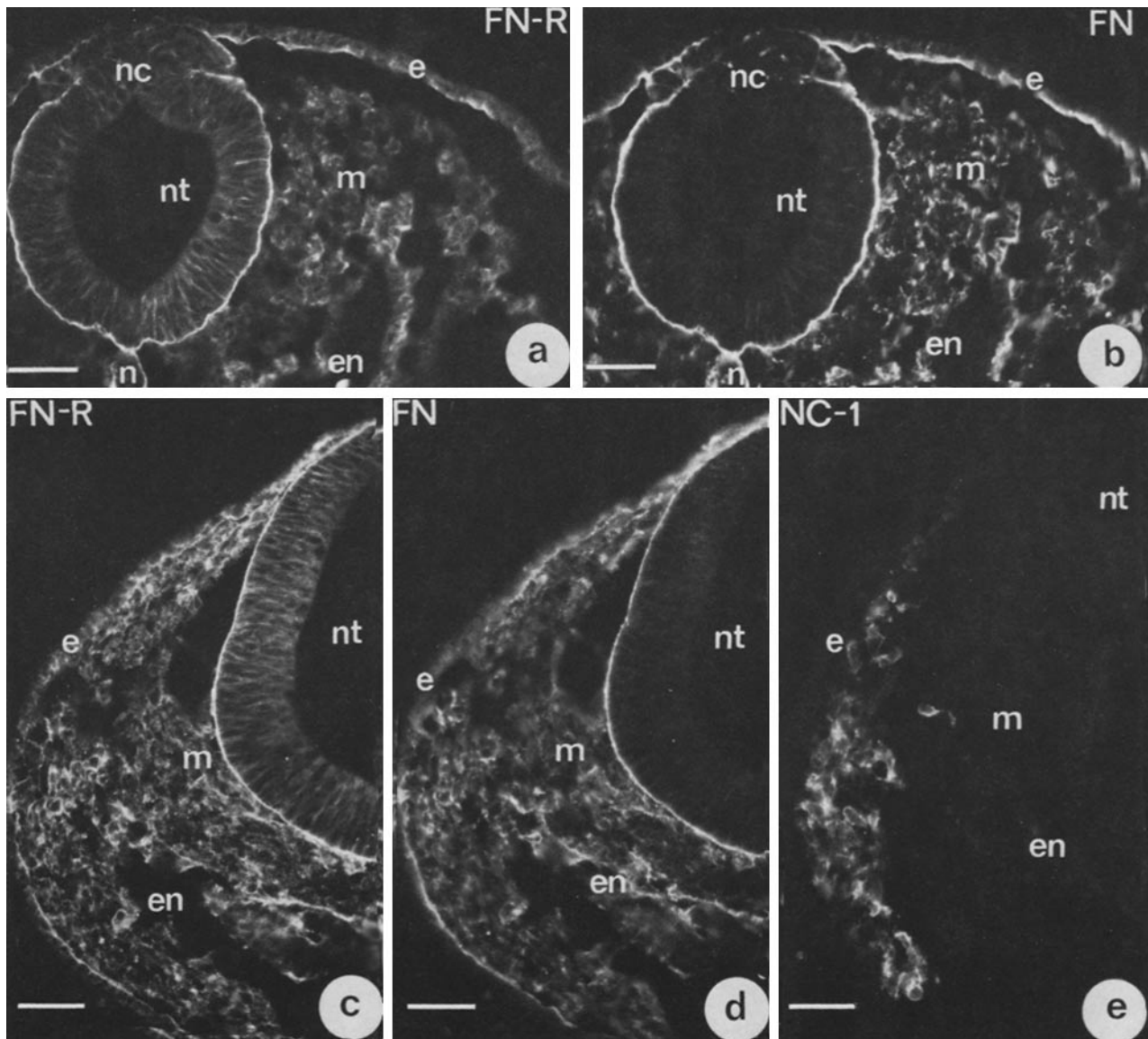


Figure 1. In situ immunofluorescent distribution of FN-receptor during cephalic neural crest cell migration based on transverse sections through the mid-mesencephalon. (a and b) 8-somite embryo double-labeled for FN-receptor (a) and FN (b). Tissues derived from each of the three primary germ layers express FN-receptor as detected by polyclonal antibodies to 140-kD glycoprotein complex. In epithelia, the FN-receptor is present on the whole cell surface, but it is enriched at the basal surface near regions where FN is present. In mesenchymes, FN-receptor co-distributes with FN at the cell surface. Neural crest cells initiating emigration from the dorsal aspect of the neural tube are delimited by FN only peripherally. They also do not stain particularly strongly for FN-receptor. (c, d, and e) 15-somite embryo double-stained for FN-receptor (c) and FN (d); e represents a similar section stained for NC-1 to indicate the location of crest cells. Crest cells migrate laterally between the ectoderm and the cephalic mesenchyme. They are strongly labeled both for FN-receptor and for FN, although not more heavily than the mesenchyme. e, ectoderm; en, endoderm; m, mesenchyme; n, notochord; nc, neural crest; nt, neural tube. Bars, 25 μ m.

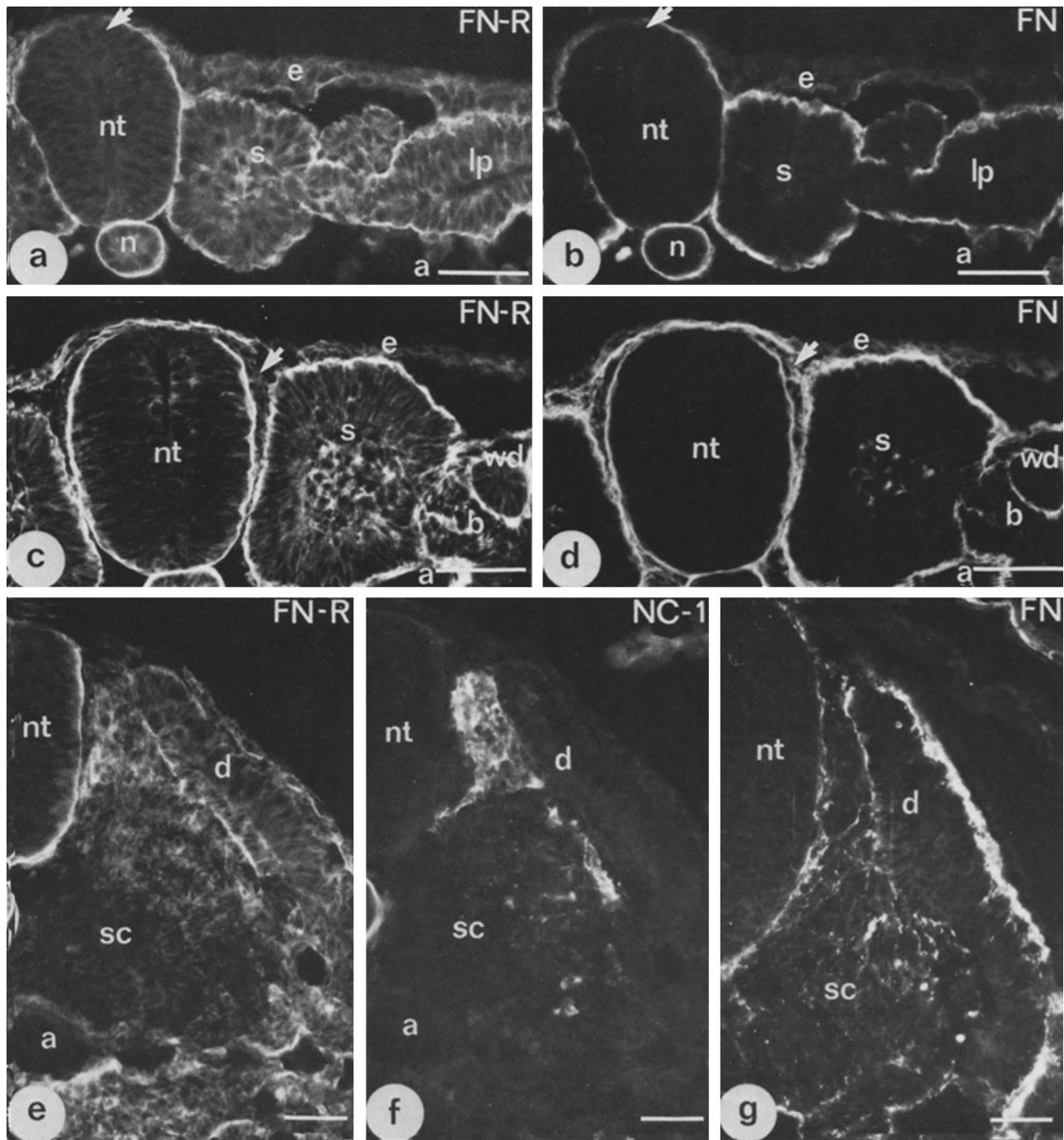


Figure 2. In situ immunofluorescent distribution of FN-receptor during neural crest cell migration based on transverse sections through the 15th somite. (a and b) 15-somite embryo double-labeled for FN-receptor (a) and FN (b). Before crest cell emigration (arrows point to premigratory crest cells in the neural tube), tissues are mainly organized into epithelia that co-express FN-receptor and FN in basement membranes. Note that the aorta, which is beginning to appear, is relatively poorly stained for either FN-receptor or for FN. (c and d) 25-somite embryo double-labeled for FN-receptor (c) and FN (d). Crest cells (arrows) undergo migration between the somite and the neural tube in a dense FN meshwork. They are stained for FN-receptor but less than the surrounding tissues. The aorta now clearly expresses both FN-receptor and FN. The Wolffian duct and the mesonephric blastema are also strongly stained for FN-receptor. (e, f, and g) 32-somite embryo double-labeled for FN-receptor (e) and NC-1 (f); g represents a similar section stained for FN. The location of crest cells was determined by staining with the monoclonal antibody NC-1. As they move between the neural tube and dermomyotome and under the myotome, the crest cells are strongly labeled for FN-receptor and FN. In contrast, the sclerotome is weakly stained for FN-receptor. a, aorta; b, mesonephric blastema; d, dermomyotome; e, ectoderm; lp, lateral plate; n, notochord; nt, neural tube; s, somite; sc, sclerotome; wd, Wolffian duct. Bars, 25 μ m.

bodies to FN-receptor and FN on cryostat sections of young quail embryos. The FN-receptor was found to be a ubiquitous protein present in tissues derived from each of the three

primary germ layers.

Mesenchyme: Mesenchymal cells, such as cephalic mesenchyme, sclerotome, mesenchyme in the limb, and connective

tissues surrounding the aorta and kidney, were all labeled for FN-receptor. The labeling was at the cell surface and generally co-distributed with FN; the staining was very similar, but not completely identical, to that for FN (Figs. 1–5). However, the intensity of the labeling varied with the tissue; for example, the cephalic mesenchyme and connective tissues around the aorta and in the limb were strongly stained (Figs. 1, *a* and *c*, 4, *a* and *c*, and 5 *b*), in contrast to the sclerotome which was only faintly stained (Figs. 2 *e* and 3, *a* and *c*).

