The avian neural crest as a model system for the study of cell lineages

JULIAN SMITH

Institut d'Embryologie Cellulaire et Moléculaire du Centre National de la Recherche Scientifique et du Collège de France, Nogent-sur-Marne, France

ABSTRACT Under the influence of environmental factors, the neural crest gives rise to numerous cell types and is therefore, by definition, a pluripotential structure. However, it was not clear until recently to what extent each individual neural crest cell possessed multiple capacities for differentiation. As a result of *in vivo* and *in vitro* approaches aimed at solving this problem, it has become apparent that the neural crest is made up of cells in different states of determination and that some lineages are segregated very early. In particular, analysis of clones obtained from single cells grown in culture has shown that, although many individual neural crest cells are pluripotential to varying degrees, others are apparently committed to give rise to only one derivative. The role of the embryonic microenvironment in the emergence of phenotypic diversity is probably complex, certain factors acting to promote the survival of selected subpopulations of fully determined progenitors, while others may direct partly committed precursors towards a specific developmental fate.

KEY WORDS: *neural crest, determination, clonal culture, cell lineage, differentiation*

Introduction

In the introduction to his now classic treatise on the neural crest, Sven Hörstadius (1950) presented this embryonic rudiment as a "very peculiar structure", by virtue of its early formation and only temporary existence. These same features made it "difficult to analyze its derivatives", which, in turn, presumably accounted for the fact that "outside the circle of investigators in the field, it is hardly known at all". Forty years later, although it remains true that "opinion still diverges on some points", the community of neural crest *aficionados* has considerably broadened, not only quantitatively, as might be expected, but also qualitatively, embracing practitioners of disciplines ranging from embryology to pathology and from neurobiology to evolution. Three recent texts (Le Douarin, 1982; Maderson, 1987; Hall, 1988) bear witness to this phenomenon and are doubtless catalyzing its extension.

That the neural crest has been thrust into prominence over the last decade and a half is in part to be explained by the arrival of new and efficient methods for studying it, both in the embryo and in culture *in vitro*, and in part by the realization that it can be used as an experimental model for the study of a number of fundamental biological problems.

The rapid dispersal of crest cells throughout the developing vertebrate animal can only be followed and analyzed if the cells have been previously labeled in a way that enables them to be identified on histological sections during the relevant periods of embryogenesis. Although a variety of more or less satisfactory marking methods have been used to elucidate the normal fate of neural crest (for early references, see Le Douarin, 1982), it is fair to claim that the introduction, by Nicole Le Douarin (1969, 1973) of a technique based on recognizable and permanent structural differences between interphase nuclei of quail and chick cells was a new point of departure for investigations of crest cell migration and differentiation *in vivo*. The experimental system wherein equivalent pieces of neural primordium are exchanged between quail and chick was initially used to substantial advantage in the laboratory of Le Douarin (and elsewhere) to study the development of the peripheral nervous system and the cranial skeleton. More recently, the creation of chick-quail chimeras has been exploited to shed light on fine details of the migration processes (Teillet *et al.*, 1987), to harvest information concerning the fate of the anterior neural folds (Couly and Le Douarin, 1987), to provide new insight into the migration of cells in the central nervous system (Balaban *et al.*, 1988; Hallonet *et al.*, 1990) and to demonstrate the transfer between species, via fragments of embryonic brain regions, of certain behavioral characteristics (Balaban *et al.*, 1988).

As far as the ontogeny of the neural crest is concerned, the usefulness of quail-chick constructs has not been limited to studies of the normal development of its derivatives: transplantations of

Abbreviations used in this paper: SMP, Schwann cell myelin protein; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor.

***Address for reprints**: Institut d'Embryologie Cellulaire et Moléculaire du CNRS et du Collège de France, 49 bis avenue de la Belle Gabrielle, F-94736 Nogent-sur-Marne, France

0214-6282/90

neural primordium to heterotopic sites have been instrumental in revealing that the embryonic microenvironment plays an essential role in the differentiation of cells issued from the neural crest. However, the interactive mechanisms involved are more conveniently investigated in model systems *in vitro*, and cultures of neural crest cells are being used to advantage in investigations of the relative importance of intrinsic and extrinsic factors in their differentiation. Particularly fruitful has been the development of culture conditions enabling single, isolated crest cells to proliferate and differentiate.

This article will briefly outline some recent contributions from our laboratory aimed at furthering our understanding of how phenotypic diversity emerges from the relatively undifferentiated and apparently uniform population of cells that compose the neural crest.

