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Receptor tyrosine kinase-dependent neural crest migration in response to differentially localized growth factors

Bernhard Wehrle-Haller and James A. Weston

Summary
How different neural crest derivatives differentiate in distinct embryonic locations in the vertebrate embryo is an intriguing issue. Many attempts have been made to understand the underlying mechanism of specific pathway choices made by migrating neural crest cells. In this speculative review we suggest a new mechanism for the regulation of neural crest cell migration patterns in avian and mammalian embryos, based on recent progress in understanding the expression and activity of receptor tyrosine kinases during embryogenesis. Distinct subpopulations of crest-derived cells express specific receptor tyrosine kinases while residing in a migration staging area. We postulate that the differential expression of receptor tyrosine kinases by specific subpopulations of neural crest cells allows them to respond to localized growth factor ligand activity in the embryo. Thus, the migration pathway taken by neural crest subpopulations is determined by their receptor tyrosine kinase response to the differential localization of their cognate ligand. Accepted 20 December 1996

Introduction
The neural crest is a transient embryonic structure which is formed by the epithelial to mesenchymal transformation of cells derived from the dorsal neural tube. In the avian and mammalian embryonic trunk, after crest cells separate from the neural epithegium, they transiently reside in an extracellular matrix-rich space, the migration staging area (MSA), bordered by the neural tube, somites and ectoderm. In the cranial region, after separation from the neural tube and initial migration, crest cells reside transiently in a space underlying the ectoderm and extending laterally towards the level of the pharynx, an area functionally equivalent to the MSA in the trunk. Neural crest cells disperse from the MSA along temporally and spatially distinct pathways and ultimately give rise to a variety of derivatives in specific embryonic locations, including neurons and glia in the peripheral nervous system, gland cells, connective tissue in branchial arch-derived craniofacial structures, and melanocytes.

In the trunk, neural crest cells begin to migrate ventrally on an intersomitic and a medial pathway through the rostral part of the sclerotome, giving rise to both neuronal and nonneuronal derivatives. About a day later, a second wave of crest cells migrates on the lateral pathway, between the dermatis and the overlying epidermis, primarily giving rise to melanocytes. In the head, the MSA extends more laterally compared to the trunk (Fig. 1A). From the MSA, an E/C8 immunoreactive cell population migrates on a dorsolateral pathway underlying the epidermis, ultimately forming the ectomesenchyme of the branchial arches and associating with the heart outflow tract. A medial population, immunoreactive to the HNK monoclonal antibody, migrates medioventrally to form the sympathetic ganglionic complex, and associates with the pharynx and gut (Fig. 1B). Significantly, as in the trunk, a small population of neural crest cells undergoes a delayed migration laterally underneath the epidermis; these cells will give rise to melanocytes in the skin and inner ear. Various reviews further discuss the unique migration, proliferation and differentiation events of developing neural crest cells.

Different mechanisms might control the migration of neural crest cells along their specific pathways. For example, it has been suggested that extracellular matrix molecules favorable for adhesion and migration form a pathway for neural crest cell migration. Recently, several members of the semaphorin family of inhibitory guidance proteins have been identified, bordering the neural crest migration and peripheral nerve pathways, suggesting that neural crest cell guidance is regulated by restricting the random

1 Abbreviations: MSA, migration staging area; RTK, receptor tyrosine kinase; NGF, nerve growth factor; SCF, stem cell factor; GDNF, glial cell line-derived neurotrophic factor; PDGF, platelet-derived growth factor.
migration of neural crest cells onto specific pathways. Although these are probably important general mechanisms that influence neural crest cell migration, they do not provide sufficient specificity to account for the observed spatial and temporal complexity of neural crest migration, nor do they account for the stereotyped differentiation of specific neural crest derivatives in precise embryonic locations.