Epithelium: Epithelia such as the ectoderm, endoderm, neural tube, somites, dermomyotomes, and kidney tubules were all stained for FN-receptor. In these epithelia in the early embryo, both the apical and the basolateral surfaces of the cells exhibited some staining for receptor. However, the labeling was greatly enriched at the basal surface, where FN was present extracellularly (Figs. 1–5). In older embryos, epithelia that are organized into tubules, e.g., kidney tubules and hepatic ducts, expressed FN-receptor only at the basolateral surfaces, and again the basal surface was strikingly preferentially labeled (Fig. 5 *d*).

Mesenchymal–Epithelial Transitions: During the condensation of mesenchymes into epithelia, for example during mesonephric tubule formation, the spatial pattern of FN-receptor on cells varied but did not follow the pattern of FN precisely. Before cell aggregation, FN was seen surrounding cells that express FN-receptor over their entire surface (Fig. 2, *c* and *d*). During aggregation, FN was confined to the basement membrane, while FN-receptor was still present over the whole cell surface, but with a preferential accumulation at the basal surface of each cell (Figs. 4 *a* and 5 *c*). When kidney tubules were fully differentiated, FN-receptor disappeared from the apical surface facing the lumen and was restricted to the basolateral surfaces (Fig. 5 *d*).

Unusual Patterns: There are also tissues that do not express FN-receptor or that express it at especially high levels. Endocardial cells during their migration in the cardiac jelly (Fig. 5 *a*), aortic cells during the formation of the aorta (Fig. 2 *a*), hematopoietic cells in the blood but not cells in hematopoietic foci (Figs. 4 *a* and 5 *b*), and chondrocytes in cartilages (Figs. 3 *f* and 4 *f*) were, so far, the only tissues that were found not to express FN-receptor. This absence generally corresponded to a concomitant absence of FN.

The neural tube was somewhat special. During formation of the neural tube, neural epithelial cells were stained over their entire surface, though with considerable accumulation at the basal surface (Figs. 1, *a* and *c*; 2, *a* and *c*; and 3 *a*). As the neural tube underwent differentiation, most of the cells

lost FN-receptor, and residual staining was confined to the periphery of the neural tube close to the basement membrane (Fig. 3 *c*). Very interestingly, a few, isolated cells within the neural tube were stained brightly both for FN and for FN-receptor (Fig. 3, *c* and *e*). These cells have not yet been identified. In older embryos, the spinal cord was completely devoid of FN-receptor.

Among tissues that stain brightly for FN-receptor, endothelial cells of the extensively developed aorta and of small capillaries are probably the most noteworthy. This intense labeling was accompanied by high levels of FN itself (Figs. 2, *c*, *d*, *e*, and *g*; 3, *c* and *e*; 4, *a*, *c*, and *e*; 5 *b*).

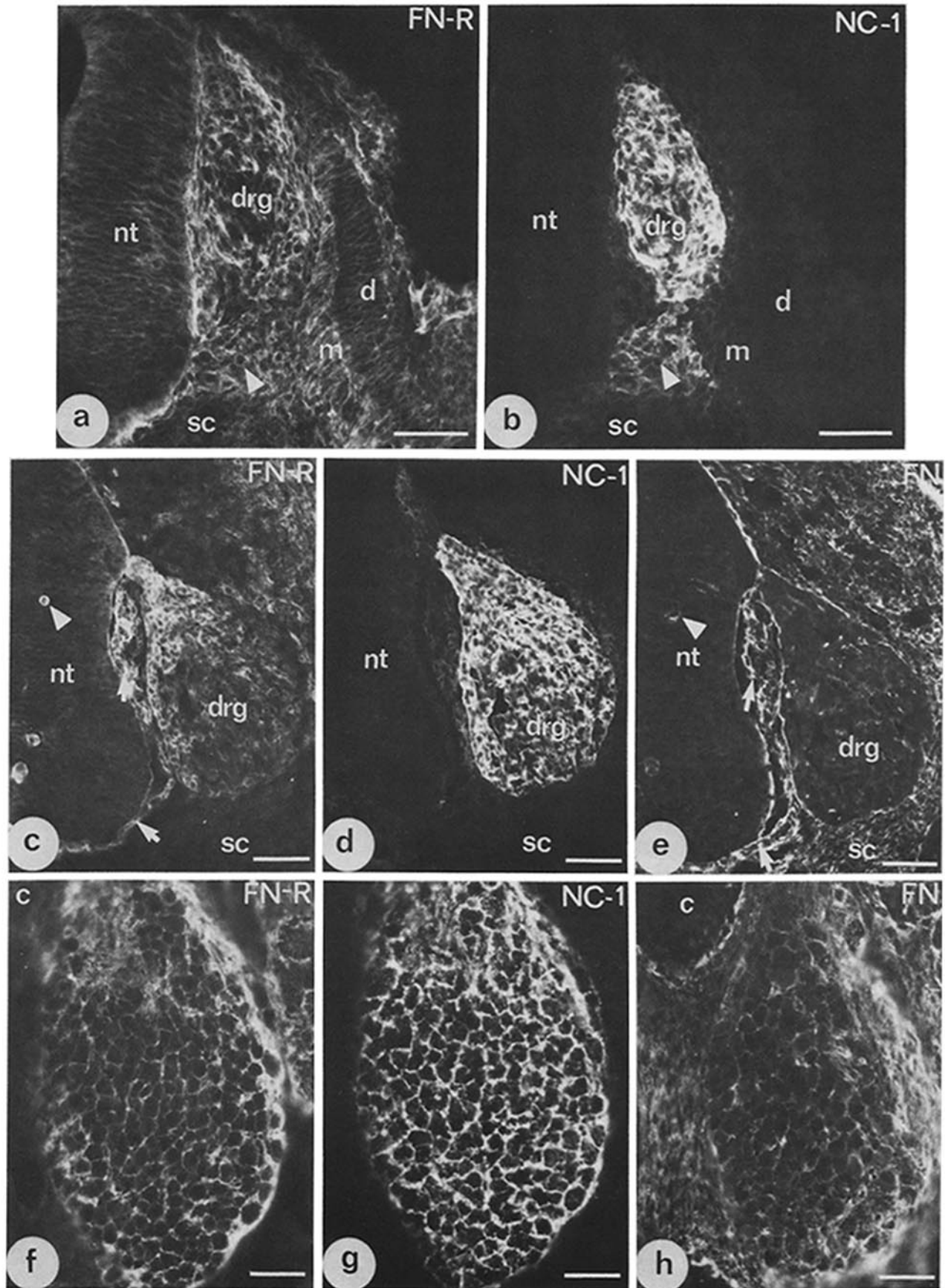
In Situ Distribution of FN-Receptor during Neural Crest Cell Migration and Differentiation

The distribution and fate of FN-receptor during the course of neural crest cell migration and differentiation was studied in detail, and its distribution was compared with that of FN. When necessary, crest cells were stained with the monoclonal antibody NC-1 to determine their precise location (61). Before their migration in the head, neural crest cells are integrated in the neural folds; the staining of these cells for FN-receptor was similar to that of the neighboring ectoderm and neural tube. As they emerged from the neural tube, crest cells formed a dense cell mass between the neural tube and the ectoderm; they were still laterally delimited by FN present in a basement membrane-like structure. Few cells in this mass were near FN, and staining for FN-receptor was faint (Fig. 1, *a* and *b*). In contrast, when crest cells were actively migrating laterally between the ectoderm and the cephalic mesenchyme, they were strongly labeled both for FN-receptor and for FN (Fig. 1, *c*, *d*, and *e*).

In the trunk, crest cells were not strongly stained, but nevertheless remained positive for FN-receptor before and during the early phases of migration, in association with high levels of FN (Fig. 2, *a–d*). As the crest cells migrated further ventrally towards the sclerotome and under the myotome, they became much more intensely labeled (Fig. 2, *e*, *f*, and *g*). When crest cells accumulated along the neural tube to form the primordium of the sensory ganglion, the staining for FN-receptor diminished in cells located in the middle of the ganglion (Fig. 3, *a* and *b*). In contrast, crest cells that accompanied motor axons during their outgrowth remained well-labeled for FN-receptor (Fig. 3, *a* and *b*).

At 4 d of incubation, as cells located in the latero-ventral region of the sensory ganglion started to differentiate, FN-receptor was nearly absent from this region; this absence

Figure 3. In situ immunofluorescent distribution of FN-receptors during the formation and differentiation of dorsal root ganglia using transverse sections through thoracic vertebrae. (*a* and *b*) 38-somite embryo double-labeled for FN-receptor (*a*) and NC-1 (*b*). The primordium of the dorsal root ganglion (*drg*) appears along the neural tube. In the *drg*, cells located in the center of the *drg* are less stained than those at the periphery. Below the *drg*, motor nerve fibers and crest cells that accompany the motor nerve fibers are well-labeled for FN-receptor (arrowheads). Note that the myotome expresses high levels of FN-receptor compared with the dermatome. (*c*, *d*, and *e*) Successive sections of a 4-d embryo stained for FN-receptor (*c*), NC-1 (*d*), and FN (*e*). The ganglion is limited by an FN-rich basement membrane; a few spots of FN can be seen among the cells of the ganglion. The medio-dorsal cells are intensely stained for FN-receptor in contrast to the latero-ventral cells, which are weakly labeled. The sclerotome, which will differentiate into cartilage, is also weakly stained. Note the very brightly stained cells in the neural tube that co-express FN-receptor and FN (arrowheads in *c* and *e*) and the strongly stained blood vessel adjacent to the neural tube (arrows in *c* and *e*). (*f*, *g*, and *h*) Successive sections of a 10-d embryo labeled for FN-receptor (*f*), NC-1 (*g*), and FN (*h*). The entire differentiated dorsal root ganglion expresses FN-receptor; the staining for FN and FN-receptor is enriched in the periphery of the ganglion. Note that the cartilage of the vertebra is negative both for FN-receptor and for FN. *c*, cartilage; *d*, dermatome; *drg*, dorsal root ganglion; *m*, myotome; *nt*, neural tube; *sc*, sclerotome. Bars, 25 μ m.



