Neural crest cell migration in the developing embryo

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In vertebrate embryos, neural crest cells migrate extensively to defined sites where they differentiate into a complex array of derivatives, ranging from neurons to pigment cells. Neural crest cells emerge uniformly from the neural tube but their subsequent migratory pattern is segmented along much of the body axis. What factors control this segmental migration? At trunk levels, it is imposed by the intrinsic segmentation of the neighbouring somitic mesoderm, while in the head, intrinsic information within the neural tube as well as extrinsic influences from the ectoderm are involved. A variety of cell–cell and cell–extracellular matrix interactions are thought to influence initiation and movement of neural crest cells. This review summarizes recent progress from both experimental embryology and cell biology approaches in uncovering the mechanisms underlying neural crest cell migration.

The neural crest, an embryonic cell population unique to vertebrates, emerges after neurulation as the neural plate folds to form the neural tube. Initiation of neural crest cell migration proceeds in a head-to-tailward (rostrocaudal) wave, shortly following tube closure. After emigration, these cells move in a highly patterned fashion through neighbouring tissues. They localize in diverse but characteristic sites within the embryo and subsequently differentiate into a wide array of derivatives, including most of the peripheral nervous system, pigment cells, the adrenal medulla and the facial skeleton. There are distinct subpopulations of neural crest cells along the embryonic axis, with cells that arise from different axial levels following distinct migratory pathways and often forming divergent derivatives.

Initiation of neural crest migration

Before the emergence of neural crest cells, the neural tube is a single-layered epithelium whose cells appear morphologically identical. Single-cell lineage analysis has shown that dorsal neural tube cells are equipotent in their ability to form neural tube and neural crest derivatives[1]. This suggests that the neural crest is not a segregated population within the neural tube and that the fate of dorsal neural tube cells is not fixed before neural crest emigration. It is conceivable that those neural tube cells closest to the dorsal midline lose cell–cell contacts by virtue of their position, emigrate and, by definition, form neural crest cells.

Several factors may render the dorsal midline the exit point for neural crest cells. First, the basement membrane surrounding the neural tube is discontinuous over its dorsal aspect[2], making this a logical site of egress. Second, changes in cell–cell adhesiveness among neuroepithelial cells appear to occur in the midline region before neural crest cell emigration. For example, adherens junctions containing N-cadherin, which connect adjacent neural tube cells[3], decrease in the dorsal midline region[4]. Third, the site of exit appears to be determined before or at neural tube closure; when the neural tube is rotated 180° dorsoventrally, neural crest cells emerge normally relative to the neural tube and independently of their relationship to other embryonic tissue[5,6].

Changes in the extracellular matrix (ECM) may stimulate neural crest emigration in some species. In the axolotl, for example, filters can be conditioned with ECM and associated molecules by transplantation onto neural crest migratory pathways at stages of active migration; when grafted into younger embryos, these elicit precocious initiation of neural crest cell migration[7]. In avian embryos, isolated dorsal neuroepithelial cells gradually increase their affinity for ECM molecules before emigrating[8]. This is consistent with the idea that premigratory neural crest cells shift the balance of their adhesions from cell–cell to cell–substrate interactions.

Cell-marking techniques and trunk migratory pathways

After emigration from the neural tube, neural crest cells enter a cell-free and ECM-rich space in which they are easily identifiable. However, they soon invade other tissues, and become morphologically indistinguishable from these. Therefore, it is necessary to mark neural crest cells to study their migration and localization. An embryonic cell marker must fulfil a number of criteria: it must be non-deleterious to the marked cells, it must not become diluted with cell division, and it must not be passed from marked to unmarked cells. Three marking techniques – neural tube transplantation, NC-1/HNK-1 antibody labelling and DiI labelling – have proved particularly useful in revealing the patterns of neural crest migration in the trunk region of avian embryos.

Neural tube transplantations have provided a wealth of information about migratory pathways and, in particular, neural crest derivatives in avian embryos[9]. Neural tubes derived from [^{3}H]thymidine-labelled chick embryos or from quail embryos are transplanted into unlabelled chick hosts. Using this approach, Weston[10] discovered that avian neural crest cells migrate along two primary pathways in the trunk (Fig. 1): dorsolaterally under the ectoderm (eventually giving rise to pigment cells) and ventrally between the neural tube and somite (eventually forming dorsal root and sympathetic ganglia, Schwann and adrenomedullary cells). The [^{3}H]thymidine marker becomes diluted in rapidly dividing

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embryonic cells and is primarily useful for short-term experiments; long-term studies are performed using donor quail neural tubes, which contain a heritable heterochromatin marker[11]. These grafts have documented the full range of neural crest derivatives arising from different regions of the neuraxis[9]. They are also useful for challenging the fates of neural crest cells by grafting them to new environments[9,12].