Advances in our understanding of receptor tyrosine kinase (RTK) expression and the activities of specifically localized growth factors such as sevenless andboss, respectively\(^{20}\), however, suggest a new cellular mechanism to explain stereotyped patterns of neural crest migration and differentiation. The aims of this review are therefore firstly, to discuss recent data on the expression and activity of RTKs in neural crest subpopulations and secondly, to suggest a general mechanism for the patterning of neural crest migration, based on differential RTK expression by neural crest cells and the requirement for localized, pathway-specific activity of cognate ligands.

**Neural crest-derived subpopulations express different receptor tyrosine kinases**

Since the discovery of nerve growth factor (NGF)\(^{21}\), it has been known that some neural crest derivatives require growth factor activity for survival\(^{22}\). RTKs have been identified in embryonic and adult neural crest derivatives. These include the neurotrophin (NGF, BDNF, NT-3, NT-4 and 5) receptors trkA, B and C\(^{22}\), the neurotrophin (glial growth factor, heregulin, ARIA) receptors erbB2, erbB3 and erbB4\(^{23}\), the receptors for PDGF, PDGFR (α and β)\(^{24}\), the receptor for Stem cell factor (SCF; also known as Steel factor, mast cell growth factor and kit-ligand)\(^{25}\) and c-ret, which responds to GDNF\(^{26}\) in the presence of GDNFR\(^{27}\).

Several mutations and gene knockouts in the mouse have demonstrated that RTK function is required for survival of subsets of neural crest derivatives, since the lack of growth factor activity or absence of growth factor receptors results in the failure of specific derivatives to persist\(^{25,29-30}\). This suggests that different neural crest-derived subpopulations develop distinct dependencies on growth factors. The presence of such distinct factor-dependent subpopulations suggests that acquiring RTK dependency is a critical step in the differentiation of multipotent neural crest precursor cells into specific neural crest derivatives.

Since the roles of RTK expression and activity in neural crest cells are largely unknown, the spatio-temporal identification of RTK activity may provide insights into the mechanism involved in early neural crest migration and segregation into various derivatives along their pathways of
migration. Accordingly, we will discuss recent discoveries concerning the role of RTK function and ligand localization in the morphogenetic behavior of several neural crest subpopulations.

**Melanocyte precursors express c-kit, which is required for their initial dispersal on the lateral pathway and subsequent survival**

Melanocyte precursors transiently depend on Steel factor (SCF), the kit-ligand, for survival of melanocytes in vitro and in vivo, neural crest-derived melanocyte precursors express the RTK, c-kit, in the MSA, and appear to depend on SCF for migration and survival on the lateral pathway almost as soon as they start to express the RTK and other melanocyte-specific markers (e.g. tyrosinase related protein-2) (summarized in Fig. 2).

SCF is transiently produced by the epithelial dermatohe and localized on the lateral migration pathway in the trunk of the mouse prior to the onset of melanocyte precursor dispersal. Subsequent expression of SCF in the overlying epidermis precedes the invasion of c-kit-expressing melanocyte precursors into the skin. Analysis of Steel mutations has shown that in the absence of SCF, melanocyte precursor cells appear in the MSA but fail to migrate. Mutant analysis has further revealed that a secreted form of SCF is

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**Fig. 2.** Graphic representation of the stages of trunk melanocyte precursor migration on the lateral crest migration pathway and perturbations in mutant environments in the mouse. (A) Premigratory crest cells (open circles) accumulate in the MSA lateral to the neural tube. Neural crest cells begin to disperse on the medial pathway by e10.5, as soon as the apical somite transforms into the medial sclerotome. (B) At that time, crest cells have not begun to disperse on the lateral pathway, but the dorsal aspect of the dermatohe begins to express SCF (small open circles). (C) Dermal mesenchyme forms by epithelial to mesenchymal transformation of the lateral aspects of the dermatohe. SCF protein is secreted dorsally and ventrally, while a subpopulation of neural crest cells located in the MSA starts to express the receptor tyrosine kinase c-kit at e11 (filled circles). (D) As soon as c-kit is expressed in melanocyte precursors, these cells start to migrate on the lateral pathway, which has completely transformed into mesenchymal dermis, and no longer expresses SCF mRNA (e11.5). Note, however, that membrane bound SCF protein (small open circles) may still be attached to mesenchyme cell surfaces. (E) By e12.5, SCF is expressed in the overlying epidermis. Melanocyte precursors begin to enter the epidermis by e13.5. (F) In the absence of SCF, in Steel (S) mutant embryos, melanocyte precursors initially differentiate (filled circles) but fail to migrate and ultimately disappear. (G) In the presence of secreted SCF only, as in S<sup> mice</sup> mutant embryos, melanocyte precursors begin to migrate on the lateral pathway but disappear subsequently. (H) As a consequence of ectopic c-kit expression in dermal fibroblasts (but not dermatohe), in Fasch (Ph) and W<sup>emas</sup> mutant embryos SCF distribution and melanocyte precursor localization is altered.

