Control of pathfinding by the avian trunk neural crest

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Summary

We have determined the pathways taken by the trunk neural crest of quail and examined the parameters that control these patterns of dispersion. Using antibodies that recognize migratory neural crest cells (HNK-1), we have found that the crest cells take three primary pathways: (1) between the ectoderm and somites, (2) within the intersomitic space and (3) through the anterior somite along the basal surface of the myotome.

The parameters controlling dispersion patterns of neural crest cells are several. The pathways are filled with at least two adhesive molecules, laminin and fibronectin, to which neural crest cells adhere tenaciously in culture. The pattern of migration through the somite may be accounted for in part by the precocious development of the basal lamina of the dermamyotome in the anterior half of the somite; this basal lamina contains both fibronectin and laminin and the neural crest cells prefer to migrate on it. In contrast, the regions into which the crest cells do not invade are filled with relatively nonadhesive molecules such as chondroitin sulphate. Some of the pathways are filled with hyaluronic acid, which stimulates the migration of neural crest cells when they are cultured in three-dimensional gels, presumably by opening spaces. Neural crest cells are also constrained to stay within the pathways by basal laminae, which act as barriers and through which crest cells do not go. Therefore, crest pathways are probably defined by several redundant factors.

The directionality of crest cell migration is probably due to contact inhibition, which can be demonstrated in tissue culture. Various grafting experiments have suggested that chemotaxis and haptotaxis do not play a role in controlling the dispersion of the crest cells away from the neural tube.

We have documented the extraordinary ability of neural crest cells to disperse in the embryo, even when they are grafted into sites in which they would normally not migrate. We have evidence that the cells' production of plasminogen activator, a proteolytic enzyme, and also the minimal tractional force that crest cells exert on the substratum as they migrate, contribute to this migratory ability.

Key words: neural crest, pathfinding, extracellular matrix, chemotaxis, haptotaxis, galvanotaxis.

Pathways of migration

If we are to understand what controls the pattern of crest cell migration, we first must know in detail what pathways they take so as to examine what physical and biochemical parameters are associated with these pathways.

Elucidating the crest cell migratory patterns has not been an easy matter. Initially the crest cells can be distinguished from other embryonic cells because they arise from a localized region, the dorsal side of the neural tube, and migrate into an extracellular space. However, as they disperse they mix with the paraxial mesoderm and then can no longer be distinguished from other embryonic cells. Thus, tracing the pathways of migration is contingent upon being able to mark the crest cells in some unambiguous way.

The earliest studies of trunk crest migration in the chick that attempted to trace the paths of migration employed $^{3}$Hthymidine as a marker for neural crest cells (Weston, 1963). Chick embryos were incubated for 24 h in $^{3}$Hthymidine and the labelled neural tubes excised and grafted into an unlabelled host in place of its neural tube. The labelled neural crest cells then migrated, presumably along their normal pathways and could be distinguished from the unlabelled host tissue. For the most part, Weston’s study mapped the final distribution of crest cells 2–3 days after they had initiated their migration. Crest cells
were observed (i) in the ectoderm, (ii) aggregated into what were presumed to be the sensory and sympathetic ganglia and (iii) dispersed in the somites.

Weston also examined some early stages of crest cell migration (20 h after grafting), but he made no attempt to trace their precise pathways. The major conclusion from examining these younger embryos was that crest migration was enhanced through the somites. Although not noted at the time, some of his figures clearly show that the predominant migration was through the anterior somite (note Fig. 3B in Weston, 1963). Hindsight has undoubtedly enhanced our ability to detect such subtleties.

Weston's study and others that were to follow (Noden, 1975; Johnston, 1966), although important, had a number of shortcomings. (1) In most early studies employing grafting of a labelled neural tube, researchers usually waited at least 20 h for the graft to heal before fixing and looking for the distribution of labelled cells. Thus, the very earliest stages of migration were not examined. In the case of the crest cell, this turns out to be a critical omission. (2) We cannot be certain that all the crest cells are initially labelled with a metabolic marker such as [3H]thymidine. Furthermore, [3H]thymidine becomes diluted as the cells divide, so its usefulness as a marker can be short-lived. In the case of the neural crest cells, we do not know how much they divide as they disperse. (3) In studies that employ grafting of labelled neural tubes, the operation itself can disrupt normal tissue associations at the very time that the crest cells are invading the paraxial mesoderm. The possibility that this could alter the normal patterns of migration is great.

The problem of dilution of the label was circumvented by Le Douarin (1973) who pioneered a permanent marking technique for the crest cells. She observed that the DNA of the closely related chick and quail stain differentially with Feulgen. Thus she could replace a chick neural tube with that of a quail, allow the crest cells to migrate from the tube, and then section and stain the chimera embryo. The nuclei of the quail crest cells can be distinguished from those of the chick host because of their darker, punctate staining. Using this technique, she confirmed what was known about migratory routes from Weston's study (Le Douarin & Teillet, 1974).

