Articles

Cell Line Segregation During Peripheral Nervous System Ontogeny

NICOLE M. LE DOUARIN

The peripheral nervous system of vertebrates arises from the neural crest and the ectodermal placodes. Construction of quail-chick chimaeras has provided significant information on the migration and fate of the neural crest and placodal cells. Transplantation of neural crest tissue to various sites in these chimaeras has demonstrated that the differentiation of neural crest cells is controlled by environmental influences during their migration and, particularly, during gangliogenesis. Experiments with in vitro and monoclonal antibody techniques have shown that these environmental cues act on a heterogeneous population of neural crest cells whose developmental potencies are partly restricted to definite differentiation pathways.

A CENTRAL PROBLEM IN DECIPHERING THE MECHANISMS that control the development of the fertilized egg to the embryo and the functional adult organism is how the developmental history of a cell influences its differentiation. The phenotype expressed by a cell is determined by two distinct, but not necessarily exclusive, mechanisms. The cell can be committed intrinsically according to its lineage derivation or extrinsically by local influences that are controlled by its position in the embryo. Changing the position of cells within the embryo can modify their destiny. However, this does not exclude the possibility that developmental decisions may result from intrinsically regulated mechanisms during cellular division. The problems are therefore to define the respective roles of cell-cell interactions and inherited genetic information in cellular differentiation and to elucidate the interplay of these two processes during development.

The neural crest of the vertebrate embryo, the main source of the cells of the peripheral nervous system (PNS), provides a good model to investigate these questions. The neural crest is a transitory embryonic structure arising from the lateral ridges of the neural primordium when they join mediodorsally during the closure of the neural tube (Fig. 1A). This structure has one striking developmental feature. Its cells have the capacity to undergo migration at precise periods of development along apparently definite pathways, and to finally settle in particular locations where they differentiate into a variety of cell types. At the trunk level, the PNS arises entirely from the neural crest, while in the head, ectodermal placodes (that is, thickenings of the superficial ectoderm which differentiate at variable distances from the main neural primordium, the neural plate) also participate in the formation of the sensory ganglia of certain cranial nerves.

Besides the PNS, the neural crest gives rise to a wide variety of structures. All the melanocytes of the body (except those of the pigmented retina and of the central nervous system) and certain endocrine and paracrine cells (the adrenal medulla, the calcitonin-producing cells, the type I cells of the carotid body) are of neural crest origin. Moreover, the cephalic neural crest yields mesenchymal cells that differentiate into the brain meninges, the entire facial and visceral arch skeleton and dermis, the musculo-connective wall of the large arterial trunks arising from the aortic arches, and the connective tissues of the buccal and pharyngeal region, including that of the salivary, thyroid, parathyroid, and thymus glands [for a review see (*1*)]. Neural crest development thus involves the establishment of different cell lineages and this necessarily raises the question of when and how the patterning of crest cell migration and diversification is established during embryogenesis.

Quail-Chick Marker System Applied to PNS Ontogeny

Much of what is known of the initial steps of PNS development is the result of in vivo and in vitro experiments with avian neural crest. The avian embryo, whose development is very close to that of mammals, is accessible to experimentation during the entire developmental period and allows the construction of embryonic (neural) chimaeras in which the fate of cells from each partner can be followed. Cells of the Japanese quail (*Coturnix coturnix japonica*) can be easily distinguished from those of the chick (*Gallus gallus*) because of the concentration of quail heterochromatin at the nucleolus in interphase nuclei (*2*). This is clearly different from the even distribution of chick heterochromatin in small chromocenters, (*3*) (Fig. 1B). Therefore quail and chick cells can be monitored throughout the duration of a coculture or graft to full differentiation. Since its discovery, the quail nuclear marker method has been used extensively in a variety of developmental systems (*4*).

By constructing neural chimaeras, the actual and potential fates of the component cells of the neural crest have been established and a fate map can be constructed. Fragments of the neural primordium from chick or quail at the level of the brain vesicles, of the spinal cord (*1*), or of the presumptive placodal ectoderm (*5*) (Fig. 2A) are transplanted into the equivalent region of an individual of the other species at the same developmental stage. These grafts are referred to as isotopic and isochronic grafts. Quail and chick embryos differ only slightly in their size and in the chronology of their development during the first days of the incubation period when most of the decisive events in embryogenesis occur, and, therefore, the chimaeras develop harmoniously. When the neural tube plus the neural folds are grafted (Fig. 2), the neural tube becomes incorporated correctly into the host axial structures, where it is covered by

N. M. Le Douarin is director of the Institut d'Embryologie du CNRS et du Collège de France, 49bis, Avenue de la Belle Gabrielle, 94130 Nogent-sur-Marne, France.

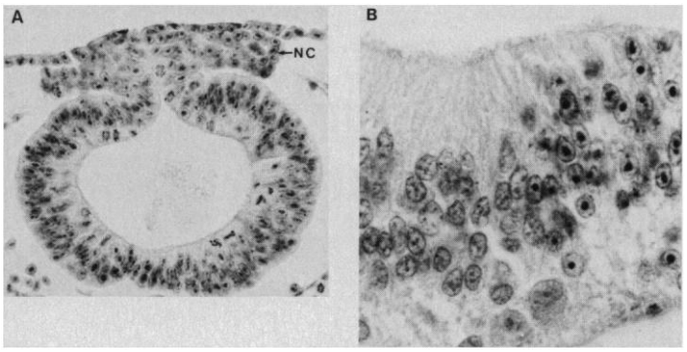


Fig. 1. (A) Transverse section of the midbrain of a seven-somite quail embryo showing the neural crest (NC). Magnification: ×535. (B) Neuroblasts of quail (right) and chick (left) stained by the Feulgen-Rossenbeck reaction. A large central mass of heterochromatin characterizes the quail nuclei. Magnification: ×1390.

