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Cell-adhesion and substrate-adhesion molecules: their instructive roles in neural crest cell migration

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Summary

The individualization of crest cells from the neural folds and neural tube involves distinct mechanisms affecting both the tissue shape and the cell environment. Though these mechanisms are not fully understood at the molecular level, the analysis of the distribution of several molecules reported to promote intercellular contacts indicates a correlation between cell detachment from the neurepithelium and the decrease of cell adhesion molecule expression. During the following phase of migration, the mode of adhesion of crest cells switches preferentially to the cell-to-substratum type. Various experiments showed that among the major extracellular matrix components, fibronectins were decisive in promoting cell attachment, spreading and motility. Additional studies on receptors for fibronectins gave new insights on the differences between a motile and a stationary cell. These results will be discussed with particular reference to the migration at the cephalic level where most perturbation experiments were performed.

The molecular biology of fibronectins provided a finer understanding of the interactions between a cell and these molecules. The tools derived from this technique will open new areas of investigation hopefully leading to a better understanding of (i) the regulation of the cell–fibronectins interaction and (ii) the specificity of the pathways of migration followed by migrating cells, like the descendants of the neural crest.

Key words: avian embryo, cell adhesion, cell migration, fibronectins.

Introduction

The ability of cells to detach from a formerly cohesive structure and to migrate and contribute to the formation of new tissues is a key event during morphogenesis. A shift from an intercellular mode of adhesion towards a preferential adhesion to an external substrate – *via* the components of the extracellular matrix (ECM) – is therefore necessary if active cell migration is to take place. The dynamic modulation of the expression of cell-to-cell or cell-to-substratum adhesion systems will dictate whether a cell will remain glued to its neighbours or venture into newly opened pathways (see Erickson, this volume). Changes in adhesion properties must accompany and somehow reflect the highly interconnected morphogenetic events. Whether these events result primarily from mechanical forces shaping the tissues or from intrinsic cellular biochemical changes is an open question. Insights into this issue can be provided by understanding the molecular basis of cell adhesion. A search for promoters of adhesion led to the isolation of three major categories of molecules: the components associated with defined junctions (like desmosomes), the cell surface molecules mediating cell–cell interactions or cell adhesion molecules (CAMs), and finally the receptors to the components of the ECM or substrate adhesion molecules (SAMs).

The neural crest (for a review, see Le Douarin, 1982; Noden, 1984) exemplifies the mechanism of modulation of cell adhesion and also provides a powerful paradigm for cell migration (Hörstadius, 1950; Weston, 1970; Le Douarin, 1976; Noden, 1978; Löfberg, Ahlfors & Fällstrom, 1980). The development of crest cells starts with the loss of their epithelial arrangement at the apex of the neural tube followed by extensive migration along well-characterized pathways through adjacent structures (Weston,

1963; Johnston, 1966; Teillet & Le Douarin, 1970; Le Douarin & Teillet, 1974; Noden, 1975; Le Lièvre, 1978; Bancroft & Bellairs, 1976; Tosney, 1978; Duband & Thiery, 1982; Thiery, Duband & Delouvée, 1982*a*; Vincent & Thiery, 1984; Le Douarin, Cochard, Vincent, Duband, Tucker, Teillet & Thiery, 1984; Rickmann, Fawcett & Keynes, 1985; Bronner-Fraser, 1986; Thiery & Duband, 1986; Teillet, Kalcheim & Le Douarin, 1987; Loring & Erickson, 1987). Since many crest cells tend to remain together in their migrating phase and aggregate at their sites of arrest (for example, the precursors of most of the peripheral nervous system) the dominant mode of adhesion must finally revert to the cell–cell type. Among the reasons that led us to employ neural crest to study cell adhesion are (i) the ability to culture crest cells and somewhat mimic their *in vivo* translocations in an *in vitro* system (Cohen & Königsberg, 1975; Rovasio, Delouvée, Yamada, Timpl & Thiery, 1983), and (ii) the possibility of perturbing directly their migration *in vivo* (Boucaut, Darribère, Poole, Aoyama, Yamada & Thiery, 1984; Bronner-Fraser, 1985; Poole & Thiery, 1986). Our studies were carried out in avian species. The current knowledge of neural crest adhesion properties will be discussed with special reference to the mechanism of cellular adhesion to fibronectins (FN).

The formation of the neural crest

Studies carried out in the avian species showed that neural crest cells individualize at the dorsal border of the neural tube after its closure (Di Virgilio, Lavenda & Worden, 1967; Karfunkel, 1974). Though this pattern does not hold for every species (rodent crest cells for instance, emerge early in the neural folds themselves: Nichols, 1981; Erickson & Weston, 1983), the sequence of events leading to the formation of the crest cell population is similar. Premigratory crest cells lose intercellular junctions like gap and tight junctions (Revel & Brown, 1975); the basal lamina formerly surrounding the neural epithelium at their site of emergence is disrupted locally (Nichols, 1981; Tosney, 1982; Duband & Thiery, 1987); cells are able to interact with the ECM components besides those of the basal lamina; their shape turns into a mesenchymal form with numerous probing filopodia (Newgreen & Gibbins, 1982; Tosney, 1982); and finally locomotion starts.

The factors responsible for the local disruption of the integrity of the neural epithelium are not known. However, it is noteworthy that morphogenesis of the neural tube involves a dramatic compression of the epithelial cells of the primitive ectoderm at the level of the apex of the neural folds (Jacobson, 1980). This region, delimiting the boundary between the future ectoderm and neurectoderm, is the site of individualization of crest cells. Recent studies have reported increased synthesis of collagenases by cells grown in collagen gels following contraction of the gel with subsequent compression of the cells (Unemori & Werb, 1986). It is tempting to suggest that the alteration of the shape of the epithelial precursors of crest cells by mechanical forces leads to the local production of proteins which degrade the basal lamina. Good candidates are collagenases and plasmin. Indeed neural crest cells were shown to produce significant amounts of plasminogen activator and plasminogen is diffusely distributed in the embryo at these stages (Valinsky & Le Douarin, 1985).