A significant advance in our determination of crest cell migratory pathways was the development of a monoclonal antibody that recognizes migratory neural crest cells (Vincent, Duband & Thiery, 1983; Vincent & Thiery, 1984; Tucker et al. 1984). This antibody has enhanced our ability to identify neural crest cells, since fluorescently labelled antibodies are easier to visualize than quail nuclei against a background of chick cells. Indeed, the distribution of crest cells becomes unmistakable. Furthermore, this technique requires no grafting, so that it is a noninvasive method that does not perturb normal development in any way.

Several laboratories have used the NC-1 antibody to trace the paths of crest migration in the trunk of the chick or quail embryo (Rickmann, Fawcett & Keynes, 1985; Bronner-Fraser, 1985; Loring & Erickson, 1987; Teillet, Kalcheim & Le Douarin, 1987). All of these studies are in agreement about several points. (1) The neural crest cells migrate in an anterior-to-posterior wave beginning in the head. (2) As the crest cells migrate off the neural tube and approach a neighbouring somite, they invade the somite, but only in its anterior half (Fig. 1). (3) Some crest cells collect in the dorsomedial sector of the sclerotome to form the sensory ganglia, whereas others migrate through the somite as far as the dorsal aorta, where they aggregate to form the paravertebral sympathetic ganglia.

There is still some disagreement about the precise pathway taken by the crest through the somite. Some reports suggest that the crest invades throughout much of the sclerotome in the anterior somite (Rickmann et al. 1985; Bronner-Fraser, 1985). We found, however, that the crest cells first enter the somite at the interface between the dermamyotome and sclerotome and migrate along this interface until they reach the dorsal aorta (Fig. 1B; Loring & Erickson, 1987). Only later are crest cells found in the sclerotome. These later-appearing crest cells may detach from the dermamyotome and entering the sclerotome, or may be intersomitic crest cells (see below) around whom the sclerotome has dispersed. Finally, other neural crest cells may enter the sclerotome by migrating along the ventral root motor fibres that also course through the anterior somite (see below).

Some of the crest cells migrate between the neural tube and somite opposite the posterior half of a somite. What happens to these cells? In a recent series of elegant grafting experiments, Teillet & coworkers (1987) have shown that these crest cells migrate either anteriorly or posteriorly along the neural tube until they arrive at the anterior side of their own, or the adjacent caudal somite, and then enter the somite at its anterior aspect.

An additional pathway taken by other crest cells is an incursion into the intersomitic spaces (Loring & Erickson, 1987; Teillet et al. 1987; Thiery, Duband & Delouvé, 1982). These crest cells are suspected to reach the dorsal aorta to become part of the paravertebral sympathetic chain. Alternatively some of the cells in the intersomitic space may mix with crest cells in the anterior somite as the sclerotome disperses and obliterates the intersomitic space.

Finally, some crest cells invade the space between
Fig. 1. (A, B). A series of paraffin sections through somite -9 (i.e. 9 somites anterior to the last-formed somite) of a 30-somite embryo. The sections were incubated with the antibody NHK-1 to identify the neural crest cells. In a section taken from the posterior portion of this somite (A), the crest cells are not found in the somite. Sections taken through the anterior portion of the same somite show the neural crest cells spread along the interface between the dermamyotome (DM) and the sclerotome (Sc). NT, neural tube, NC, neural crest, DA, dorsal aorta, PCV, posterior cardinal vein, PD, pronephric duct. Bar, 100 μm. (C). Whole mount preparation of a 28-somite chick embryo labelled with the HNK-1 antibody photographed at an axial level where neural crest cell migration into the somite is well underway. The anterior border of each somite is marked by an arrowhead and the anterior–posterior axis is noted. The crest cells are found only in the anterior half of each somite and are generally aligned in parallel. Bar, 100 μm. (From Loring & Erickson, 1987).

In summary, trunk crest cells migrate (1) laterally in an extracellular space to form the pigment cells of the skin, (2) ventrally through an extracellular space above and alongside the neural tube until the crest cells enter the anterior portion of the somite and (3) in the intersomitic space between adjacent somites. This initial pattern of migration, which limits crest invasion to the anterior half of each somite, appears to be responsible for the metameric arrangement of the sensory and sympathetic ganglia.

I have not considered in this section the pathways of head crest migration in the chick, since less is known about the precise pathways taken by these cells. The studies of Noden (1975, 1978) and Johnston (1966) employing [3H]thymidine or chick–quail chimaeras as markers for the crest may be inaccurate for the reasons outlined above and should be reexamined using the more recent antibody markers.

the somites and ectoderm and ultimately invade the ectoderm between day 5 and 6 to become the pigment cells of the skin (Teillet & Le Douarin, 1970; Teillet, 1971). It has never been determined how far laterally the pigment cell precursors move before they actually invade the ectoderm. Weston (1963) has suggested that the crest cells enter the ectoderm just above the neural tube soon after they begin to migrate. His results may be due to an artifact of the grafting technique, since the crest cells may prematurely enter a breach in the ectoderm made during surgery.