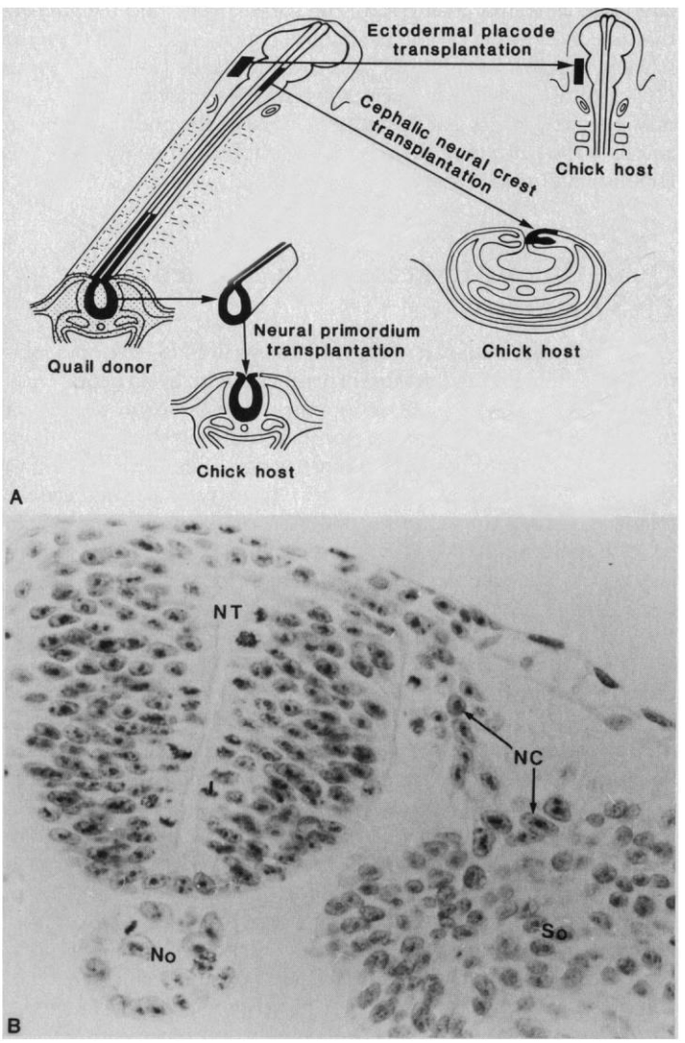


Fig. 2. (A) Experimental design used to trace the cells of neural crest and placodal origin during PNS ontogeny. (B) Migration of neural crest cells (NC) visualized by the quail nuclear marker characterized by the large mass of heterochromatin in the nucleus, in a 3-day (E3) chick embryo into which a quail neural primordium (neural tube plus neural folds) was grafted at the level of somites 23 to 28 at the 28-somite stage. The embryo was observed 18 hours after surgery. No, notochord; NT, neural tube; So, somite. The section was subjected to Feulgen staining. Magnification: ×947.

ectoderm within 12 hours, regardless of the species chosen as host or donor. Even single quail neural crest cells can be identified when migrating in chick tissues (Fig. 2B). In addition, the development of neural crest derivatives was normal in these birds. The correct maturation of the chimaeric central nervous system (CNS) was strikingly demonstrated by allowing the host to reach the stage of hatching (*6*). The hatched quail-chick spinal cord chimaeras can stand, walk, and fly, and their behavior is the same as that of normal chickens of the same age (Fig. 3).

Fate Map of the Neural Crest

The fate of neural crest cells was studied by grafting quail neural primordia (the neural tube together with the neural folds) into chick embryos (and vice versa) at all levels of the neuraxis. Neural crest cell migration and ganglion formation were then followed during development. The stability of the labeling in the quail-chick marker system allowed a correspondence to be established between the level of the graft and the location of the ganglion cells. This fate map of the autonomic nervous system (ANS) on the neural crest is represented in Fig. 4A.

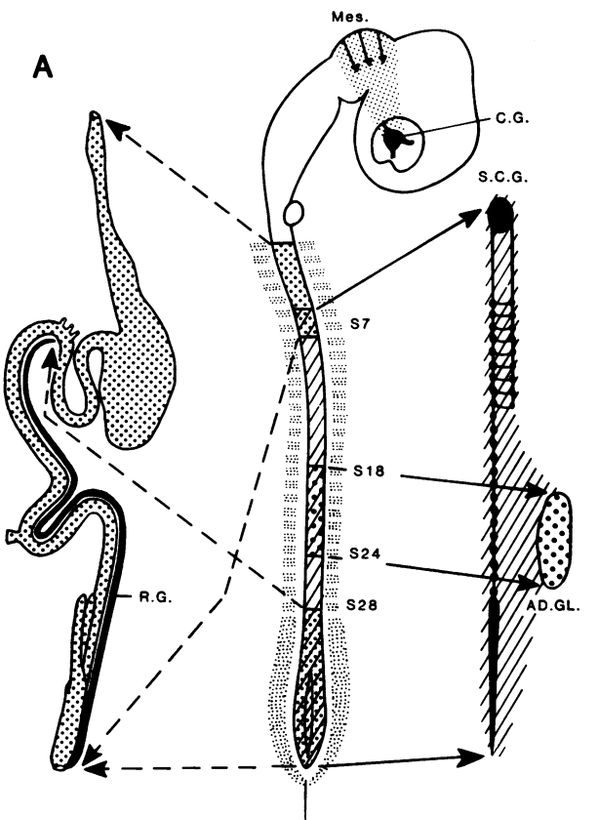
In the area of the trunk between somites 7 and 28, neural crest cell migration is strictly confined to the dorsal mesenchyme derived from the somites since the only crest derivatives in this area are the sensory and sympathetic chain ganglia, the aortic and adrenal plexuses, and the adrenomedullary cords. No crest cells penetrate the dorsal mesentery except for the Schwann cells that follow the nerve bundles to the periphery. In contrast, orthotopic grafts at the vagal and lumbosacral levels of the neural primordium result in colonization of the dorsal mesenchyme, the mesentery, and the splanchnopleure where the neural crest cells contribute to the enteric ganglia of the postumbilical gut. At the vagal level, the cells migrate lateroventrally under the superficial ectoderm. They reach the lateral wall of the pharynx and become incorporated into its mesodermal wall. Thereafter, they expand craniocaudally by migrating and dividing actively and form most of the intrinsic gut innervation. The levels of origin of the cells supplying the enteric, parasympathetic, and sympathetic ganglia roughly parallel the areas of the intermediate motor column from which the preganglionic fibers of the parasympathetic (vagal and sacral) and sympathetic (thoracolumbar) systems arise.