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Table 1. Growth factor ligands discussed in the text, and their receptor tyrosine kinases expressed in crest-derived subpopulations

<table>
<thead>
<tr>
<th>Growth factor ligand</th>
<th>Ligand source/location</th>
<th>Receptor</th>
<th>Migrating neural crest subpopulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF (Steel factor)</td>
<td>Dermatome, epidermal/lateral pathway</td>
<td>c-kit</td>
<td>Melanocyte precursor</td>
</tr>
<tr>
<td>NT-3 (neurotrophin-3)*</td>
<td>Neural tube/medial pathway</td>
<td>trk C</td>
<td>Subset of medial migrating trunk neurogenic crest cells</td>
</tr>
<tr>
<td>GDNF</td>
<td>Gut mesenchyme</td>
<td>GDNFRα/βc-ret</td>
<td>Subpopulation of cranial crest cells (enteric precursor)</td>
</tr>
<tr>
<td>Neuregulin</td>
<td>Motorneurons/ventral roots; rhombomeres 2, 4 and 6; migrating cranial crest and distinct branchial arch cells</td>
<td>erbB3/erbB3</td>
<td>Schwann cell precursor; subpopulation of cranial and trunk neural crest cells</td>
</tr>
<tr>
<td>PDGF (AA, AB, BB)</td>
<td>Epidermis</td>
<td>PDGFRα</td>
<td>Subpopulation of cranial neural crest cells (ectomesenchymal)</td>
</tr>
</tbody>
</table>

*Other neurotrophins and their receptors are expressed later in neural crest derivatives[22, 28].
1erbB4 is not expressed in migrating neural crest cells[24].

sufficient to direct initial melanocyte precursor migration onto the lateral pathway, whereas a membrane-bound form of SCF is required for the survival of melanocyte precursors on that pathway[40] (Fig. 2A-E).

Essentially then, the localization of SCF on the lateral pathway forms a prepattern, which is reflected by the subsequent distribution of c-kit-expressing melanocyte precursors on that pathway. When the distribution of SCF activity (SCF protein) on the lateral pathway is perturbed either by ectopic expression of c-kit in dermal mesenchyme, as in the Ws/m(m) and Patch mutations[42, 44], or by altering SCF production, as in different Steel alleles, melanocyte migration pattern is changed and subsequent survival is affected[42, 44] (Fig. 2F-H). This demonstrates that c-kit-expressing melanocyte precursors not only require SCF activity for survival, but also change their migration pattern in response to altered SCF availability in the tissue. This suggests that SCF exerts a chemotactic signal toward c-kit-expressing melanocyte precursors. This model is further supported by studies demonstrating that SCF can elicit chemotactic responses by c-kit expressing cells in vitro[45, 46].

Thus, the control of pathway selection, migration and survival of melanocyte precursors by c-kit/SCF signaling, represents a clear example of RTK control of neural crest cell behavior. We suggest that the control of differential expression of RTKs in neural crest cells, combined with localized growth factor activity, may also provide the necessary specificity for the specific migration of other neural crest-derived subpopulations.