Fate and potentialities of the neural crest

As *in vivo* tracing studies (extensively reviewed elsewhere, e.g. Le Douarin, 1982; Le Douarin and Smith, 1983, 1988) have amply underlined, the neural crest possesses a remarkably wide-ranging capacity for differentiation, giving rise to a multiplicity of derivatives that include, in addition to diverse categories of neurons and non-neuronal cells found in the ganglia of the peripheral nervous system, melanocytes, endocrine and paraendocrine cells and connective, skeletal and muscular tissue of the head and neck. A similar *in embryo* approach, involving the heterotopic transposition of fragments of neural primordium between the chick and the quail, has revealed that the differentiation of neural crest-derived cells is in great part conditioned by the embryonic microenvironment that they encounter during and following their migration from the dorsal neural tube. In fact, throughout its length, the neural crest possesses essentially identical potentialities, at least as far as its neural derivatives are concerned. Although its full range of developmental capacities is not normally realized at all levels of the neural axis, virtually all of them can be elicited experimentally by subjecting the cells to appropriate environmental influences *in vivo* or *in vitro*. These facts raise fundamental embryological issues relating to the state of determination, or commitment to a particular developmental fate, of individual crest cells when they leave the neural primordium en route for the sites in which they will ultimately differentiate. Does the global pluripotentiality of the neural crest population reflect a similar state of affairs at the level of each cell, or is the neural crest a composite structure, already comprising differently committed subsets of cells each of which is determined to follow a particular pathway of differentiation? According to the answers to these questions, it can be envisaged that the role of environmental factors in neural crest ontogeny consists in the imposition of a particular developmental option on toti- or multipotent progenitors or in the selective promotion of the survival and multiplication of particular subpopulations of cells whose subsequent differentiation would follow a genetically determined plan.

During recent years, much research effort has been spent in our laboratory trying to determine at what stages different neural crest-derived cell lineages become segregated and, in particular, whether premigratory or migrating cells include developmentally restricted precursors of any of them. The approaches we have used include attempts to identify lineage markers on neural crest cells, analysis of the potentialites of individual crest cells in clonal culture and studies on the limitation of the developmental capacities of neural crest derivatives with time. As a result of such investigations, it is becoming increasingly clear that the neural crest is not homogeneous with respect to the potentialities of its constituent cells.

In vivo *analysis of the neural crest cell population: evidence for heterogeneity of developmental potentialities*

As a general rule, no phenotypic features characteristic of any of their derivatives are expressed before neural crest cells have reached their final sites of arrest. However, it has proved possible to label neural crest cells *in situ* with monoclonal antibodies raised against determinants expressed by peripheral ganglion cells and, in some cases, this approach has revealed individual differences between cells in the migrating population. For instance, GIN1, obtained by immunization with homogenates of embryonic quail nodose ganglia, was found to decorate the surface of only one migrating crest cell in four at the mesencephalic level (Barbu *et al.*, 1986). It is implied, although it has not been demonstrated experimentally, that the immunoreactive cells are determined precursors of derivatives that express the same antigenic determinant at a later developmental stage (i.e., virtually all satellite and Schwann cells in peripheral ganglia and nerves as well as minor subsets of neurons). In any event, this result clearly reveals a heterogeneity at the molecular level in cranial neural crest cells well before they have reached the sites at which they will differentiate into recognizable phenotypic categories.

The heterotopic grafting studies, briefly mentioned earlier, demonstrate that the developmental capacities of the crest cell population are (with the exception of its neurectodermal potentialities, which are confined to the cephalic region) qualitatively equivalent at all levels of the neuraxis. But these, and other experiments, also indicate that their distribution is quantitatively nonuniform. For instance, trunk crest is a less abundant potential source of enteric ganglia than is cranial crest (Le Douarin and Teillet, 1973; Smith *et al.*, 1977; Newgreen *et al.*, 1980). Conversely, trunk crest has a greater aptitude than cranial crest to differentiate into adrenergic cells and melanocytes (Newgreen *et al.*, 1980; our unpublished results).

Heterogeneity revealed by in vitro *culture of neural crest*

Indirect evidence from mass cultures

Cultures of neural crest are being used increasingly as model systems for studying differentiation. In addition, they have provided some information on the state of commitment of the cells at the time they are put into the culture dish. It has become apparent, as a result of work from a number of laboratories (for a review, see Le Douarin and Smith, 1988), that most of the cell types that the neural crest gives rise to *in vivo* can differentiate *in vitro*. A striking point raised by these studies is the dissimilar response of neural crest cells to a given set of culture conditions. For instance, only a fraction of them ever expresses properties that are characteristic of neuronal differentiation (Cohen, 1977; Maxwell *et al.*, 1982; García-Arrarás *et al.*, 1987; Ziller *et al.*, 1987) and the populations of neurons that do develop can be shown to be neurochemically heterogeneous (Maxwell *et al.*, 1984; Maxwell and Sietz, 1985; García-Arrarás *et al.*, 1986). It should not, however, be immediately concluded that this fact necessarily reflects the existence of an equivalent number of precommitted precursor types in the explanted crest, for, as will be shown in the next section, similarly