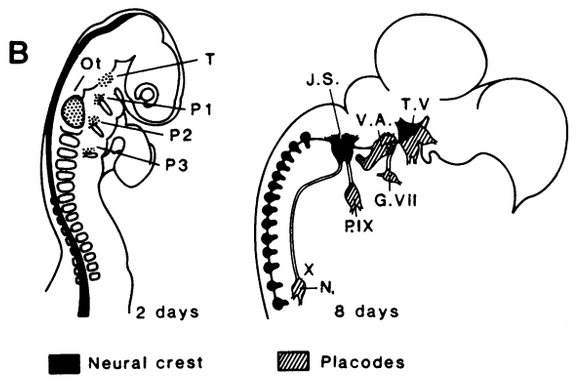
The sensory ganglia in the PNS are formed by a pair of dorsal root ganglia (DRG) which arises at the level of each somite, the more cranial pair corresponding to somite 6 in both quail and chick embryos. The ontogeny of the sensory ganglia in the head is complicated because the neural crest is not the only source of ganglion cells. The ectodermal placodes also participate in the formation of certain sensory ganglia and, until appropriate techniques were used, their ontogeny remained controversial. The isotopic labeling of the neural crest cells with [^{3}H]thymidine contributed to understanding this area (*7–9*). Subsequently the quail-chick chimaera system allowed the respective roles of the placodes and of the neural crest in the ontogeny of the cephalic PNS to be defined (*5, 10–12*). As a general rule, sensory neurons arise both from the neural crest and the ectodermal placodes, while the glial cells of the head sensory ganglia and nerves are all derived from the crest with no participation of the placodal ectoderm. The respective distribution of crest and placodal neurons in the cranial sensory ganglia is indicated in Fig. 4B. The fate map of the head PNS ganglia in a ten-somite embryo as drawn by D'Amico-Martel and Noden is shown in Fig. 4C. (*5*).

The nature of the migration pathways followed by the neural crest cells when they leave the neural primordium has been well docu-

Fig. 3. Quail→chick spinal cord chimaeras at 2 weeks of age. The quail neural primordium was grafted at the 25-somite stage at the brachial level (somites 17–21). The animals display normal behavior. Note the quail-chick pigmented feathers of the wings which result from migration into the chick skin of presumptive melanocytes from quail neural crest.

mented during the last few years and extensively reviewed recently (*13*). The role of glycosaminoglycans (particularly hyaluronic acid) and of cell-associated glycoproteins such as fibronectin in the migration process has been emphasized, but the puzzling problem of the specification of the final positioning of the neural crest derivatives remains largely unanswered. Detailed analysis of the pathways followed by neural crest cells in the various regions of the body pointed to the dependence of crest cell migratory behavior on the general morphogenetic processes taking place in the embryo at this stage of development. At the trunk level the metameric segmentation of the dorsal mesoderm into somites plays a crucial role in the patterning of DRG and sympathetic chain ganglia. The latter derive mainly from cells which migrate first between two consecutive somites and then laterally between the two initial somitic components, the sclerotome (future vertebral cartilage) and the dermomyotome (from which dermis and striated muscles arise). The DRG, in contrast, are formed at the level of each somite by cells which aggregate at the top of the neural tube, as if they were prevented from descending further by the somite itself (*14*). At the cephalic and vagal levels, the neural crest cells mainly take a superficial migration route under the ectoderm (*14*) and populate





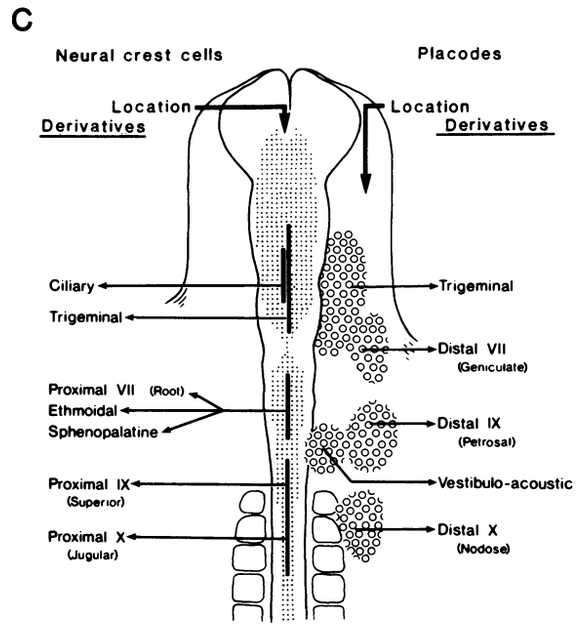


Fig. 4. (A) The origin of adrenomedullary and autonomic ganglion cells. The spinal neural crest caudal to the level of the fifth somite gives rise to the ganglia of the sympathetic chains. The adrenomedullary cells originate from the spinal neural crest between the level of somites 18 and 24. The vagal neural crest (somites 1–7) gives rise to the parasympathetic enteric ganglia of the preumbilical region. The ganglia of the postumbilical gut originates from both the vagal and lumbosacral neural crest. The ganglion of Remak (R.G.) is derived from the lumbosacral neural crest (posterior to the level of somite 28). The ciliary ganglion (C.G.) is derived from the mesencephalic crest (Mes.). AD.GL., adrenal gland; S.C.G., superior cervical ganglion. (B) (Left) Distribution of placodes in a 2-day-old embryo. T, trigeminal; Ot, otic; P1, P2, P3, epibranchial placodes. (Right) Sensory spinal DRG and cranial ganglia at 8 days, the neurons of which are either of crest (■) or placodal (▨) origin. V, VII, IX, and X refer to the number of the cranial nerve associated with the sensory ganglia; T, trigeminal; G., geniculate; J., jugular; S., superior; P., petrosal; N., nodose; and V.A., vestibuloacoustic. (C) Schematic drawing of a stage-9.5 chick embryo (about ten somites) indicating positions of neural crest and placodal anlagen for cranial sensory and autonomic ganglia [from (*5*) with permission].

the ventral regions of the head and visceral arches (*15*). Some of them later differentiate into bones, cartilage, and dermis, while others become incorporated into the developing cephalic PNS ganglia or, at the level of somites 1 to 7, form most of the innervation of the gut.