The recent discovery of various CAMs (for a review, see Edelman & Thiery, 1985; Obrink, 1986) prompted us to look at their distribution during crest cell individualization. Primary CAMs are defined as molecules mediating cell–cell adhesion and which appear quite early during development, regardless of the differentiation program the cells undergo. The neural cell adhesion molecule N-CAM and the liver cell adhesion molecule L-CAM belong to this family (Crossin, Chuong & Edelman, 1985). Another widely distributed CAM is N-cadherin which may be closely related to the adherens junction-specific cell adhesion molecule A-CAM (Volk & Geiger, 1986*a*,*b*; Hatta, Takagi, Fujisawa & Takeichi, 1987). During later stages in development, secondary CAMs are expressed with a restricted distribution on a limited number of cell types. A given cell type may express several of these molecules thus enlarging the repertoire of the adhesive response. Moreover there are various ways of altering the adhesive efficiency. Calcium dependence (influencing L-CAM and N-cadherin, but not N-CAM), cell surface density (a twofold increase of N-CAM induces a 30-fold increase in the rate of aggregation, Hoffman & Edelman, 1983), polar distribution (Hoffman, Friedlander, Chuong, Grumet & Edelman, 1986), *cis*-interactions with other molecules (Hoffman *et al.* 1986) and chemical alteration (the large amount of sialic acid on the embryonic form of N-CAM hinders the formation of the homophilic interaction between the molecules on opposite cell membranes, Rothbard, Brackenbury, Cunningham & Edelman, 1982), all participate in the modulation of the cellular interactions. L-CAM is predominantly expressed in a variety of epithelia but progressively disappears from the surface of the neural epithelium, as revealed by immunofluorescence studies (Thiery, Delouvée, Gallin, Cunningham & Edelman, 1984).

The ectoderm at the border of the presumptive territory of the neural crest still expresses L-CAM. Concomitantly the level of N-CAM, expressed in the three primordial layers, dramatically increases in the

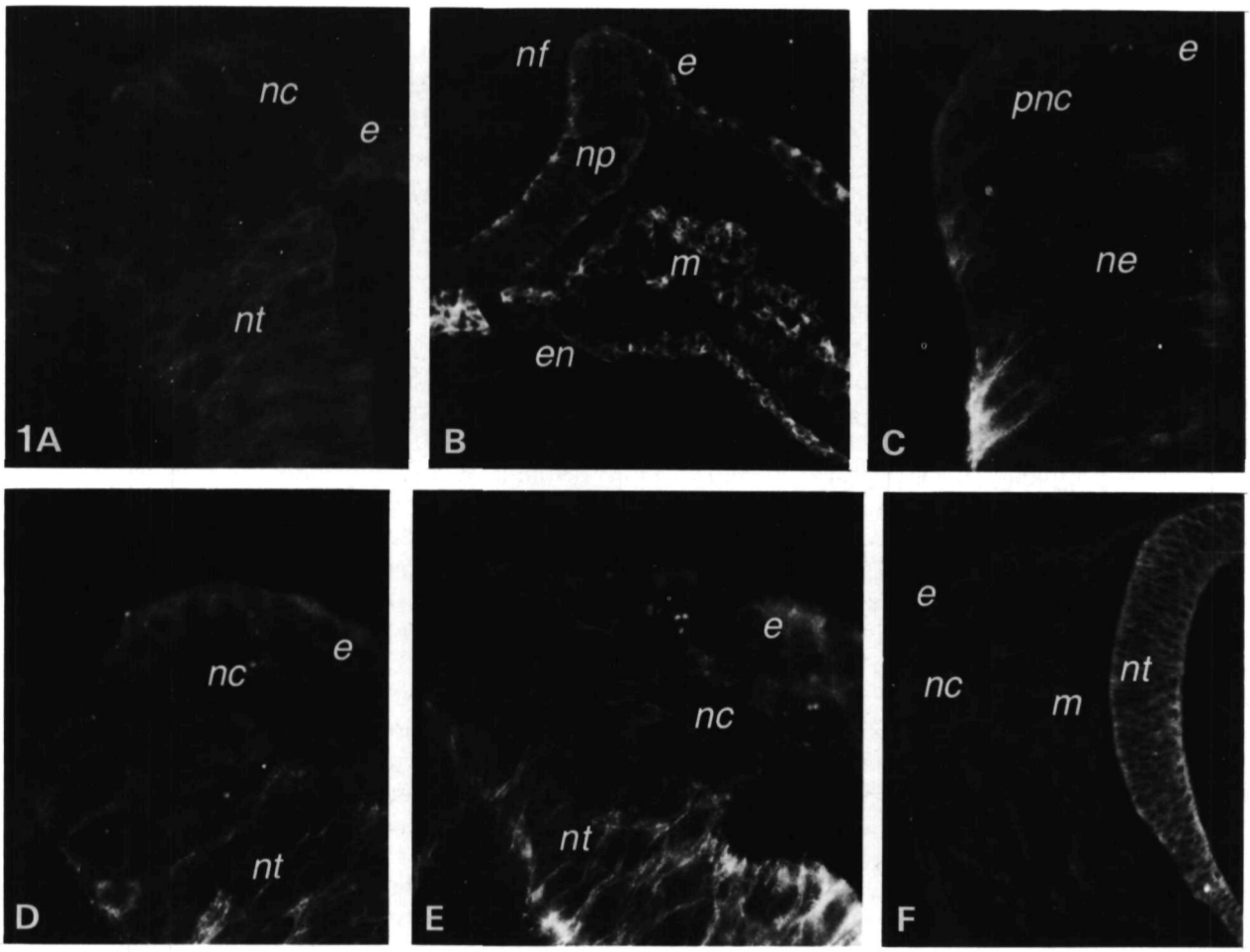

Fig. 1. Distribution of N-CAM (A), and A-CAM or N-cadherin (B–F) during mesencephalic neural crest cell migration as revealed by immunofluorescence studies. 6-somite-stage chick embryo at the time of the fusion of the neural folds. (A) The staining level for N-CAM is about the same in the neurepithelium proper and on the premigratory crest cells (though slightly lower on the latter). (B) Overall staining for A-CAM of the mesencephalon of a 4-somite-stage embryo prior to neural fold fusion. (C) Same stage as in B at the apex of the neural fold. Premigratory crest cells are weakly stained as compared to the intense apical and basal staining of neural epithelial cells. (D) 6-somite-stage embryo. A-CAM level increases on neural epithelial cells whereas individualizing crest cells are devoid of staining. (E) 8-somite-stage embryo. The neural tube closure is almost complete and A-CAM staining expands into the epithelial cells at the apex of the tube. Crest cells leaving the neural tube are not stained. (F) 12-somite-stage embryo. Migrating crest cells are devoid of staining, but the neurepithelium in contrast is intensely stained. Compare with the distribution of crest cells on Fig. 4C at a similar level and stage. *e*, ectoderm; *en*, endoderm; *m*, mesenchyme; *nc*, neural crest; *ne*, neural epithelium; *nf*, neural folds; *np*, neural plate; *nt*, neural tube; *pnc*, premigratory neural crest.

