Developmental potential of neural crest-derived cells migrating from segments of developing quail bowel back-grafted into younger chick host embryos

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Summary

The technique of back-transplantation was used to investigate the developmental potential of neural crest-derived cells that have migrated to and colonized the avian bowel. Segments of quail bowel (removed at E4) were grafted between the somites and neural tube of younger (E2) chick host embryos. Grafts were placed at a truncal level, adjacent to somites 14–24. Initial experiments, done in vitro, confirmed that crest-derived cells are capable of migrating out of segments of foregut explanted at E4. The foregut, which at E4 has been colonized by cells derived from the vagal crest, served as the donor tissue. Comparative observations were made following grafts of control tissues, which included hindgut, lung primordia, mesonephros and limb bud. Additional experiments were done with chimeric bowel in which only the crest-derived cells were of quail origin. Targets in the host embryos colonized by crest-derived cells from the foregut grafts included the neural tube, spinal roots and ganglia, peripheral nerves, sympathetic ganglia and the adrenals, but not the gut. Donor cells in these target organs were immunostained by the monoclonal antibody, NC-1, indicating that they were crest-derived and developing along neural or glial lineages. Some of the crest-derived cells (NC-1-immunoreactive) that left the bowel and reached sympathetic ganglia, but not peripheral nerves or dorsal root ganglia, co-expressed tyrosine hydroxylase immunoreactivity, a neural characteristic never expressed by crest-derived cells in the avian gut. None of the cells leaving enteric back-grafts produced pigment. Cells of mesodermal origin were also found to leave donor explants and aggregate in dermis and feather germs near the grafts. These observations indicate that crest-derived cells, having previously migrated to the bowel, retain the ability to migrate to distant sites in a younger embryo. The routes taken by these cells appear to reflect, not their previous migratory experience, but the level of the host embryo into which the graft is placed. Some of the population of crest-derived cells that leave the back-transplanted gut remain capable of expressing phenotypes that they do not express within the bowel in situ, but which are appropriate for the site in the host embryo to which they migrate.

Key words: neural crest, neural crest migration pathways, quail-chick chimeras, developing bowel, back-transplantation, enteric nervous system.

Introduction

A common feature of the pattern of development of all of the derivatives of the neural crest is a migration of precursors from the neuraxis to a variety of locations in the embryo in which these cells assume their terminally differentiated phenotype (Le Douarin, 1982; 1986; Noden, 1988; Weston, 1982). Both before and during migration crest-derived cell populations are developmentally heterogeneous; some cells are multipotential (Bronner-Fraser and Fraser, 1988; Anderson, 1989) while others appear to be fully determined (Sieber-Blum and Cohen, 1980; Baroffio et al. 1988; Dupin et al. 1990). Neural precursors in particular, are subjected to a severe selection process so that only some survive to form neurons, while others disappear or remain in a resting state (Le Douarin and Smith, 1987). The pathways along which crest-derived cells migrate in embryos are organized such that particular axial levels of the crest colonize characteristic target organs (Le Douarin
and Teillet, 1973; 1974). When crest cells are grafted from one axial level to another, they migrate to novel destinations, which are appropriate, not for the site from which the graft is obtained, but for the level of the site into which the graft is placed. Phenotypes expressed by crest-derived cells in these novel locations also are appropriate for the target organ to which they migrate and not the original axial level of the donor’s crest (Le Douarin and Teillet, 1974; Le Douarin et al. 1975; Smith et al. 1977; Fontaine-Pérus et al. 1982; Rothman et al. 1986). These experiments thus establish that the local environment of migratory pathways or target organs plays an important role in determining which derivatives of the crest are ultimately expressed in particular locations.

Crest-derived precursor cells, which are capable of expressing more than a single terminally differentiated phenotype, appear to be retained in ganglia throughout the entire developmental period. The presence of these cells has been demonstrated in back-transplantation experiments in which quail ganglia are introduced into the migration pathways of younger chick host embryos (Le Douarin et al. 1978; Dupin, 1984; Erickson et al. 1980; Le Lièvre et al. 1980; Ayer-Le Lièvre and Le Douarin, 1982; Schweizer et al. 1983; Coulombe and Bronner-Fraser, 1986). Certain of the grafted ganglion cells recover the ability of their crest-derived predecessors to move along migratory pathways and colonize organs to which these pathways lead. Back-transplantation experiments, moreover, have revealed that there are differences between ganglia with respect to the developmental potentialities of the uncommitted cells they retain (Fontaine-Pérus et al. 1988). Autonomic, nodose and dorsal root ganglia, which have previously been analyzed by back-transplantation, have each revealed differences in the types of cell to which they can give rise in host embryos.