miscellaneous derivatives can be obtained when single crest cells develop in an appropriately permissive medium. A mere cataloguing of the phenotypes expressed in mass cultures of neural crest cannot therefore provide much information concerning the state of determination of the cells when they are removed from the embryo. On the other hand, results of experiments involving the sequential culturing of neural crest in different media plead strongly in favor of the hypothesis of an early segregation of two neuronal lineages whose precursor cells have demonstrably different requirements for differentiation. Thus, Ziller *et al.* (1983) noted that neuron-specific markers (neurofilament proteins and tetanus toxin-binding sites) were expressed by a small subset of quail mesencephalic neural crest cells that displayed a neuronal morphology within 24 hours of being placed in a fully defined medium. These neurons, which differentiated without dividing in culture, were later shown to contain the neuropeptide substance P (Ziller *et al.*, 1987), and may be of sensory type. Replacing the artificial medium by another containing serum and a high concentration of chick embryo extract resulted in the death and detachment of the substance P-containing neurons, followed a few days later by the differentiation, from mitotically active precursors, of cells that expressed a catecholaminergic phenotype, characteristic of the majority of neurons of the sympathetic subdivision of the autonomic nervous system.

Direct evidence for heterogeneity obtained from analysis of clonal cultures

Multipotent cells can be made to divulge the full content of their developmental repertoire only if they are subjected experimentally to an environmental situation permissive enough to allow them to realize all of their predispositions for differentiation. Furthermore, experiments of this kind are only meaningful if they are performed with single cells.

It is possible, by microinjecting a fluorescent tracer, to mark selectively single neural crest cells (or rather their precursors in the dorsal neural tube) in the living embryo, and analysis of their progeny has provided valuable information on the developmental fate of individual cells *in vivo* (Bronner-Fraser and Fraser, 1988, 1989). However, no conclusions can be drawn concerning their phenotypic potentialities, i.e., what alternative or additional derivatives they could have given rise to under other circumstances, for instance, had they followed a different migration pathway and/or settled in a different site. For this reason, the study of descendents of single cells *in vivo* is complementary to, but cannot replace, a detailed analysis of the capacities of individual cells to differentiate *in vitro*.

Clonal cultures of neural crest were first performed, using the limiting dilution technique, by Sieber-Blum and Cohen (1980), who identified three different kinds of clone in the colonies they obtained. Recently, Baroffio *et al.* (1988) and Dupin *et al.* (in press) have initiated a comprehensive investigation in which they have employed a technology that guarantees that only one cell is seeded. Using culture conditions that have proved to be extremely favorable for the proliferation and the differentiation of isolated crest cells and exploiting a large range of markers to identify the cell types that develop, they have considerably extended the earlier studies. In so doing, they have clarified a number of important problems relating to neural crest cell ontogeny.

The experimental protocol involves the isolation of single cells by carefully controlled proteolysis of mesencephalic neural crest dissected out of 10- to 13-somite stage quail embryos while it is in the process of migrating laterally under the surface ectoderm. Cells are individually picked out of the suspension with an elongated pipette, under microscopic control, and placed in separate culture wells on top of a feeder layer of growth-inhibited mouse 3T3 cells, in a complex medium including serum, chick embryo extract and growth factors. In preliminary experiments, these conditions were found to be suitably permissive not only for the survival and multiplication of crest cells seeded at low density, but also for the expression of phenotypes representative of the major lineages arising from the neural crest.

Clones derived from single neural crest cells were analyzed after 10-16 days in culture using a variety of phenotypic markers to identify differentiated cells. Among these were: antibodies directed against neurofilament proteins, against tyrosine hydroxylase and against vasoactive intestinal polypeptide (to define subpopulations of neurons); a monoclonal antibody recognizing a surface glycoprotein ("Schwann cell myelin protein, SMP") found on all Schwann cells, but not on ganglionic satellite cells or on neurons (Dulac *et al.*, 1988); melanin, a marker of terminal differentiation of cells of the melanocyte lineage; and cartilage, diagnostic for certain mesectodermal derivatives.