neural epithelium. Thereafter crest cells leaving the neural tube progressively lose N-CAM immunoreactivity (Fig. 1A; Thiery, Duband, Rutishauser & Edelman, 1982*b*; Edelman, Gallin, Delouvée, Cunningham & Thiery, 1983; Duband, Tucker, Poole, Vincent, Aoyama & Thiery, 1985). N-cadherin is present in the neurepithelium but its disappearance from the emigrating crest cells is more rapid and striking than for N-CAM (Fig. 1B–F; Hatta *et al.* 1987; Duband, Volberg, Sabanay, Thiery & Geiger, 1988). The pattern of disappearance of the CAMs from the neural crest cell surface thus correlates with individualization and migration, and suggests their involvement in the adhesive behaviour of these cells. Interestingly, N-CAM and N-cadherin were detected *de novo* in many neural crest derivatives after aggregation of the cells (Thiery, Duband, Rutishauser & Edelman, 1982*b*; Duband *et al.* 1985, 1988; Hatta *et al.* 1987). The secondary CAM, neurone–glia cell adhesion molecule (Ng-CAM) was also expressed (Thiery, Delouvée, Grumet & Edelman, 1985). Molecular probes for CAMs messengers will permit an

early detection of their expression and help to understand the regulation and modulation of cell adhesion (see Kintner & Melton, 1987).

Interaction with the ECM

Direct access to the components of the ECM has been shown to promote epithelial cell migration (Greenburg & Hay, 1982). Ultrastructural studies revealed the presence of ECM in the spaces colonized by crest cells (and also prior to their migration) and along their pathways of migration (Bancroft & Bellairs, 1976; Ebendal, 1977; Tosney, 1978; Hay, 1978). Various authors characterized this ECM composition: glycosaminoglycans (especially hyaluronate) and chondroitin sulphate are found (Derby, 1978; Pintar, 1978; Pratt, Larsen & Johnston, 1975), together with collagens of various subtypes (von der Mark, von der Mark & Gay, 1976; Duband & Thiery, 1987), and glycoproteins such as laminin, cytotactin and fibronectins (Newgreen & Thiery, 1980; Mayer, Hay & Hynes, 1981; Crossin, Hoffman, Grumet, Thiery & Edelman, 1986; Duband & Thiery, 1987; Tan, Crossin, Hoffman & Edelman, 1987). Some of these components are found exclusively in the basal lamina surrounding the structures encountered by crest cells (like laminin and type IV collagen). Not only the adjacent tissues (e.g. neural tube, ectoderm, notochord and somites) but also the neural crest cells have been shown to deposit this ECM (Manasek & Cohen, 1977; Greenberg & Pratt, 1977; Pintar, 1978; Solursh, Fisher, Meier & Singley, 1979; Newgreen & Thiery, 1980).

Several reports showed the importance of many of these molecules in promoting cell spreading and locomotion (Newgreen, Gibbins, Sauter, Wallenfels & Wütz, 1982; Rovasio *et al.* 1983; Tucker & Erickson, 1984; Bee & Newgreen, this volume). However, fibronectins (FN, see next section) either alone or associated with other ECM components, enhance neural crest cell attachment to the substrate and promote crest cell motility. Hyaluronate, chondroitin sulphate, collagens, laminin, and serum proteins do not enhance substrate attachments and instead may favour cell aggregation (Erickson & Turley, 1983; Newgreen *et al.* 1982; Rovasio *et al.* 1983; Tucker & Erickson, 1984). The preferential adhesion to FN can be demonstrated when trunk crest cells are cultured on a substrate consisting of FN stripes. Crest cells spread and migrate on FN while they stop and aggregate on stripes devoid of FN (Rovasio *et al.* 1983). Clearly, the adhesion properties of cranial crest cells merit closer examination. Using immunofluorescence for FN, Newgreen & Thiery (1980) studied the *in vitro* ability of crest cells from all levels of the neural axis to synthesize and deposit FN (Fig. 2). Crest cells from sacral levels and peripheral cells from cranial neural crest explants were found to contain cytoplasmic or extracellular fluorescence after FN-antibody treatment and immunofluorescence visualization. The synthesis and deposition of FN by a cell may prevent its extensive locomotion because a network of FN fibres develops around the cell and tends to immobilize it (Couchman, Rees, Green & Smith, 1982). Cranial crest cells migrate in a relatively large cell-free space (Pratt *et al.* 1975) whereas trunk crest cells migrate in narrow pathways between adjacent tissues and basal laminae where population pressure may be critical (Thiery *et al.* 1982*a*; Rovasio *et al.* 1983). The relevance of FN synthesis by a subpopulation of cranial crest cells *in vitro* might be related to their migration into a cell-free space where FN could form a scaffolding for pluridirectional migration.

FN and crest cell migration

Fibronectins are multifunctional glycoproteins involved in haemostasis, cell differentiation, phagocytosis and malignancy (see Hynes & Yamada, 1982; Furcht, 1983; Akiyama & Yamada, 1983; Yamada, Akiyama, Hasegawa, Hasegawa, Humphries, Kennedy, Nagata, Urushihara, Olden & Chen, 1985; Hynes, 1985; Dufour, Duband & Thiery, 1986 for reviews) in addition to their role in cellular migration. Two forms are produced: soluble or plasma FN and the tissue FN found in ECM. The FN molecules consist of two similar chains (approximately 220×10^3 M_r) linked at their carboxy terminal ends by disulphide bridges (Fig. 3). Schematically, each chain presents a series of domains characterized by the macromolecules they bind (heparin, fibrin, collagen). One of these domains (75×10^3 proteolytic fragment) was initially characterized as the cell-binding part and the peptide sequence involved was reduced to the so-called RGDS binding site (Arg–Gly–Asp–Ser) (Pierschbacher & Ruoslahti, 1984). Early experiments showed the involvement of FN in neural crest cell motility by directly perturbing *in vivo* migration after injection of monovalent antibodies against the RGDS cell-binding domain. The pattern of migration was thus dramatically altered when the antibodies were injected into the cell-free space of the cephalic region (Poole & Thiery, 1986). A similar effect was observed if small peptide fragments containing the RGDS sequence were injected (Fig. 4), thus directly correlating the presence of FN in the cranial pathways with the migratory ability of cephalic crest cells *in vivo* (Boucaut *et al.* 1984). Crest cells must differ from nonmotile neighbour cells in the way they interact with the ECM substrate. Investigation of potential differences in the distribution of receptors to FN on