The developmental potential of crest-derived cells that have migrated to the bowel has not previously been investigated. Moreover, no previous experiments have investigated the ability of crest-derived cells in ganglia that are, like those of the ENS, embedded in their target organ, to leave that organ and re-migrate following back-transplantation. Enteric ganglia differ structurally and chemically from other types of autonomic ganglia and resemble the CNS more than they do the remainder of the PNS (Gershon, 1981; Furness and Costa, 1987). The current work was thus designed to use back-transplantation experiments to evaluate whether crest-derived cells that have previously migrated to the bowel can leave the gut and migrate again to reach other sites in a host embryo. Segments of gut were back-transplanted between the neural tube and somites in the truncal region of younger host embryos. Enteric neurons and glia are not normally derived from this level of the neural crest. The experiments were thus designed to ascertain whether crest-derived cells, leaving the donor bowel, would migrate to non-enteric ganglia of the trunk, the host’s gut, or both. Since the experiments revealed that crest-derived cells from the back-transplanted bowel reached sympathetic ganglia in the host embryos, the ability of these enteric-derived cells to express tyrosine hydroxylase (TH) immunoreactivity was also investigated. TH is never expressed by crest-derived cells in the avian gut (Smith et al. 1977), but is a marker for cells developing along the sympathico-adrenal lineage. Chick–quail interspecies grafts were made so that the quail nuclear marker could be used to follow the donor cells in host embryos.

**Materials and methods**

**Donor tissue**

Fertilized quail eggs (*Coturnix coturnix japonica*) were incubated at 37°C in a forced-air incubator for 4–5 days. The embryos were removed from the eggs and staged according to Zacchei (1961). The gastrointestinal tract was then extirpated, immersed in Tyrode’s solution and dissected. The ganglion of Remak was carefully removed from the dorsal surface of the bowel and segments of presumptive duodenum, which has at this age been colonized by vagal crest-derived cells, or of hindgut (colorectum or caecal appendage), which have not, were removed and minced. As controls, segments of lung primordia (E6), mesonephros (E5–6) and limb bud (E4) were also removed. The resulting small pieces of minced tissue were used for grafting into host embryos.

**Host embryos**

Chick embryos (White Leghorn) were incubated at 37°C. Embryos were incubated until they had attained 14–22 somites (stages 11–14 Hamburger–Hamilton [H–H; 1951]). 3 ml of albumin were removed from each egg. Embryos were visualized by injecting a suspension of carbon particles in Tyrode’s solution beneath the blastoderm. A portion of the vitelline membrane was removed and a unilateral cut was made in the embryonic ectoderm, dorsal to the most caudal 1–3 somites. A small portion of the dorso-medial part of the somite, corresponding to the segment of neural tube next to which the graft was to be placed, was deleted. A segment of donor bowel was then inserted between the somites and neural tube (Fig. 1). The eggs were sealed with Scotch™ brand cellophane tape and the embryos were returned to the incubator. Host embryos were killed when they reached E6–10. The embryos were fixed overnight, at 4°C with 4% formaldehyde (from paraformaldehyde, in phosphate-buffered saline at pH 7.4), Zenerk’s, or Carnoy’s solutions. Most embryos were then dehydrated in a graded series of ethanol and embedded in paraffin; however, for immunocytochemical investigations (see below), embryos were cryo-

![Fig. 1. A diagram depicting the procedure used in back transplantation of segments of gut. Note that a segment of quail bowel was placed unilaterally between the neural tube and somites in the truncal region of host chick embryos.](image-url)
protected with 30% sucrose, embedded in OCT medium (Miles Laboratories, Naperville, IL), frozen with liquid N2, and frozen sectioned with a cryostat-micromate. Paraffin sections were cut at 8–10 μm and placed on gelatin-coated slides. These sections were stained with the Feulgen–Rosenbeck (1924) procedure, counterstained with carmine green and coverslipped.

Construction of chimeric foregut

In order to prepare segments of foregut for back-transplantation in which only the crest-derived cells would bear the quail nuclear marker (n=6), chimeric bowel was produced. For this purpose, chick hosts were initially prepared by excising the neuraxis from the vagal region of embryos at stages 9–10 [H-H; 7–10 somites]. This procedure deletes the vagal crest before crest cell migration has been initiated at this axial level (Le Douarin and Teillet, 1973). Isochronic segments of neuraxis were then removed from the vagal region of quail embryos. The quail neural rudiments were isolated from adherent tissue by incubation with Pancreatin™ (Gibco; diluted 1:4 with Tyrode’s salt solution) and placed into the groove formed in the previously prepared chick host embryos by the removal of the neuraxis. The proximo-distal orientation of the quail neuraxis was maintained in the grafts. The chimeric embryos resulting from this surgery were then incubated for an additional 3–5 days so that the foregut would become populated by crest-derived cells, all of which would bear the quail nuclear marker; moreover, the quail marker would not be found in endoderm- or mesoderm-derived cells. These segments of bowel in which only the crest-derived cells were specifically marked were then used for back-transplantation into chick host embryos as described above.