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What, then, are the various factors that determine these pathways?

Control of pathways

One could conceive of two very different methods of controlling the migratory pathways taken by neural crest cells. Either (1) each crest cell is predetermined to develop into a particular phenotype and it follows particular cues to get to the correct site or (2) the crest cells are pluripotential and all crest cells follow the same cues; those crest cells that migrate first and get to their particular site become the most distal crest derivatives.

In their classic experiments, Le Douarin & Teillet (1974) showed that trunk crest cells grafted from one axial level to another follow the correct pathways for that axial level. Such experiments suggest that there are predetermined pathways that the population as a whole follows. Similarly, Noden (1975) exchanged crest cells between various levels of the brain and found that the pattern of crest cell migration depended upon the environment through which the grafted cells dispersed.

At present, no evidence supports the notion that predetermined crest cells follow their own specific migratory cues. This is not to say that some crest cells are not predetermined prior to, or soon after, they initiate migration, since clearly some are (see Barald, 1982; Ciment & Weston, 1982; Girdlestone & Weston, 1985; Barbu, Ziller, Rong & Le Douarin, 1986). But even in the head crest, where crest cells that will give rise to skeletal elements are clearly patterned prior to migration (Noden, 1984), heterotopically grafted cephalic crest cells will still follow the stereotypical pathways for the axial level in which they have been grafted, even though ectopic or misplaced skeletal elements form.

The preponderance of evidence indicates that the environment is primarily responsible for determining the patterns of migration. Environmental factors believed to dictate the migratory pathways are (1) extracellular spaces that provide a passage way of least resistance, (2) an adhesive substratum on which to migrate. For example, the metencephalic crest cells migrate laterally and then split into preotic and postotic streams when they are obstructed by the invaginating otic placode (Anderson & Meier, 1981). The anterior mesencephalic crest and diencephalic crest migrate around the developing optic stalk but not over the top of it (Noden, 1975), presumably because of the tight apposition of the overlying ectoderm.

Most of the spaces into which the crest cells migrate are filled with the glycosaminoglycan hyaluronic acid (Toole & Trelstad, 1971; Pratt, Larsen & Johnson, 1975; Derby, 1978). This is a hydrophilic molecule that expands in water owing to the high density of negative charges that prevent its compaction. Hyaluronic acid (HA) is responsible, at least in part, for the development of many spaces in the embryo since it appears concomitant with spaces and the removal of HA with hyaluronidase collapses the spaces (Anderson & Meier, 1982; Fisher & Solursh, 1977).

The role of HA in facilitating crest migration is supported by the observation that when HA is added to collagen gels, it increases the spaces between collagen fibrils and increases the speed of movement of neural crest cells embedded in the gels (Tucker & Erickson, 1984).

Although spaces are undoubtedly important factors in guiding neural crest cells, the following points should be kept in mind. (1) Spaces may appear concomitant with extracellular matrix molecules that are necessary for sustaining neural crest cell migration. Thus, the stimulus to migration may not be the space, but rather the appearance of an adhesive molecule on which to move. (2) Crest cells possibly do not move into some areas because inhibitory molecules there prevent crest migration and not because the space is too small. (3) Finally, crest cells can get into very small spaces, if given the appropriate substratum on which to migrate. For example, the space between the dermamyotome and sclerotome is not large, but crest cells still use this as the primary path of migration. Thus, it is not clear how large a migratory 'space' needs to be for a crest cell.

Extracellular matrix substratum

Spaces may very well facilitate migration, but they are not sufficient, since the cells must be able to adhere to a substratum in order to generate the tractional force to move.

Several types of extracellular matrix molecules that
might facilitate crest cell adhesion have already been localized in the crest migratory pathways. These include fibronectin (Newgreen & Thiery, 1980; Thiery et al. 1982; Mayer, Hay & Hynes, 1981), laminin (Rickmann et al. 1985; Rogers, Edson, Letourneau & McLoon, 1986), collagen I and III (von der Mark et al. 1976; our unpublished results), cytotactin (Grumet, Hoffman, Crossin & Edelman, 1985) and the glycosaminoglycans hyaluronic acid and chondroitin sulphate (Derby, 1978; Pintar, 1978; Kvist & Finnegan, 1970). Furthermore, the distribution of fibronectin and laminin is temporally and spatially correlated with the migration of the crest cells through the anterior somite. These molecules appear in the basal lamina of the dermamyotome on which the crest cells appear to move. Basal lamina develops first, however, in the anterior somite (Fig. 2; Loring & Erickson, 1987), the site of initial crest migration.

Although the presence of these various molecules is spatially and temporally correlated with crest migratory pathways, such correlative evidence does not predict the behaviour of the cells on particular matrix molecules.

