Significance of the Cranial Neural Crest

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The cranial neural crest has long been viewed as being of particular significance. First, it has been held that the cranial neural crest has a morphogenetic role, acting to coordinate the development of the pharyngeal arches. By contrast, the trunk crest seems to play a more subservient role in terms of embryonic patterning. Second, the cranial crest not only generates neurons, glia, and melanocytes, but additionally forms skeletal derivatives (bones, cartilage, and teeth, as well as smooth muscle and connective tissue), and this potential was thought to be a unique feature of the cranial crest. Recently, however, several studies have suggested that the cranial neural crest may not be so influential in terms of patterning, nor so exceptional in the derivatives that it makes. It is now becoming clear that the morphogenesis of the pharyngeal arches is largely driven by the pharyngeal endoderm. Furthermore, it is now apparent that trunk neural crest cells have skeletal potential. However, it has now been demonstrated that a key role for the cranial neural crest streams is to organise the innervation of the hindbrain by the cranial sensory ganglia. Thus, in the past few years, our views of the significance of the cranial neural crest for head development have been altered. *Developmental Dynamics 229:5–13, 2004.* © 2003 Wiley-Liss, Inc.

INTRODUCTION

The established view of the cranial neural crest was that it played a pivotal role in vertebrate head development. It was thought that the cranial neural crest carried the cues for organising the morphogenesis of the head and, uniquely, was the source of much of the head skeleton. However, it is now becoming apparent that we must reassess our views of the significance of the cranial neural crest, and it is with this reassessment that this review is concerned.

MIGRATION OF THE CRANIAL NEURAL CREST

The migration of the cranial neural crest is quite distinct from that of the trunk. In the trunk, the neural crest cells are subjected to the alternating permissive and inhibitory halves of somites, which result in their segmental organisation (Rickmann et al., 1985). In the head, the neural crest cells pour out from the developing brain into a periphery that is devoid of somites. Yet, the cranial crest cells do not migrate as a single mass but are organised into streams, three of which can be identified in the developing head of all vertebrate embryos: trigeminal, hyoid, and post-otic (Fig. 1). The trigeminal crest arises from the midbrain and rhombomeres 1 and 2 of the hindbrain and forms neurons within the trigeminal ganglion and the components of the orofacial prominences and mandibular arch—the skeleton of the lower and upper jaw (Lumsden et al., 1991; Schilling and Kimmel, 1994). The second crest stream, the hyoid, arises primarily from rhombomere 4 of the hindbrain and forms neurons of the proximal facial ganglion as well as the constituents of the second pharyngeal arch—the hyoid skeleton (Lumsden et al., 1991; Schilling and Kimmel, 1994). Finally, the post-otic crest is generated by rhombomeres 6 and 7 of the hindbrain and forms the neurons of the proximal and jugular ganglia, and the skeletal components of the posterior pharyngeal arches (Lumsden et al., 1991; Schilling and Kimmel, 1994).