Growth of developing bowel in organotypic tissue culture

Segments of quail foregut (E4; presumptive gizzard and most proximal small intestine) were dissected. The gut was then cut into small (0.25 mm³) pieces and explanted onto collagen-coated or polylysine-laminin-coated coverslips. Just enough culture medium was added to prevent the explants from turbid for 1 day. Fresh medium was then added in an amount sufficient to cover the explants of bowel. The cultures were specifically marked were then used for back-transplantation into chick host embryos described above. Using p-phenylenediamine in glycerol (Johnson and de C. Nogueira Araujo, 1981). NC-1 immunoreactivity was also demonstrated with goat anti-mouse IgM labeled with alkaline phosphatase (diluted 1:50; Kirkegaard and Perry). When this procedure was employed, alkaline phosphatase activity was visualized in tissues by using an alkaline phosphatase substrate kit (Vector Laboratories; Kit III) that produces a blue reaction product. The staining was performed in the presence of 1.5 mM levamisole (Sigma Chemical Co.), which inhibits the endogenous alkaline phosphatase activity of the neural tissue (Ponder and Wilkinson, 1981). In addition, 10 mM MgCl₂, which enhances the enzyme activity of alkaline phosphatase (DeJong et al. 1985) was added to the solution containing the substrate. Preparations were incubated with the substrate in the dark for 30–60 min or until the blue reaction product was seen. Tyrosine hydroxylase (TH) immunoreactivity was demonstrated using a polyclonal anti-TH serum (diluted 1:1000; Eugene Tech, Allendale, NJ). Sites of bound anti-TH antibodies were visualized using a biotinylated goat anti-rabbit serum and avidin–horseradish peroxidase (HRP; Vectastain Elite ABC™ kit (PK-6101, Vector Laboratories). Peroxidase activity was visualized with H₂O₂ and 3, 3’-diaminobenzidine (DAB). DNA was then demonstrated by the Feulgen procedure following post-fixation overnight in Zenkers solution. Combining the localization of sites of NC-1 immunoreactivity with alkaline phosphatase and those of TH with peroxidase permitted simultaneous visualization (triple labeling) of TH and NC-1 immunoreactivities with the quail nuclear marker. Preparations were dehydrated, cleared in Histocontrol (National Diagnostics), coverslipped with Permount and examined by bright-field microscopy.

Results

Migration in vitro of enteric crest-derived cells out of explants of gut

The ability of intra-enteric crest-derived cells to migrate out of the bowel wall was investigated by growing explants of embryonic foregut, removed from E4–8 quail and chick embryos, in organotypic tissue culture. Cultures were grown for 10–14 days, fixed and AChE activity was demonstrated in the explants. In addition to dense plexuses of cells and fibers that displayed AChE activity within the explants, many small clusters of cells that were a considerable distance away from the explants themselves also expressed this marker (Fig. 2). These extra-enteric (satellite) clusters sent neuritic projections back to the gut proper and/or also to one another. The presence of neurons in the bowel and in the satellite ganglia away from it confirms that the preumbilical (E4) chick and quail gut contains crest-derived precursor cells that can leave the bowel and migrate away to give rise to distant ganglia.

Backtransplantation of quail foregut into chick embryos

Segments of E4 quail foregut were inserted between the most caudal 1–3 somites and the neural tube of E2 chick host embryos (14–22 somites; stages 12–14 [H-H]; Fig. 1). As demonstrated by the culture experiments described above, the E4 quail foregut contains crest-derived cells that are the precursors of the ENS. These cells develop and mature in the mesoderm-derived
mesenchyme that later becomes the musculoconnective tissue of the bowel wall. Host embryos were examined after a further 4–8 days of incubation (total time of incubation: 6–10 days). The grafts survived well and cells displaying the quail nuclear marker could easily be recognized in the host embryos in dense aggregates with a fibro-muscular appearance (Fig. 3). When grafts included endoderm, a blind cyst, or a sinus that opened to the surface ectoderm was formed. The epithelium lining this cyst or sinus was always entirely made up of quail cells and was surrounded by a condensation of mesenchyme consisting mostly of quail cells, but also containing some cells of host origin. Quail cells from the back-transplanted bowel were also observed in a variety of different sites in the host embryo (Table 1). Since the age of the host embryo at the time of back-transplantation might be expected to affect the migration of cells from the donor bowel, destinations were separately identified in embryos that were greater or less than 20 somites at the time of back-transplantation. The distribution of quail cells in host embryos was subjected to a $\chi^2$ analysis. The observed distribution differed significantly from one that might have occurred by chance ($P<0.001$). The destinations reached by grafted cells were always ipsilateral to the grafts and included sympathetic ganglia (Fig. 4A), spinal nerve roots (Fig. 4B), the adrenal glands (Fig. 4C), dorsal root ganglia (Fig. 4D) and peripheral nerves (Fig. 4E).