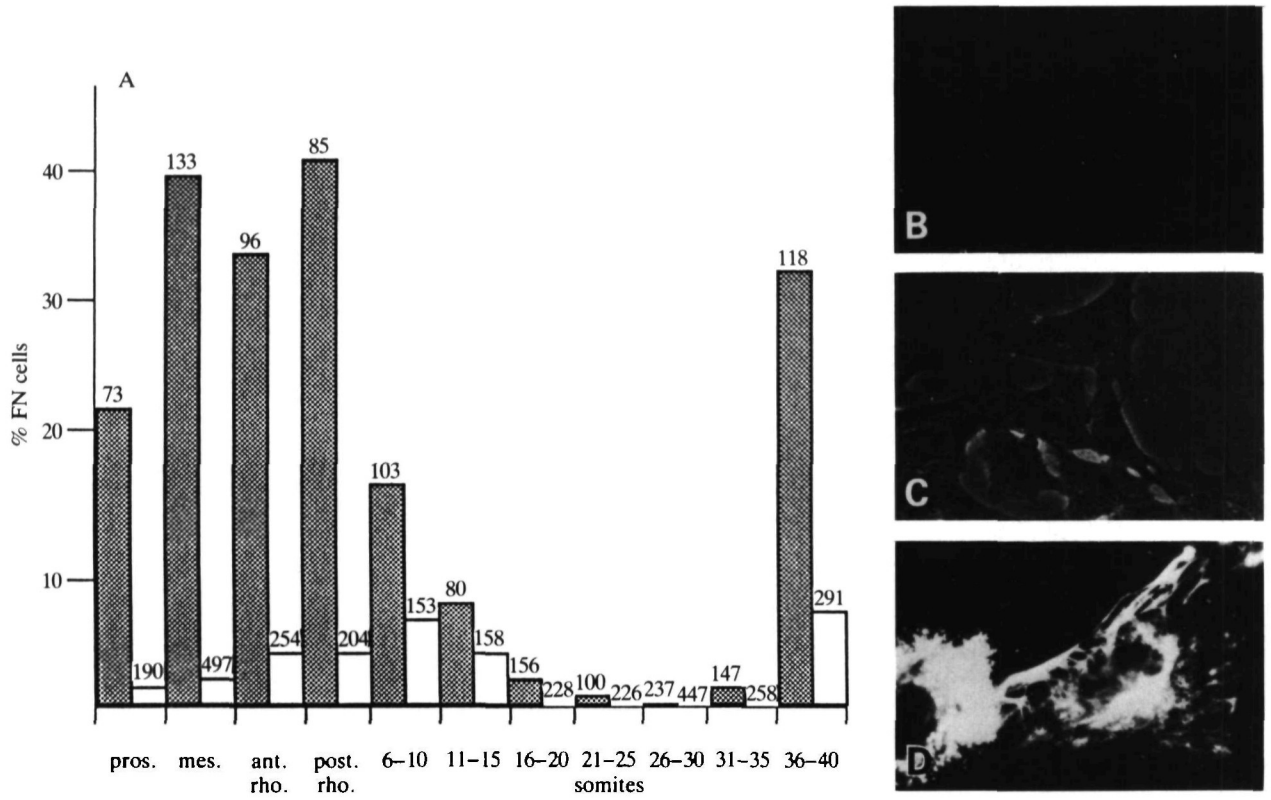

Fig. 2. (A) Plot of the frequency of crest cells with cytoplasmic anti-fibronectin fluorescent granules against the level of origin of the neural tube explant at the respective times of crest cell emigration. Cells were cultured for 72 h. Hatched columns represent cells at the periphery and open columns show central cells of the crest cell halo. The numbers above the columns refer to the total number of cells counted in each case. Fluorescence granules were predominantly found in peripheral cranial and sacral crest cells whereas almost all intermediate levels contain crest cells with few fluorescent granules. (B, C) Immunofluorescence and phase contrast of trunk crest cells. No reactivity is found. (D) Two peripherial cranial crest cells showing cytoplasmic perinuclear fluorescent granules.