NC-1/HNK-1 antibodies that recognize neural crest cells make it possible to follow the earliest routes of neural crest migration. Monoclonal antibodies NC-1 and HNK-1[13] both recognize a carbohydrate epitope on the majority of migrating neural crest cells. The epitope is first detectable shortly after emigration and persists on various derivatives. By examining sections through embryos fixed and stained at different stages, pathways of neural crest migration can be inferred. Neural crest cells migrate through the sclerotomal portion of the somite ventrally towards the aorta. Interestingly, they move in a segmental fashion through the rostral half of each somite, but do not move through the caudal half[14] (Fig. 1). This metameric pattern of migration has important consequences for formation of the peripheral nervous system, since the peripheral ganglia also are aligned with the rostral portion of each somite[15,16]. In addition to being absent from the caudal half of the sclerotome, avian neural crest cells are never observed in a space of approximately 85 μm width surrounding the notochord[17].

Although antibody labelling has enhanced our understanding of neural crest migratory pathways, this approach has several pitfalls. Antibodies are not entirely specific; they recognize numerous cell adhesion molecules associated with many non-neural crest cells[18] and do not recognize all neural crest populations. Furthermore, we can gain only a static picture of neural crest migration by examining cell distribution in fixed embryos. An alternative marking technique for labelling neural crest is to inject the lipophilic dye DiI into the lumen of the neural tube or directly into the neural folds[19,20]. Because the dye is hydrophobic and lipophilic, it intercalates into all cell membranes that it contacts. Injection into the neural tube marks all neural tube cells, including presumptive neural crest cells within its dorsal aspect. Injected embryos can be examined either in whole mounts or in sections. Because the time and location of injection can be controlled, this provides a direct approach for following migratory pathways. In addition, the dye can be used to follow neural crest pathways in a number of species, including chick, mouse and frog[19–23].

Despite the differences in the nature of the techniques involved, DiI labelling and antibody staining provide similar pictures of the ventral neural crest migratory pathway. In addition, DiI-labelling reveals that neural crest cells that migrate dorsally under skin do so in an unsegmented manner and, in avian embryos, appear to initiate migration much later than ventrally moving cells. Because injection of DiI at progressively later stages of development labels only those neural crest cells that are premigratory at the time of injection, this approach can be utilized