Developmental Potential of Neural Crest Cells

The fate maps described above led to the distinction in the neural axis of regions characterized by different fates (sympathetic, parasympathetic, enteric, or mixed) and migratory behavior of their component crest cells. The developmental potentials of these crest cells were tested by heterotopic transplantation of fragments of the neural primordium from quail embryos to chick recipients (*16–18*).

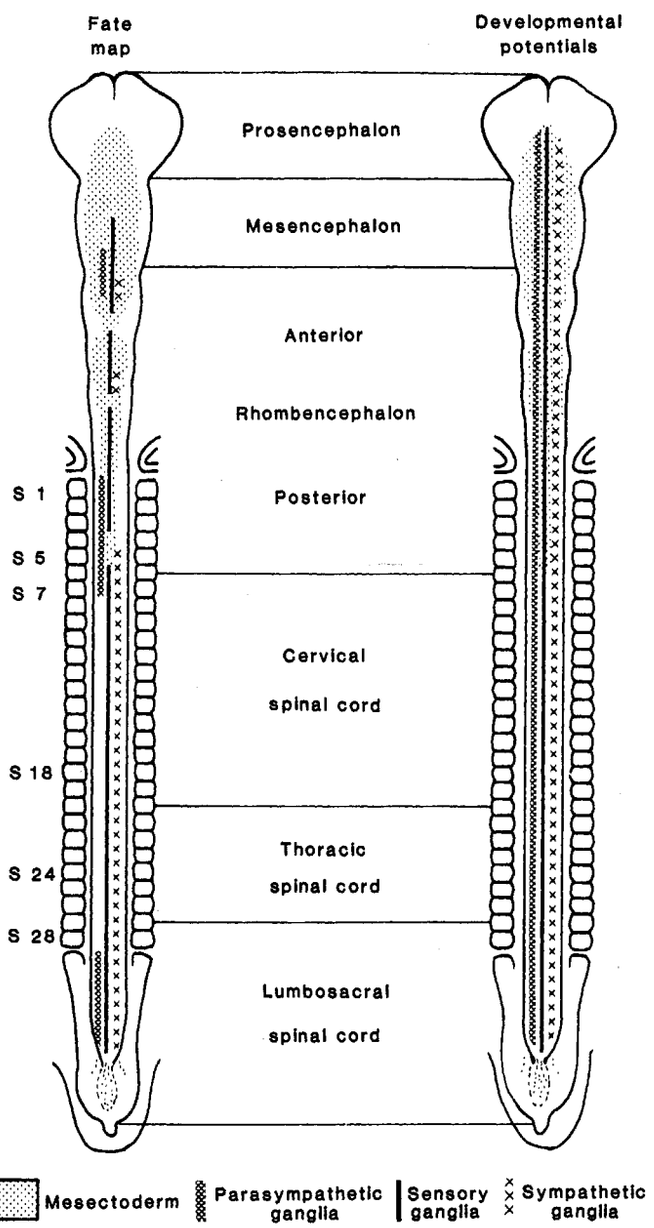


Fig. 5. Fate map of the presumptive territories along the neural crest yielding the mesectoderm, the sensory, parasympathetic, and sympathetic ganglia in normal development (left). Developmental potentials for the same cell types as shown in the fate map are indicated (right). In fact, if neural crest cells from any level of the neural axis are implanted into the appropriate site of a host embryo, they can give rise to almost all the cell types forming the various kinds of PNS ganglia. This is not true, however, for the ectomesenchymal cells (also called mesectoderm) whose precursors are confined to the cephalic area of the crest down to the level of somite 5. S, somite.

The cephalic and vagal neural crest, when transplanted at the level of somites 18 to 24 (adrenomedullary level of the crest), yielded adrenergic cells that colonized the host sympathetic ganglia and suprarenal glands. Conversely, cells from the cervicotruncal neural crest, which normally do not penetrate the mesentery and gut, did so when the neural tube was transplanted prior to crest cell migration in the vagal area of the neuraxis (*16*). These truncal crest cells which migrated into the gut did not follow their normal differentiation pathways. Not only did they become distributed into myenteric and submucosal plexuses, but they failed to express the adrenergic phenotype characteristic of their normal fate. Instead they differentiated, like the vagal crest, into cholinergic (*19*) and peptidergic (*20*) neurons.

These findings were confirmed in a different experimental system. Pieces of chick hindgut which had not received their supply of neural crest cells were transplanted onto the chick chorioallantoic membrane where they were associated with quail neural crest. Enteric plexuses that displayed cholinergic characteristics differentiated in the hindgut, irrespective of whether the crest cells would normally have given rise to enteric ganglia, to sympathoblasts, or to adrenomedullary cells (*1*).

Initial pluripotentiality of premigratory neural crest cells was also shown in a study by Noden (*10*) which involved heterotopic transplantation of different regions of the cranial neural crest. Thus, the forebrain crest, which normally does not yield PNS derivatives (Fig. 4C), formed normal ciliary and trigeminal ganglia when grafted to the mesencephalic-metencephalic region. In subsequent experiments (*18*), forebrain crest transplanted to the 18- to 24-somite area produced not only Schwann cells and glia but also sensory DRG neurons, sympathetic, and adrenomedullary cells.