The results to date, which are based on the analysis of more than 500 clones, lead to a number of interesting conclusions. The first is that neural crest cells taken at the migrating stage have widely differing abilities to proliferate: after 10 days in culture, some clones contain fewer than 10 cells while others are made up of more than 20,000. The second is that a great many individual neural crest cells are multipotential, since the overwhelming majority of clones that they give rise to contain more than one identifiable derivative (up to six have been found, in unequal and variable proportions). Thirdly, cells in the migrating neural crest are heterogeneous in their developmental potencies. The majority may be pluripotential, but they are not identically so; well over a dozen categories of clone have been identified according to the phenotype, or combination of phenotypes, that they express. Furthermore, although clones displaying multiple phenotypes predominate, some are composed of only one kind of cell. Rare colonies containing fewer than 10 cells have been found to consist entirely of neurons; others, larger and more common, are exclusively made up of Schwann cells.

Several additional interesting points have been brought to light in the course of this investigation. For instance, neurons were identified in somewhat less than half of the clones and were almost always associated with non-neuronal cells that were both of the Schwann (SMP^+) and non-Schwann (satellite; SMP^-) type. This result clearly implies that common precursors for non-neuronal cells and neurons are prevalent in the migrating neural crest. Interestingly, a sizeable proportion of clones contained only Schwann cells, indicating that committed progenitors of this cell type also exist in the same population. Another very recent series of observations (Baroffio *et al.*, submitted for publication) sheds new light on the segregation of neuronal and mesectodermal lineages. Of nearly 300 clones examined, 8 were shown to contain cartilage plus a neural derivative (neurons and Schwann or satellite cells). This is the first demonstration that neurons, glia and mesenchymal cells can derive from the same precursor in a vertebrate embryo and shows that mesectodermal and neural lineages are not completely segregated by the time crest cell migration is under way, as was suggested earlier (Le Lièvre and Le Douarin, 1975) following the

results of heterotopic transplantation of the cranial crest to the trunk region (in fact, the *in vivo* experiments show that this kind of precursor exists only at the cephalic level, but that it can express its mesectodermal potentialities in ectopic sites).

Finally, a single clone (0.3% of the total number examined) was found to contain the entire spectrum of phenotypes that could be identified by means of the available markers, i.e., cartilage together with neuronal, Schwann, satellite and pigment cells. It can be proposed that the corresponding founder cell is a representative of a totipotent population occurring at low frequency, much like the stem cell from which lymphoid and hemopoietic cells arise during formation of the blood system (Anderson, 1989; Baroffio *et al.*, submitted for publication).

In sum, the global analysis of over 500 clones reveals *a posteriori* a population of neural crest cells that are extremely heterogeneous in terms of their potentialities for differentiation. In addition to the "totipotent" cell, the existence of 16 different types of pluripotent progenitor and 4 types of monopotent precursor (approximately 20% of the total number of clones) could be inferred.

Implications of the results obtained in clonal cultures

The above data constitute a direct demonstration of the non-equivalence of the migrating neural crest population with respect to the abilities of individual cells to divide and differentiate under conditions that provide factors necessary for proliferation and signals required for the development of most of their derivatives. This is consistent with a model in which, as they migrate and divide, subpopulations of neural crest cells become rapidly, but not synchronously, committed to different and increasingly limited developmental fates. The relationships between the different types of precursor, i.e., their respective positions on a lineage tree, have not yet been clarified, but the absence of any coherent pattern in the combinations of potentialities intermediate between the totipotent progenitor and the monopotent precursors suggests that restriction does not proceed in an ordered, sequential fashion and that stochastically occurring events contribute extensively to the segregation of cell lineages.

Among the probable consequences of the early developmental heterogeneity of the neural crest population would be the accumulation, at the sites in which differentiation occurs, of a mixed population of precursors corresponding to different lineages, only some of which have potentialities that are normally expressed in a given location. An essential role of the environment would therefore be to select from this heterogeneous population only those progenitors whose potentialities "correspond" to a particular site; inappropriate precursors would remain in a state of latence or ultimately disappear, according to how strict their requirements for survival are.

Experimental support for these notions has been obtained from studies designed to determine whether peripheral ganglion cells retain any of the developmental potentialities possessed by their forebears in the neural crest.