In the trunk, the first migrating neural crest cells move ventrally from the neural tube and migrate through the anterior half sclerotome.
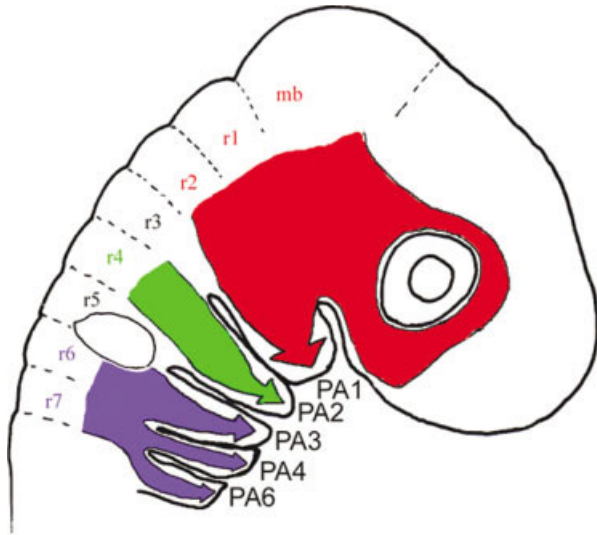
Fig. 1. Cranial neural crest migrates in three streams. The trigeminal stream (red) emanates from the midbrain (mb), rhombomeres (r) 1 and 2. It migrates into the upper jaw primordia, underneath the eye, and into the first pharyngeal arch (PA1), which forms the lower jaw. The hyoid stream (green), from rhombomere 4, migrates into the second pharyngeal arch, forming the jaw support. The post-otic stream (purple) emerges from rhombomeres 6 and 7. It populates the caudal pharyngeal arches, of which there are three in the chick embryo depicted here.
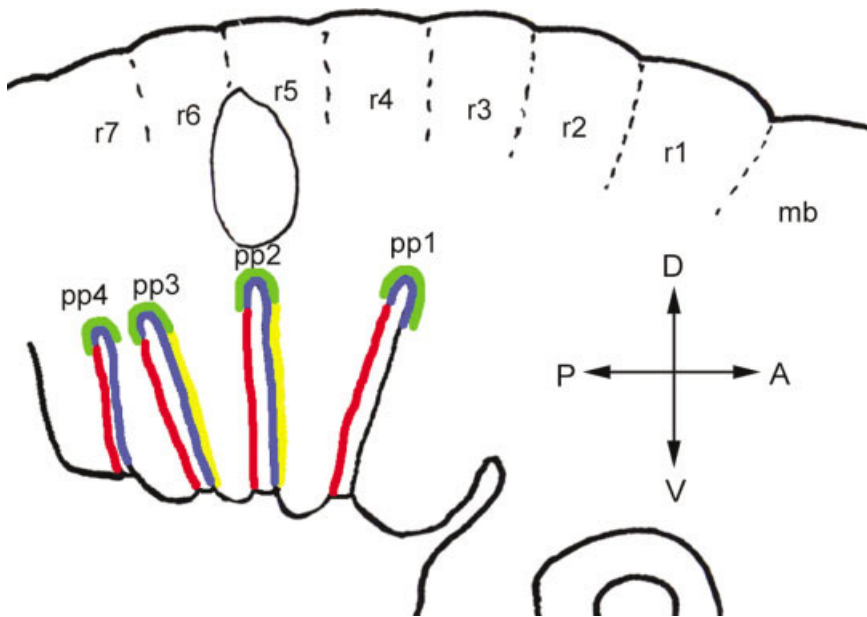

Fig. 2. Regionalisation of the pharyngeal endoderm. Endodermal pharyngeal pouches (pp) sit between the pharyngeal arches and express a range of markers that demonstrate distinct regionalisation. Pax-1 (green) is expressed at the dorsal tip of each pouch. BMP-7 (blue) is expressed in the posterior endodermal margin, while Fgf8 (red) is expressed in the anterior endodermal margin. Shh (yellow) is expressed at the posterior margin of pouches 2 and 3 only. D, dorsal; V, ventral; P, posterior; A, anterior; mb, midbrain; r, rhombomere.
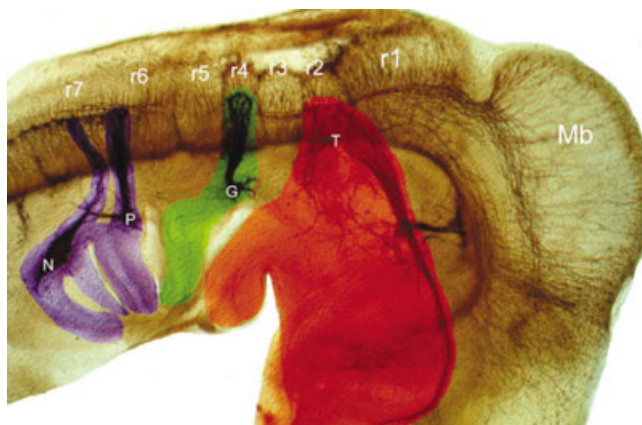
Fig. 3. Segregation of cranial neural crest establishes correct afferent innervation in the vertebrate head. Whole-mount chick embryo labeled with neurofilament antibody to visualise axonal projections. The trigeminal axons (T) extend toward the hindbrain along the first or trigeminal crest stream (red), entering at rhombomere (r) 2. Axons of the geniculate (G) extend along the hyoid crest stream (green), into rhombomere 4. Axons from the petrosal (P) and nodose (N) extend along the post-otic crest stream (purple) into rhombomeres 6 and 7, respectively. Mb, midbrain.

These cells have a neuronal fate. Those that move furthest ventrally form the sympathetic ganglia, while the remainder cease migration in the anterior sclerotome and form the dorsal root ganglia. The later migrating neural crest cells, however, move dorsolaterally between the dermamyotome and the overlying ectoderm, and these cells will form the melanocytes (Weston and Butler, 1966; Serbedzija et al., 1990; Erickson et al., 1992). In the head, there is also a correlation between the timing of crest migration and the fates the crest cells follow, but it is significantly different. Here, the early migratory cells populate the pharyngeal arches and facial prominences, and these groups will generate ectomesenchymal derivatives: bone, cartilage, and connective tissue, while the later migrating cells stay closer to the developing central nervous system and generate neurons and glia of the cranial ganglia (Baker et al., 1997).