An approach used by our laboratory and others to study crest cell behaviour on extracellular matrix (ECM) molecules is to culture neural crest cells on tissue culture plastic to which individual or combinations of known ECM components have been adsorbed or conjugated (Fig. 3). Such studies have shown that crest cells flatten and spread more on fibronectin and laminin than on any other ECM component yet tested (Newgreen et al. 1982; Rovasio et al. 1983; Erickson & Turley, 1983; Newgreen, 1984). In fact, when 'lanes' of either fibronectin (Rovasio et al. 1983) or laminin (Newgreen, 1984) are painted on a tissue culture plastic dish, the crest cells will stay on the fibronectin or laminin lanes and not venture onto the less-adhesive plastic. Furthermore, the crest cells migrate faster and are more directionally persistent on fibronectin than any other ECM molecule tested (Erickson & Turley, 1983).

Crest cells also adhere to, and migrate on, denatured collagen in vitro (Newgreen, 1982; Erickson & Turley, 1983) as well as through native collagen gels (Davis, 1980; Tucker & Erickson, 1984). The glycosaminoglycans, on the other hand, are either nonadhesive or lowly adhesive for trunk neural crests. Crest cells are unable to adhere to hyaluronic acid (Fisher & Solursh, 1979; Erickson & Turley, 1983), but, as mentioned above, hyaluronic acid can enhance neural crest cell migration when it is added in low concentration to collagen gels (Tucker & Erickson, 1984). Thus hyaluronic acid may function...
Fig. 3. Neural crest cells grown for 18 h on individual matrix components that have been adsorbed or derivatized to the plastic substratum with no serum or embryo extract in the medium. The neural tube, from which the neural crest cells have immigrated, is located at the margin of each micrograph. (A) Neural crest cells on tissue culture plastic. This is a relatively less adhesive substratum than others such as fibronectin and the cells are somewhat rounded. (B) Neural crest cells grown in medium containing 25 μg fibronectin (FN). The cells are considerably flatter and have dispersed further away from the neural tube than when grown on plastic alone. (C) Neural crest cells grown on chondroitin sulphate (CS) that has been derivatized to a tissue-culture plastic substratum. Cell morphology and behaviour is similar to that on plastic alone. (D) Only a few neural crest cells have migrated from the neural tube when hyaluronic acid (HA) has been derivatized to a plastic substratum. ×990. (From Erickson & Turley, 1983).

not as a substratum for migration, but as a facilitator of migration by opening spaces.

Chondroitin sulphate and chondroitin sulphate proteoglycan (CSPG) when tested alone or in combination with other ECM molecules, reduce spreading and adhesiveness of neural crest cells to their substratum (Newgreen et al. 1982; Newgreen, 1982; Erickson & Turley, 1983; Tucker & Erickson, 1984). Interestingly, regions that are avoided by the crest in the trunk (Newgreen et al. 1982) and the head (Brauer, Bolender & Markwald, 1985) are high in CSPG. Newgreen, Scheel & Kastner (1986) have direct evidence from tissue culture studies that the CSPG present in the perinotochordal space is probably responsible for inhibition of crest invasion into that region. These data suggest that ECM molecules may function not only to stimulate neural crest migration, but also to inhibit it. Thus, an effective means of controlling the pathways of migration may be to prevent the cells from invading into regions adjacent to the pathway (see Morris, 1979).

A recent approach to identifying the role of various ECM components is to perturb their function by the injection of specific antibodies or peptides into the embryo that compete with the binding of cells to a particular ECM molecule. Such studies have at least corroborated the conclusions derived from tissue culture studies (see Tucker, this volume). Boucaut et al. (1984) injected a decapeptide that competitively inhibits fibronectin function into the mesencephalon at the time that neural crest cells in that region initiate migration and found that morphogenetic movement ceases. Similarly, Bronner-Fraser (1985) injected the CSAT antibody, which recognizes a receptor for fibronectin and laminin, into the mesencephalon and showed a marked reduction in cranial crest migration.
Curiously, none of these injections appears to affect trunk crest migration as dramatically as in the head, if at all. This could be because the controls of crest migration in the trunk are different to those in the head. Alternatively, because the embryonic architecture is so different in the trunk, the trunk crest may simply not encounter enough antibody to affect their migration.

**Neurones**

Crest cells may also associate with other cell types that act as migratory pathways. During the early stages of crest migration in the trunk, a large population of crest cells begin to accumulate in the dorsomedial corner of the sclerotome. Since dorsal root motor neurones are also known to grow into the anterior portion of the sclerotome (Keynes & Stern, 1984; Rogers et al. 1986), they were a logical choice for directing crest migration into the sclerotome and perhaps even trapping the presumptive dorsal root ganglion cells there.

Double-labelling experiments identifying both neural crest cells and ventral root motor fibres show that the two are co-distributed (Rickmann et al. 1985; Loring & Erickson, 1987). Indeed the crest cells appear enmeshed in the tangle of ventral motor neurones (Fig. 4). Furthermore, the ventral root fibres grow into the sclerotome just hours prior to the time that crest cells accumulate in the sclerotome.