Heterogeneity of ganglion cell precursors

Early segregation of autonomic and sensory lineages

Fragments of diverse quail peripheral ganglia were subjected to the environment normally encountered by migrating neural crest cells *in vivo* by implanting them between the somites and the neural tube of 2-day chick embryos (Le Douarin *et al.*, 1978; Le Lièvre *et al.*, 1980; Dupin, 1984). The sites in which the grafted cells finally settled were localized by means of the quail marker and their phenotypes were identified by the application of other appropriate cytochemical techniques. In essence, a population of grafted non-neuronal ganglion cells (the neurons were shown not to survive the transplantation) was able to migrate, colonize the sensory and autonomic structures of the host and differentiate there. However, the sites colonized by the transplanted cells depended on the type of ganglion grafted. Thus, cells were seen to invade the host dorsal root ganglia and develop there into sensory neurons and satellite cells only when young sensory ganglia were transplanted. On the other hand, regardless of their age, both dorsal root and autonomic ganglia were found to contain cells capable of differentiating in autonomic sites. This is consistent with the hypothesis that sensory and autonomic lineages are segregated prior to the formation of the peripheral ganglia and supports the idea that sensory ganglia (neurons and glia) normally develop from a mixed collection of progenitors of sensory and of autonomic type. It is not yet clear exactly when this segregation occurs and, as suggested by the results of the clonal culture experiments, the decisive event probably does not take place simultaneously in all precursors. Experiments in which single cells in the dorsal neural tube were injected with a fluorescent dye *in vivo* (Bronner-Fraser and Fraser, 1989) have indicated that progenitor cells with an exclusively sensory fate are present even before the neural crest is formed; in the majority of embryos in which labeled cells were located in the spinal ganglia, no other peripheral sites were colonized. On the other hand, Sieber-Blum (1989), after applying antibodies directed against a sensory lineage-specific marker, SSEA-1, to clonal cultures of crest cells that had migrated from the neural tube *in vitro*, concluded that dual sensory/autonomic potentialities were still present at this stage.

Limited distribution of sensory ganglion cell precursors in the young embryo

The apparent lack of sensory neuron precursors in developing autonomic ganglia of any age is at first sight contrary to the idea that all peripheral ganglia arise from a heterogeneous population of cells with different commitments. However, this hypothesis does not necessarily require that all types of precursor cell survive equally well at inappropriate sites. In this respect, it was noted (Le Douarin, 1986) that those sensory ganglia whose neurons derive from the neural crest (in the head, neurons of certain distal sensory ganglia differentiate from the ectodermal placodes) develop in close proximity to the neural tube. Indeed, ablation of the neural tube just after the migration of the neural crest had been shown to prevent development of the ganglia of the dorsal root but not of the sympathetic chain (Teillet and Le Douarin, 1983). It was conjectured that the neural tube produces a factor, diffusible over only a short distance, that is required for the survival of sensory neuron precursors before their axons have reached peripheral targets. Any such precursor migrating further away (to the sites of autonomic ganglion formation, for instance) would rapidly succumb in the absence of the necessary trophic support.

In experiments performed to validate this hypothesis (Kalcheim and Le Douarin, 1986), a silastic membrane was placed between the neural tube and the newly forming dorsal root ganglia of a young

chick embryo, thus effectively isolating the neuronal precursors from all central influences. As a result, the presumptive sensory ganglion cells died within 10 hours of the operation, whereas autonomic ganglion cell precursors accumulated apparently normally in the sympathetic chains. The suggestion that the neural tube liberates soluble factors required for early sensory gangliogenesis was reinforced when it was shown that an extract of embryonic neural tube, incorporated into the membrane, was able to rescue the sensory ganglion cell precursors distal to it; 36 hours after the operation, some of them had even differentiated into identifiable neurons. As a result of attempts to identify the active factor(s) in neural tube extract, it was demonstrated that "brain-derived neurotrophic factor" (BDNF), isolated from pig brain on the basis of its ability to promote the survival of embryonic chick sensory neurons in culture (Barde *et al.*, 1982), is a promising candidate. Incorporation of BDNF, together with laminin, into the silastic membrane significantly increased survival of the cells in the dorsal root ganglion rudiments, whereas NGF, laminin alone or an extract of embryonic liver had no positive effects (Kalcheim *et al.*, 1987). It can be concluded that the existence of a factor that selectively promotes the survival of sensory ganglion cell precursors can reasonably account for their topologically restricted distribution within the developing embryo.

Demonstration of the presence of autonomic ganglion cell precursors in sensory ganglia

Autonomic precursors are found throughout embryonic development and beyond not only in various autonomic structures, but also within dorsal root ganglia. This conclusion was initially based entirely on data obtained from the *in vivo* transplantation of ganglia but has since been amply confirmed in an *in vitro* system, developed by Xue *et al.* (1985), in which dorsal root ganglia, grown as dissociated cells under conditions favorable for the proliferation of the non-neuronal population, are monitored periodically for the appearance of autonomic nerve cells, defined by their expression of catecholaminergic features. (During normal development of the peripheral nervous system in the avian embryo, the catecholaminergic phenotype is expressed exclusively by sympathetic neurons and adrenal medulla and can therefore be considered as a reliable "autonomic-type" marker.) When chick embryo extract was added to the medium, cells expressing tyrosine hydroxylase immunoreactivity and capable of synthesizing and storing noradrenaline appeared after 2-3 days; after 6 days, they amounted to approximately 2% of the cells initially put into culture. Several lines of evidence suggested that the cells expressing this autonomic phenotype arose from precursors that were distinct from primary sensory neurons; in particular, whereas all the sensory neurons were post-mitotic at the time they were removed from the embryo for culturing, many of the noradrenergic cells were shown to have divided before they differentiated. Subsequent results suggested that the latent autonomic progenitors consitute a discrete subpopulation of non-neuronal cells that can be recognized in uncultured dorsal root ganglia by virtue of their high affinity uptake system for noradrenaline (Xue and Smith, 1988).