Fig. 2. Satellite ganglia formed in an organotypic tissue culture of an explant of E4 quail foregut. AChE activity has been demonstrated. Neurons and processes are stained darkly. The explant itself lies just out of the field at the top. The locations of satellite ganglia are shown by the →. Scale bar=50 μm.

Fig. 3. The main body of a back-transplanted segment of bowel displays a dense fibromuscular stroma. The boundary between the graft and the surrounding host tissue is shown by the →. Scale bar=20 μm.

Table 1. Targets reached by quail cells migrating from backgrafts of foregut (E4) placed into the truncal region of host embryos

<table>
<thead>
<tr>
<th>Target</th>
<th>&lt;or=20 somites</th>
<th>&gt;20 somites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal gland</td>
<td>0/9</td>
<td>6/9</td>
</tr>
<tr>
<td>Sympathetic ganglia</td>
<td>7/9</td>
<td>7/7</td>
</tr>
<tr>
<td>Dorsal root ganglia</td>
<td>7/9</td>
<td>9/9</td>
</tr>
<tr>
<td>Spinal roots and nerves</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>Gut</td>
<td>6/9</td>
<td>0/9</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>7/9</td>
<td>7/9</td>
</tr>
<tr>
<td>Meninges adjoining spinal cord</td>
<td>8/9</td>
<td>6/9</td>
</tr>
<tr>
<td>Dermis, feather germs, and dorsal mesenchyme</td>
<td>6/7</td>
<td>8/9</td>
</tr>
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</table>
Moreover, cells from the back-grafts of foregut also invaded the adjacent neural tube (Fig. 4F) and were later observed in both white and gray matter of the spinal cord. Some of the targets reached by these enteric-derived cells, such as spinal roots and ganglia, and neural tube were only colonized by quail cells in the region adjacent to the grafts. Other targets, including sympathetic ganglia, the adrenal gland and peripheral nerves were located at some distance from the site of the implants. In addition, quail cells were also found in the feather germs (Fig. 5A), dermis, dorsal mesenchyme, the walls, but not the endothelium, of blood vessels (Fig. 5B), and the meninges, but donor cells never entered the bowel of the host embryos or produced pigment. Moreover, quail cells from back-grafts of bowel reached all the same destinations in host embryos whether or not endoderm was included in the grafted tissue; however, no cyst or sinus was formed by endoderm-free grafts of donor gut.

Back-transplantation of quail lung primordia, mesonephros, limb buds and hindgut into chick embryos

Since the grafts of quail foregut contained both crest- and mesoderm-derived mesenchymal cells, experiments were done to ascertain which of these was responsible for colonizing various sites in host embryos. One such experiment was to back-transplant segments of control tissues that contain few or no crest-derived cells. Lung primordia, mesonephros and limb buds were selected as sources of mesenchyme likely to contain rare, if any, crest-derived cells. No cells were observed to leave grafts of mesonephros (5 cases); however, many quail cells did exit from grafts of lung primordia or limb buds to enter the dermis, feather germs (Fig. 6A) and dorsal mesenchyme of the host embryos. Occasionally (2 of 9 cases), when a graft of lung or limb bud happened to be positioned so as to lie apposed to a dorsal root or sympathetic ganglion, small numbers of donor cells were found in the peripheral portion of an adjoining root or ganglion. (Fig. 6B). These cells appeared to lie in ganglionic connective tissue. In contrast to adjacent ganglia, however, quail cells were never seen in the adrenal gland, the spinal cord, or peripheral nerves. None of the cells leaving back-grafts of lung primordia or limb bud entered more than a single dorsal root or sympathetic ganglion immediately adjacent to the graft. No pigment-containing cells were seen in embryos that received back-grafts of lung primordia, limb bud or mesonephros.