We believe that crest cells adhere to and perhaps are even arrested in their migration by the neurones. Of course, neural crest cells and ventral root neurones could be independently following the same environmental cue and may not be guided by each other. In support of a causal relationship, we have preliminary evidence that removal of the ventral root neurones prior to their emergence from the neural tube results in a reduction in size of the crest-derived sensory ganglia and a loss of neural crest cells along the ventral root fibres at that axial level (S. A. Scott, J. F. Loring and C. A. Erickson, unpublished data; see also Landmesser & Honig, 1986). Conversely, ablation of neural crest cells prior to their exit from the neural tube does not affect motor neurone outgrowth (Rickmann et al. 1985). We do not know why the crest cells might have a stronger affinity to the neurones than the surrounding environment.

**Boundaries**

Crest cells may be restrained to particular highways by adhesive molecules on which they prefer to adhere and by borders of nonadhesive or less adhesive molecules, such as chondroitin sulphate proteoglycan, into which they will not go. In addition, most of the crest pathways are bordered by epithelia that rest on basal laminae. Such epithelia include the epidermis, the neural tube, the undersurface of the dermomyotome, a portion of the medial surface of the somite and the larger embryonic blood vessels such as the dorsal aorta. It has been suggested that the basal lamina delimits early crest migration by acting as an impenetrable barrier (e.g. see Weston, 1982; Erickson & Weston, 1983).

To test this idea, neural crest cells were cultured on basal laminae derived from placental amnions (see also Gehlsen & Hendrix, 1987) or grafted into chick neural tubes or epidermis, where they were forced to confront a basal lamina. Under no circumstance did a neural crest cell penetrate a basal lamina. Rather, the crest cells flatten and spread upon contact with the basal lamina, suggesting that the basal lamina is indeed a barrier to migration. Alternatively, the basal lamina may represent a substratum to which crest cells prefer to adhere and will not detach once they have made contact, since it is composed of ECM molecules that are particularly adhesive for the neural crest, notably fibronectin and laminin (Newgreen, 1984). In either case, the basal lamina may act as yet another element, perhaps redundant, in the environment, that determines boundaries for crest cell migratory pathways.

**Control of directionality**

Neural crest cells must receive some directional cues as they migrate, because the crest pathways, as they have just been described, define position only. Specifically, some stimulus must direct the neural crest cells to disperse from their point of origin; furthermore, once crest cells enter a pathway they must know whether to turn right or left.

Many mechanisms may impose directional movement on embryonic cells. These include positive and negative chemotaxis, haptotaxis, galvanotaxis and contact inhibition. Each of these has been tested for its possible role in neural crest morphogenesis. Such studies in toto suggest that contact inhibition is the most probable control of directional movement.

**Haptotaxis and chemotaxis**

At least two mechanisms employ gradients that could direct crest cells laterally and ventrally: these are haptotaxis and chemotaxis.

Haptotaxis is the ability of cells to move up an adhesive gradient (Carter, 1967), owing at least in part to the more rapid detachment from the less adhesive end of the substratum (Harris, 1973). A gradient of adhesiveness extending from the neural tube, both laterally and ventrally, is an attractive mechanism for directing the crest to lateral and ventral positions.
Alternatively, a diffusible chemotactic molecule, which has its source in either lateral or ventral positions, might attract crest cells. Chemotaxis has been implicated in other developmental phenomena, including the migration of primordial germ cells (DuBois, 1968) and growing axons (Lumsden & Davies, 1983; Gundersen & Barrett, 1979).

The presence of a preestablished gradient, such as the two suggested above, was tested by a series of grafting experiments (Erickson, 1985). When neural crest cells were placed either ventrally (Fig. 6) or laterally (Fig. 7) in the crest migratory pathways, the grafted cells dispersed radially and some of them migrated back toward the neural tube. This migration toward the neural tube ceases when the grafted crest cells reach an oncoming stream of host crest cells.

Since crest cells can move in the reverse direction along established migratory pathways, it is probable that a preestablished gradient of either an adhesive or chemotactic nature, does not exist in the chick embryo.

Negative chemotaxis

Negative chemotaxis has been proposed to direct dispersion of amphibian neural crest cells away from the neural tube (Twitty, 1949). Studies by Twitty and his associate Niu (Twitty & Niu, 1948, 1954) suggested that neural crest cells produce a factor that causes their mutual repulsion when it accumulates in high enough concentrations. They observed, for example, that if aggregates of neural crest cells were sucked into a capillary tube and one end sealed, the crest cells at the sealed end dispersed, whereas those at the open end did not move. In another experiment, crest cells grown beneath a coverslip were more dispersed.