These data and others (Xue *et al.*, 1987) show that avian sensory ganglia contain a contingent of cells possessing features expected of autonomic sympathetic precursors. They do not express their catecholaminergic potentialities within the dorsal root ganglia *in vivo*, but can be induced to do so when exposed to appropriate conditions *in vitro*. In the course of a series of experiments directed at identifying the factors responsible for initiating their differentiation, Xue *et al.* (1988) found that the requirement for chick embryo extract could be partially replaced by a chemically defined medium supplemented with various growth factors and hormones. The testing of each of these in turn led to the intriguing finding that low concentrations of insulin and insulin-like growth factor-I mimicked quantitatively and qualitatively the differentiation-triggering action of chick embryo extract. It is tempting to suggest that the same polypeptides play a role *in vivo* during the normal development of the sympathetic nervous system; however, although insulin (De Pablo *et al.*, 1982) and its receptor (Hendricks *et al.*, 1984) have been detected in the chick embryo at the relevant stages, it is not known whether the spatial and temporal distribution of these molecules is consistent with this proposition.

Concluding remarks

In the light of new data, obtained over the past few years by means of a combination of experimental resources, there is now little room for doubt that the neural crest is a mosaic structure in terms of the properties of its individual cells; some appear to be totally, others partially, committed before, or soon after, they leave the neural primordium. The experimental evidence briefly presented here supports the idea that certain broad developmental options, such as the choice between sensory and autonomic lineages, are taken early. This is doubtless an oversimplification and recent results are consonant with the idea that an additional segregation occurs within the autonomic lineage, resulting in an unequal distribution along the neural axis of enteric and sympathetic precursors (Fontaine-Pérus *et al.*, 1988).

Nevertheless, it would be unwarranted to jump to the conclusion that the entire program of development is irrevocably determined before the migrating cells reach their destination. Only a minority of individual cells are apparently restricted to a single developmental fate while they are migrating, as the clonal studies have shown, and, although information is lacking on this point, some crest cells may retain multiple potentialities for differentiation when they arrive at the sites of gangliogenesis. This eventuality would not modify the conclusion that peripheral ganglia develop from a heterogeneous assortment of cells; however, it may imply the existence of environmental factors, in addition to those that exert a selection on appropriate sets of determined progenitors, that interact with still pluripotent precursors in a more "instructive" manner - by modifying the probability of commitment to a specific fate, or by triggering and/or stabilizing the expression of a particular phenotype. Identifying these factors and understanding how they work are important priorities for future research on the developmental biology of the neural crest.

Acknowledgments

Acknowledgment is due to Nicole Le Douarin, Anne Baroffio and Elisabeth Dupin for collective reflection on the lineage problem. Financial assistance was obtained from the Centre National de la Recherche Scientifique, the Association pour la Recherche sur le Cancer, the Fondation pour la Recherche Médicale and by Basic Research Grant N° 1-866 from March of Dimes Birth Defects Foundation.

References

ANDERSON, D.J. (1989). The neural crest cell lineage problem: neuropoiesis? *Neuron*

3: 1-12.

BALABAN, E., TEILLET, M.-A. and LE DOUARIN, N.M. (1988). Application of the quail-chick chimaera system to the study of brain development and behavior. *Science 241:* 1339-1342.

BARBU, M., ZILLER, C., RONG, P.M. and LE DOUARIN, N.M. (1986). Heterogeneity in migrating neural crest cells revealed by a monoclonal antibody. *J. Neurosci. 6:* 2215-2225.

BARDE, Y.A., EDGAR, D. and THOENEN, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO J. 1:* 549-553.

BAROFFIO, A., DUPIN, E. and LE DOUARIN, N.M. (1988). Clone-forming ability and differentiation potential of migratory neural crest cells. *Proc. Natl. Acad. Sci. USA 85:* 5325-5329.

BRONNER-FRASER, M. and FRASER, S. (1988). Cell lineage analysis shows multipotentiality of some avian neural crest cells. *Nature 335:* 161-164.

BRONNER-FRASER, M. and FRASER, S. (1989). Developmental potential of avian trunk neural crest cells *in situ*. *Neuron 3:* 755-766.

COHEN, A.M. (1977). Independent expression of the adrenergic phenotype by neural crest cells *in vitro*. *Proc. Natl. Acad. Sci. USA 74:* 2899-2903.