SEGREGATION OF THE CRANIAL CREST STREAMS

The segregation of the cranial crest is evident as soon as they are generated by, and emerge from, the neural tube, and it is events within the developing hindbrain that are responsible for establishing this segregation. Studies in chick have shown that one mechanism that helps enforce the segregation of the crest is the focal depletion of crest cells from the two territories that lie between the streams, rhombomeres 3 and 5. These two rhombomeres do produce some neural crest cells. However, these cells do not migrate laterally but rather move anteriorly and posteriorly to join the streams of crest migrating from the adjacent even-number rhombomeres (Sechrist et al., 1993; Birgbauer et al., 1995; Kulesa and Fraser, 2000). The majority of the neural crest cells produced by rhombomeres 3 and 5, however, are lost by means of apoptosis, which is induced in these crest cells by signals from the neighbouring segments (Graham et al., 1993). One of the main consequences of this inductive interaction is to promote the expression of the signalling molecules Bmp-4

in the crest primordia of rhombomeres 3 and 5, which in turn acts to sponsor the apoptotic elimination of these cells (Graham et al., 1994). In vitro application of Bmp-4 can also promote crest depletion of rhombomeres 2 and 6, which correlates with the finding that much of the hindbrain neural crest expresses the necessary receptors and intracellular effectors to respond to this factor (Farlie et al., 1999; Smith and Graham, 2001). Bmp-4 will not induce, however, cell death in rhombomere 4 neural crest, which corresponds with the finding that these crest cells express the Bmp-4 antagonist *Noggin* in addition to the Bmp receptors and intracellular transducers (Smith and Graham, 2001). Thus, the neural crest cell death is localised to rhombomeres 3 and 5 both by restricting the expression of the *Bmp-4* ligand to these segments as a result of an inductive interaction from the flanking segments and through the expression of *Noggin* in the neural crest cells of rhombomere 4, which are juxtaposed to sites of *Bmp-4* expression.

Studies in other species have suggested that the establishment of the cranial neural crest streams may be somewhat different to that observed in chick. In mammalian embryos, cranial neural crest migration commences before neural tube closure and occurs over a protracted period of time (Morriss-Kay and Tucket, 1991). In the chick, however, cranial neural crest cells only begin migrating before neural tube closure at mesencephalic levels, with most cranial crest cells migrating after tube closure (Tosney, 1982). Another difference between chick and mammals is that it has long been established that, in mammals, the cranial crest do not migrate in a strict rostrocaudal sequence. Indeed, there is also variation in the timings and patterns of migration between mammalian embryos (Tan and Morris-Kay, 1985). Recent studies have also suggested that the mechanisms underlying the streaming of the cranial crest in the mouse differ significantly from those uncovered in the chick, although the precise nature of these mechanisms has yet to be elucidated (Trainor et al., 2002). Similarly, we know little about the mechanism by which cra-

nial neural crest streaming is maintained in other species where focal cell death in rhombomeres 3 and 5 is not observed, such as in zebrafish (Schilling and Kimmel, 1994) and *Xenopus* embryos (Hensey and Gautier, 1998).

Other work has shown that there are additional mechanisms that could contribute to the streaming of the neural crest. Several studies seem to suggest that there are inhibitory influences emanating from rhombomeres 3 and 5, which restrict the movement of cells across these segments. For example, if the neural crest primordium of rhombomere 4 is grafted dorsally in rhombomere 3, even though many neural crest cells are now produced they are deflected anteriorly and posteriorly (Niederlander and Lumsden, 1996). This effect could be due to the expression in both rhombomeres 3 and 5 of *Sema3A*, a molecule which is known to inhibit neural crest migration (Eickholt et al., 1999). Similarly, it has been suggested that the mesenchyme opposite these two segments is inhibitory to neural crest migration (Farlie et al., 1999) and that this finding is due to the function of *ErbB4* in the hindbrain (Golding et al., 2000). These studies help explain why the few crest cells generated by rhombomeres 3 and 5 do not move laterally from these rhombomeres but migrate anterior and posterior. Furthermore, work in *Xenopus* has also suggested that interactions between the ephrins and their receptors play a role in streaming the crest in the periphery (Smith et al., 1997). Given these results, it seems likely that the segregation of the neural crest into streams is in part due to events in the hindbrain, including, in chick, the induction of focal neural crest apoptosis in rhombomeres 3 and 5, as well the action of subsequent cues in the periphery, which act to reinforce and stabilise this segregation.