The crest-derived cells in the foregut are known to be of vagal origin (Le Douarin and Teillet, 1973; Smith et al. 1977; Allan and Newgreen, 1980). In contrast to the foregut at E4, no vagal crest-derived cells are present at this time in the hindgut. Cells from the vagal crest do not colonize the hindgut until E7. The hindgut itself at E4 contains no cells that express neurofilament immunoreactivity (Payette et al. 1984; Pomeranz and Gershon, 1990), although it does contain a population of NC-1 immunoreactive cells that ascends within the bowel wall (Pomeranz and Gershon, 1990). Following grafts of hindgut, as after grafts of foregut, lung primordia and limb bud, quail cells were found in the dermis (Fig. 6C), feather germs and dorsal mesenchyme. Quail cells from hindgut grafts were also observed in dorsal root and sympathetic ganglia and the spinal cord; however, only ganglia adjacent to the grafts were colonized and quail cells from the hindgut were never seen in the adrenal gland. No pigment-containing cells were seen following back-transplantation of the E4 hindgut.

Back-transplantation of chimeric foregut into chick embryos

Since quail cells were found to leave back-transplanted organ rudiments in chick host embryos, even when these rudiments contained few if any cells of crest origin, the locations reached by cells leaving back-transplants of tissue do not by themselves establish whether or not these cells are crest-derived. In order to determine which sites in host embryos are reached by crest-derived cells emigrating from back-transplanted segments of bowel, chimeric foregut was constructed. The chimeric bowel, used for back-grafting, contained endodermal and mesodermal cells of chick origin and quail cells derived from the vagal level of the neural crest; therefore, detection of the quail marker in host embryos receiving these grafts revealed, not only that the marked cells were derived from the back-transplanted bowel, but that they were of crest origin. Mesoderm- or endoderm-derived cells leaving the gut could not be distinguished from cells of the host. Quail cells from chimeric back-grafts were found in the adrenal gland, sympathetic ganglia (Fig. 7A), peripheral nerves (Fig. 7B), dorsal root ganglia (Fig. 7C), spinal roots, and the spinal cord (Fig. 7D). Although quail cells did not enter feather germs, they were occasionally found in the dermis or dorsal mesenchyme, apparently within a peripheral nerve or blood vessel wall (Fig. 7E). The restriction of quail cells in the dermis and their failure to enter feather germs suggests that enteric crest-derived cells do not, following back-transplantation, give rise to mesectoderm. No pigment-containing cells were found.

Demonstration of NC-1 immunoreactivity in cells emigrating from enteric back-grafts

The monoclonal antibody, NC-1, has been observed to mark most crest-derived cells in avian embryos that are migrating or developing along neural or glial lineages (Vincent et al. 1983; Vincent and Thiery, 1984). Crest-derived cells colonizing the bowel have been found to be NC-1-immunoreactive (Tucker et al. 1986) although this immunoreactivity is lost from those crest-derived cells that develop as mesectoderm or as melanocytes. NC-1 immunoreactivity was investigated in embryos that had received back-transplants of E4 quail foregut. In these experiments, the quail nuclear marker was simultaneously demonstrated either by propidium iodide fluorescence (Rothman et al. 1986) or the Feulgen reaction. Many cells in the peripheral nerves (Fig. 8A), sympathetic ganglia, adrenal glands (Fig. 8B), and
spinal cord (Fig. 8C) of the host embryos, in which foregut had been back-transplanted, were doubly labeled by the NC-1 and quail markers. There were also many NC-1 immunoreactive cells in these sites that were of host origin. In addition, ganglia, made up of NC-1-immunoreactive cells, developed within the fibromuscular stroma of the grafts themselves (Fig. 8D).

The dermis, feather germs and dorsal mesenchyme were also investigated with the NC-1 antibody. In this case, the reagent was applied to animals that had
received back-transplants of E4 foregut or hindgut. Although many quail cells migrated from either type of graft to the dermis, feather germs and dorsal mesenchyme, most of these cells were not NC-1-immunoreactive. NC-1 immunoreactivity in the skin was confined to scattered small nerve fibers that were innervating the areas invaded by quail cells from the grafts of foregut or hindgut (Fig. 8E).