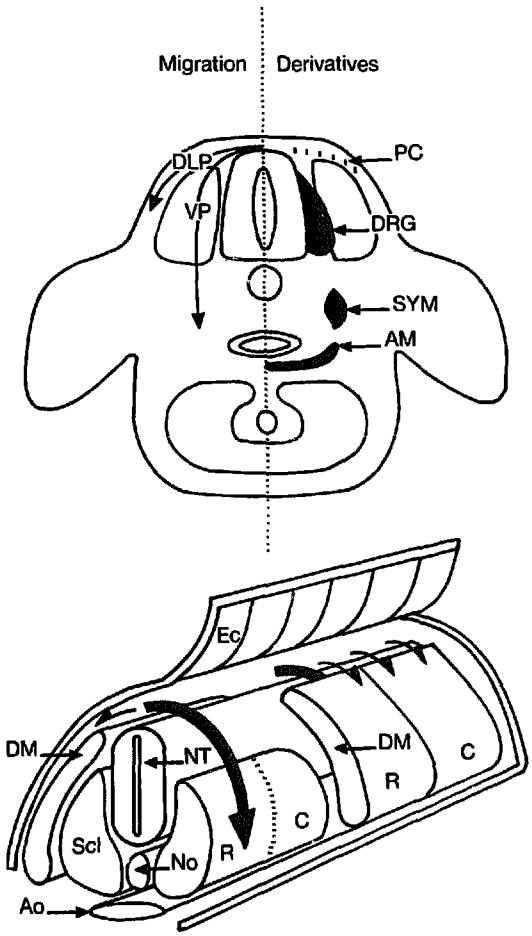

FIGURE 1

(top) Cell-marking techniques have revealed that trunk neural crest cells migrate along two pathways: a dorsolateral pathway (DLP) whose cells form pigment cells (PC), and a ventral pathway (VP) whose cells form the dorsal root ganglia (DRG), sympathetic ganglia (SYM), adrenomedullary cells (AM) and aortic plexuses. (bottom) In the rostrocaudal dimension, neural crest cells migrate through the rostral (R), but not caudal (C), half of each somitic sclerotome (Scl). However, their migration on the dorsolateral pathway between the ectoderm (Ec) and dermomyotome (DM) is unsegmented. NT, neural tube; No, notochord; Ao, aorta.

to follow the timing and order of neural crest migration. Avian neural crest cells continue to emerge from the neural tube for approximately 24 h. Interestingly, neural crest cells that emigrate at progressively later times contribute to progressively more dorsal derivatives, suggesting that the derivatives become assigned in a ventral-to-dorsal order.

Inhibitory influences on trunk neural crest migration

What controls the segmental pattern of neural crest migration such that the cells move through the rostral but not the caudal half of each somite? There may be inherent segmentation within the neural tube that results in neural crest cells emerging in a

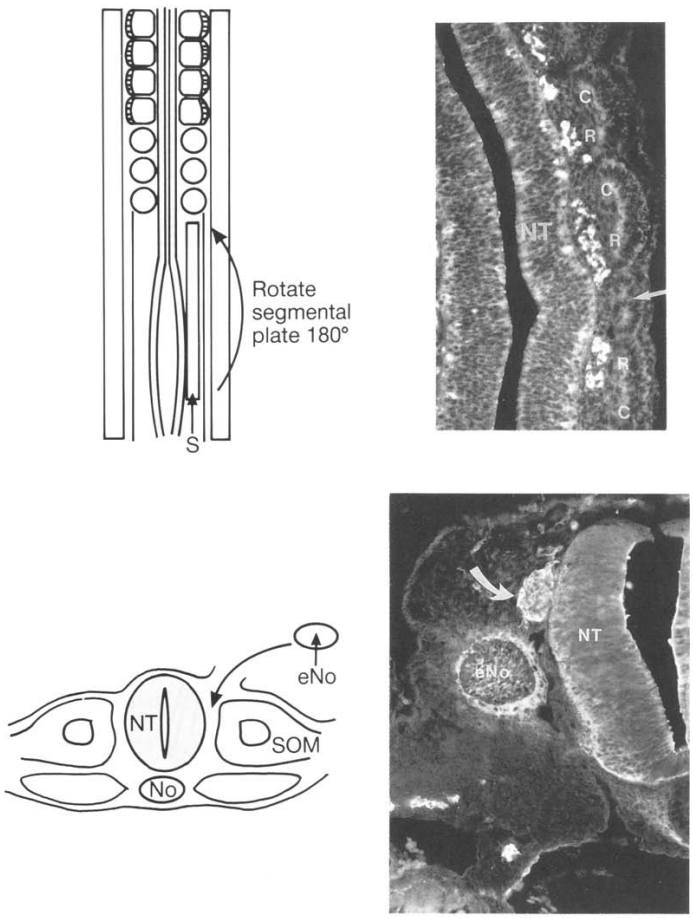

FIGURE 2

Effects of tissue manipulations on neural crest cell migration. (top left) Schematic diagram illustrating the procedure of rotating the segmental plate (S) 180°. This operation results in the formation of somites with inverted rostrocaudal polarity. (top right) Longitudinal section through an embryo one day after segmental plate rotation. Below the small somite (arrow) marking the graft border, neural crest cells migrate through the rostral (R), but not the caudal (C), half of each somite. In the grafted region above the arrow, neural crest cells migrate through the original rostral half of each rotated somite (data from Ref. 24). NT, neural tube. (bottom left) Schematic diagram illustrating the procedure of implanting an ectopic notochord (eNo) between the neural tube (NT) and somites (SOM). (bottom right) A transverse section through an embryo fixed 1.5 days after implantation of an ectopic notochord on the left side. HNK-immunoreactive neural crest cells (arrow) fail to approach the ectopic notochord (data from Ref. 26).