MORPHOGENETIC ROLE FOR THE CRANIAL NEURAL CREST

It has in the past been argued that the segregation of the cranial neural crest into these streams was essential for ensuring correct morphogene-

sis—creating skeletal elements of the correct size, shape, and orientation in the appropriate location. A key piece of evidence supporting this viewpoint comes from studies in which neural crest cells were forced to populate an inappropriate pharyngeal arch (Noden, 1983). Thus, if presumptive first arch crest is grafted in place of presumptive second arch crest, then the grafted crest cells fill the second arch, rather than the first, and, furthermore, although these transposed crest cells generate skeletal derivatives, they differentiate as components of the lower jaw, i.e., first arch, rather than second arch elements. These transposed neural crest cells also caused the musculature of the second arch to form skeletal attachments typical of the first arch (Noden, 1983). The conclusion that emerged from these studies was that it was the neural crest cells that were acting to organise the development of the pharyngeal arches. Furthermore, as these grafts were of neural tube origin before the emergence of the neural crest, it was also suggested that the neural crest cells acquired their sense of identity when they were within the neural primordium. Thus, the segregation of the cranial crest into stream would act to ensure that first arch crest, carrying cues for jaw development, did not intermingle with hyoid crest, carrying cues for the patterning of the second arch; the streaming of the crest would act to guarantee the faithful transfer of patterning information from the neural primordium to the periphery.

The idea that the segregation of the cranial crest into streams was important for the morphogenesis of the head was further enforced by a long-term fate map of the cranial crest, which revealed a highly constrained pattern of skeletomuscular connectivity in the head (Kontges and Lumsden, 1996). In this study, it was found that head muscle connective tissues derived from neural crest from a particular axial level were always exclusively anchored to skeletal domains derived from the same origin. This finding occurred even within skeletal elements, such as the jaw or the tongue skeleton, which are compounds of crest cells

populations from different axial levels. Furthermore, this even holds true if one of the attachment points is on the mesodermally derived basal cranial capsule; these attachment points were shown to derive from discrete clusters of crest cells embedded on the skull.

In many ways, the hypothesis that it is the neural crest cells that pattern the pharyngeal arches is very attractive. First, although this patterning mechanism is seemingly different from that observed in the rest of the embryo, there is a commonality in that, in all cases, the skeletogenic tissue is the repository of the patterning information. In the trunk, the paraxial mesoderm-derived somites, which generate the vertebra, direct motor neuron patterning in the adjacent spinal cord (Ensini et al., 1998). The lateral plate mesoderm, which forms the appendicular skeleton, acts to attract muscle precursors from adjacent somites and subsequently directs their patterning into specific muscle types (Chevallier and Kieny, 1982; Francis-West et al., 2003). In the head, the neural crest is the prime source of skeletal tissue, and here these cells are thought to pattern the arches. Second, if the neural crest cells do pattern the arches, then this also provides an explanation of how the different tissues of the developing head can be coordinated. For example, the neural crest cells that populate and organise the first arch arise from the same axial level as the trigeminal motor neurons that innervate that arch, and thus one can imagine how a correspondence is achieved between sensorimotor innervation, centered on the hindbrain, and the musculoskeletal effectors in the periphery.

ENDODERM PLAYS A KEY ROLE IN PHARYNGEAL MORPHOGENESIS

Several studies, however, have now suggested that this crest-centric view of pharyngeal patterning needs to be reassessed. More specifically, it is now apparent that the pharyngeal endoderm plays a key role in organising the development of the pharyngeal arches. The for-

mation of endodermal pharyngeal pouches is the first indication of pharyngeal arch development (Veitch et al., 1999). Furthermore, the post-otic crest that fills the posterior arches is split by the formation of the pharyngeal pouches. In keeping with this view, it has been shown that, if the neural crest is ablated, through removal of the neural tube before crest production, pharyngeal arches do form and are regionalised (Veitch et al., 1999). In normal pharyngeal arches, Bmp-7 is expressed at the posterior endodermal margin, FGF-8 in the anterior endoderm, and Pax-1 in the most dorsal endoderm of the pharyngeal pouches, and these expression domains are unchanged in arches that are devoid of neural crest cells (Veitch et al., 1999; Fig. 2). Also, these crest-free arches have a sense of individual identity. Normally, sonic hedgehog is a prominent early feature of the posterior endoderm of the second arch, following later in the posterior endoderm of the third arch, and again in the absence of crest the spatial and temporal dynamics of sonic hedgehog is unchanged in the absence of crest (Veitch et al., 1999; Fig. 2). Thus, the endoderm can form a segmented series that is appropriately regionalized in the absence of neural crest.