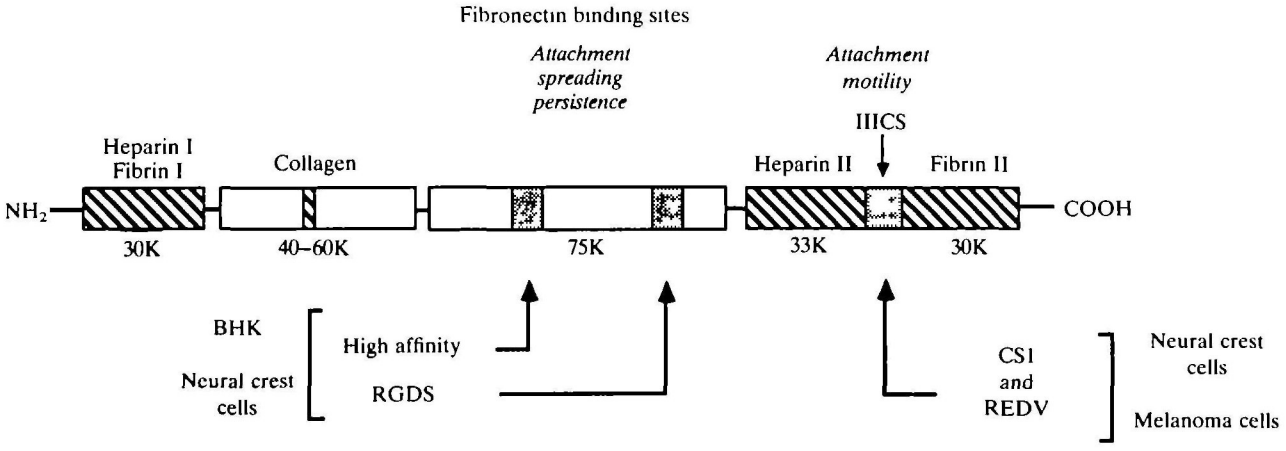

Fig. 3. Schematic representation of the FN molecules. Only one chain of FN is represented. Hatched areas indicate the binding domains for several macromolecules. Thin lines in the chain represent the protease-sensitive sequences. Grey areas indicate the approximate location of the cell recognition sites. Baby hamster kidney cells (BHK) use the Arg–Gly–Asp–Ser (RGDS) and the high-affinity site. B16–F10 melanoma cells use the low affinity Arg–Glu–Asp–Val (REDV) binding site in conjunction with the CS1 site located in the type III homology connecting segment (IIICS). Neural crest cells can utilize all sites except REDV (unpublished observations). The presumptive roles of these sites are also indicated in the upper part of the figure.

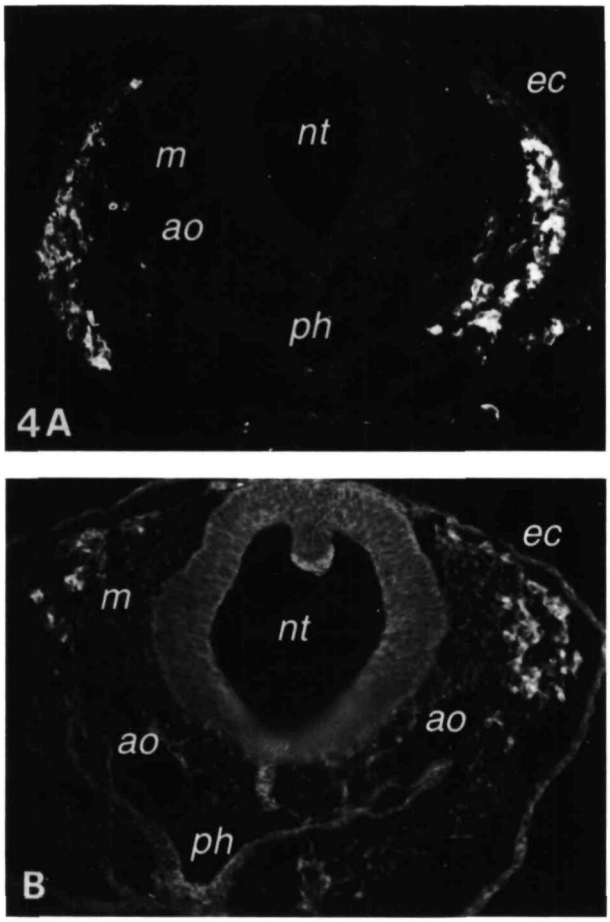

Fig. 4. Perturbation of cephalic neural crest cell migration *in vivo*. The embryo was injected *in ovo* 8 h earlier with either a buffer solution (A) or with a decapeptide containing the RGDS sequence (B) in the cell-free space at the midmesencephalon level prior to crest cell migration. The sections were stained with a monoclonal antibody recognizing migrating avian crest cells (Vincent & Thiery, 1984). (A) Section through the site of injection at the 11-somite stage. Crest cells have reached the lateroventral part of the embryo near the pharynx. (B) The injected embryo reveals a dramatic inhibition of crest cell migration and cell proliferation in the cell-free space. Most crest cells were not able to leave the neural tube and were forced into the lumen. The neural tube and the trapped crest cells both exhibit a positive staining typical of the antibody (see Vincent & Thiery, 1984 and Tucker, Delarue, Zada, Boucaut & Thiery, 1988 for further details). *ao*, aorta; *ec*, ectoderm; *m*, mesenchyme; *nt*, neural tube; *ph*, pharynx.

the surface of stationary *versus* motile cells was a logical experimental progression.

The family of FN-receptors

Several molecules have been shown to exhibit the properties of FN-receptors (see Leptin, 1986, or Hynes, 1987 for a review) and exhibit different affinity or specificity for FNs. In birds, FN-receptors correspond to the described 140×10^3 (140K) complex or CSAT antigen and consist of two alpha chains (140K and 160K) and one beta chain (120K). The three chains appear to be essential for the binding to FN (Buck, Shea, Duggan & Horwitz, 1986). This receptor may recognize other ECM molecules like laminin, vitronectin or collagens (Horwitz, Duggan, Greggs, Decker & Buck, 1985). The location of this 140K FN-receptor complex was studied during neural crest individualization and migration (Fig. 5 and Duband, Rocher, Chen, Yamada & Thiery, 1986). These investigators concluded that the receptor complex was mainly located in FN-rich regions and not particularly expressed on neural crest cells. The complex is found on epithelial cells (predominantly at the basal surface where FN is concentrated) as well as on mesenchymal cells (where it codistributes with FN at the cell surface). The differentiation of the behaviour of stationary *versus* motile cell types is, therefore, not an expression of the FN-receptors being restricted to the surface of migrating cells. At all levels, crest cells, like other cell types, exhibit higher amounts of FN-receptors when they encounter FN.

Could there be a difference in the FN-receptor complex distribution on the cell surface? When crest cells are cultured on FN from a neural tube explant, they migrate away from the explant and develop close adhesions to the surface. Relatively few focal contact sites are found under the cell processes, as revealed by interference reflection images (Duband *et al.* 1986). The 140K FN-receptor complex was localized evenly over the whole surface of the cell (Fig. 6A). Young somitic fibroblasts are motile and exhibit a similar distribution of focal contacts and FN-receptors. On the other hand, somitic fibroblasts or crest cells cultured for several days, or ectoderm cells (cells that are not, or no longer, motile and present numerous focal contacts), show that the FN-receptors accumulate in specific areas close to the cell-to-substratum contact sites (Fig. 6B). Also in stationary cells, the cytoskeletal filament actin is organized into microfilament bundles and stress fibres radiating all over the cell area. Talin and vinculin, two other cytoplasmic molecules involved in transmembrane association with FN (Singer & Paradiso, 1981), codistribute with the numerous focal contacts. In contrast, motile cells lack microfilament bundles and talin and vinculin are diffuse throughout the cytoplasm. In addition, vinculin can be detected within the few focal contacts made by the cell processes. Finally, stationary cells are able to synthesize and deposit FN on the substratum. The FN-receptor serves as a link between the ECM and the cytoskeleton and recent experiments measured the lateral mobility of this FN-receptor complex in the membrane. The results show