metameric pattern. Alternatively, the adjacent mesoderm may be responsible for segmental migration; the rostral portion of the somite may be permissive and/or the caudal portion may be inhibitory or non-permissive for neural crest cell migration. To distinguish between these alternatives, we rotated either the neural tube or the adjacent mesoderm. Rostrocaudal rotation of the segmental plate mesenchyme (which forms the somites; Fig. 2) results in morphologically normal somites. However, the migratory pattern of neural crest cells through the sclerotome is reversed such that they now traverse the caudal (original rostral) halves of the rotated sclerotomes[24]. This suggests that the segmented pattern of neural crest cell movement is due to cues inherent to the somitic mesoderm, and that these cues are fixed at the segmental plate stage even before differentiation of the somites. At a molecular level, there may be inhibitory cues in the caudal sclerotome, attractive cues in the rostral sclerotome or both. Although their function remains to be established, possible candidates for inhibitors within the caudal portion of the somite include T-cadherin, chondroitin sulphate proteoglycans (CSPGs) and molecules recognized by peanut lectins[25]. Other molecules are distributed selectively in the rostral portion of the somite, including butyrlcholinesterase[26] and tenascin, although the latter appears as a consequence rather than a cause of neural crest migration[27].

Another embryonic region lacking neural crest cells is the perinotochordal space. To test whether the notochord inhibits neural crest migration, a length of quail notochord can be grafted into the chick sclerotome at the time of neural crest cell migration (Fig. 2). Neural crest cells appear to avoid the implanted notochord, in a chondroitinase- and trypsin-sensitive manner[28]. The perinotochordal region contains abundant extracellular matrix molecules, including CSPG, consistent with the possibility that CSPGs are responsible for inhibition of neural crest cells. Similarly, CSPGs are thought to result in the delayed entry of neural crest cells along the dorsal presumptive pigment pathways[29], and are implicated in the migration of endocardial cushion cells[30] and in inhibition of motor axons[31]. The inhibitory effects of the notochord appear to depend on age; notochords from 2–3-day-old embryos give optimal inhibition, which declines thereafter (Z. Pettway and M. Bronner-Fraser, unpublished). Curiously, other migratory cell populations, such as sclerotomal cells, do not avoid notochord and actually move towards it, suggesting that distinct mechanisms may govern the migration of different cell types.

These results suggest that trunk neural crest cells are highly migratory and invade many available sites and that their distribution is controlled primarily by inhibitory factors that exclude them from the caudal portion of the somites and the perinotochordal region. This leads to the interesting possibility that negative regulators may play an important role in patterning neural crest cells.

Neural crest migratory pathways in the head

The cranial neural crest can be subdivided into caudal forebrain, midbrain and hindbrain, with each population following different migratory pathways and forming a different array of derivatives. Similar cell-marking approaches to those described above have been used to study migratory patterns in the head. Neural crest cells emerging from the forebrain and midbrain move primarily as a broad, unsegmented sheet of cells under the ectoderm. They contribute to the skeleton and connective tissue of the face, and some peripheral ganglia[9]. In the hind-

brain, neural crest cells migrate as three broad streams that populate the first, second and third branchial arches, respectively[32]. This segmental migration correlates with the segmental appearance of the hindbrain neural tube, which is divided into a series of eight swellings called rhombomeres (Fig. 3). No neural crest cells are obvious in the mesenchyme lateral to rhombomere 3 (R3) and between the neural tube and the otic vesicle lateral to rhombomere 5 (R5).

This metameric pattern could arise from a segmental generation of neural crest cells controlled by intrinsic properties of the neural tube. Alternatively, as in migration at trunk levels, extrinsic factors in the environment could result in the segmented migration of neural crest cells from the hindbrain. In support of an intrinsic mechanism of patterning, Lumsden and colleagues[32] have proposed a non-uniform origin of neural crest cells in the hindbrain; based on dye labelling studies, they suggested that R3 and R5 fail to produce crest cells. However, a careful repeat of these experiments with focal injections of DiI has shown that all rhombomeres, including R3 and R5[20], generate neural crest cells, although cell death[32] probably leads to somewhat fewer cells arising from R3. Rather, labelled cells that originate in R3 and R5 deviate rostrally or caudally, failing to enter the adjacent paraxial mesoderm or otic vesicle region as if to avoid specific neighbouring domains (Fig. 3). In a preliminary series of experiments, we have examined the relative importance of intrinsic versus extrinsic influences on neural crest migration from the hindbrain by altering the rostrocaudal positions of either the neuroectoderm or the adjacent mesoderm. Whereas mesoderm grafts had no apparent effects, transposition of the neural tube resulted in labelled neural crest cells following pathways generally appropriate for their new position after grafting. After neural tube rotation, small ectopic otic vesicles formed in the ectoderm adjacent to R4. Surprisingly, the neural crest cells moved directionally towards and around these ectopic otic vesicles (J. Sechrist, T. Scherson and M. Bronner-Fraser, unpublished). These experiments suggest that environmental signals from an ectodermal placode contribute to the patterning of neural crest migration in the hindbrain.