Fig. 4. Double label with HNK-1 (A,C) and E/C8 (B,D) to identify neural crest cells and ventral root fibres, respectively. (A,B) A section through the anterior portion of somite 17 of a 30-somite chick embryo. Neural crest cells are found along the undersurface of the dermamyotome as well as in the dorsomedial portion of the sclerotome (arrowheads). (A) The emerging ventral root fibres are superimposable on the neural crest cells found in the sclerotome (arrowhead). (C,D) A section through the axial level two somites anterior to that pictured in A,B. More HNK-1-stained cells are seen in the developing sensory ganglion, on the ventral root and along the dorsal aorta. The ventral root fibres (B, arrowheads) have now reached the dermatome/myotome. Again, crest cells in the sclerotome are colocalized with these fibres. Bar, 100 μm. (From Loring & Erickson, 1987).
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Fig. 5. (A) Transmission electron micrograph of a thin section through a chick neural tube fixed 24 h after receiving a graft of pigmented neural crest cells into the lumen of the neural tube. x720. (B) Higher magnification of the area indicated by arrow b in A, showing that a pigmented neural crest cell has reached the basal surface of the neural tube. x9600. (C) High magnification of B, demonstrating that the grafted cell has spread on the basal lamina of the neural tube but has not penetrated it. x24800. (From Erickson, 1987).

than the cells that escaped out the sides. In both situations, the capillary tube and the coverslip presumably ‘trapped’ the putative repulsive molecule or limited its diffusion away.

Attempts to duplicate these experimental results with either quail (Erickson & Olivier, 1983) or amphibian crest cells (R. E. Keller, personal communication) have not been successful. We found, for example, that quail crest cells are less dense under a coverslip because of accelerated cell death. In addition, there appeared to be no difference in the extent of dispersion of crest cells dependent upon their position in a capillary tube. Finally, we drew aggregates of neural crest cells into capillary tubes of varying diameters. As the diameter of the tube is decreased, one would predict that the chemotactic factor would accumulate faster and the cells would be more dispersed; in fact, the opposite occurred.

Galvanotaxis
Recently, there have been numerous reports that embryonic cells, such as neural crest cells and sclerotome cells, respond to an imposed electrical field by migrating toward the negative pole (Nuccitelli & Erickson, 1983; Stump & Robinson, 1983; Cooper & Keller, 1984). The threshold for this response is surprisingly low, as little as 10 mV mm⁻¹ for somite cells (Erickson & Nuccitelli, 1984). It is also known that embryonic epithelial cells can create electrical fields (e.g. Jaffee & Stern, 1979; Lindemann & Voute, 1976) by pumping ions across themselves.

We have found that currents emanate from isolated quail neural tubes that are in the correct orientation and of sufficient magnitude to direct crest cell migration. Furthermore, they arise at the appropriate axial levels and at the correct time (Fig. 8; Erickson & Nuccitelli, 1986). However, we do not yet know if these same currents exist in the intact embryo and, more importantly, if they have any role in neural crest motility. Even if they do exist, the field strength must drop off dramatically some distance from the neural tube, perhaps owing to resistivity in the extracellular matrix, because our grafting experiments described
above show that crest cells can move counter to their usual direction of migration until they meet the oncoming host crest.

**Contact inhibition**

Still the most attractive explanation for directed motility of neural crest cells is contact inhibition, since at the very least observed crest cell behaviour or experimental manipulations do not disprove it. Contact inhibition of movement is the result of contact between two locomotory cells, in which the contacting cell ceases lamellipodial activity (so-called contact paralysis) at the point of contact, and generally retracts and moves off in another direction (Abercrombie & Heapman, 1954; Abercrombie, 1970).

In tissue culture, this phenomenon has two effects on population dynamics: (1) cells will move radially away from regions of highest concentration to regions where cells are sparsest and (2) elongate cells will align in parallel arrays (Erickson, 1978). Crest cells in the embryo similarly disperse from their point of highest concentration at the neural tube toward the periphery where there are no other cells occupying their pathway. Furthermore, crest cells are often aligned in parallel during their early stages of migration when we can observe them best (Bancroft & Bellairs, 1976; Tosney, 1978).

Several investigators (Newgreen et al. 1979; Rovasio et al. 1982) have clearly shown that neural crest cells in tissue culture migrate directionally away from the point of highest concentration although neither study demonstrated contact paralysis owing to the magnification of their filming conditions. Similarly, Gooday & Thorogood (1985) provide indirect evidence that crest cells display contact inhibition using the nuclear overlap assay developed by Abercrombie & Heaysman (1954). I have used time-lapse cinemicrography combined with differential interference optics to observe contact behaviour of neural crest cells. These studies have unequivocally shown that the lamellipodium of a neural crest cell displays contact paralysis when it contacts the side of another crest cell (Erickson, 1985).

There is at least one instance in which contact inhibition cannot explain the directional migration of neural crest cells. Keller & Speith (1984) filmed pigment cells as they migrated beneath the skin of the axolotl embryo. These cells are unerringly persistent in their directional migration and yet they do not apparently contact each other. Perhaps galvanotaxis can explain this directional migration.

With the advent of a variety of fluorescent vital dyes, it may now be possible to label premigratory neural crest cells in chick or mammalian embryos and actually observe neural crest migration in vivo.