As implied above, differential expression of RTKs accompanies initial differentiation of specified cells, inducing partially restricted subpopulations. As these subpopulations arise, the ligands for their differentially expressed RTKs often appear concurrently in the embryonic environment. Several mechanisms may regulate ligand availability, including localized synthesis of ligand, dominant negative expression of receptors, differential expression of splice variants and presentation or sequestration by extracellular matrix. Below, we will suggest examples of such mechanisms as they affect development of other neural crest derivatives (Table 1).

trkC function is required for proliferation, migration and survival of some crest-derived neurogenic precursors

As early as e10.5 (embryonic day 10.5) of mouse development, embryos lacking NT-3, the ligand for trkC, exhibit an increase in cell death in the nascent sensory ganglia compared to wild type[47]. Consistent with this result, the application of neutralizing antibodies to NT-3 in avian embryos induces the loss of a subpopulation of neural crest cells in developing sensory ganglia as early as e3[48]. These results suggest that a subpopulation of neural crest-derived cells has acquired dependence on trkC function at the earliest stages of gangliogenesis and possibly even prior to gangliogenesis, while the crest-derived precursors are still dispensing on the medioventral pathway.

Consistent with that notion, trkC is expressed by subsets of premigratory and migratory neural crest cells[49, 50]. Interestingly, NT-3 has several different effects on neural crest cells including proliferation, survival and differentiation[50, 51]. These pleiotropic effects might be explained by differential expression of trkC isoforms[52] with respect to developmental stages of the embryo and developmental state of neural crest derivatives. For example, different isoforms of trkC expressed in cultured neurons appear to mediate different responses to NT-3[52].

Alternatively, pleiotropic effects of NT-3 in trkC-dependent neural crest cells could also be mediated by different concentrations of NT-3. Consistent with this notion, application of pharmacological concentrations of NT-3 to e3 chick embryos during the formation of sensory and nodose ganglia, results in a severe reduction of ganglia size[53]. This might be explained by a premature differentiation of trkC-dependent neuronal precursors[53]. These results suggest that regulation of the levels of growth factor activity available to crest cells in precise embryonic locations is important for their normal behavior. That growth factor dosages influence cell
behavior in RTK-expressing cells has also been demonstrated in c-kit-expressing mast cells. Thus, low concentrations of SCF are sufficient for survival and induce spreading on extracellular matrix, whereas a 100-fold higher concentration of SCF is required for mast cell proliferation\(^{54,56}\). We suggest that low concentrations of NT-3 and other growth factors are sufficient to ensure initial dispersal and survival of neurogenic precursors, whereas higher local concentrations lead to overt neuronal differentiation and subsequent dependence on target-derived growth factor signaling. Elevated levels of NT-3 may therefore affect the normal differentiation program of neurogenic precursors\(^{53}\). It will be interesting to learn whether NT-3 application or overexpression before the start of neural crest cell migration influences the location and behavior of trkC-expressing neural crest cells in vivo.

Neurogenic neural crest-derived cells disperse primarily on the medial pathway. If neurotrophin activity plays a role in directing the choice of pathway by these cells, such activity would need to be localized to the medial and absent from the lateral pathway. It is not immediately clear, however, how local differences in NT-3 activity can be established and maintained when NT-3 appears to be diffusible and uniformly released from the neural tube\(^{51}\). Two possible mechanisms are plausible. First, differential distribution of NT-3 binding extracellular matrix components\(^{12}\) may create local differences in concentration and availability of NT-3. Second, it has recently been shown that besides a subpopulation of medial-migrating neural crest cells, trkC is also expressed in the dermatome, which borders the lateral crest migration pathway. At this time trkC-positive neural crest cells migrate solely on the medial pathway\(^{46,56}\). Based on our observation that ectopic c-kit expression reduces the levels of SCF-immunoreactivity and activity\(^{42}\), we suggest that the expression of the trkC receptor on the lateral pathway reduces NT-3 availability to neural crest cells in that location, and thereby limits the location where NT-3-dependent (trkC-expressing) neural crest cells can migrate, survive and proliferate in response to NT-3.