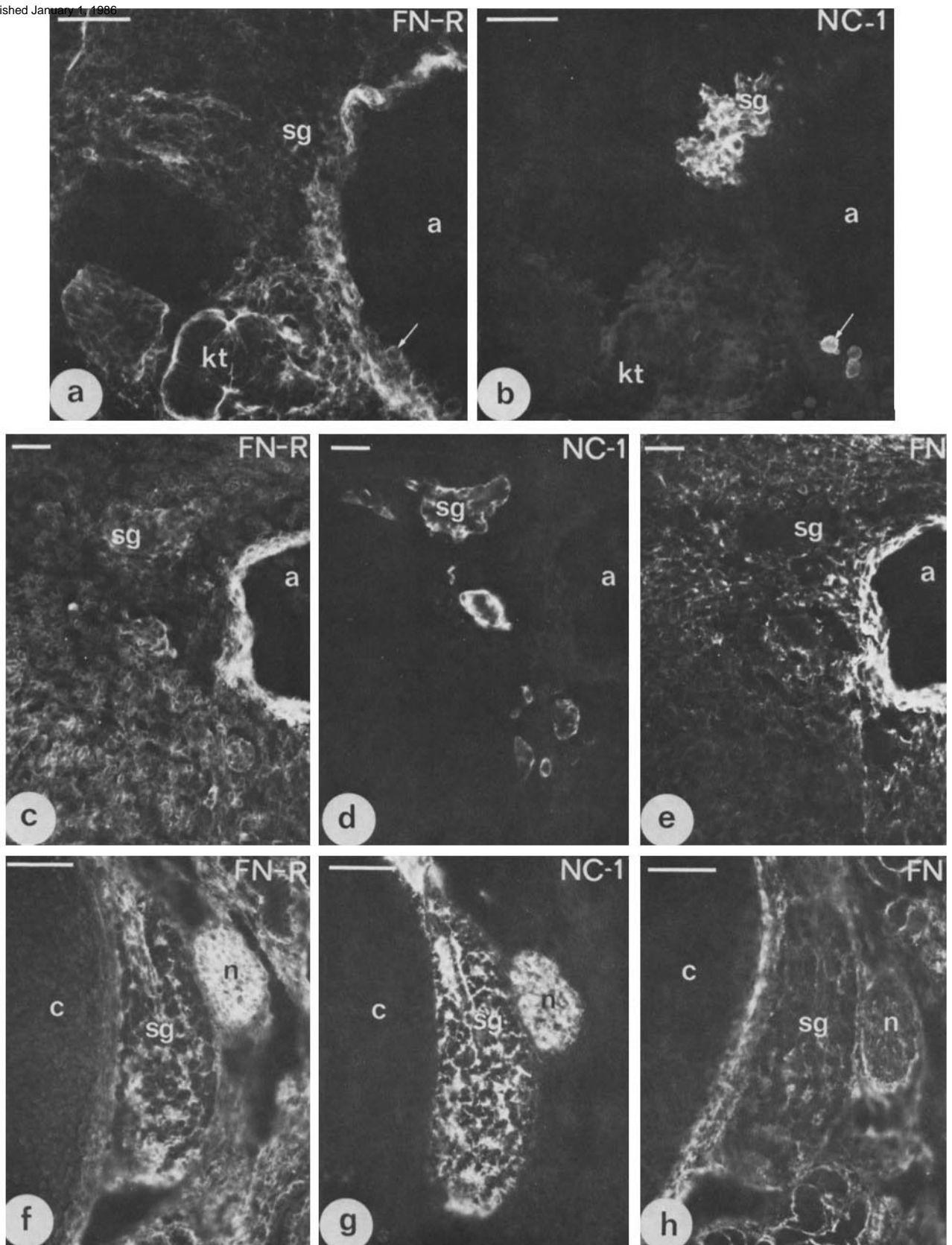


Figure 4. In situ distribution of FN-receptor during the formation and differentiation of sympathetic ganglia in transverse sections at the thoracic level. (*a* and *b*) 38-somite embryo double-stained for FN-receptor and NC-1. Crest cells accumulating along the aorta to form the sympathetic ganglion are faintly labeled for FN-receptor, in contrast to the well-labeled aorta and kidney tubules. Note that hematopoietic stem cells detaching into the lumen of the aorta are brightly stained both for NC-1 and for FN-receptor (arrows), while the cells in the blood are entirely negative, though visualizable by phase contrast microscopy. (*c*, *d*, and *e*) Successive sections of a 4-d embryo labeled for FN-receptor (*c*), NC-1 (*d*), and FN (*e*). The sympathetic ganglion is well-defined, but only the cells at the border of the ganglion are well-labeled for FN-receptor. In contrast to the sensory ganglia, the sympathetic ganglia are devoid of FN and are not surrounded by a basement membrane. (*f*, *g*, and *h*) Successive sections of a 10-d embryo labeled for FN-receptor (*f*), NC-1 (*g*), and FN (*h*). Many, but not all of the cells in the ganglion, are stained for FN-receptor and FN. A nerve adjacent to the ganglion is intensely stained. Note that the cartilage is devoid of FN-receptor and FN. *a*, aorta; *c*, cartilage; *kt*, kidney tubules; *n*, nerve; *sg*, sympathetic ganglion. Bars, 25 μ m.

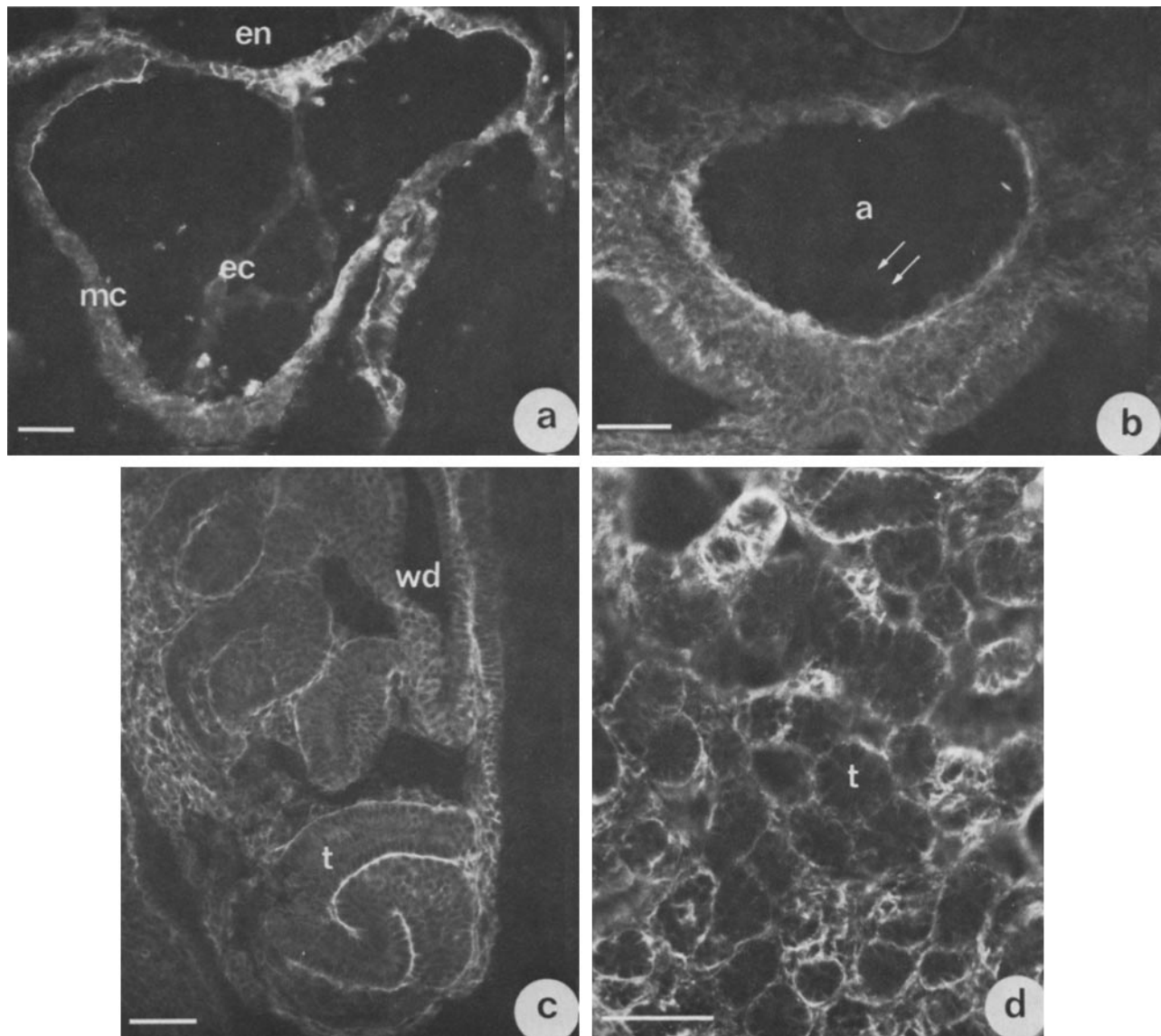


Figure 5. In situ immunofluorescent distribution of FN-receptor in non-neuronal tissues. (a) Transverse section through the heart of a 10-somite embryo labeled for FN-receptor. Endocardiac cells migrating in the cardiac jelly are virtually devoid of FN-receptor, whereas myocardial cells are labeled. *ec*, endocardium; *en*, endoderm; *mc*, myocardium. (b) Transverse section through the aorta of a 35-somite embryo labeled for FN-receptor. The endothelium of the aorta is strongly stained. Arrows indicate unstained blood cells. *a*, aorta. (c and d) Transverse sections through the mesonephron of a 4-d (c) and 10-d embryo (d) stained for FN-receptor. At 4 d, mesonephric cells associated into tubules exhibit staining for FN-receptor over the entire cell surface with a substantial enrichment at the basal surface. At 10 d, staining has disappeared from the apical surfaces of cells. *t*, tubule; *wd*, Wolffian duct. Bars, 25 μ m.

correlated with the absence of FN. In contrast, the medio-dorsal region of the ganglion stained brightly, even though very little FN was seen in this region (Fig. 3, *c*, *d*, and *e*). When the sensory ganglion was fully differentiated at 10 d of incubation, the whole ganglion was stained for FN-receptor. However, it was impossible to determine whether glial or neuronal cells were labeled (Fig. 3, *f* and *g*). Nerve fibers emerging from the ganglion were intensely labeled for FN-receptor. The cells at the periphery of the ganglion were strongly stained for FN-receptor, correlating with an intense labeling for FN (Fig. 3, *f* and *h*). The pattern of distribution and fate of FN-receptor during the genesis of the sympathetic ganglia was very similar to that of the sensory ganglia, but the decrease and disappearance of FN-receptor from the surface of the cells was more obvious (Fig. 4, *a-e*). The differentiation

of cells into neurons and glia was also characterized by the appearance of FN-receptor on the surface of some cells, which also stained for FN (Fig. 4, *f-h*).

Morphology and Behavior of Neural Crest Cells, Somitic Fibroblasts, and Ectodermal Cells Cultured on FN Substrates

To examine the function of the putative fibronectin receptor more analytically, we focused in detail on several selected cell types displaying a wide spectrum of migratory activities in vitro. We compared highly migratory neural crest cells and migrating somitic fibroblasts with nonmigratory somitic and ectodermal cells.