**Demonstration of tyrosine hydroxylase immunoreactivity in cells emigrating from enteric back-grafts**

The observation that cells of vagal crest origin leave back-grafts of bowel and reach sites that are normally targets of migration of trunk crest-derived cells raises the question as to whether the enteric cells in their novel destinations express phenotypes appropriate for...
Fig. 6. (A) Quail cells (→) from a graft of E4 limb bud have infiltrated the mesenchyme of a feather germ of a chick host embryo. (B) Quail cells (→) from an adjacent graft of limb bud have entered the peripheral region of an adjoining dorsal root ganglion of a chick host embryo. These cells appear to be located in ganglionic connective tissue. Note that similar appearing quail cells are also found in the mesenchyme surrounding the ganglion (arrowhead). (C) Quail cells from a graft of E4 hindgut have colonized the dermis of a chick host embryo. No quail cells have entered the epidermis. Scale bars=20μm.

the vagal or truncal levels of the crest. No cells in the avian bowel express TH immunoreactivity in situ, but this enzyme is expressed in sympathetic ganglia and adrenomedullary cords. Experiments were therefore performed to determine whether crest-derived cells leaving back-grafts of bowel and entering sympathetic ganglia were TH-immunoreactive. Following back-transplantation of segments of foregut, many TH-immunoreactive cells were found in sympathetic ganglia that were doubly labeled by the quail nuclear marker (Fig. 8F). When NC-1 immunoreactivity was simultaneously demonstrated with that of TH, cells were observed that were triply labeled, displaying the quail nuclear marker, TH, and NC-1 immunoreactivities (Fig. 8G). In contrast to sympathetic ganglia, no TH-immunoreactive quail cells were found in dorsal root ganglia or within the grafts themselves; nevertheless, many small quail cells in dorsal root ganglia (Fig. 8H) and within the grafts (Fig. 8D) expressed NC-1 immunoreactivity, indicating that enteric cells of vagal crest origin were present in both of these locations.

Discussion

Observations made on explants of E4 quail foregut grown in organotypic tissue culture provided the first evidence that crest-derived precursor cells are able to leave the bowel. At this developmental age, the foregut is known to have been colonized by cells derived from the vagal region of the neuraxis (Le Douarin and Teillet, 1973; Allan and Newgreen, 1980; Payette et al. 1984; Tucker et al. 1986). Clusters of cells that displayed AChE activity were found in the culture dishes a considerable distance away from the enteric explants. Both the neuritic connections established between these clusters and their expression of AChE indicated that they contained neurons. AChE is a marker for enteric neurons; it is expressed by all of the neurons of the mature ENS (Schofield, 1968; Gunn, 1971) and all of the cells that develop in explants of avian foregut that express AChE co-express NC-1 and neurofilament immunoreactivities (Pomeranz and Gershon, 1990). The clusters thus represent satellite ganglia. Since neurons do not themselves migrate, the formation of satellite ganglia in the cultures indicates that at least some of the crest-derived cells that colonize the E4 quail foregut are neural precursors that are not permanently fixed in place, but able to dissociate themselves from the surrounding bowel wall and migrate out of the gut. Back-transplantation was then used to investigate the developmental potential and migratory routes followed by cells exiting from the wall of the gut. In these experiments, quail gut was grafted into chick embryos so that donor cells could be identified in the hosts by recognizing the quail nuclear marker. Endodermal derivatives were identified by comparing the results of back-transplanting segments of gut that did or did not contain mucosa. Vagal crest derivatives were identified by comparing the patterns of migration in host embryos of cells leaving back-grafts of foregut with those of cells leaving control back-grafts of mesonephros, lung primordia, limb bud or E4 hindgut, none of which contain substantial numbers of vagal crest-derived cells. Two
Fig. 7. Targets in chick host embryos reached by crest-derived cells of quail origin (→) migrating from chimeric grafts of foregut (E5–E6). (A) Sympathetic ganglion. (B) A peripheral nerve. Note the abundance of crest-derived quail cells in the nerve and their absence from the surrounding mesenchyme. (C) Dorsal root ganglion. Note that most of the donor cells in the ganglion are small. (D) Spinal cord. Note that the quail cells are found in a cluster (arrowhead). They are located at the junction of the gray (g) and white matter (w). (E) Linear aggregates of cells in the dorsal mesenchyme. These aggregates appear to be associated with developing blood vessels and may represent Schwann cells in perivascular nerves. Scale bars=20 μm.
additional techniques were utilized to distinguish crest-derived from other enteric emigrés in the host embryos. Most important of these was to examine back-transplanted chimeric bowel, in which vagal crest-derived cells were quail and mesoderm-derived cells were chick. The other technique was to demonstrate NC-1 immunoreactivity, which marks crest-derived cells that are migrating or developing along Schwann or neural lineages (Vincent et al. 1983). In the developing chick foregut specifically, both the neurofilament-immunoreactive cells and the glial cells are NC-1-immunoreactive (Pomeranz and Gershon, 1990), although the glial cells do not express the immunoreactivity of glial fibrillary acidic protein in situ until hatching (Payette et al. 1984). Crest-derived cells migrating out of the donor bowel, therefore, are doubly labeled in NC-1-immunostained preparations, displaying both the quail nuclear marker and NC-1 immunoreactivity.