c-ret function is required for colonization of the gut by crest-derived enteric ganglion precursors

Subpopulations of cranial neural crest cells begin to express c-ret between e9 and e10 of mouse development. Some of these cells make contributions to the enteric ganglia in the gut and ganglia in the head\(^{57,58}\). In the absence of a functional c-ret gene, the superior cervical ganglia is absent, and the enteric ganglia and myenteric plexus lack both neuronal and glial contributions at mid and hindgut levels\(^{54,56}\). Thus, although c-ret is expressed very early in different subsets of migrating neural crest cells, its activity appears to be required only by cells in the superior cervical ganglia, and by crest-derived cells that enter the gut and eventually give rise to neuronal and glial cells of enteric ganglia\(^{58}\).

Disruption of GDNF expression yields the same developmental defects as seen in c-ret-deficient mice\(^{33,35,36}\), suggesting that secreted GDNF could be a ligand of c-ret. It has recently been shown, however, that the functional ligand for c-ret is probably a complex of GDNF with a GPI-anchored receptor, GDNFRI\(^\alpha\)\(^{27,28}\). This GDNF/GDNFRI\(^\alpha\) complex can remain bound to the cell surface or be released\(^{27}\). In principle, therefore, the complex can activate c-ret expressed at the surface of the same cell or on adjacent cells\(^{27,28}\). Thus, neural crest cells that express c-ret will respond to GDNF only if GDNFRI\(^\alpha\) is co-expressed in the same cell, or produced by other cells in the vicinity, which in the case of neural crest cells, is not yet known.

GDNF is normally expressed in the gut mesenchyme at high levels in close proximity to the enteric ganglia\(^{26,36}\). It has been reported that GDNFRI\(^\alpha\) is expressed in the smooth and striated muscles, surrounding the enteric ganglia at e15.5 of mouse development\(^{38}\); however, the expression pattern of GDNFRI\(^\alpha\) in enteric precursors themselves has not yet been clearly established. Thus, it is not known whether GDNF/GDNFRI\(^\alpha\) is co-expressed with c-ret in neural crest-derived cells or in surrounding tissue prior to the dependence of crest-derived cells on c-ret/GDNF signaling. Likewise, it is not known whether GDNF-dependence is acquired when GDNFRI\(^\alpha\) is expressed in enteric precursors.

Based on our model that specific crest-derived subpopulations respond to differentially localized growth factor, we speculate that tissue expressing GDNFRI\(^\alpha\) influences the local level of GDNF activity and thereby locally affects behavior of cells that depend on c-ret signaling. Thus, the fate of c-ret-expressing neural crest cells that do not coexpress GDNFRI\(^\alpha\) may be influenced by released and membrane anchored GDNF/GDNFRI\(^\alpha\), reminiscent of c-kit-expressing melanocyte precursors, which normally encounter both secreted and membrane-bound SCF on the lateral pathway. Specifically, the migration and proliferation of c-ret expressing enteric precursors, which are known to depend on localized GDNF expression by the gut mesenchyme\(^{33,35,56}\), may be influenced by GDNFRI\(^\alpha\) expressing cells in the gut. Experimental manipulation of the localization of GDNF activity in the gut, either by changing GDNF levels or by ectopic expression of GDNFRI\(^\alpha\), has not yet been attempted. Nevertheless, GDNF expression in the target tissue of c-ret-expressing neural crest cells strongly suggests that both tropic and trophic roles of c-ret-mediated GDNF signaling might be regulated by localized GDNFRI\(^\alpha\) expression during enteric ganglion precursor migration into the gut.