Therefore, at each axial level, the neural crest is potentially able to give rise to the various cell types of the PNS (*21*). In this respect, the fate map of the neural crest cells differs strikingly from the distribution of their developmental potentials along the neuraxis (Fig. 5). Since each axial level of the body has a specific set of PNS structures, the various embryonic microenvironments must play a decisive role in gangliogenesis (as seen above), as well as in choosing among the large array of potential phenotypes that characterize neural crest cell populations.

Developmental Potentials of Peripheral Ganglion Cells

Back-transplantation of embryonic quail peripheral ganglia into the chick neural crest migration pathway (described in detail in Fig. 6) subjected the ganglion cells to microenvironments which elicit various phenotypes in neural crest cells. In the dorsal trunk structures of 2- to 4-day embryos, certain unexpected properties of peripheral ganglion cells appeared.

Soon after implantation, the ganglion lost cohesiveness and its component cells became dispersed in the host somitic structures. Twenty-four hours after grafting there were many single cells or small clusters of quail cells in the host somite region, many of which incorporated [^{3}H]thymidine (*22*). There were also pycnotic figures indicating the death of certain implanted cells. The dispersion phase was followed by entry of the progeny of the grafted cells into the host neural crest–derived structures. Localization of the grafted cells varied with the nature (sensory or autonomic) and age of the grafted ganglia. Quail cells populating the host DRG and differentiating there into sensory neurons and glia were found only after grafts of

DRG. In contrast, Schwann cells and autonomic derivatives (sympathetic neurons, chromaffin cells and, in some cases, enteric ganglia) were obtained after both DRG and autonomic ganglion grafts. When grafted under similar conditions, autonomic ganglia, such as the ciliary, Remak, and sympathetic ganglia, never gave rise to sensory neurons in the host DRG (*1, 23*). Moreover, the capacity of quail DRG cells to populate the host sensory ganglia was restricted to the time when sensory neuroblasts were still mitotic. When all the sensory neurons had withdrawn from the cell cycle, which was completed at the end of the seventh day (E7) of incubation in the quail, DRG grafts gave rise exclusively to ganglion cells of the autonomic type (*18*).

This suggested that (i) postmitotic neurons did not survive when implanted in a host embryo before E7 and (ii) neuroblasts able to populate the host DRG and to differentiate into sensory neurons disappeared from quail spinal ganglia after E7. It has been shown, mainly in tissue culture experiments, that survival and neurite regeneration of peripheral neurons are dependent upon growth factors such as nerve growth factor (NGF). It is conceivable that in the 2- to 3-day embryo growth factors are not yet produced or do not attain sufficient concentrations to ensure survival and neurite outgrowth of the implanted neurons. If postmitotic neurons die after transplantation, the cells that divide and differentiate in the host must belong to the non-neuronal cell population of the ganglion.

The possible non-neuronal origin of the dividing and differentiating cells in the ganglia was tested as follows. Because of the mixed placodal and crest origin of the petrosal and nodose ganglia, it is possible to label either their neuronal or non-neuronal (destined to become glial) component cells. Labeling these non-neuronal cells is achieved by making an isotopic and isochronic graft of a quail neural primordium at the rhombencephalic level. The chick epibranchial placodes provide the ganglia with neuronal cells and the implanted quail crest cells give rise to the whole population of non-neuronal cells. The reverse labeling is achieved with the quail as host and the chick as donor.

Back-transplantation of a 7- to 10-day nodose or petrosal ganglia containing quail neurons and chick non-neuronal cells into the migration pathways of the chick crest cells resulted in no colonization of the host crest derivatives by quail cells. In contrast, many quail cells invaded the host when the grafted chimaeric nodose ganglion contained a quail non-neuronal cell population. The invasiveness of the cells derived from the graft was considerable. Numerous quail neurons and glia were found in adrenal medulla, sympathetic ganglia, and gut. No quail-derived neurons, however, were found in the DRG of the host (*12*).

The Dual Cell Line Segregation Model

To account for these experimental results, I proposed a model in which two cell lines of peripheral ganglion precursor cells become segregated early during neural crest ontogeny (*24*) (Fig. 7A). These are the precursors which give rise, respectively, to sensory and autonomic neurons. In the migrating crest or in the very early ganglia, developmental potentials of the two precursors are already restricted to the autonomic and sensory pathways. However, choices concerning their terminal differentiation can still take place under the influence of definite environment cues, as demonstrated in sympathetic nerve cells of the newborn rat (*25*).

According to this hypothesis, two types of precursors, autonomic and sensory, exist in the emigrating crest cells. In the back-transplantation experiments no sensory neurons could be obtained from either distal sensory or autonomic ganglion grafts, while autonomic derivatives arose from all types of grafted peripheral embryonic ganglia. I propose that this reflects different survival requirements for the two types of precursors. Sensory precursors are able to survive and differentiate only in ganglia situated in close proximity to the CNS, while autonomic precursors remain alive in all types of PNS ganglia, at least until hatching. Moreover, since sensory neurons of graft origin did not arise in the host when the implant of DRG was removed from embryos older than E7, it can be deduced that neuroblasts of the sensory type disappear from quail sensory ganglia after this stage. This corresponds to the time when all the DRG neurons of the quail have withdrawn from the cell cycle (*18*). Therefore, sensory neuronal precursors either become postmitotic neurons or disappear in the DRG after E7 in quail embryos. In contrast, autonomic precursors remain in all types of peripheral ganglia even when neuronal differentiation is complete, since their development can be triggered by the microenvironment of a younger host in the back-transplantation experimental system.