When foregut was used as the donor tissue, quail cells were found in dorsal root ganglia, sympathetic ganglia, the adrenal glands, peripheral nerves and spinal roots. These sites were also colonized in host embryos by quail cells when the grafts used for back-transplantation were themselves chimeric and only the crest-derived cells were of quail origin. The quail cells leaving grafts of foregut and reaching these targets in the host embryos also expressed the NC-1 marker. These observations thus indicate that crest-derived cells leave back-grafts of bowel and migrate again in younger host embryos. The targets of the host to which the bulk of these crest-derived cells from the gut migrate are appropriate destinations for cells emigrating from the crest at the level of the trunk (Le Douarin, 1982). Some of these sites, such as sympathetic ganglia and the adrenal gland, are not appropriate targets for cells derived from the vagal crest, the original source of the crest-derived cells in the donor bowel. Moreover, in none of the embryos receiving truncal grafts of gut did any donor cells reach the host’s bowel. When some of the enteric crest-derived cells recover the ability of their ancestors to migrate as a result of back-transplantation, therefore, they follow pathways that reflect neither their axial level of origin nor their previous migratory history. Instead, the path they follow is determined by the location of the graft. These observations show that following its colonization the bowel retains a population of crest-derived cells that are not yet terminally differentiated and which have the ability to migrate when placed in a permissive environment. In culture, these cells migrate randomly to give rise to satellite ganglia scattered around an explant of bowel. In an embryo, however, the crest-derived cells evidently follow defined routes and thus are able to respond to directional cues or signals provided by the host embryo. The marked tendency of crest-derived cells to leave the bowel, manifested in both the culture and back-transplantation situations, indicates that a mechanism must exist in the intact gut to retain these migratory cells and prevent the exodus that takes place when the bowel is removed from its natural location. The nature of this mechanism remains to be determined.

Sites in host embryos where mesodermal cells from back-transplanted bowel were found were different from those to which crest-derived cells migrated. For example, donor cells were found in the dermis of the skin, feather germs and the dorsal mesenchyme of the hosts, no matter whether foregut, hindgut, lung primordia or limb bud were back-transplanted. Moreover, most of the quail cells in these locations failed to exhibit NC-1 immunoreactivity. Most importantly, very few quail cells were found in the dermis, feather germs and dorsal mesenchyme when chimeric back-grafts of gut were made, in which only crest-derived cells carried the quail nuclear marker. The vast majority of the donor cells in the dermis of the skin, feather germs and dorsal mesenchyme, therefore, were from the mesoderm-derived enteric mesenchyme. The small numbers of crest-derived cells reaching these destinations were probably Schwann cells associated with peripheral nerves. Small numbers of mesoderm-derived cells, especially from limb buds, were occasionally found within dorsal root ganglia and peripheral nerves adjacent to grafts. These cells appeared to contribute to the connective tissue at the periphery of these structures. It is probable that the mesodermal cells entered ganglia only by direct extension because no non-crest-derived cells from any source appeared to be able to follow migration pathways to reach sympathetic ganglia, the adrenal glands, or ganglia and nerves in segments distant from the sites of the grafts.

Certain cells from the small intestine that migrated...
following back-transplantation in host embryos so as to colonize sympathetic ganglia were found to express TH immunoreactivity. Since these cells were also NC-1 immunoreactive, it can be concluded that they were crest-derived. TH immunoreactivity is not expressed during development or in adult animals within the avian small intestine (Mackey et al. 1989); the expression of this phenotype thus is inappropriate for crest-derived cells colonizing the bowel. TH expression, however, is appropriate for crest-derived cells in sympathetic ganglia. In contrast to sympathetic ganglia, none of the crest-derived cells that left donor back-grafts of bowel to reach dorsal root ganglia, spinal roots or peripheral nerves, expressed TH. Expression of TH by cells migrating from gut back-transplants is thus limited in occurrence to cells developing in a site in which TH expression normally occurs. Phenotypic expression by these secondarily migrating cells, therefore, is affected by their ultimate location within the host. It can be concluded that the population of crest-derived cells in the avian bowel contains cells that not only are able to migrate in a host embryo, but to express certain target-specific phenotypes that are not displayed in the enteric environment. The enteric crest-derived population, therefore, contains precursor cells that are either uncommitted with regard to phenotype or, alternatively, committed to a catecholaminergic phenotype, which is not expressed unless the cells reach a micro-environment that is permissive for catecholaminergic expression.