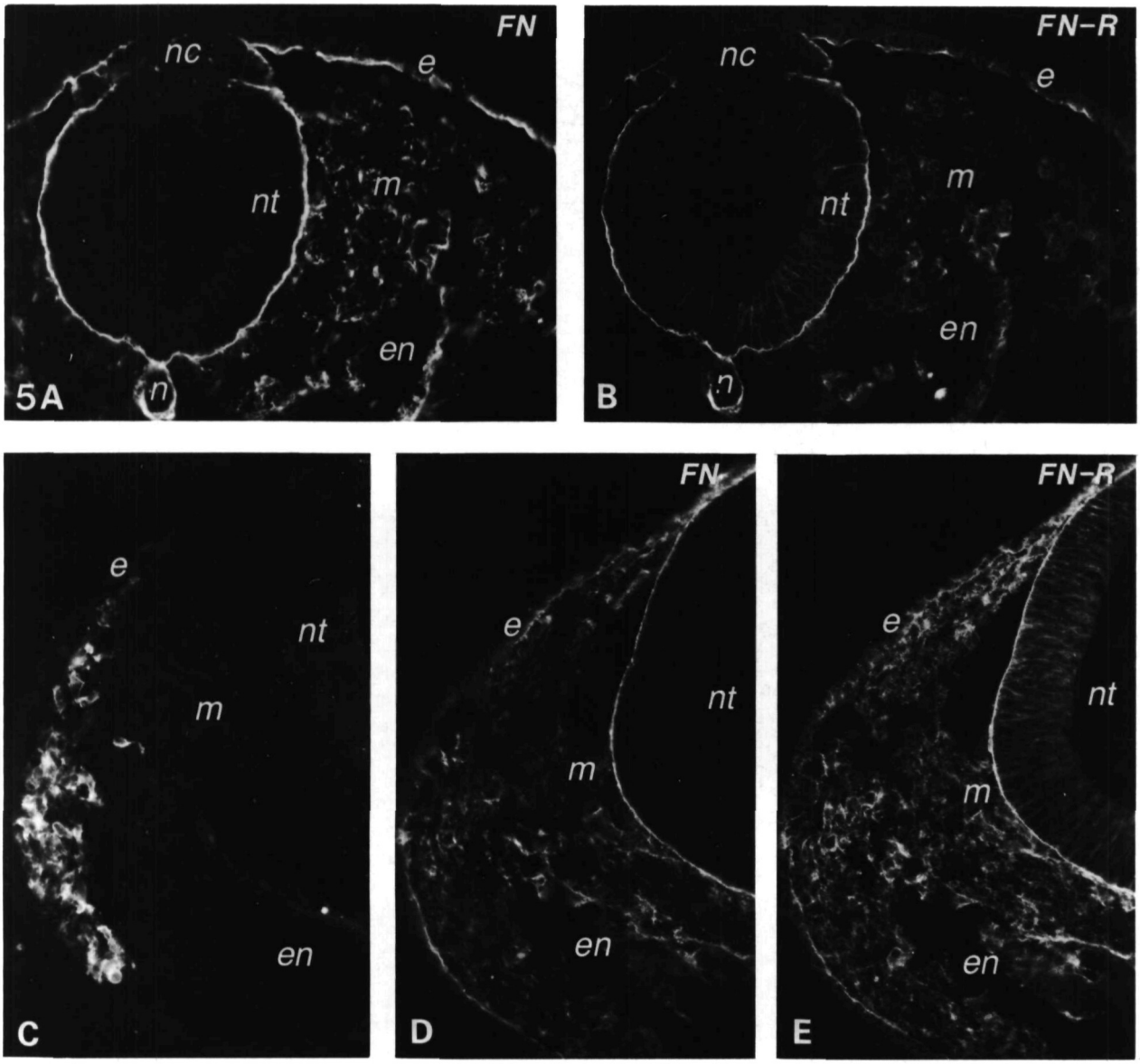

Fig. 5. Distribution of FN and their receptor during avian cephalic crest cell migration. Transverse sections at the mid-mesencephalon level were double-stained for FN (A,D) and the 140K glycoprotein receptor complex (B,E). Crest cells were revealed by staining adjacent sections (C) with the same antibody as in Fig. 4. (A,B) 8-somite-stage chick embryo. Crest cells starting to migrate from the neural tube are delimited by FN and express FN-receptor. (C–E) 15-somite embryo. Crest cells are migrating laterally between the ectoderm and the cephalic mesenchyme (C) in FN-rich environment (D) and are labelled for the FN-receptor (E). At both stages, FN is present in the mesenchyme and in the basement membrane of epithelia. Cells derived from the three primary germ layers express the fibronectin receptor at their surface. Neural crest cells do not exhibit a higher reactivity than adjacent cells. *e*, ectoderm; *en*, endoderm; *m*, mesenchyme; *n*, notochord; *nc*, neural crest; *nt*, neural tube.

that more than 70% of the receptors can diffuse readily in motile crest cells. In contrast, less than 20% remain mobile when cells arrest and develop microfilament bundles and the receptors are included in focal contacts (J. L. Duband *et al.* unpublished data).