In support of this view is the observation that all sensory ganglia whose neurons originate from the neural crest develop in contact with the CNS. This is true for the DRG, for the proximal part of the trigeminal ganglion (situated on cranial nerve V), and for the superior-jugular complex corresponding to the root ganglia of nerves IX and X. In addition, as soon as they withdraw from the cell

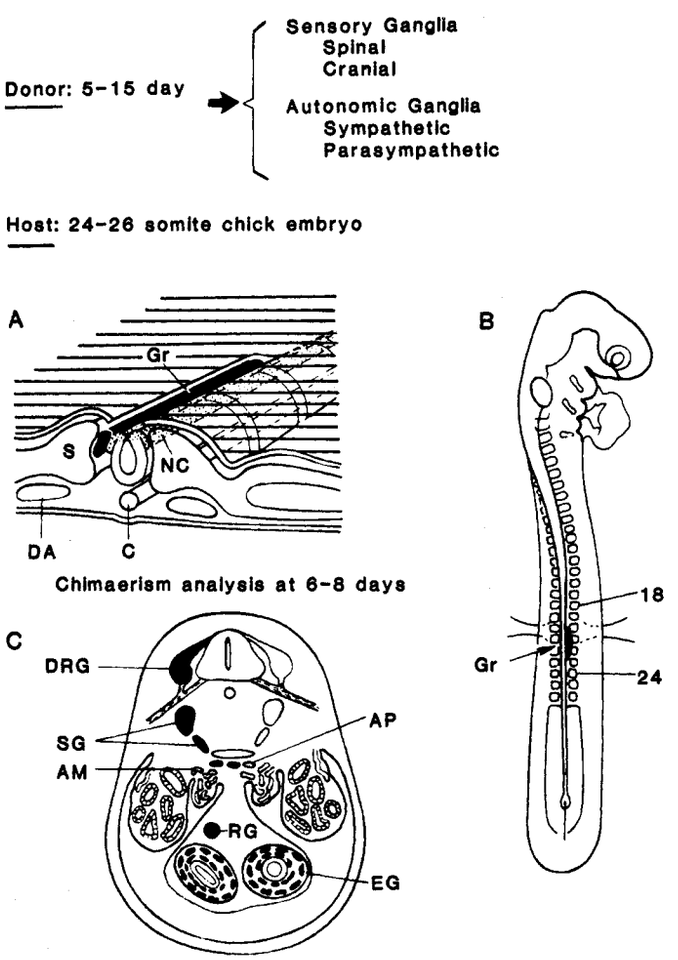


Fig. 6. Diagram showing the experimental procedure followed in the back-transplantation of quail PNS ganglia into the chick neural crest migration pathway. (A and B) The position of the graft (≅2000 cells). (C) The host crest derivatives in which quail cells are found in the 6- to 8-day chick host. AM, adrenal medulla; AP, aortic plexus; C, notochord; DA, dorsal aorta; DRG, dorsal root ganglion; EG, enteric ganglia; Gr, graft; NC, neural crest; RG, ganglion of Remak; S, somite; and SG, sympathetic ganglia.

cycle, sensory neurons readily extend neurites toward the CNS, as can be seen with antibodies directed against neurofilament proteins (*26*). It can be proposed that the CNS exerts a short-range positive effect on the survival, and perhaps the differentiation, of precursor cells of the sensory lineage (Fig. 7B) via production of a growth factor at these early stages. One candidate for this function might be the factor recently extracted and purified from adult pig CNS by Barde *et al.* (*27*) since it enhances survival of and neurite outgrowth from chick DRG neurons in culture. Another candidate is NGF, for which DRG are a natural target. Moreover, NGF-like immunoreactivity has recently been demonstrated in the CNS (spinal cord and brain) of certain mammalian fetuses (*28*), as has retrograde transport of ^{125}I-labeled NGF from the spinal cord of newborn rats to the DRG via the dorsal roots (*29*).

Cell Line Segregation in Vitro

The differentiation of neural crest cells in culture has been reported to be dependent on the composition of the culture medium and substrate. Moreover, the results obtained in my laboratory suggest that the differentiation in the various media is related to the degree of commitment of the progenitor cells present in the neural crest during the migratory phase.

When cultured in serum-containing medium (Dulbecco's minimal essential medium supplemented with 15 percent fetal calf or horse serum), isolated mesencephalic or trunk neural crest cells grew rapidly but did not exhibit a morphologically identifiable neuronal phenotype. However, acetylcholine (Ach)-synthesizing activity, already existing in vivo, was significantly higher in these cultures than in the freshly removed crest (*30*). Under these conditions, catecholamine (CA) biosynthesis was very low or absent, which suggested that proliferation but not differentiation of the undifferentiated crest cells was triggered.

In contrast, when equivalent neural crest explants were cultured in a fully defined serum-free medium which contained hormones, growth factors, and transferrin [Basic Brazeau Medium (BBM)] (*31*), a subpopulation of crest cells readily differentiated into neurons within a few hours without dividing. These cells extended neuronal outgrowths and expressed tetanus toxin binding sites and neurofilament proteins. A pulse of depolarizing current applied to the cells, which had typical neuritic processes, revealed their capacity to generate action potentials from day 4 in culture (*32*), thus confirming their neuronal nature. Catecholamines were neither synthesized nor stored in the neurons which differentiated in serum-free medium, and only very low levels of ACh synthesis could be detected in these neurons. The neurotransmitter(s) associated with these neurons is thus unknown. Therefore, serum exerted a strong inhibitory effect on neurite outgrowth, and conditions stimulating proliferation of crest cells were incompatible with the expression of a neuronal phenotype by these cells.

Culture of neural crest cells can, however, yield neuron-like cells (*33*) containing CA and somatostatin (*34*) even in the presence of serum if 10 to 15 percent chick embryo extract (CEE) is added to the medium. These cells appear in the culture only after several days during which time many of them incorporate [^{3}H]TdR (*34*).