Subpopulations of cranial and trunk neural crest cells express erbB2/erbB3 and require localized neuregulin signaling for migration and survival

Neural crest-derived Schwann cell precursors respond to neuregulin signaling on ventral root nerve fibers

Neuregulin, also known as glial growth factor\(^{29}\), is the ligand which activates the erbB family of receptor tyrosine
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kinases. ErbB4 binds neuregulin and induces tyrosine phosphorylation. ErbB2 is necessary for tyrosine phosphorylation, but not sufficient for signal transduction. It does not bind neuregulin; conversely, erbB3 binds neuregulin, but lacks a functional tyrosine kinase domain. Thus, a heterodimeric complex of erbB2/3 or erbB2/B4, or a homodimeric complex of erbB4, is necessary for signal transduction of the cognate ligand neuregulin.

In the trunk, many neural crest cells migrating on the medial pathway express erbB3. Subsequently, some of these cells are found in the sensory ganglia, and others associate as Schwann cell precursors with the ventral nerve root and the growing peripheral nerve. Mice carrying a deletion in the neuregulin or the erbB2 gene lack erbB3-expressing Schwann cell precursors associated with the ventral root and peripheral nerves in e10.5 motor nerves, but still express erbB3 in the sensory ganglia. This suggests that the regulatory system involving erbB2/B3 is necessary to attract Schwann cell precursors to the peripheral nerves. At e10.5, erbB3-expressing cells in the sensory ganglia are not affected in embryos lacking erbB2 and neuregulin. Therefore, these cells must either lack neuregulin signal transduction machinery (erbB2) or neuregulin dependence. Conversely, this result suggests that only the Schwann cell precursors coexpress erbB2 and erbB3 and are dependent on neuregulin signaling. We suggest, therefore, that localized neuregulin activity may be required for the initial association, proliferation and migration of neural crest cells along peripheral nerves.

Neuregulin is expressed in various differentially spliced forms, and is expressed both as a secreted and a membrane-bound growth factor. In the trunk, migrating neural crest cells do not express neuregulin, nor is it expressed by tissues bordering their migration pathways. In contrast, spinal motor neurons located in the ventral horn and forming axonal projections express neuregulin beginning at e10.5. Later in development, neuregulin is also expressed in neurons of the sensory ganglia, where it might be involved in glial/neuron specification, as suggested by in vitro experiments. It is likely, therefore, that neuregulin protein is localized to or secreted from the surfaces of growing motor and sensory nerves, where it regulates migration, survival and proliferation of neural crest derived-subpopulation of neuregulin-dependent Schwann cell precursors.

A subpopulation of cranial crest cells requires differential erbB2 expression and localized neuregulin activity to promote its contribution to cranial ganglia

Cranial ganglia consist primarily of neurons of placodal origin and of neural crest-derived glial cells. In the head, a subpopulation of cranial neural crest cells expressing erbB3 emerge from rhombomeres 2, 4 and postotic levels of the hindbrain at e9 of mouse development. At e10.5, erbB3-expressing cells are associated with the cranial ganglia and afferent nerves. Mice that lack neuregulin or erbB2, lack the neural crest cell-derived neuronal and nonneuronal contributions to cranial ganglia at e10.5. Defects are apparent as early as e9.5 of development, suggesting that neuregulin dependence (and, by inference, coexpression of erbB2) begins in erbB3-expressing neural crest cells as soon as they emerge from the neural tube. Other cranial neural crest cells which express a general neural crest marker, AP-2, and neurons of placodal origin, are not affected in mutant embryos.

Neuregulin is expressed by cells in rhombomeres 2, 4 and 6 and in a subpopulation of neural crest cells emanating from these levels. In addition, neuregulin is expressed in a distinct subset of cells of the branchial arches in e9 mouse embryos prior to emigration of erbB3-expressing crest cells. 1 day later (e10), neuregulin is strongly expressed in a subpopulation of cells in cranial ganglia. Although neurons of placodal origin normally express neuregulin, they do not require it for survival, since they survive in neuregulin-negative embryos. Thus, the development of the distally located placode-derived neurons in the cranial ganglia is not affected in neuregulin- or erbB2-deficient embryos. We suggest, therefore, that placode-derived neurons support the development of erbB2/3-expressing neurons in the proximal portion of the cranial ganglia, and the non-neurogenic crest-derived cells in the distal portion of the ganglia. This conclusion suggests an important role for localized paracrine neuregulin signaling between neural and glial precursors within a developing cranial ganglia.