**Migratory capability**

One of the extraordinary features of neural crest cells is that they are migratory no matter where they are placed in the early chick embryo, even in ectopic sites.
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Fig. 7. Sections through a 27-somite chick embryo that received a graft of pigmented crest cells lateral to the last somite (X marks the site of graft placement) and was then fixed 7 h later. These sections are taken through the middle of the somite (A), near the anterior margin (B) and through the intersomitic space (C). The grafted cells moved both medially and laterally along the somite (S) and ectoderm (E) and are spread along the intersomitic blood vessel (BV) in C. NT, neural tube; M, mesonephric duct; DA, dorsal aorta. Bar, 50 μm. (From Erickson, 1985).

Fig. 8. Current pattern measured around the tube of a chick embryo at stage 12 using the vibrating probe. Somites were carefully removed with sharpened tungsten needles to allow access to the neural tube epithelium. The current pattern detected along the length of the neural tube and in cross section is shown in the lower figure. A, anterior; P, posterior; D, dorsal; V, ventral. (From Erickson & Nuccitelli, 1986).

Of the dispersal properties of the neural crest is a mouse tumour cell line, sarcoma 180 (S–180). After grafting S-180 cells into the crest pathways, they were found in many of the same areas to which the crest would normally migrate (Fig. 10; Erickson et al. 1980), although some of this dispersal may be passive in nature (see Bronner-Fraser, 1982). In fact, these experiments testing the migratory potential of S-180 cells should be reinvestigated in light of our current understanding of neural crest cell pathways.

Such experiments demonstrate that crest cells have migratory capabilities that exceed those of almost all other embryonic cells. The basis for this exceptional dispersal capability is not yet known, but I will present a few possibilities.

Protease activity
Numerous tumorigenic cells lines that are notoriously invasive produce high levels of proteolytic enzymes,
Fig. 9. Low magnification of a chick embryo fixed 48 h after receiving a graft of pigmented neural crest cells into a stage-21 hindlimb bud. (B) High magnification of boxed area in A. Arrowheads indicate pigmented crest cells that have migrated from the limb into the ventral crest pathway and are now distributed in the dorsal mesentery and around the developing kidneys and adrenal cortex. Bars, 25 μm. (From Erickson et al. 1980).

Fig. 10. (A) Low magnification through a section of a chick embryo fixed 24 h after receiving a graft of sarcoma-180 cells into the crest pathway at the level of somite 26 in a 27-somite host. (B) High magnification of box 1 in A. The sarcoma-180 cells (arrowheads) are distributed along the ventral root fibres and in the region of the dorsal root ganglion. (C) High magnification of boxed area 2 in A. Sarcoma-180 cells (arrowheads) are distributed ventrally around the dorsal aorta and in the dorsal mesentery. Bars, 25 μm. (From Erickson et al. 1980).
including collagenases and plasminogen activator (PA; e.g. Mignatti, Robbins & Rifkin, 1986). PA is a neutral serine protease that converts plasminogen to plasmin. Both plasmin and PA itself can degrade extracellular matrix components (Quigley, 1979; Fairbairn et al. 1985; Sheela & Barrett, 1982; Laug, de Clerck & Jones, 1983; Sullivan & Quigley, 1986). Degradation of the ECM might allow cells to tunnel through a dense matrix. Furthermore, Chen, Olden, Bernaud & Chu (1985) have shown that proteases are locally secreted in adhesion plaques and may be important for releasing cell adhesions, allowing the cell to take another step forward. The secretion of proteases might allow the crest cells to move more readily. Finally, a number of morphogenetic events are associated with the localized action of the PA/plasmin system, including the colonization of the bursa of Fabricius by haemopoietic precursors (Valinsky, Reich & Le Douarin, 1981) and the involution of mammary epithelium (Ossowski, Biegel & Reich, 1979).

Valinsky & Le Douarin (1985) have identified PA activity in cephalic neural crest cells. Similarly, we have found that trunk crest cells in chick do indeed produce higher levels of PA activity than somite cells isolated from the same age embryos. Furthermore, as the crest cells differentiate into pigment cells, they produce an increasingly higher level of PA activity. PA, in preliminary experiments, appears to be important in migration, since inhibitors of PA activity (NPGB, $10^{-5}$ M; benzamidine, $2 \times 10^{-5}$ M; leupeptin, $5 \times 10^{-5}$ M) reduce crest migration in tissue culture (Fig. 11). We are presently using antibodies to chicken PA to perturb crest migration in the embryo.

**Reduced tractionsal force**

Harris & co-workers (1980, 1981) demonstrated that fibroblasts exert a tractional force on their substratum as they locomote and that this force is sufficiently strong to deform a silicone rubber sheet or a collagen lattice. Indeed this force can be so strong that a fibroblast will tear its deformable substratum and consequently be immobilized (Tucker, Edwards & Erickson, 1985). However, cells that are normally locomotory in the embryo or in the adult, such as neurones, lymphocytes and macrophages, do not exert a strong or even a measurable tractional force. These investigators suggest that low tractional force permits migration and that the excessive tractional forces exerted by fibroblasts may have a role in tissue modelling and patterning, rather than migration.