Two generations of neurons can be successively obtained from the same explanted mesencephalic neural crest if the conditions are modified during the course of the culture. In cultures from mesencephalic crest initiated in BBM, the bipolar neurons readily differentiate within the first 24 hours and disappear rapidly if serum is added at 48 hours. The non-neuronal cells divide actively but no morphologically recognizable neurons develop. In contrast, if CEE prepared from E9 chicks is added to BBM along with the serum, a novel generation of neuron-like cells containing CA arises from about day 6 onward from cycling precursors (*35*). Thus, the adrenergic phenotype develops in culture only when precursors are allowed to divide. This is consistent with the inhibition by antimitotic agents of

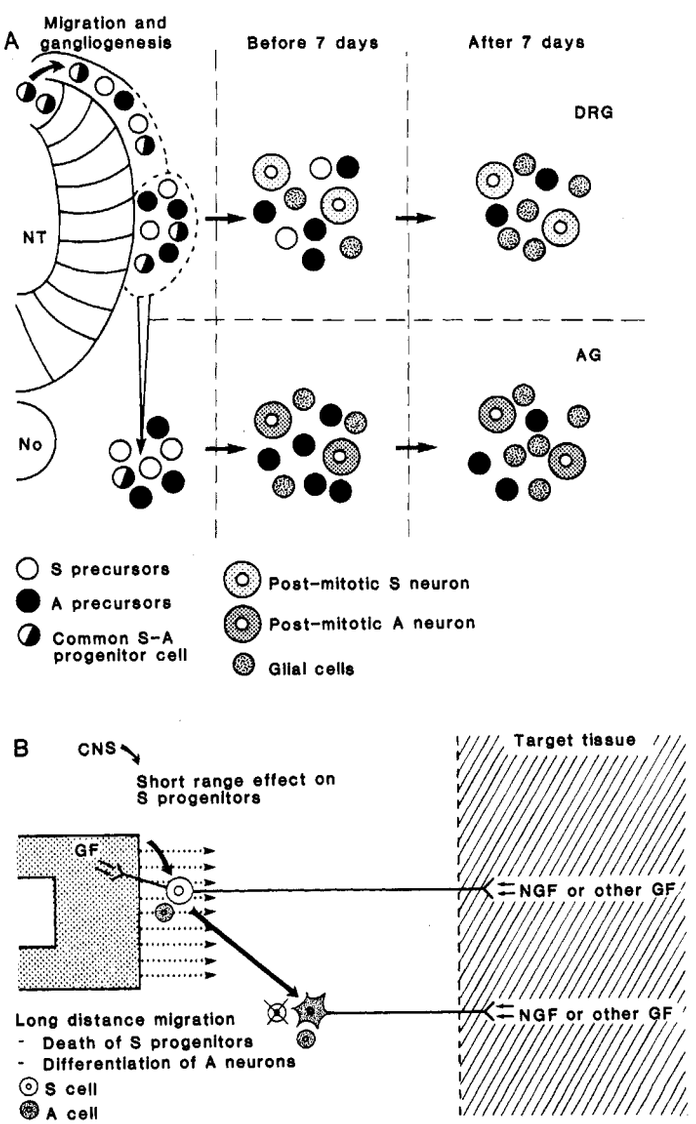


Fig. 7. (A) Hypothesis accounting for cell line segregation during PNS ontogeny. Two types of precursors, sensory (S) and autonomic (A), arise from a common progenitor during neural crest cell individualization and/or migration and in the early steps of gangliogenesis. During gangliogenesis, the S precursors can survive only in ganglia developing in close proximity to the CNS, from which they benefit from the effect of a growth factor. They rapidly become exhausted in their proliferation capacities and, by 7 days, they have reached the postmitotic state. In the non-neuronal cell population of these sensory ganglia, A progenitor cells subsist while glial cells differentiate. In the autonomic ganglia (AG) developing at a distance from the CNS, the S progenitors disappear rapidly while the A precursor cells survive at least until hatching. (B) Diagram showing the different survival requirements of the sensory (S) and autonomic (A) progenitor cells of the PNS at the time of early gangliogenesis. S progenitors need a growth factor (GF) from the CNS to survive and extend neurites. It is only later in development that they find in the target organ NGF and/or other GF able to fulfill their needs. A progenitors can survive and differentiate at a long distance from the CNS. In sensory ganglia, they survive for long periods of time but neither extend neurites nor differentiate in quail and chick. S precursors do not survive if they happen to migrate to ganglia distant from the CNS.

CA-containing cell differentiation in crest cultured with both serum and CEE (*36*).

These results support the contention that the developing neural crest contains at least two types of neuronal precursors with distinct behavior and differentiation requirements. More work is needed to relate more closely the data obtained in vivo and in vitro, and to identify the two types of progenitors.

The conclusions reached as a result of in ovo transplantation experiments, which indicated the presence of autonomic precursors in the non-neuronal cells of the dorsal root and nodose ganglia, have been further substantiated by the in vitro culture approach (*37*). The DRG were removed from E9 to E15 quail embryos (stages at which all sensory neurons are postmitotic), dissociated to single cells, and grown in tissue culture. We examined the cultures for cells with autonomic features by monitoring properties associated with the adrenergic phenotype after having confirmed that no cells with such features were detectable during normal embryonic development of the ganglia. When the medium contained CEE, numerous cells exhibited tyrosine hydroxylase (TH) immunoreactivity from day 4 of culture onward. They contained endogenous CA, as demonstrated by glyoxylic acid–induced histofluorescence, and the cultures also converted exogenous tyrosine to norepinephrine. A large proportion of the TH-positive cells divided before and after differentiating. In contrast, sensory neurons never displayed adrenergic properties and did not multiply. Results obtained with dissociated nodose ganglion cultures were qualitatively similar. These results confirm the presence in the non-neuronal cell population of the sensory ganglia of potential autonomic precursor cells which are able to divide and to differentiate along the adrenergic pathway under the influence of environmental factors, here provided by CEE.