Information intrinsic to the neural tube is also important for the patterning of hindbrain neural crest cells. For example, rhombomere boundaries correspond to borders of gene expression for several regulatory transcription factors, presumed to be determinants of both rhombomere and the associated neural crest phenotype[33]. Consistent with this possibility, Noden[34] found that grafting first-arch neural crest into the second arch resulted in a duplication of first-arch structures, suggesting that premigratory neural crest cells already have some positional identity.

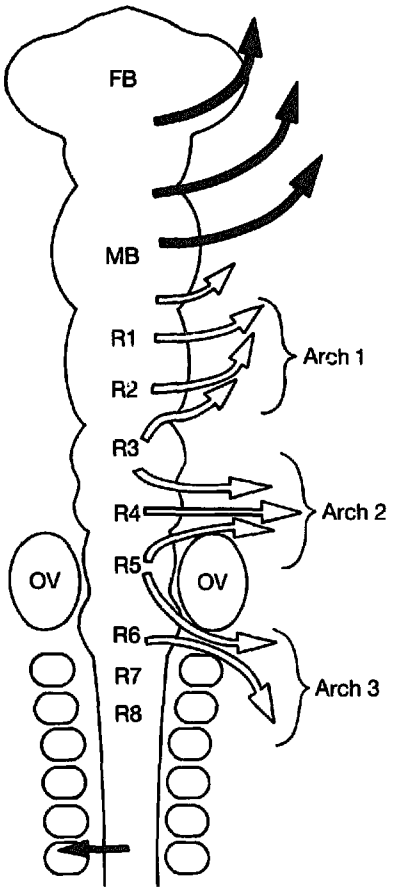

FIGURE 3

Schematic diagram illustrating patterns of neural crest cell migration in the head. Neural crest cells originate from all axial levels but do not enter the region adjacent to rhombomere 3 (R3) or the otic vesicle (OV) lateral to R5. Cells arising from R3 contribute to branchial arches 1 and 2, whereas cells arising from R5 contribute to arches 2 and 3. By contrast, R4 contributes only to branchial arch 2. (Modified from Ref. 20.) FB, forebrain; MB, midbrain; black arrows, midbrain neural crest; grey arrows, hindbrain neural crest.

Receptors and their extracellular matrix molecules on neural crest pathways

Extracellular matrix molecules, including fibronectin, laminin, tenascin/cytotactin, various collagens and proteoglycans, are abundant along neural crest migratory pathways[35], although relatively little is known about their role in guiding these cells. Neural crest cells possess several integrin receptors for extracellular matrix molecules. *In vitro*, antibodies against β_1 integrins block the attachment of neural crest cells to fibronectin, laminin and collagens, suggesting that β_1 integrins are the primary mediators of their attachment[36]. However, little is known about the α subunits associated with β_1 integrins on neural crest cells. By using antisense oligonucleotides to disrupt neural-crest–ECM interactions, we have shown that trunk neural crest cells utilize at least three integrin subunits[37], including the α_1 subunit[38]. Although the identity of the others remains unknown, antibody labelling experiments suggest that they do not correspond to α_5, α_6 or α_7 (M. Bronner-Fraser, unpublished).