Neural crest-derived cells do not migrate (or fail to survive) adjacent to rhombomeres 3 and 5. Although neuregulin expression correlates well with cranial crest migration, it is not apparent whether or how the activity of secreted neuregulin can be restricted to the destination of these migrating cells. We suggest that due to their binding activity for neuregulin, either erbB3 or erbB4, can act as dominant negative regulators of neuregulin activity. Therefore, tissue or cells which express either erbB3 or erbB4 could negatively regulate the availability of neuregulin to dependent cells and thereby affect their dispersal and localization.

It is interesting, therefore, that erbB3 is expressed in rhombomeres 3 and 5, alternating with neuregulin expression in rhombomeres 2, 4 and 6. Due to the expression of erbB4 in rhombomeres 3 and 5, we suggest that neuregulin activity might be depleted adjacent to rhombomeres 3 and 5, resulting in the inability of erbB2/3-expressing, neuregulin-dependent neural crest cells to migrate in that region in vivo. According to this notion, removal of erbB4 from rhombomere 3 would result in a change of migration pattern of erbB2/3-expressing neural crest cells over rhombomere 3, reflecting the unrestricted availability of neuregulin protein released from the hind-
brain. Neural crest cells originating in rhombomeres 2 and 4 would spread over the area of rhombomere 3, effectively reducing the normal spatial separation between the trigeminal and facial/auditory ganglia. In fact, fused trigeminal and facial/auditory ganglia are exactly what has been observed by Gassmann and coworkers in mice deficient in erbB4[64]. A similar pattern of fused ganglia has been reported in mice lacking Krox-20, which is normally expressed in rhombomeres 3 and 5[55,56]. Although the early pattern of cranial neural crest cell migration has not been studied in either erbB4- or Krox-20-deficient embryos, we suggest that Krox-20 regulates the pattern of cranial ganglion distribution through the expression of erbB4 in rhombomeres 3 and 5.

**PDGF receptor function is required for the development of ectomesenchymal derivatives**

Cells associated with the cranial crest express PDGF receptor at e9 of mouse development[24,30] and give rise to branchial arch mesenchyme. However, in *Patch* mutants[30], which are deficient in PDGF receptor[67], developmental defects are first manifested 2 days later when branchial arches fail to fuse. In addition, cells from the cardiac crest which normally migrate to the heart and form the septation of the outflow tract[5], fail to do so in *Patch* mutant embryos[30].

PDGF receptor is able to bind to both forms of PDGF (A and B) expressed in the embryo[69]. During craniofacial development, PDGF A mRNA is expressed by the overlying epidermis, whereas PDGF B is expressed in branchial arch mesenchyme[69]. PDGF A is produced as two splice variants with identical receptor binding domains, but differential abilities to interact with cell surface or extracellular matrix heparan sulfate proteoglycan[70]. This suggests that one form of PDGF A is normally associated with epidermal basement membranes, and is contacted by migrating ectomesenchymal cells[16] that express PDGF receptors. It is not known whether the claret or phenotype results from a failure of ectomesenchymal crest cells to reach the branchial arches during their early migration or their inability to proliferate in their appropriate locations. However, since PDGF receptor has been shown to elicit trophic responses in vitro[71], it is tempting to speculate that such a mechanism directs ectomesenchymal neural crest precursor dispersed from the MSA into their target areas (Fig. 1B).

**Discussion**

In this review we suggest a new mechanism for regulating neural crest cell migration patterns. We postulate that developmentally distinct subpopulations of crest-derived cells arise after pluripotent crest cell precursors undergo epithelial-mesenchymal transformation and enter the MSA. Within the MSA, we suggest that crest-derived subpopulations begin to express specific RTKs, in response to presently unknown intrinsic or extrinsic cues, which thereby allow them to respond to differentially localized growth factor ligands in the embryo. Thus, the MSA would be the embryonic location where neural crest cells make their first specific pathway choices, informed by the presence of specific ligand activity on the different pathways, and the nature of the RTKs expressed by specific neural crest cells there.