COULY, G. and LE DOUARIN, N.M. (1987). Mapping of the early primordium in quail-chick chimaeras. II. The prosencephalic neural plate and neural folds: implications for the genesis of cephalic human congenital abnormalities. *Dev. Biol. 120:* 198-214.

DE PABLO, F., ROTH, J., HERNANDEZ, E. and PRUSS, R. (1982). Insulin is present in chicken eggs and early chick embryos. *Endocrinology 111:* 1909-1916.

DULAC, C., CAMERON-CURRY, P., ZILLER, C. and LE DOUARIN N.M. (1988). A surface protein expressed by avian myelinating and nonmyelinating Schwann cells but not by satellite or enteric glial cells. *Neuron 1:* 211-220.

DUPIN E. (1984). Cell division in the ciliary ganglion of quail embryos *in situ* and after back-transplantation into the neural crest migration pathways of chick embryos. *Dev. Biol. 105:* 288-299.

DUPIN, E., BAROFFIO, A., DULAC, C., CAMERON-CURRY, P. and LE DOUARIN, N.M. Schwann cell differentiation in clonal cultures of the neural crest, as evidenced by the anti-SMP monoclonal antibody. *Proc. Natl. Acad. Sci. USA* (In press.)

FONTAINE-PERUS, J., CHANCONIE, M. and LE DOUARIN, N.M. (1988). Developmental potentialities in the nonneuronal population of quail sensory ganglia. *Dev. Biol. 128:* 359-375.

GARCIA-ARRARAS, J.E., CHANCONIE, M., ZILLER, C. and FAUQUET, M. (1987). *In vivo* and *in vitro* expression of vasoactive intestinal polypeptide-like immunoreactivity by neural crest derivatives. *Dev. Brain Res. 33:* 255-265.

GARCIA-ARRARAS, J.E., FAUQUET, M., CHANCONIE, M. and SMITH, J. (1986). Co-expression of somatostatin-like immunoreactivity and catecholaminergic properties in neural crest derivatives; co-modulation of peptidergic and adrenergic differentiation in cultured neural crest. *Dev. Biol. 144:* 247-257.

HALL, B.K. (1988). *The Neural Crest*. Oxford University Press, New York.

HALLONET, M.E.R., TEILLET, M-A. and LE DOUARIN, N.M. (1990). A new approach to the development of the cerebellum provided by the quail-chick marker system. *Development 108:* 19-31.

HENDRICKS, S.A., DE PABLO, F. and ROTH, J. (1984). Early development and tissue-specific patterns of insulin binding in chick embryo. *Endocrinology 115:* 1315-1323.

HÖRSTADIUS, S. (1950). *The Neural Crest: its Properties and Derivatives in the Light of Experimental Research*. Oxford University Press, London.

KALCHEIM, C., BARDE, Y.A., THOENEN, H. and LE DOUARIN, N.M. (1987). *In vivo* effect of brain-derived neurotrophic factor on the survival of neural crest precursor cells of the dorsal root ganglia. *EMBO J. 6:* 2871-2873.

KALCHEIM, C. and LE DOUARIN, N.M. (1986). Requirement of a neural tube signal(s) for the differentiation of neural crest cells into dorsal root ganglia. *Dev. Biol. 116:* 451-466.

LE DOUARIN, N.M. (1969). Particularités du noyau interphasique chez la caille japonaise (*Coturnix coturnix japonica*). Utilisation de ces particularités comme "marquage biologique" dans les recherches sur les interactions tissulaires et les migrations cellulaires au cours de l'ontogenèse. *Bull. Biol. Fr. Belg. 103:* 435-452.

LE DOUARIN, N.M. (1973). A Feulgen-positive nucleolus. *Exp. Cell Res. 77:* 459-468.

LE DOUARIN, N.M. (1982). *The Neural Crest*. Cambridge University Press, Cambridge.

LE DOUARIN, N.M. (1986). Cell line segregation during peripheral nervous system ontogeny. *Science 231:* 1515-1522.

LE DOUARIN, N.M. and SMITH, J. (1983). Differentiation of avian autonomic ganglia. In *Autonomic Ganglia* (Ed. L.G. Elfvin). John Wiley and Sons, Chichester, pp. 427-452.

LE DOUARIN, N.M. and SMITH, J. (1988). Development of the peripheral nervous system: cell line segregation and chemical differentiation of neural crest cells. In *Handbook of Chemical Neuroanatomy, Vol. 6, The Peripheral Nervous System* (Eds. A. Björklund, T. Hökfelt and Ch. Owman). Elsevier, Amsterdam, pp. 1-50.