Neural crest cells emigrate vigorously from neural tubes cultured on FN-substrates. After 24 h, they have developed a

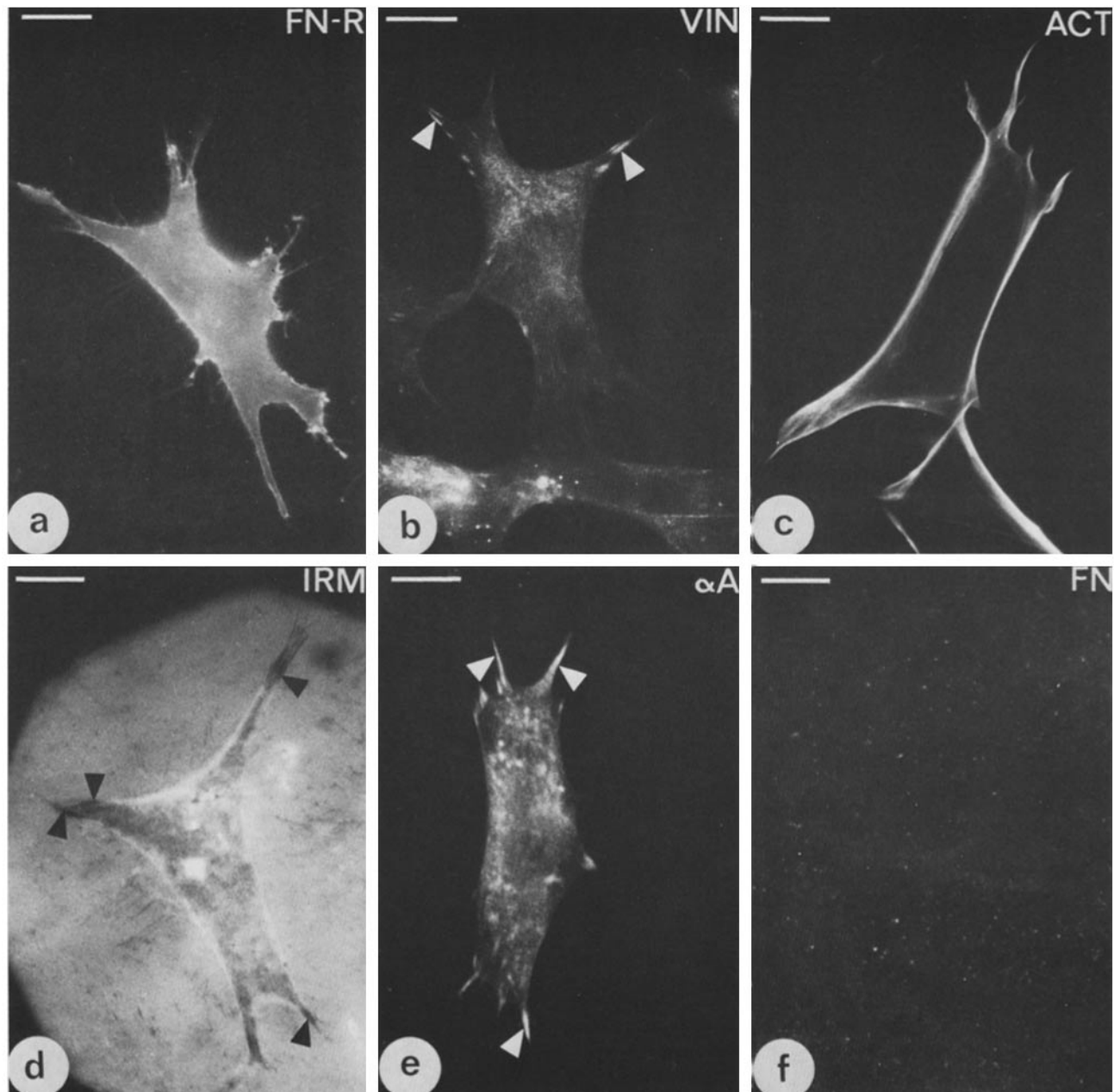


Figure 6. Immunofluorescent detection of FN-receptor (*a*), vinculin (*VIN*) (*b*), actin (*ACT*) (*c*), α -actinin (αA) (*e*), and FN (*f*) on cultured neural crest cells. *d* shows interference reflection microscopy image (*IRM*). The FN-receptor labeling is diffuse on the cell surface membrane (*a*). The microfilament bundles tend to be concentrated towards the lateral edges of neural crest cells as well as in the cell processes (*c*). Both vinculin (*b*) and α -actinin (*e*) show labeling restricted to the ends of the cell processes (arrowheads in *b* and *e*). These regions frequently correspond to dark areas in interference reflection microscopy images (arrowheads in *d*). No synthesis and deposition of FN as a meshwork is observed (*f*). The spots observed in *f* represent staining of apparent aggregates in the FN coating on the coverslip. Bars, 1 μ m.

halo of crest cells forming a dense monolayer of ~3,000–5,000 cells. Crest cells were highly motile and displayed a stellate morphology with several long and active cell processes (Fig. 6).

Somatic fibroblasts cultured on FN-substrates displayed different morphologies and behavior depending on the duration of culture. When cultured for only 12–24 h, somitic fibroblasts were similar in size to crest cells (10–15- μ m long); these “young” somitic cells were locomotory, and they had a bipolar morphology with a wide leading edge and a thin

trailing edge (Fig. 7). Within 3 or 4 d, most of the somitic fibroblasts lost their polarity and became stationary; these older cells became large (more than 50- μ m long) and well-spread with a polygonal morphology (Fig. 8). These two types of somitic fibroblasts were termed young and old somitic fibroblasts, respectively. Similar behavior has been described for fibroblasts emerging from chick heart explants (16). In contrast to neural crest and somitic cells, ectodermal cells showed little locomotory activity. They formed small epithelial clusters of tightly juxtaposed cells (not shown).

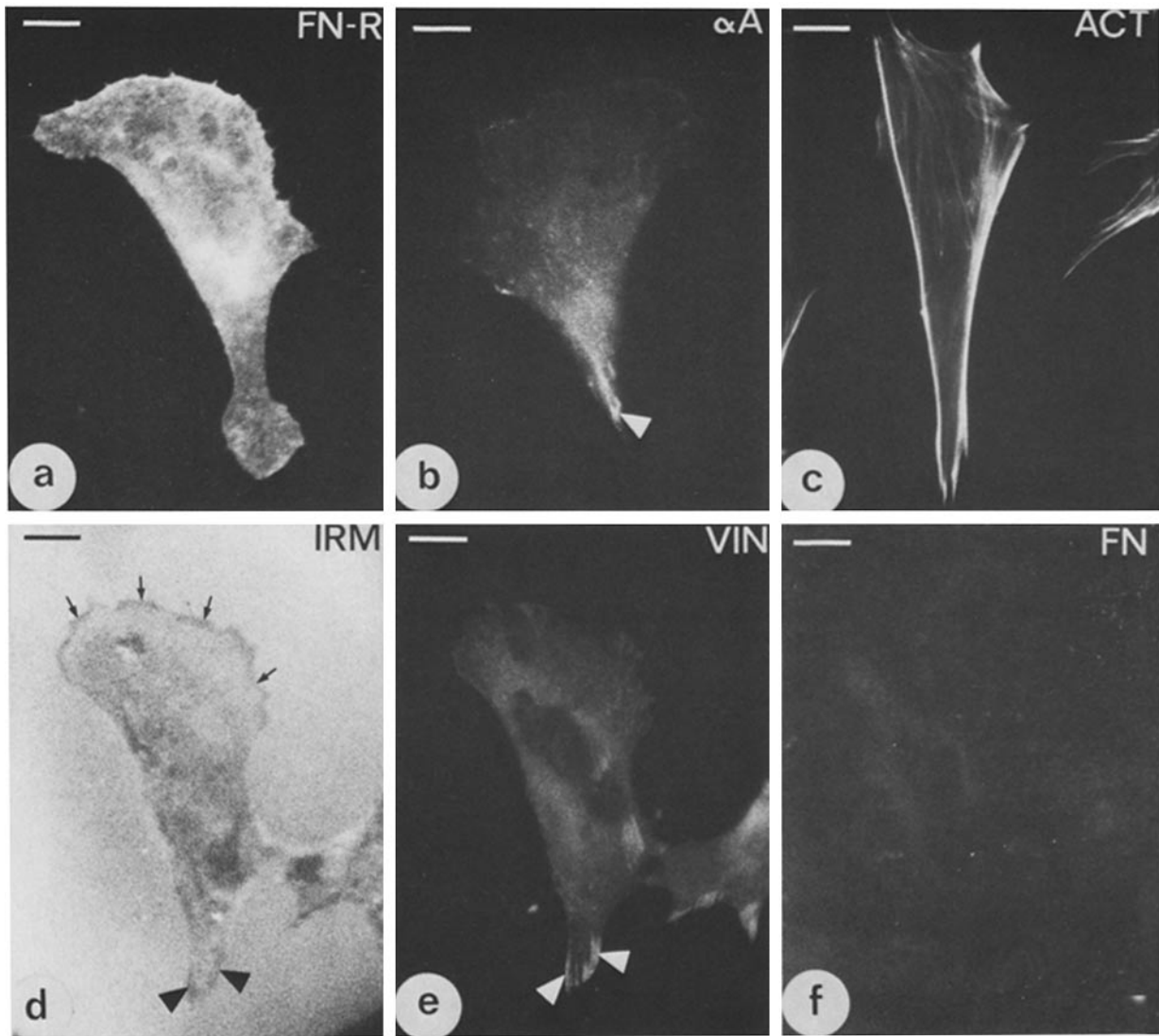


Figure 7. Immunofluorescent detection of FN-receptor (*a*), α -actinin, (αA) (*b*), actin (*ACT*) (*c*), vinculin (*VIN*) (*e*), and FN (*f*) on young somitic fibroblasts cultured for 24 h. *d* shows an interference reflection image (*IRM*). Migratory fibroblasts are bipolar with a broad leading edge characterized by ruffles (short arrows in *d*) and a narrow trailing edge. The interference reflection images reveal dark grey zones, and no structures corresponding to focal contacts are detectable except at the tip of the trailing edge (arrowheads in *d*). The trailing edge area is preferentially labeled for both α -actinin and vinculin (arrowheads in *b* and *e*). The FN-receptor labeling is diffuse on the cell surface membrane (*a*). Actin bundles are relatively poorly organized and are oriented along the cell axis (*c*). No extracellular FN deposition is observed (*f*). Bars, 1 μ m.

Structure of Cell-Substratum Adhesion Sites in Cultured Embryonic Cells

Cultured cells were examined for cell-to-substratum adhesion sites using interference reflection microscopy and immunofluorescence labeling. The distribution of FN-receptor was compared with that of two molecules known to be enriched in cell substratum adhesion sites, vinculin and α -actinin.

Neural crest cells displayed a uniform, diffuse, bright labeling for FN-receptor over the entire cell surface (Fig. 6*a*). Interference reflection images showed predominantly dark-grey zones corresponding to close adhesions; focal contact sites were rare under the cell body, and when present, they were found under cell processes (Fig. 6*d*). These focal contacts frequently corresponded to areas labeled both for vinculin (Fig. 6*b*) and for α -actinin (Fig. 6*e*).