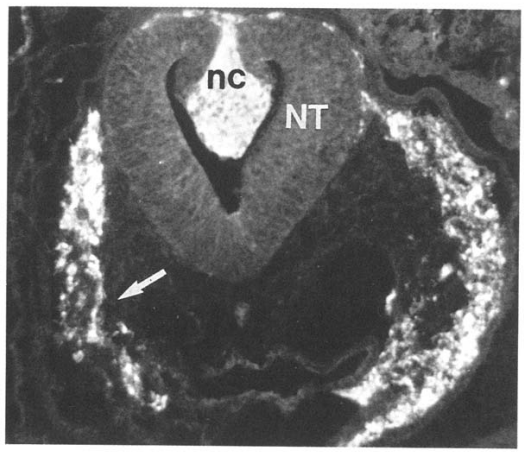

FIGURE 4

A transverse section through an embryo one day after injection of antibodies against the β_1 subunit of integrin into the cranial mesenchyme on the left side. HNK-1-positive neural crest cells on the injected side (arrow) appear fewer in number than on the control side. Furthermore, numerous neural crest cells (nc) are trapped within the lumen of the neural tube (NT). Reproduced, with permission, from Ref. 39.

In vivo perturbation experiments suggest that some extracellular matrix molecules play a functional role in cranial neural crest migration. By microinjecting antibodies lateral to the cranial neural tube adjacent to neural crest migratory pathways, antibodies can be used to 'knock-out' selected cell–matrix interactions (Fig. 4). Using this approach, numerous molecules have been shown to be essential for proper cranial neural crest cell emigration. These include the β_1 subunit of integrin, fibronectin, laminin–heparan-sulphate-proteoglycan complex, tenascin and galactosyltransferase[35,39]. Antibodies to these reagents cause a build up of neural crest cells within or adjacent to the neural tube, together with various neural tube abnormalities (Fig. 4). Thus, it is likely that many of these molecules are required for proper emigration of neural crest cells. Those neural crest cells that are able to emigrate appear to migrate normally, suggesting that many of their interactions remain intact. The necessity for many rather than single molecules suggests that the interactions involved in cranial neural crest migration are complex and, perhaps, multivalent. Moreover, in contrast to their effect on neural crest cells in the head, none of these antibodies has detectable effects on trunk neural crest cells, although they do affect migration of myoblast cells[40] in this region of the embryo. Therefore, different guidance mechanisms may be involved for neural crest cells at different axial levels; cell–matrix interactions may play a predominant role in the initiation of cranial neural crest migration, while cell–cell interactions of trunk neural crest cells with somite and notochord cells (described above) may dominate in the trunk. In addition, different cell types in the same embryonic region (e.g. neural crest cells and myoblast cells) appear to use different guidance mechanisms.

Conclusions and insights

Because of its ease of manipulation, accessibility, migratory ability and differentiation capacity, the neural crest provides an excellent model system for studying complicated cell movements. The mechanisms underlying neural crest cell migration are beginning to be understood thanks both to new methods for cell marking together with grafting approaches, and to tissue culture studies utilizing cellular and molecular markers. In the trunk, cell–cell interactions may predominate, such that the mesodermal somites control the rostrocaudal patterning of neural crest cells and the notochord prevents neural crest cells from crossing the midline. In the head, the segmental migration of neural crest cells in the hindbrain may be influenced by both intrinsic and extrinsic cues; all rhombomeres generate neural crest cells, but their subsequent migration may be influenced by environmental cues. Although both trunk and cranial neural crest cells utilize integrin receptors, antibodies to these receptors affect cranial but not trunk neural crest cell emigration, highlighting the possibility that different neural crest cell populations utilize different interactions in their migration. It is important to note that, without further functional evidence, the presence of a particular molecule along neural crest migratory pathways does not necessarily imply a guiding or regulatory role. Future experiments must address the molecular nature of the interactions that guide neural crest cells.

On a final note, it is worth considering the similarities and differences between neural crest cells and other migratory cell types. The concept of attractive and inhibitory signals presented here may represent general mechanisms in the guidance of migrating cells. As an example, both motor axons and trunk neural crest cells follow similar pathways and may respond to similar environmental cues. Furthermore, some molecules like CSPGs inhibit movement of numerous cell types, including neural crest cells, endocardial cushion cells and motor axons. However, different migratory cell types have different cell surface properties and, consequently, unique cell–cell and cell–matrix interactions. Thus, the migratory response of sclerotomal cells and myotomal cells is distinct from that of neural crest cells under identical environmental conditions. A balance between cell–cell and cell–matrix interactions may dictate migratory pathway choice and directionality in a cell-type-specific manner. By contrast to some other cell types described in this issue, neural crest cells tend to migrate in groups rather than as individual cells. Thus, interactions among migrating neural crest cells themselves could be important for their movement. It is important to bear in mind both the similarities and differences between cell types when investigating the general mechanisms underlying embryonic cell movement.

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