LE DOUARIN, N.M. and TEILLET, M-A. (1973). The migration of neural crest cells to the wall of the digestive tract in avian embryo. *J. Embryol. Exp. Morphol. 30:* 31-48.

LE DOUARIN, N.M., TEILLET, M-A., ZILLER, C. and SMITH, J. (1978). Adrenergic differentiation of cells of the cholinergic ciliary and Remak ganglia in avian embryo following *in vivo* transplantation. *Proc. Natl. Acad. Sci. USA 75:* 2030-2034.

LE LIEVRE, C.S. and LE DOUARIN, N.M. (1975). Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. *J. Embryol. Exp. Morphol. 34:* 125-154.

LE LIEVRE, C.S., SCHWEIZER, G.G., ZILLER, C and LE DOUARIN, N.M. (1980). Restriction of developmental capabilities in neural crest cell derivatives as tested by *in vivo* transplantation experiments. *Dev. Biol. 105:* 362-378.

MADERSON, P.F.A. (Ed.) (1987). *Development and Evolutionary Aspects of Neural Crest*. Wiley-Interscience, New York.

MAXWELL, G.D. and SIETZ, P.D. (1985). Development of cells containing catecholamines and somatostatin-like immunoreactivity in neural crest cultures: relationship of DNA synthesis to phenotypic expression. *Dev. Biol. 108:* 203-209.

MAXWELL,G.D., SIETZ, P.D. and JEAN, S. (1984). Somatostatin-like immunoreactivity in embryonic sympathetic ganglia. *J. Neurosci. 4:* 576-584.

MAXWELL, G.D., SIETZ, P.D. and RAFFORD, C.E. (1982). Synthesis and accumulation of putative neurotransmitters by cultured neural crest cells. *J. Neurosci. 2:* 879-888.

NEWGREEN, D.F., JAHNKE, I., ALLAN, I.J. and GIBBINS, I.L. (1980). Differentiation of sympathetic and enteric neurons of the fowl embryo in grafts to the chorioallantoic membrane. *Cell Tissue Res. 208:* 1-19.

SIEBER-BLUM, M. (1989). Commitment of neural crest cells to the sensory neuron lineage. *Science 243:* 1608-1611.

SIEBER-BLUM, M. and COHEN, A.M. (1980). Clonal analysis of quail neural crest cells: they are pluripotent and differentiate *in vitro* in the absence of non-crest cells. *Dev. Biol. 80:* 96-106.

SMITH, J., COCHARD, P. and LE DOUARIN, N.M. (1977). Development of choline acetyltransferase and cholinesterase activities in enteric ganglia derived from presumptive adrenergic and cholinergic levels of the neural crest. *Cell Differ. 6:* 199-216.

TEILLET, M-A., KALCHEIM, C. and LE DOUARIN, N.M. (1987). Formation of the dorsal root ganglia in the avian embryo: segmental origin and migratory behavior of neural crest progenitor cells. *Dev. Biol. 120:* 329-347.

TEILLET, M-A. and LE DOUARIN, N.M. (1983). Consequences of neural tube and notochord excision on the development of the peripheral nervous system in the chick embryo. *Dev. Biol. 98:* 192-211.

XUE, Z.G., LE DOUARIN, N.M. and SMITH, J. (1988). Insulin and insulin-like growth factor-I can trigger the differentiation of catecholaminergic precursors in cultures of dorsal root ganglia. *Cell Differ. Dev. 25:* 1-10.

XUE, Z.G. and SMITH, J. (1988). High-affinity uptake of noradrenaline in quail dorsal root ganglion cells that express tyrosine hydroxylase immunoreactivity *in vitro*. *J. Neurosci. 8:* 806-813.

XUE, Z.G., SMITH, J. and LE DOUARIN, N.M. (1985). Differentiation of catecholaminergic cells in cultures of avian sensory ganglia. *Proc. Natl. Acad. Sci. USA 82:* 8800-8804.

XUE, Z.G., SMITH, J. and LE DOUARIN, N.M. (1987). Developmental capacities of avian embryonic dorsal root ganglion cells: neuropeptides and tyrosine hydroxylase in dissociated cell cultures. *Dev. Brain Res. 34:* 99-109.

ZILLER, C., DUPIN, E., BRAZEAU, P., PAULIN, D. and LE DOUARIN, N.M. (1983). Early segregation of a neuronal precursor cell line in the neural crest as revealed by culture in a chemically defined medium. *Cell 32:* 627-638.

ZILLER, C., FAUQUET, M., KALCHEIM, C., SMITH, J. and LE DOUARIN, N.M. (1987). Cell lineages in peripheral nervous system ontogeny: medium-induced modulation of phenotypic expression in neural crest cultures. *Dev. Biol. 120:* 101-111.