In neural crest cells, actin bundles were often not well-organized; they were concentrated at the lateral edges of the cells and in the cell processes, orientated along the long axis of the cells (Fig. 6*c*). Finally, as already shown (38, 42, 51), crest cells did not synthesize and deposit FN extracellularly (Fig. 6*f*).

Young somitic fibroblasts showed patterns of distribution of FN-receptor, vinculin, α -actinin, and actin very similar to those of crest cells. FN-receptor was uniformly distributed over the entire cell membrane (Fig. 7*a*). There was often local staining for α -actinin and vinculin in the trailing edge (Fig. 7, *b* and *e*). As for crest cells, interference reflection images of young somitic fibroblasts showed predominantly dark grey-zones (Fig. 7*d*), indicating that these cells were in close association with the substratum. Few structures corresponding to focal contacts could be detected, except in the trailing edge

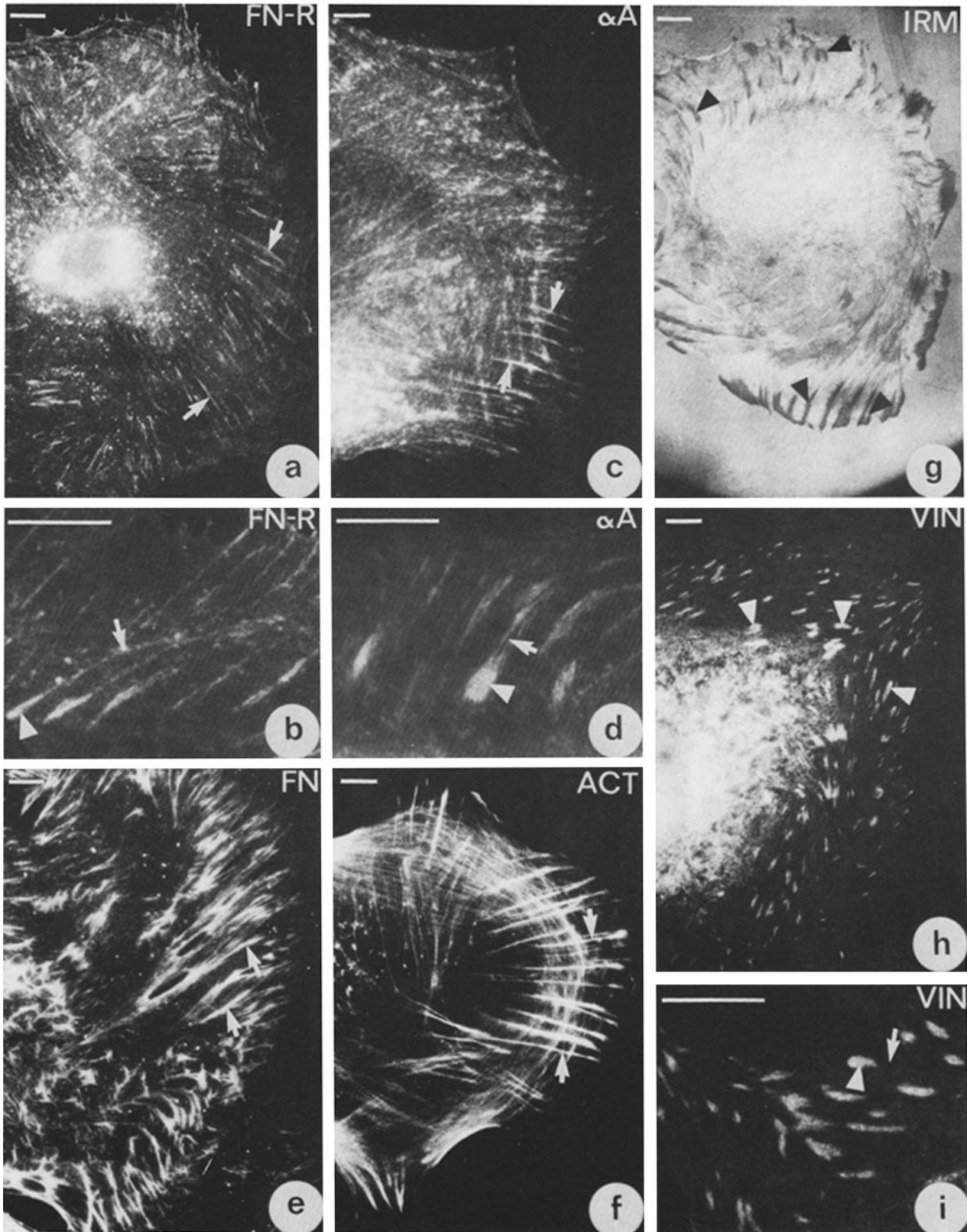


Figure 8. Single-labeled immunofluorescent detection of FN-receptor (*a* and *b*), α -actinin (αA) (*c* and *d*), FN (*e*), actin (*ACT*) (*f*), and vinculin (*VIN*) (*h* and *i*) on old somitic fibroblasts cultured for 4 d. *g* shows an interference reflection image. Stationary fibroblasts are large and flattened with numerous focal contact sites as shown by interference reflection microscopy (arrowheads in *g*). These focal contact sites are labeled for vinculin (arrowheads in *h*). In areas of focal contacts, FN-receptor (*a* and *b*) and α -actinin (*c* and *d*) are excluded from the focal contacts and form needle-eye type structures. Labeling for actin (*f*) shows a much more highly ordered network of microfilament bundles and stress fibers that correspond to some extent to the labeling for FN (*e*), FN-receptor (*a*), and α -actinin (*c*). *b*, *d*, and *i* indicate the localization of FN-receptor, α -actinin, and vinculin in stress fibers and near focal contacts. Arrows, stress fibers; arrowheads, focal contacts. Bars, 1 μ m.

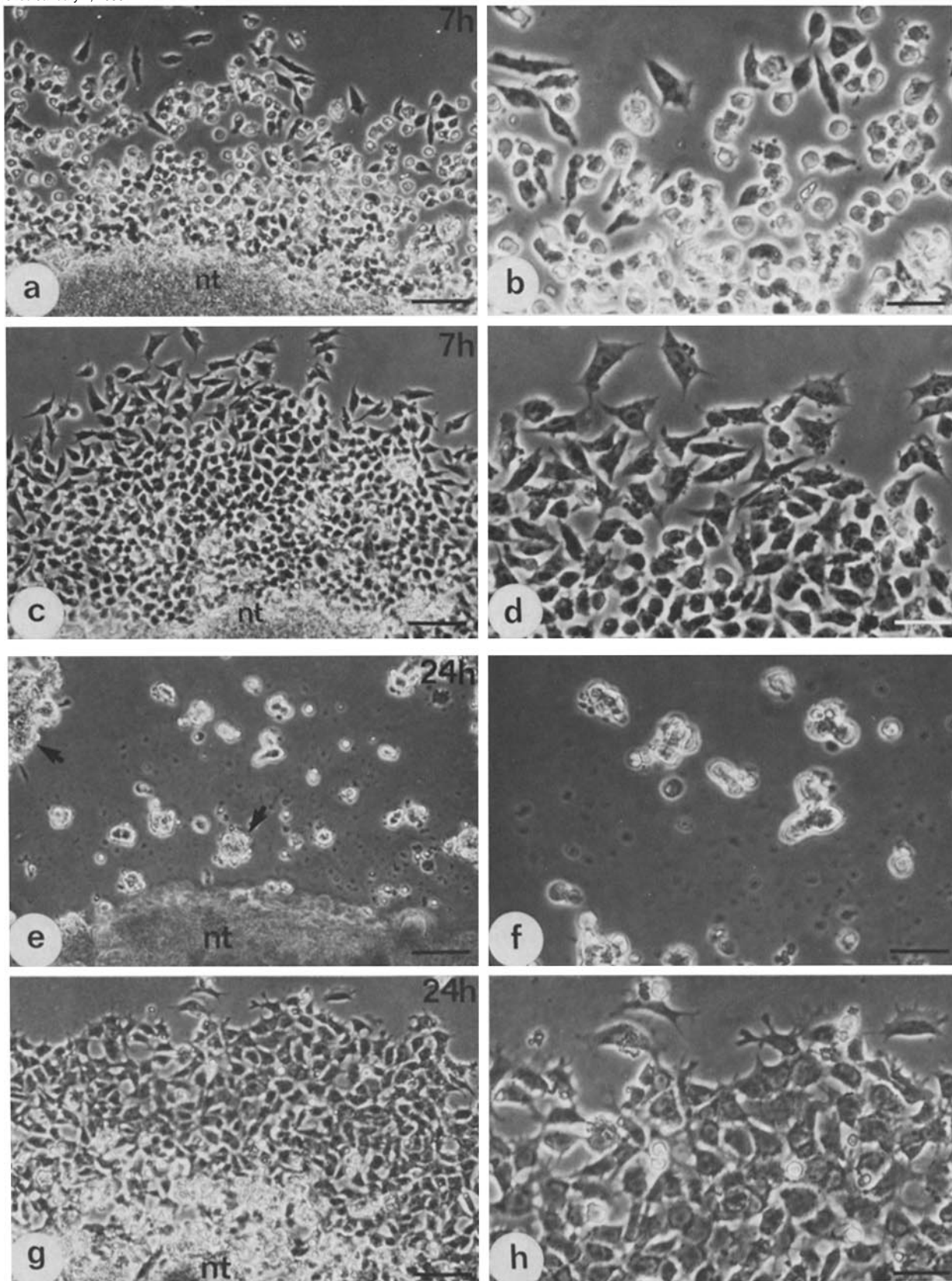


Figure 9. Effect of monovalent (Fab') antibodies to FN-receptor on migrating neural crest cells in vitro. Neural crest cells were allowed to emigrate from the neural tube onto a FN-coated substrate for 12 h. At that time, monovalent antibodies to FN-receptor (*a*, *b*, *e*, and *f*) or control preimmune monovalent antibodies (*c*, *d*, *g*, and *h*) were added to the medium at a final concentration of 2.5 mg/ml. Cultures were examined for the behavior of crest cells 2, 7, and 24 h later. *a*, *c*, *e*, and *g* show general views of the halos, and *b*, *d*, *f*, and *h* are higher magnifications showing the morphology of the cells. Within 7 h, a great majority of crest cells are found in the presence of anti-FN-receptor Fab' fragments; only a few cells at the migration front are still flattened (*a* and *b*). In contrast, crest cells in the presence of control Fab' fragments develop a normal halo after 7 h (*c* and *d*) very similar to that in normal medium (compare with Fig. 14, *c* and *d*). After 24 h of incubation in the presence of anti-FN-receptor Fab' (*e* and *f*), many crest cells have detached from the substratum, and the remaining cells frequently form aggregates of variable sizes (arrows in *e*). In control experiments (*g* and *h*), the halo is fairly normal, even though occasional cells are round. Bars: (*a*, *c*, *e*, and *g*) 10 μ m; (*b*, *d*, *f*, and *h*) 5 μ m.