We tested the possibility that neural crest cells might also exert a low tractional force that could explain their migratory capability (Tucker et al. 1985). When crest cells are grown on a silicone rubber sheet they do not deform their substratum. Likewise they do not deform a collagen gel unless the concentration of collagen falls below $250 \mu g \, ml^{-1}$. On the other hand, those embryonic cell types that do not migrate in the embryo, such as somite cells, not only...
Stage of neural crest migration

<table>
<thead>
<tr>
<th>Stage</th>
<th>Length of pathway*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crest cells at medial edge of somite</td>
<td>128 ± 19</td>
</tr>
<tr>
<td>Crest cells distributed along dermatome/myotome</td>
<td>131 ± 36</td>
</tr>
<tr>
<td>Crest cells at dorsal aorta</td>
<td>143 ± 23</td>
</tr>
</tbody>
</table>

**Fig. 12.** Path length does not change during migration under dermatome/myotome. Camera-lucida tracings showing the distribution of neural crest cells at successive stages in their dispersal across the width of the somite and the average length of the neural crest pathway (from medial edge of the dermamyotome to the dorsal aorta) at each stage of migration. There is no statistical difference in the length of the pathway as the crest cells move from one end to the other, suggesting that the crest cells must move actively in relationship to the somite, rather than being transported passively due to the growth of the somite. (From Loring & Erickson, 1987).

deform such substrata, but can even tear a collagen gel and their dispersal is arrested.

An interesting possibility is that crest cells are able to use various extracellular matrices as migratory substrata owing to their minimal tractional force, whereas other embryonic cells may destroy the substratum because of their excessive tractional force. While this remains a fascinating possibility, we have no further data to support this notion, and it remains a difficult one to test.

**Passive movement**

Recent experimentation suggests that not all of the crest dispersion is due to active, lamellipodial driven migration (Noden, 1984; Bronner-Fraser, 1982). Such studies have pointed out that the enormous distances traversed by the crest are partially due to the growth of the surrounding mesenchyme in which the crest cells disperse.

In a review, Noden (1984) has summarized three different studies in which host chick ectoderm, paraxial mesoderm or neural crest cells were replaced with the equivalent quail tissue. When the grafted cells were followed in successive stages, the lateral expansion of the ectoderm, underlying mesoderm and the crest cells progressed at the same rate. Thus, in the head, once the crest cells have managed to spread over the mesoderm, they may continue their ventral dispersion by more or less riding along on the underlying mesoderm as it grows. Similarly, Bronner-Fraser (1982, 1985) has shown that Latex beads injected into the cavity of the somites are passively dispersed and accumulate in the sclerotome lateral to the dorsal aorta, where the sympathetic ganglia develop. This dispersion of the beads is probably due to the growth and expansion of the sclerotome (Gasser, 1979).

The situation in the trunk is quite different to that in the head. In the trunk, crest cells migrate into the somite and have even traversed the somite and reached the dorsal aorta during a short period when the somite itself has not grown (Fig. 12; Loring & Erickson, 1987). Thus, although the growth of embryonic structures undoubtedly accounts for the large distances that crest derivatives are found from the dorsal neural tube, the patterns of migration that are established early are likely due to active movement.

**Conclusions**

The patterns of crest cell migration in the trunk of the avian embryo have been delineated recently owing to the development of a monoclonal antibody that recognizes migratory neural crest cells. The detailed
analysis of these pathways should now allow us to ascertain the environmental controls for these pathways.

Still unknown are all the environmental macromolecules that may guide neural crest cells. A particularly fruitful avenue of investigation, in this regard, may be embryonic mutants in which the environment through which the crest cells move is atypical. For example, recent investigations of the mutant lethal-spotting (Is), in which the bowel is developmentally abnormal due to the absence of the crest-derived enteric ganglia, suggest that the environment of the mutant's terminal gut cannot support invasion of neural crest cells (Rothman, Nilaver & Gershon, 1982; Rothman & Gershon, 1984). If this is true, the Is/Is mutant may allow us to identify the extracellular matrix components that sustain neural crest cell migration (Gershon & Rothman, 1987).

Likewise the perturbation of the crest pathways with monoclonal antibodies to specific matrix molecules may prove useful. Results from such studies, however, need to be interpreted cautiously since a negative result does not necessarily discount the role of that molecule in crest morphogenesis.

The pathways of crest migration in the head are not nearly as defined as those in the trunk but we now have the tools to study cephalic crest migration as well. It will be particularly intriguing to see if the paraxial mesoderm organized as somitomeres has the same ability to organize crest migration (see Anderson & Meier, 1983) as the somites do in the trunk.

Finally, we know little about what confers the extraordinary migratory abilities on the neural crest cells. Indeed we have little information about the invasive behaviour of many cells, including lymphocytes and metastasizing cancer cells. The rapidly expanding list of cellular oncogenes that are associated with cellular transformation may eventually provide the clues for the controls of invasive behaviour of normal cells during development.

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References


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