During neural crest cell differentiation, migration represents one aspect of a complex web of developmental events. It is important to emphasize, however, that since the diversification of the crest cell population occurs soon after it segregates from the neural/pigment epithelium, each subpopulation is likely to respond to unique environmental cues, and each has to be analyzed specifically. It is also likely, however, that irrespective of the differentiated state of a cell, the event of migration is linked to RTK expression and the consequent response to localized cognate ligand activity. For example, c-ret is expressed in enteric precursors before they undergo neuron/glia restriction[58,72]. Likewise, some multipotent cells appear to be transiently present at early stages of dispersal on the lateral pathway[14]. It is not known whether these multipotent cells express RTKs during migration, and if so, which ones. We do know, however, that although neurogenic cells appear very transiently on the lateral pathway (Y. Waga, M. Mochii, K. S. Vogel and J. A. Weston, unpublished; see also ref. 14), no neuronal crest derivatives persist there. This suggests that appropriate neuronal survival cues are absent or unavailable on this pathway[49].

Interestingly, the onset of migration of many precursor populations correlates very well with their dependence on RTK activity for survival. Thus, enteric ganglion precursors, cranial and trunk neuron and glial precursors, Schwann cell precursors, and melanocyte precursors all express and depend on specific RTKs and seem to rely on ligands uniquely active in specific embryonic locations. Since crest-derived cells, and especially melanocyte precursors, represent an unusually invasive cell type[73], the tight control of pathway choice and cell survival from the onset of migration from the MSA may therefore serve an important function to assure that derivatives of these invasive cells normally differentiate only in appropriate embryonic locations.

In summary, the neural crest cell distribution pattern established through RTK/ligand interaction could be exerted either by tropic or by trophic stimuli, or a combination of both. The persistent tight regulation of survival by the levels of available growth factors, however, makes it difficult to identify a tropic function in control of neural crest cell migration. Nevertheless, two models to explain differential migration and localization of subpopulations of neural crest cells in response to localized growth factors can be imagined (Fig. 3). First, neural crest cell subpopulations that acquire growth factor dependence migrate randomly through the embryo. Specific localization into the target area would be achieved by target-derived trophic support and apoptosis of
Fig. 3. Two different models explain growth factor-induced accumulation of RTK-expressing subpopulations of neural crest cells in ligand-expressing target areas. In model I (A-C), subpopulations of neural crest cells begin to express a specific RTK while residing in the MSA (A). From this location they disperse randomly within the adjacent tissue (B). Cells acquire growth factor dependence and undergo apoptosis when they are localized ectopically, but survive and proliferate when they are localized where their respective growth factor is expressed (C). In model II (D-F) growth factors released from the target areas form a gradient in the tissue while subpopulations of crest cells begin to express specific RTKs in the MSA (D). As soon as the growth factor gradient reaches the MSA, RTK-expressing cells will disperse up the gradient of their cognate ligand (E). Subsequently, the cells reach the source of their cognate ligand (target area) and begin to proliferate (F).

ectopically located cells outside of the target area (Fig. 3A-C). Alternatively, a gradient of growth factor might be released from the target tissue (Fig. 3D-F), which directs neural crest subpopulations towards their specific target organ. This could occur by some tropic mechanism, either chemotaxis or, if specific substrates are involved, some sort of haptotaxis[12,10]. The first model is based on classical evidence of cell death in peripheral ganglia associated with matching the number of neurons with the size of the target tissue and the amount of released growth factors. In contrast, the second model, which we suggest for consideration and further analysis, postulates that neural crest-derived cells migrate directly in response to a gradient of tropic signal provided by localized growth factor activity in the environment. Further tests of this hypothesis would be appropriate.

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