(Fig. 7*d*). These dark zones were also usually the only areas of the cells that were stained for vinculin (Fig. 7*e*). Labeling for actin revealed only a few microfilament bundles, usually lateral and oriented along the cell axis (Fig. 7*c*). Finally, like crest cells, most young somitic fibroblasts lacked the ability to synthesize and deposit FN on the substrate (Fig. 7*f*); only a small amount of FN meshwork could be observed under some isolated fibroblasts.

The pattern of distribution of cell-substratum adhesion sites and the organization of microfilament bundles was strikingly different on old somitic fibroblasts, which instead closely resembled the distribution previously reported (15) for chick embryo fibroblasts. Both displayed FN-receptor labeling concentrated in linear patterns (Fig. 8, *a* and *b*) along prominent actin microfilament bundles (Fig. 8*f*). The linear FN-receptor staining pattern was similar to that for α -actinin (Fig. 8, *c* and *d*), but quite distinct from vinculin staining in focal contacts (Fig. 8, *g*, *h*, and *i*).

As somitic cells became stationary, small focal contact sites appeared at the leading edge of the cell that were stained for vinculin, α -actinin, and FN-receptor. Next, FN fibers were deposited on the substratum. Finally, the cells acquired a polygonal shape, microfilament bundles became ordered, and the density of extracellular FN fibers was markedly increased. Ectodermal cells displayed microfilament organization and cell-substratum adhesion sites very similar to those of old somitic fibroblasts (not shown).

When cells were labeled for FN-receptor without any fixation and permeabilization, crest cells and young somitic fibroblasts showed an intense patchy staining for FN-receptor covering the entire cell surface. In old somitic fibroblasts, the staining was restricted to limited areas that co-localized with stress fibers. Unfixed epithelial cells did not show any fluorescence, suggesting that FN-receptor molecules were polarized to the surface contacting the substratum (data not shown).

In order to exclude the possibility that the apparent absence of focal contacts in crest and young somitic cells was due to problems of accessibility to antibodies or artifacts of fixation, we isolated focal contacts using saponin treatment of cells (44). While focal contacts could be obtained easily from old somitic fibroblasts, saponin treatment of crest and young somitic cells left only vesicle-like structures on the substratum that were stained both for FN-receptor and for FN, and that did not resemble focal contacts (data not shown).

Involvement of FN-Receptor in Spreading and Displacement of Neural Crest and Somitic Cells

The possible role of FN-receptor in cell spreading and motility was tested using several different types of experiments: (*a*) Cells were allowed to initiate migration on FN-coated substrates, then monovalent Fab' fragments of antibodies for FN-receptor were added to the culture medium. (*b*) Cells were deposited on FN-coated substrates in the presence of monovalent antibodies to FN-receptor to examine for inhibition of initial cell adhesion. (*c*) Cells were deposited on dishes coated only with anti-FN-receptor antibodies, and their ability to adhere or to migrate on this substratum was measured.

In the first set of experiments, neural crest cells were allowed to migrate outward from the neural tube onto FN substrates. There was a dose- and time-dependent inhibition of cell migration and spreading (Figs. 9 and 11*a*). After 24 h in the presence of antibodies, very few crest cells remained spread,

and many cells detached from the substratum; the remaining cells were rounded and tended to form aggregates (Fig. 9, *e* and *f*).

Young somitic fibroblasts responded similarly to monovalent antibodies to FN-receptor, but the initial response was slower than for crest cells (Figs. 10 and 11*b*). In contrast to young somitic fibroblasts, spreading of old somitic fibroblasts was not disturbed by the presence of anti-FN-receptor Fab' fragments, even at a concentration of 2.5 mg/ml (Fig. 10, *d-f*).

In a second type of experiment to examine initial cell attachment, FN-mediated adhesion and spreading were strongly inhibited when monovalent antibodies to FN-receptor were added to neural crest cell suspensions during adhesion assays. Increasing the concentration of antibodies increased the inhibition, and control monovalent antibodies were without significant effect (Fig. 12). Similar results have been obtained with young somitic cells (data not shown).

In a third series of experiments, we tested the ability of crest cells and somitic cells to adhere to antibodies to FN-receptor coated at various concentrations on plastic. The results were compared with the effect on all cell adhesion of FN, type I collagen, and control IgG.

As shown in Fig. 13, antibodies to FN-receptor can mediate cell attachment and spreading for both somitic and neural crest cells in a dose-dependent manner. FN itself had a similar effect as substrate-absorbed antibodies to FN-receptor using both crest and somitic cells. It is interesting to note that anti-FN-receptor antibodies were even more effective on a weight basis than FN as a mediator of crest cell adhesion, and had a similar effect as FN on somitic cells (Fig. 13). Type I collagen and control IgGs were poor substrates for cell spreading. We also examined the time course of spreading of crest and somitic cells (Fig. 13). Crest cells spread more rapidly on anti-FN-receptor and FN than somitic cells, but within 2 h the proportion of spread somitic cells was similar to that of crest cells.

Finally, we tested the ability of neural crest cells to migrate on antibodies to FN-receptor coated on the substratum; the results were compared with the migratory behavior on FN and type I collagen (Fig. 14). On substrates coated with 10-1,000 μ g/ml anti-FN-receptor antibodies, neural crest were able to leave the neural tube but migrated poorly. Crest cells are unusually flattened, polygonal, and adhered to each other; their organization resembled an epithelium (Fig. 14, *a* and *b*). In contrast, crest cells organized into a large halo and exhibited the usual stellate morphology in parallel cultures on FN substrates (Fig. 14, *c* and *d*). As shown elsewhere (43, 48), type I collagen was a poor substratum for crest cell migration at any concentration (Fig. 14, *e* and *f*).

Discussion

In the present study, we have analyzed the localization of the 140-kD glycoprotein complex thought to represent the FN receptor in embryonic cells, with particular emphasis on its function in cell motility. Our major findings are: (*a*) FN-receptor is widely distributed in the avian embryo, but is generally markedly enriched near regions containing FN; (*b*) motile cells differ strikingly from stationary cells with respect to the distribution of the receptor on the cell surface and to

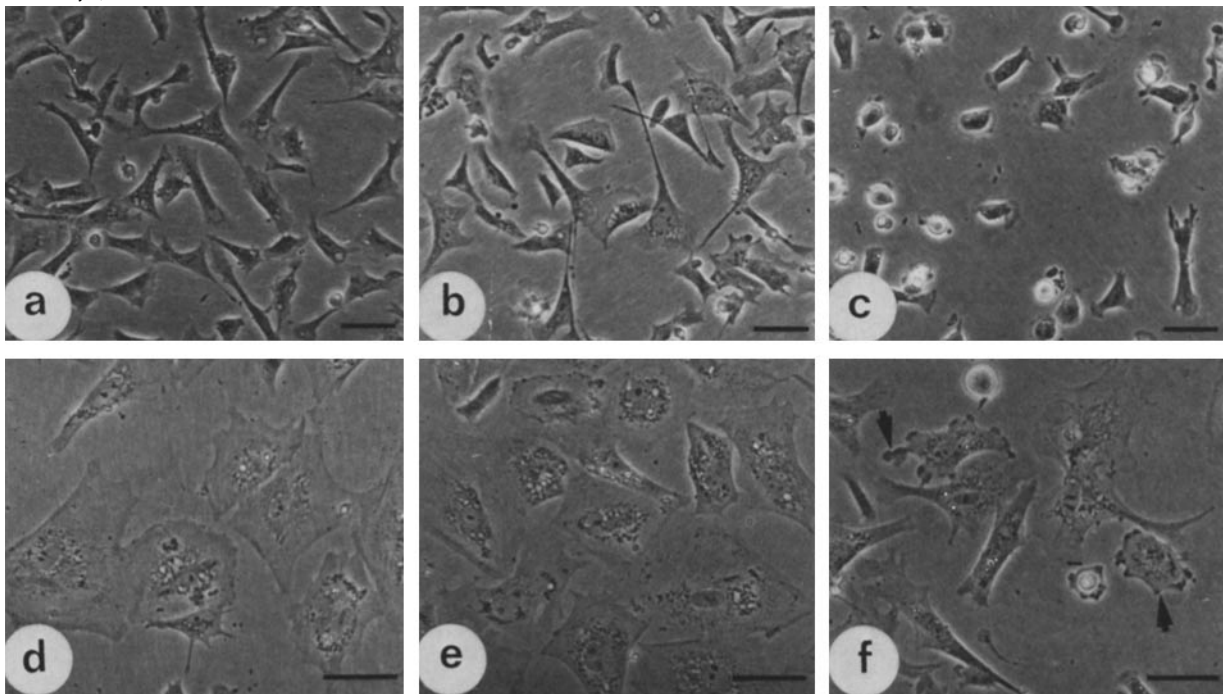


Figure 10. Effect of monovalent antibodies to FN-receptor on young (*a-c*) compared with old (*d-f*) somitic cells. Somitic cells were cultured on FN for 12 h (*a-c*) or 4 d (*d-f*), and monovalent antibodies were added to the culture medium at a final concentration of 2.5 mg/ml. After 7 h of culture in the presence of anti-FN-receptor Fab' (*c*), young somitic cells are frequently round, while in normal medium (*a*), they exhibit the typical triangular shape. In the presence of control Fab' fragments (*b*), young somitic cells may be less spread than in control medium but they never detach from the substratum. Old somitic cells remain flattened after 24 h in normal medium (*d*) or in medium containing control Fab' (*e*). In the presence of anti-FN-receptor Fab' for 24 h, they are less flattened and sometimes show blebs, but they are rarely round and never detach from the substratum (*f*). Bars: (*a-c*) 5 μ m; (*d-f*) 10 μ m.