Schematically, a motile cell would present diffuse mobile FN-receptors many of which are not linked to FN and nonorganized microfilament bundles. Conversely, the FN-receptors of stationary cells cluster near focal contacts and microfilament bundles and serve as a direct link between the FN fibres they bind and the cytoskeleton (Fig. 7). The transmembrane link comprises FN fibres on the external side and cytoplasmic molecules on the intracellular side. Among these, vinculin, alpha-actinin and talin have

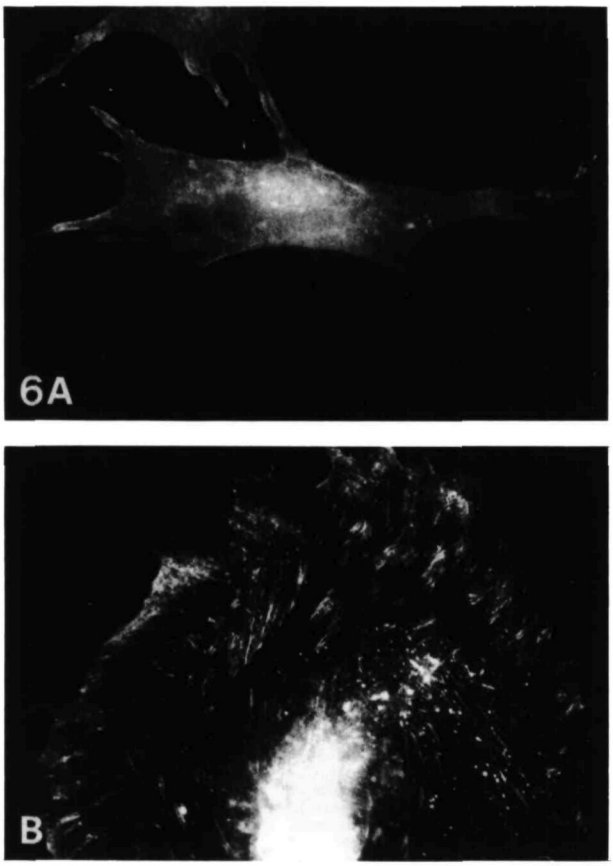

Fig. 6. Immunofluorescence distribution of FN-receptors on motile (A) and stationary (B) cells. (A) Migratory neural crest cells after 24 h in culture. The FN-receptor pattern of expression is diffuse with local concentrations restricted to the ends of the cell processes. (B) Stationary 'old' somitic fibroblast (4 days in culture). In contrast to the migrating crest cells, this cell has developed numerous focal cell contacts, where the FN-receptors are concentrated, colocalized with stress fibres. Higher magnification would reveal a needle-eye-type pattern of localization for both FN-receptor and alpha-actinin around the areas of focal contacts (not shown).

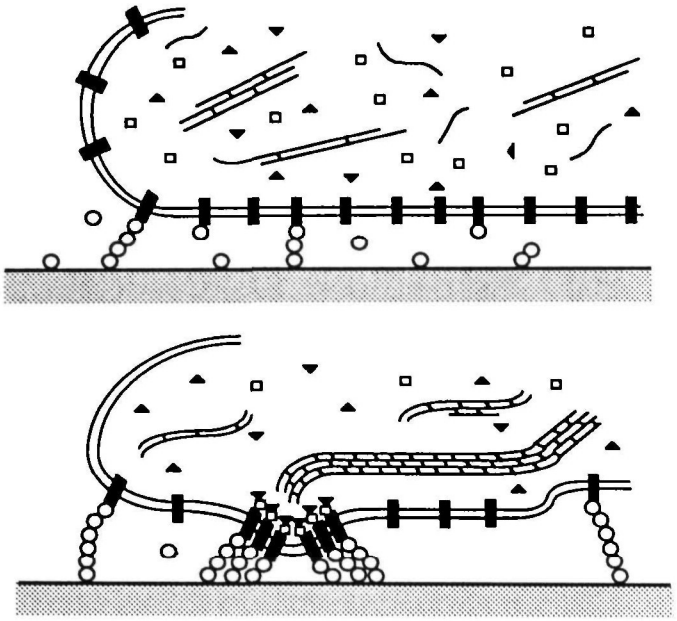

Fig. 7. Diagrams depicting the differences between a motile (A) and a stationary cell (B). A migratory cell exhibits nonorganized microfilament bundles (long lines for actin and short ones for alpha-actinin), vinculin (open squares) and talin (triangles) as soluble molecules and a diffuse distribution of mobile FN-receptors at the cell surface. Many FN-receptors are not bound to extracellular FN. Stationary cells, on the other hand, display clusters of FN-receptors concentrated close to the focal contacts and microfilament bundles. The FN-receptors have a poor membrane mobility and build a transmembrane link between the extracellular FN and the cytoskeletal compartment via talin, vinculin and other uncharacterized cytoplasmic molecules.

been shown to participate in the attachment of actin filaments to the internal side of the membrane (for a review, see Burridge, 1986). In particular, talin binds to the FN-receptor (Horwitz, Duggan, Buck, Beckerle & Burridge, 1986) and phosphorylation of the latter may prevent this binding (Hirst, Horwitz, Buck & Rohrschneider, 1986; A. Horwitz, personal communication). The state of the neural crest cell FN-receptors with respect to phosphorylation is currently under investigation.

The ability to synthesize and deposit FN could also modulate the distribution of the FN-receptors at the surface of the cell and favour the appearance of FN-receptor clusters. It is noteworthy that transformed cells exhibit similar characteristics to motile cells including no production of FN, a disorganized cytoskeleton and a diffuse distribution of the FN-receptors (Yamada, Ohanian & Pastan, 1976; Willingham, Yamada, Yamada, Pouyssegur & Pastan, 1977; Ali, Mautner, Lanza & Hynes, 1977; Hynes, 1981; Chen, Wang, Hasegawa, Yamada & Yamada, 1986). After addition of FN to the culture medium, transformed cells temporarily resume the normal phenotype characteristic of a stationary cell (Chen *et al.* 1986).

Antibodies to the FN-receptors perturb both cell spreading and cell motility when in suspension in the culture medium (Duband *et al.* 1986). Migration of cephalic neural crest cells was prevented *in vivo* by injection of antibodies to the FN-receptors (Bronner-Fraser, 1985), thus presenting more evidence of the involvement of the FN and FN-receptor molecules in crest cell migration.

However, since both stationary and motile cells have FN-receptors and encounter FN strands, why is it that motile cells do not rapidly stop migration after FN linkage and display the stationary phenotype? A low affinity for FN could be a possible explanation.

This, coupled to a diffuse cell surface distribution of a mobile receptor, would allow a motile cell to establish reversible binding to the substrate. A low FN affinity is not inconsistent for a stationary cell, since the FN-receptors tend to cluster and thus promote stronger local cell attachment. The binding affinity of cells to FN is about $10^6 mole^{-1}$, i.e. 100 times less than the affinity of antibodies to their antigens. When crest cells are cultured onto anti-FN-receptors adsorbed to the substratum, cell attachment and spreading still occur (and are even more pronounced) but cell migration is considerably reduced as opposed to migration on a FN substrate (Duband *et al.* 1986). This work indicated that a low affinity for FN might be the key to effective migration. In fact, the interaction of a cell with FN is not as simple as the recognition of the RGDS binding site. Other sites on FN molecules and the RGDS-mediated interaction are necessary, but not sufficient, to promote migration and some cells may even by-pass this interaction.