Heterogeneity of Neural Crest Indicated by Monoclonal Antibodies

As cells differentiate, certain surface and cytoplasmic antigens either appear or undergo developmental changes. Monoclonal antibody technology provides unique tools to reveal and study such differentiation antigens. Several laboratories have detected specific antigenic determinants on differentiated peripheral ganglia and traced their cell lineage to their early precursors in the neural crest. This has revealed that a certain degree of cellular heterogeneity is established very early in the neural crest. For example, the monoclonal antibody EC8 directed against DRG ganglion cell identifies a cytoplasmic polypeptide associated with the neuronal phenotype in PNS ganglia and is first detectable at the end of neural crest cell migration in the branchial arch region (*38*). Another indication of the early commitment of certain cells is the demonstration of immunoreactivity against neurofilament protein (a marker for the neuronal phenotype) in early migrating crest cells of the chick (*39*).

The monoclonal antibodies CG1 and CG4 prepared from mouse spleen by Barald (*40*) using chick ciliary ganglia as the immunogen are more restricted in their cellular specificity. These antibodies stained only ciliary ganglion neurons and a small subpopulation of cultured mesencephalic neural crest cells. Their reaction with the crest in situ has not, however, been reported. Recently, immunization of a mouse with quail nodose ganglia yielded a monoclonal antibody, GlN1, which reveals a surface and cytoplasmic marker present on about 25 percent of early migrating crest cells and which is subsequently expressed by all peripheral glial cells and a neuronal subpopulation. This antibody immunoprecipitates a polypeptide of 80 kilodaltons from PNS ganglia membrane extracts. When compared to other markers of antigenic heterogeneity of the neural crest cells GlN1 exhibits the most striking pattern.

Conclusions

The migrating neural crest cell population is not composed of a homogeneous population of cells that are all endowed with the entire range of developmental capacities expressed in neural crest derivatives. Certain developmental restrictions are already imposed on crest cells before they reach their target sites in the developing embryo. These restrictions, which apply to crest cells all over the neural axis, are probably acquired through the successive cleavages of neural epithelium-derived cells. Clones that vary in their expression of the pigment cell phenotype can be obtained from cultured truncal neural crest (*41*), thus demonstrating an initial heterogeneity in the neural crest. The cell lineage yielding the crest-derived mesenchymal cells (mesectoderm) also segregates early from the other neural crest stem cells (*1, 16*). During PNS development, the cells that differentiate into sensory neurons (type S progenitors) or into autonomic cell types (type A progenitors) form two distinct lineages that are isolated early during neural crest ontogeny. Further differentiation choices take place during the development of the S and A lineages and are regulated by environmental influences. These influences may already operate during the migration process but seem to be particularly effective at the sites where the crest cells become localized at the term of their migration.

Among these environmental cues, survival and growth factors specific for certain cell types at definite developmental stages must play an important role in the selection among the numerous crest cell developmental potentials. The local absence of such factors may explain the disappearance of sensory neuronal precursors in autonomic ganglia. Other factors of non-neuronal cell origin have also been demonstrated to influence the choice of neurotransmitter and the cellular phenotype in neural crest–derived neurons (*42*). Such factors act on partly committed precursors in which certain developmental options are subjected to environmental regulations.

According to this view, a mixture of cells, with variable degrees of commitment, migrate to their destination via predetermined pathways whose pattern and temporal availability depend upon embryonic morphogenesis. If transplanted into a given embryonic region, the PNS precursor cells contained in the neural crest appear to have basically the same migratory behavior irrespective of their level of origin along the neuraxis and their fate in normal development. They yield ganglia whose nature and distribution are appropriate to the target tissue they reach rather than to their normal fate.

These mechanisms reveal considerable plasticity in the early neural primordium. The presence in the developing peripheral ganglia until hatching of resting stem cells capable of differentiating into autonomic neurons also shows that a large number of developmental potentialities remain unused in the ontogeny of the vertebrate nervous system.

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Elementary Particle Physics and the Superconducting Super Collider

CHRIS QUIGG AND ROY F. SCHWITTERS

The present status and future prospects of elementary particle physics are reviewed, and some of the scientific questions that motivate the construction of a major new accelerator complex in the United States are summarized.

ELEMENTARY PARTICLE PHYSICS, THE SCIENCE OF THE ULtimate constituents of matter and their interactions, has undergone a remarkable development during the past two decades. A host of experimental results made accessible by the present generation of particle accelerators and the accompanying rapid convergence of theoretical ideas have brought to the subject an unprecedented coherence. This clarity, however, brings into sharp focus fundamental limitations in the current picture that raise fresh possibilities and set new goals for advancing our understanding of nature. The progress in particle physics has been more dramatic and more thoroughgoing than could have been imagined only a dozen years ago. Many of the deep issues then current have been addressed, and many of the opportunities then foreseen have been realized. This progress and the profound questions emerging from it have brought particle physics to an intellectual turning point comparable to the synthesis of classical physics in the late nineteenth century that preceded the discovery of relativity and quantum mechanics.

Experimental pursuit of some of the fundamental questions in elementary particle physics requires energies higher than those provided by any accelerators now in operation or under construction anywhere in the world. For this reason, physicists in the United States are now preparing a proposal for a very high energy superconducting proton-proton collider, the Superconducting Super Collider (SSC) (*1*). This major new accelerator complex would be based on the accelerator principles and technology that were developed in connection with the construction of the Fermilab Tevatron (*2*) and on extensive work on superconducting magnets in the United States over the past 20 years (*3*). The proposed SSC would have an energy about 20 times that of the Tevatron collider recently tested at Fermilab. The high energy of SSC is needed to answer some of today's pressing questions in elementary particle physics. In addition, such a large increase in energy will open up new and uncharted territory. Historically, such openings lead to revolutionary advances for entire fields of science.

In this article, we summarize current understanding of the basic constituents and forces, which can be expressed entirely by what has come to be known as the "standard model" of particle physics. We describe recent progress, both theoretical and experimental, and we review the questions and problems raised by the standard model.

Chris Quigg is head of the Theoretical Physics Department at the Fermi National Accelerator Laboratory, Post Office Box 500, Batavia, IL 60510, and professor of physics at the University of Chicago, Chicago, IL 60637. Roy F. Schwitters is professor of physics at Harvard University, Cambridge, MA 02138, and visiting scientist at Fermilab where he is coleader of the Collider Detector project.