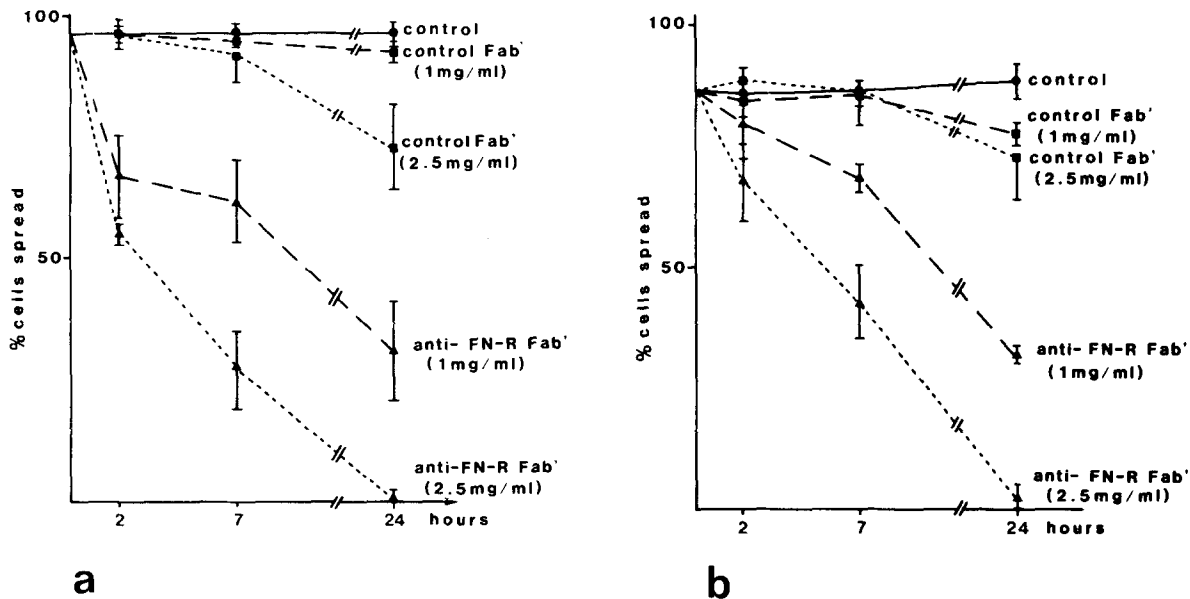


Figure 11. Dose-response curves for detachment of neural crest cells (*a*) and somitic cells (*b*) by monovalent antibodies to FN-receptor. Crest cells and somitic cells were precultured for 12 h on FN. They were subsequently incubated in normal medium or in medium containing control preimmune Fab' fragments or anti-FN-receptor Fab' fragments at final concentrations of 1 mg/ml and 2.5 mg/ml. After 2, 7, and 24 h of incubation, flattened and rounded cells were counted in different areas of the explants. The results are expressed as the percentage of total cells that remained spread at the time indicated.

the organization of cell-substratum contacts; and (*c*) FN-receptor is necessary for both cell spreading and cell motility.

140-kD proteins in several types of cells appear to function as FN receptors involved in binding of FN and adhesion to

FN-coated substrates. The evidence includes immunological inhibition studies (9, 11, 14, 15, 20, 24, 26, 33, 34, 41) and affinity purification of such molecules by FN (47). In avian systems, a 140-kD glycoprotein complex has been character-

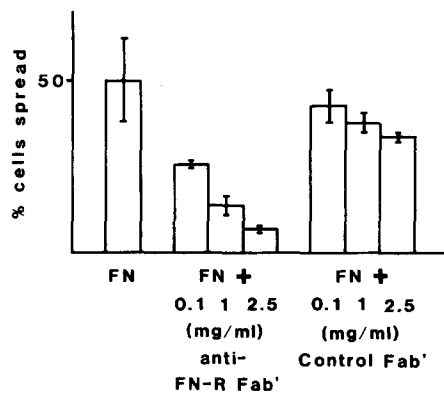


Figure 12. Inhibition of initial adhesion of crest cells to FN by monovalent antibodies to FN-receptor. Cells were incubated for 1 h in Terasaki wells coated with 10 μ g/ml FN in the presence of normal medium or of medium containing either control monovalent antibodies or monovalent antibodies to FN-receptor at final concentrations of 0.1, 1, and 2.5 mg/ml. A total of 100 cells in each well was counted.

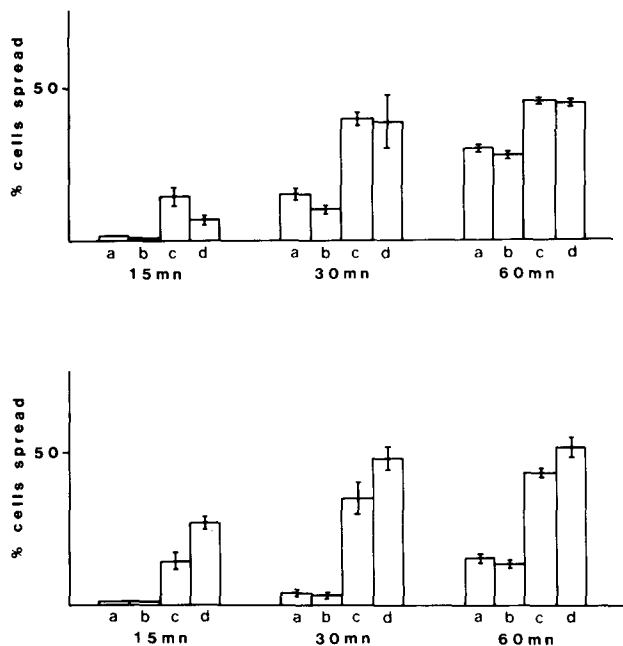


Figure 13. Adhesion of young somitic cells (*top*) and neural crest cells (*bottom*) to substratum-adsorbed antibodies to FN-receptor as a function of time. Cells were deposited in Terasaki wells coated with collagen (a), 0.1 mg/ml control IgG from preimmune rabbits (b), 0.1 mg/ml FN (c), and 0.1 mg/ml antibodies to FN-receptor (d). A total of 100 cells in each well was counted, and the results are expressed as the percentage of original cells that had spread. Note that crest cells adhere to antibodies to FN-receptor more rapidly and effectively than do somitic cells.

ized independently by several laboratories (9, 15, 26, 33, 47). Monoclonal and polyclonal antibodies to this complex inhibit cell adhesion to FN (9, 15, 24, 33, 41), and the complex has recently been shown to bind to FN with modest affinity (2a, 29a). Although we refer to this protein complex as FN-receptor for conciseness, it is important to stress that other molecules such as gangliosides and heparan sulfate may also

be associated with FN receptor functions, and that the 140-kD complex might also sometimes serve as a receptor for other molecules.

In Situ Distribution of FN-Receptor during Avian Embryogenesis

The FN-receptor was found to be nearly ubiquitous in distribution, characterized by strikingly increased quantities on portions of cells in close proximity to areas rich in FN. FN-receptor may thus be available for potential use on most cells in these embryos, but it appears to be redistributed and concentrated at sites of tissue contact with FN. Its functions were examined experimentally *in vitro* using several different cell types cultured from these embryos (see below).

FN-receptor was generally present *in vivo* in cells undergoing morphogenesis, including neural crest cells. The loss of FN-receptor from hematopoietic stem cells after entry into the circulatory system is consistent with a report describing loss of FN receptor activity during erythroid differentiation, as measured by a biological assay (45, 46). In most cases, however, there was no obvious evidence for direct regulation of cell behavior or FN distribution by altered quantities of FN-receptor.

For example, the aggregation of mesonephric blastema cells into tubules was not accompanied by a rapid polarization of the receptor to the basal surface; the redistribution of the receptor only occurred when the tubules were fully differentiated. On the other hand, the emigration of crest cells from the neural tube could be correlated with an increase in staining for the receptor, but only in the head. In the trunk, an increase in labeling for FN-receptor on crest cells was detectable only long after their departure from the neural tube. In addition, while the aggregation of crest cells was accompanied by a disappearance of FN among crest cells, the FN-receptor decreased more slowly than the FN. It is thus possible that the locations and amounts of FN-receptor complex are partially regulated by local concentrations of FN, or that it possesses other functions besides that of an FN receptor. For example, an additional role as a laminin receptor for fibroblastic cells has been suggested for this complex (29a).

Localization of FN-Receptor and Structure of Cell Substratum Linkage Complexes in Nonmotile Embryonic Cells

The organization of cell-substratum adhesion sites has been studied in detail previously in stationary fibroblasts, using either immunoelectron microscopy (13, 52) or a combination of interference reflection microscopy and immunofluorescent labeling (14, 15, 53, 62) on cultured cells. It appears that anchorage of a cell to FN is a complex phenomenon that involves numerous molecules. Vinculin is present at the termini of microfilament bundles and co-distributes with focal contacts (10, 13, 23). α -Actinin is associated with actin in microfilaments and is enriched at the periphery of focal contacts. Outside of the cell, fibers of FN are deposited in areas adjacent to, but not directly in, focal contacts, as well as in sites that correspond to stress fibers (12, 13, 29, 30, 52, 53). In a few cases, FN has been shown to co-localize with linear vinculin-rich sites (52-54).

The distribution of FN-receptor has been described on chicken embryonic fibroblasts (14, 15, 19), myogenic cells