It is of interest that no cells from back-grafts of bowel ever contained pigment and none appeared to give rise to mesectodermal derivatives. The absence of melanocytic or mesectodermal expression implies either that none of the crest-derived cells that colonize the gut remain capable of melanocytic or mesectodermal expression, or that none of the crest-derived cells that leave back-grafts of bowel reach sites in the host embryos that are permissive for expression of these phenotypes. Melanocytes are known to develop from cells leaving the neural crest at all levels of the neuraxis (Teillet, 1971) including the vagal one (Ciment et al. 1986), and mesectodermal derivatives are normally produced by vagal crest-derived cells (Le Douarin, 1982); therefore, cells capable of differentiating as melanocytes or mesectoderm are present in the crest population from which the cells that colonize the gut are derived. Melanocytes are not normally present in the bowel of quail embryos; nevertheless, the gut has been shown to be able to support the development of melanocytes when truncal crest-derived cells are induced to invade explants of bowel in chorioallantoic membrane grafts (Smith et al. 1977) or organotypic tissue cultures (Coulter et al. 1988). It thus seems likely that crest-derived cells that are capable of melanocytic expression are filtered out of the population that colonizes the gut before these cells reach the bowel. It has been reported that crest-derived cells lose their ability to give rise to melanocytes within the branchial arches (Ciment et al. 1986) and it is known that crest-derived cells migrating to the bowel pass through the caudal branchial arches (Ciment and Weston, 1983; Payette et al. 1984; Tucker et al. 1986). The branchial arches could thus act to remove cells capable of melanocytic (and perhaps also mesectodermal) expression from the population of crest-derived cells that colonizes the bowel. If so, the failure of crest-derived precursors in back-grafts of gut to give rise to pigment or connective tissue cells in host embryos would be due to an absence of progenitors with a melanocytic or mesectodermal potential in the population of crest-derived cells that colonizes the gut, rather than to an inappropriate environment.

Cells from the enteric back-grafts were found to be capable of invading the neural tube of the host embryos. This phenomenon has previously been reported (Rothman et al. 1987). Since migration of donor cells into the CNS only occurred when grafts were made of gut and not of limb buds, lung primordia, or other types of peripheral ganglia (Le Douarin et al. 1978; Ziller et al. 1979; Erickson et al. 1980; Le Lièvre et al. 1980; Ayer-Le Lièvre and Le Douarin, 1982; Schweitzer et al. 1983), it is likely that the cells that invade the neural tube are crest-derived. This conclusion is strongly supported by the observations that quail-marked crest-derived cells from chimeric back-grafts were found in the CNS and that the enteric cells that invade the CNS are NC-1-immunoreactive. It is intriguing to note that although a similar invasion of the neural tube by crest-derived cells has not been seen following the back-transplantation of other peripheral ganglia (Le Douarin et al. 1978; Ziller et al. 1979; Erickson et al. 1980; Le Lièvre et al. 1980; Ayer-Le Lièvre and Le Douarin, 1982; Schweitzer et al. 1983), this phenomenon does occur when the migratory properties of cells in dorsal root ganglia are changed by exposure of the cells to a phorbol ester (Sears and Ciment, 1988). It is not clear why enteric crest-derived cells enter the neural tube. In doing so they clearly are not following an established neural crest migration pathway; however, it is of interest in this regard to recall that the normal structure of the ENS resembles that of the CNS more than it does extraenteric peripheral nerve (Gershon, 1981; Gabella, 1987). Conceivably, therefore, an affinity exists between the precursors of enteric neurons and/or glia and the CNS, which enables enteric vagal crest-derived cells to enter the neural tube when these structures are artificially placed in close proximity.

In summary, the current results are consistent with the view that the quail foregut at the time of its colonization contains crest-derived precursor cells that can leave the bowel and follow neural crest migratory pathways to reach ganglia, nerves and adrenal glands. When grafts are placed in the trunk, the pathways followed are appropriate for crest cells in the trunk. In addition, some of the enteric crest-derived cells appear to be able to express a phenotype that is not expressed within the gut, but which is appropriate for the site in which these migratory cells develop; thus, enteric crest-derived cells exhibit a site-specific expression of TH immunoreactivity in sympathetic ganglia. On the other hand, some of the potentialities of the neural crest, such...
as the ability to give rise to melanocytes and to mesoderm, are not expressed by back-transplanted enteric crest-derived cells; therefore, although the population of crest-derived cells in the foregut is multipotential, a restriction of their developmental potential has clearly occurred. The extent of this restriction, its progression with age, and its relation to the maturation of the bowel remains to be established.

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