FN are also known to be very sensitive to destruction by protease activity. A possible strategy for a motile cell to prevent its arrest in or on a FN network would consist of degrading the FN molecules themselves thus minimizing the risk of strong interactions through clustering of FN-receptors. Transformed cells have been reported to synthesize surface metalloproteases that degrade FN (Chen, Olden, Bernard & Chu, 1984; Chen & Chen, 1987). The motile neural crest cells interacting with FN may also express localized proteolytic activities at their surfaces.

Is FN-binding sufficient to explain the specificity of the neural crest cell pathways of migration?

The importance of the RGDS-binding site in cell adhesion to FN derived from the study of the interactions of different FN proteolytic fragments. Other approaches can be devised to investigate this interaction, including the use of synthetic peptides spanning part of the FN sequence or fusion proteins having a fragment of the FN molecule. Using several of these approaches, Humphries & colleagues (1986, 1987) showed two additional sites involved in the selective adhesion of B16-F10 melanoma cells to FN. Baby hamster kidney cells did not recognize these two additional FN binding sites. A particularly interesting feature of these sites is their location in the type III homology connecting segment (III CS region, see Fig. 3). This region is known to be alternatively spliced and to give rise to the different variants of FN chains. The differences in the subunits of one FN molecule arise mainly from this differential splicing at the RNA level in the III CS region (Kornblihtt, Vide-Pedersen & Baralle, 1984). Moreover, the pattern of interaction with FNs requires three distinct sequences: (i) attachment, (ii) spreading and (iii) motility in the case of a migrating cell. The RGDS-binding site appears to be important in the first two phases (see Pierschbacher & Ruoslahti, 1984; Yamada & Kennedy, 1984; Singer, Kawka, Scott, Mumford & Lark, 1987; Streeter & Rees, 1987). Motility *per se* could be the result of the interaction with RGDS and another binding site and the two distinct sites found for melanoma cell interaction are good candidates. That some cell types (like baby hamster kidney cells) do not recognize the additional FN sites could result from the lack of proper receptors and argues for more complexity in cell-to-FN interactions. This process is further complicated by the heterogeneity of FN molecules themselves due to differential RNA splicing. The binding sites described by Humphries and colleagues could well be relevant to neural crest migration since melanoma cells are derived from the neural crest. Obviously the preferential deposition of FN molecules with or without a given cell binding site along possible crest cell migration pathways would provide a consistent explanation for the choice made by crest cells. Fibronectins are known to be ubiquitous (as revealed by polyclonal antibodies) and can be detected in possible pathways of migration not used by crest cells (Duband & Thiery, 1982; Thiery *et al.* 1982*a*; Krotoski, Domingo & Bronner-Fraser, 1986). The recent developments of the molecular biology of FN will soon enable the detection of one given type of FN. Hopefully, the use of molecular probes or specific antibodies will shed new light on the preferential migration patterns of neural crest cells. Though additional mechanisms may also account for cell motility besides FN, crest cell migration has now developed new perspectives with more sophisticated examination of the mechanisms of FN binding to their external membrane receptors.

Conclusion

Studies on receptors to FN have provided new insights into the differences between a motile and a stationary cell. The factors responsible for the arrest of the cell, i.e. the shift from a motile to a stationary state, are still under current investigation as well as those responsible for the inverse condition. Undoubtedly the morphology and the biochemical composition of the environment are both crucial to these processes. Interestingly the presence of FN is not always sufficient to explain neural crest motility and the choice of directionality. Early experiments revealed FN *in vivo* in putative pathways of migration

not known to be reached by crest cells. One interpretation could be that other factors alter or prevent the interaction of the crest cell with FN. Possible candidates are the cytotactin molecule and its proteoglycan receptor, as reported by Tan *et al.* (1987). Alternatively the different forms of FN variants, with distinct cell-binding activities, may be distributed in specific patterns contributing to an overall ubiquitous pattern. The molecular biology of FN will provide the tools to test this hypothesis. Advances in the cell-binding domains have revealed a far more intricate way of interaction of a cell with FN than thought before. Cells like B16-F10 melanoma interact with FN through sites located in the III-CS connecting segment. Crest cells can also interact with FN through these sites in addition to the RGDS-mediated interaction. The binding to FN is thus probably further complicated by the existence of other cell surface receptors. Even the mere interaction of crest cells with the RGDS sequence is not sufficient to promote locomotion. The affinity of this peptide alone or coupled with adjacent sequences of FN for the cell is far less than that of the whole molecule (Akiyama, Hasegawa, Hasegawa & Yamada, 1985). It has been reported (Yamada & Kennedy, 1987; Humphries & Yamada, 1987) that other molecules with RGDS sequences or similar peptides, do not exhibit adhesive properties. The search for additional FN recognition sites on FN molecules led to the discovery of a high affinity binding site that is necessary to promote cell attachment and migration (Fig. 3). In cell attachment and spreading tests using fusion proteins of FN spanning these sites, the activity coupled with that of the RGDS site is almost equivalent to that of intact fibronectin. A fusion protein containing only the RGDS site was 50-times less active (Obara, Kang, Rocher-Dufour, Kornblihtt, Thiery & Yamada, 1987).

Altogether, these results reveal many intricate ways available for a cell to interact with one given molecule. The interactions with or between several other molecules whose relative ratios could dictate the amount of spreading, attachment and locomotion provide additional mechanisms of modulation of the cell behaviour. Evidence has been obtained to acknowledge the role of at least one component in neural crest cell migration, but even for fibronectins there are many unanswered questions. In particular, in the case of cephalic crest cell migration, the influence on the migration pattern of the synthesis and deposition of FN by a subpopulation of cells, is not clear. Could it be that the deposition of FN by a cell modulates the locomotory behaviour of the same cell using the molecule as a substrate? If so, then is the deposition permissive or inhibitory for cell migration? Much has been achieved in the field of cell locomotion and in the understanding of neural crest cell migration in particular, but the probes that will become available in the near future will certainly allow a finer understanding of the specificity of the pathways of migration followed by the descendants of the neural crest at the different levels of the neural tube.

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