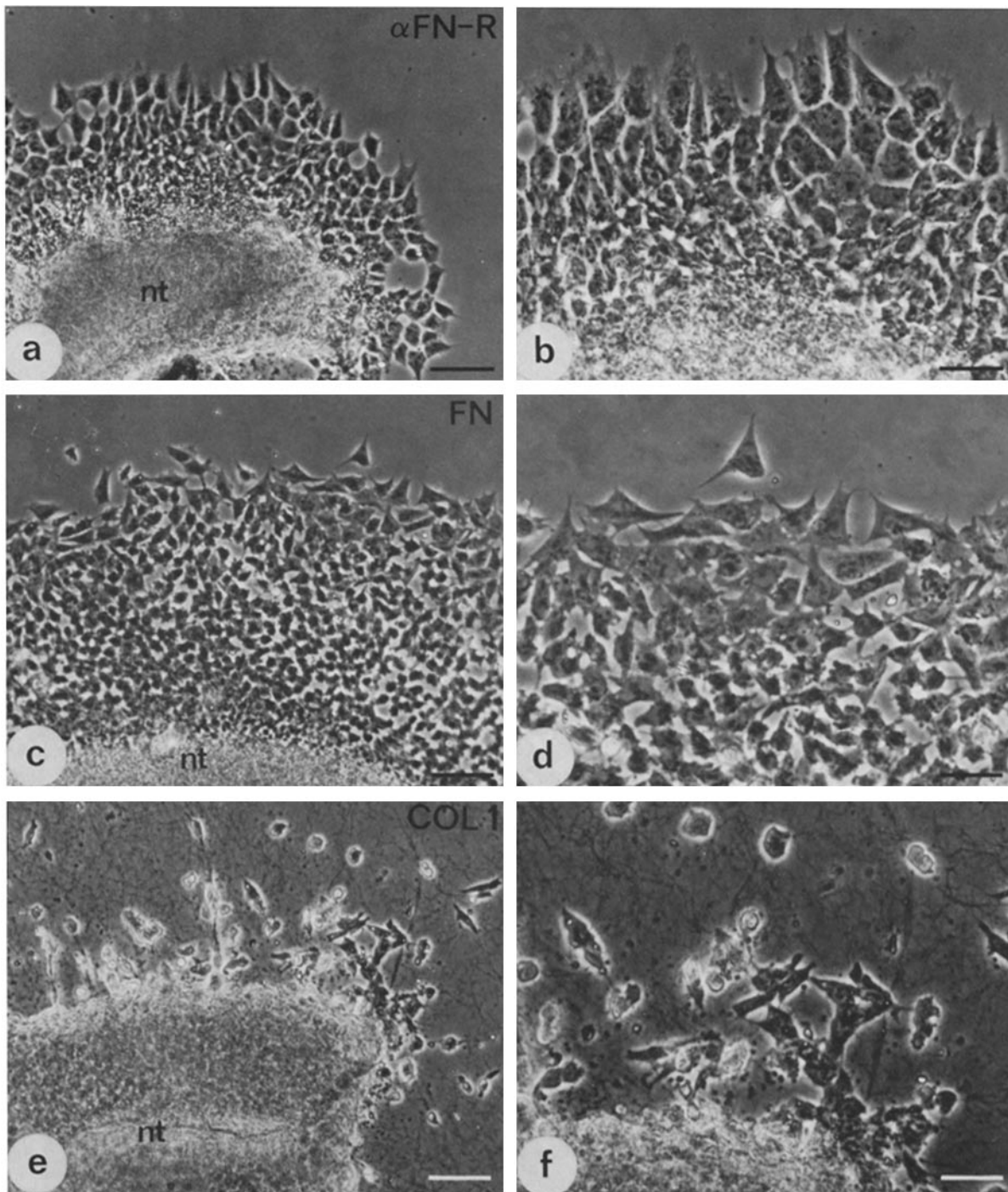


Figure 14. Defective migration of neural crest cells on antibodies to FN-receptor coated on the substratum. Neural tube explants were deposited on coverslips coated with 0.1 mg/ml antibodies to FN-receptor (*a* and *b*), 0.1 mg/ml FN (*c* and *d*), and 0.1 mg/ml type I collagen (*e* and *f*). Crest cells were allowed to migrate on these substrata for 24 h and then photographed, *a*, *c*, and *e* show general views of the halos, and *b*, *d*, and *f* are higher magnifications showing the morphology of the cells. While crest cells on FN develop a large halo and exhibit a typical stellate morphology (*c* and *d*), they form a smaller halo and are more flattened and polygonal on antibodies to FN-receptor (*a* and *b*). On collagen, crest cells migrate poorly and are frequently round (*e* and *f*). Note that for *c* and *d*, the neural tube was mostly (*c*) or entirely (*d*) below the photographic field because of the greater degree of migration on FN. Bars: (*a*, *c*, and *e*) 10 μ m; (*b*, *d*, and *f*) 5 μ m.

(14, 19), and other cell types (14). The present report provides complementary information on its distribution and on the organization of cell surface linkages in nonmotile, early embryonic somite and ectoderm cells. The organization of the

cytoskeleton and the structure of cell-substratum adhesion sites are very similar to those of cultured embryonic fibroblasts. FN-receptor is strikingly enriched at sites aligned with both α -actinin and FN.

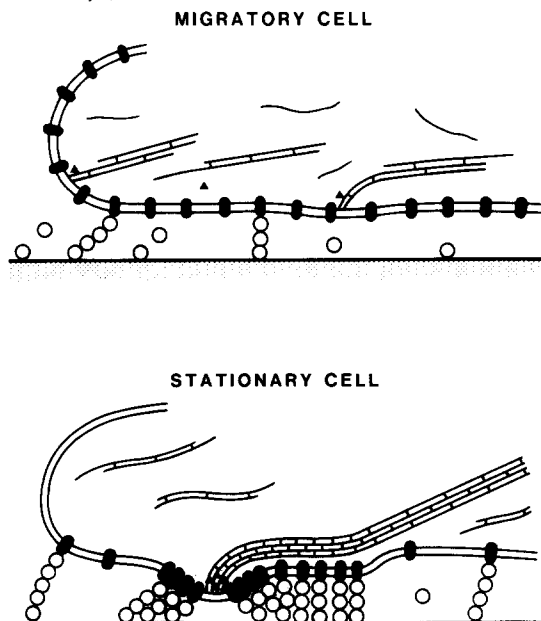


Figure 15. Schematic speculative diagrams comparing modes of cell-to-substratum adhesion of migratory and stationary cells. Migratory cells are characterized by non-organized microfilament bundles and numerous FN-receptors organized diffusely on the membrane. The receptors may be mobile, and a number of them are not bound to FN. In contrast, in stationary cells, FN-receptors are concentrated in clusters close to focal contact sites and microfilament bundles. They are linked directly or indirectly both to the cytoskeleton and FN fibers. These receptors appear to have poor membrane mobility, and most of them are bound to FN fibers. O, fibronectin; ●, FN-receptor; ▲, vinculin; long lines, actin; cross-links between lines, α -actinin.

This concentration and localization of FN-receptor at specific sites in the membrane of stationary cells may provide strong and stable anchorage of the cell to the substratum. This hypothesis is supported by several findings. The staining of living stationary fibroblasts or of ectodermal cells was frequently negative on the upper surface of cells and, when present, was associated with stress fibers at the cell periphery (see also reference 14), indicating its concentration and perhaps immobilization on the lower surfaces of cells and near stress fibers. Saponin treatment of stationary cells permitted the isolation of focal contacts that remained firmly attached to the substratum and were still positive at their periphery for FN-receptor. Finally, stationary cells were more refractory to detachment from the substratum by streams of medium or by trypsin than the motile cells described (unpublished data).

Localization of FN-Receptor and Structure of Cell-Substratum Contacts on Motile Cells

Neural crest cells are highly motile both *in vivo* and *in vitro*. It has been shown previously that FN greatly enhances their motility and that direct interactions between neural crest cells and FN are crucial for their migration (42, 43, 48). Like chick heart fibroblasts, immediately after explantation (17), neural crest cells lack the ability to synthesize FN, and they instead use exogenous FN for their movement (38, 42, 51). This mode of interaction with FN is thought to be important for rapid cell motility (17, 42).

In the present study, we demonstrate that the distribution of FN-receptor is distinctly different on motile cells compared with stationary cells, and that this difference is associated with differences in adhesive site and cytoskeletal organization. In contrast to its accumulation in specific sites on stationary cells, the FN-receptor on motile cells is strikingly uniform and diffuse in its distribution over the entire cell surface of both neural crest and young somitic fibroblasts, even though they differ in morphology and modes of locomotion. In addition, both exhibit very similar cytoskeletal organization; the lack of actin microfilament bundles resembles that reported for rapidly migrating chick heart fibroblasts, which instead display microfilament meshworks (16, 11a). We also established that α -actinin and vinculin localization to bundles was minimal in these motile cells in comparison with their classical specialized localization in stationary cells.

Interference reflection microscopy of these highly migratory cells showed few focal contact sites, accompanied by poor organization of actin microfilament bundles, confirming previous observations in crest cells (60). Interference reflection microscopy also revealed regions of close apposition to the substratum in close adhesions. Attempts to prepare focal contacts from intact motile cells yielded only aggregates or vesicles that labeled for both FN and FN-receptor. In addition, staining of living cells for FN-receptor showed substantial amounts of patched fluorescence on the upper surface of the cells. This finding indicates the presence of numerous receptor molecules that are not redistributed and immobilized on the ventral cell surface (as in stationary cells), and suggests mobility of the receptor within the plane of the membrane (see also reference 25). The presence of free and mobile receptors on the cell surface could be important to permit a labile adhesion of the cell membrane to the substratum and the rapid establishment of new contacts.

Evidence that the FN-receptor is actually involved in cell motility is provided by the perturbation experiments. Blocking the interaction of the receptor with FN inhibits both cell spreading and cell migration. Interestingly, although increasing the concentration of antibodies increases the inhibition of crest cell adhesion (see Fig. 9), increasing the concentration of anti-receptor antibodies does not increase inhibition of stationary fibroblasts (15). This result may reflect the existence of independent adhesive mechanisms as suggested by Decker et al. (20), but it could also be the direct consequence of the establishment of aggregated, polyvalent, high-affinity adhesive complexes that are resistant to antibody inhibition. In support of this hypothesis, we have found that the inhibition of early cell attachment and spreading by anti-FN receptor antibodies could be overcome by increasing the amount of FN absorbed to substrata (unpublished results); this competitive type of relationship between an inhibitor and FN has been attributed to decreasing effectiveness of a competitive inhibitor in the face of increasing multivalent interactions between FN and the FN-receptor (2, 66).

While this manuscript was under review, Bronner-Fraser (8a) reported results complementary to ours using JG22E monoclonal antibodies *in vivo*. Cranial neural crest cell migration *in vivo* was markedly inhibited by the antibodies (8a), producing a pattern of inhibition similar to that obtained previously with inhibitory synthetic peptides from the cell-binding sequence of FN (7). She also reported preliminary results establishing the existence of the JG22 antigen in neural

crest cells, somites, notochord, and neural tube (8a).

In contrast to the inhibitory effects of anti-FN-receptor antibodies present in excess in solution, we find that when they are absorbed to the substratum, these antibodies can effectively mediate the adhesion and spreading of crest cells (see also reference 15). However, the adsorbed antibodies are much less effective in promoting migration. This unexpected deficit in supporting migration may be due to different affinities for the receptor. The affinity of antibodies to antigens roughly averages 10^8 M^{-1} ; this affinity is much higher than the binding affinity of cells to FN, which is 10^6 M^{-1} (1, 2a, 29a). It appears reasonable that binding of such high-affinity ligands to the FN-receptor would produce paralysis of crest cells. Such a result suggests that cell motility may be favored by low-affinity, reversible binding of cell surface receptors to FN and other substrates for cell migration.

Taken together, our results suggest that the putative 140-kD FN-receptor is involved in both cell adhesion and cell migration during development. In motile cells (Fig. 15), FN-receptors are diffusely organized on the cell surface. A low affinity constant, mobility in the plane of the membrane, and the fact that not all of them are bound to FN would permit labile adhesion of the cell to the substratum and an ability of the cell to rapidly establish new contacts with the substratum. The low affinity of the receptor for FN may be important in that it prevents paralysis of the cell, and establishing higher-affinity interactions with another ligand such as anti-FN-receptor antibody absorbed onto the substrate also permits attachment and spreading, but inhibits locomotion.

In contrast, in nonmotile cells (Fig. 15), FN-receptors tend to be immobilized in well-defined areas of cells close to the cell-to-substratum contact sites; the architecture of the cytoskeleton and its linkage with the cell membrane and with the extracellular matrix appears to be highly ordered and stable. Such multivalent fibrillar or plaque-like structures may compensate for the low affinity of the receptor for FN, providing strong anchorage to the substratum. One area for future investigation will be to determine the precise role of each of the three proteins that comprise the avian FN-receptor complex in cell anchorage and cell motility.

The authors thank Dr. Beat A. Imhof for useful discussions, Dr. Daniel Louvard (Institut Pasteur, Paris) for advice and help with interference reflection microscopy, Dorothy Kennedy, Dr. Takayuki Hasegawa, Etsuko Hasegawa, and Susan Yamada for generous help and advice in antibody preparation and characterization, and Lydie Obert, Stephane Ouzounoff, and Sophie Tissot for their valuable help in the preparation of this manuscript.

This work was supported by the Ministère de la Recherche et de la Technologie (MRT 84-C1312), the Centre National de la Recherche Scientifique (ATP 950 906), the Institut National de la Santé et de la Recherche Médicale (CRE 83-4017), and the Association pour la Recherche sur le Cancer (ARC 6455), as well as the U.S. Public Health Service (Intramural Program, and grant HL33711).

Received for publication 31 July 1985, and in revised form 23 September 1985.

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