That pharyngeal segmentation is not dependent upon the crest is also significant, because it reflects the evolutionary history of the pharyngeal arches. Endodermal pharyngeal segmentation is a feature of all chordates (Schaeffer, 1987) and, thus, originated before the evolution of the neural crest. The regionalisation of the pharyngeal endoderm also preceded the emergence of the neural crest, and it has been shown in Amphioxus that the *Pax-1/9* gene is expressed within the pharyngeal pouches in a similar manner to vertebrates (Holland and Holland, 1996). Thus, the evolution of the vertebrate pharynx must have involved an integration between the neural crest cells, which generate the pharyngeal skeleton, and a more ancient segmentally organised pharyngeal endoderm.

Further evidence for a role for endoderm in directing pharyngeal

arch development has come from the zebrafish mutant *vgo* in which the pharyngeal pouches fail to form (Piotrowski and Nusslein-Volhard, 2000). In this mutant, arch development is severely perturbed and the neural crest-derived pharyngeal cartilages are disorganised, often fusing with each other, or in the more posterior arches, failing to form altogether. This failure is not due to defects in the crest but results from the failure of pouch formation. Other mutants that display endoderm defects, such as *bon* and *cas* which do not produce endoderm, also show defects in the formation of the pharyngeal arches, and in these animals, the pharyngeal cartilages fail to form at all. It has also now been demonstrated that the neural crest cells are induced to form cartilage by means of the action of FGF-3 emanating from the endoderm (David et al., 2002). Furthermore, it has been definitively demonstrated, in chick embryos, that neural crest cells respond to patterning cues produced by the pharyngeal endoderm to generate distinct skeletal components (Couly et al., 2002). The ablation of specific domains of the pharyngeal endoderm at early stages of development results in the specific loss of particular neural crest-derived skeletal elements. Moreover, if rostral neural crest cells are exposed to an ectopic piece of pharyngeal endoderm, then they will generate a supernumerary jaw. Of interest, the orientation of the ectopic strip of endoderm determines the orientation of the skeletal elements of the additional jaw.

PHARYNGEAL PATTERNING IS CONSENSUAL

These studies clearly highlight that the endoderm plays a pre-eminent role in the patterning of the pharyngeal arches; however, the neural crest cells are not completely passive in this process. Rather, several studies suggest that the response of different populations of the neural crest to endodermal cues is dependent upon the transcription factors that they express, most notably their Hox gene repertoire. During normal development, each of the cranial neural crest streams expresses different Hox genes (Hunt et al., 1991). The trigeminal stream is Hox negative, the hyoid expresses Hox-a2, and the post-otic crest express members of third paralogous Hox group—Hox-a3, Hoxb3, Hox-d3. Of interest, in mice lacking Hox-a2, the second arch exhibits a transformation, and jaw elements form within it (Gendron-Maguire et al., 1993; Rijli et al., 1993). These duplicated jaw elements form as a mirror image to the first arch elements, which would be consistent with these cells also responding to cues emanating from the first pharyngeal pouch. However, if Hox-a2 expression is forced in the neural crest cells of the first arch, then jaw development is inhibited and an ectopic hyoid forms, and again this is mirror imaged (Grammatopoulos et al., 2000; Pasqualetti et al., 2000). That Hox genes allow neural crest cells to respond differentially to endoderm cues is also supported by the observation that, if Hox-positive crest were exposed to an ectopic strip of rostral pharyngeal endoderm, then, unlike the rostral crest which do not express Hox genes and form a jaw in this situation, these cells do not form a jaw (Couly et al., 2002; Creuzet et al., 2002).

It has become clear that other transcription factors, in addition to Hox genes, can similarly act to modulate the way in which crest cells interpret the peripheral patterning cues. In particular, the Dlx family, which display nested expression domains along the proximodistal axis of the arches seems to function in this way (Simeone et al., 1994; Qiu et al., 1997a). Normally, the crest-derived mesenchyme of the maxillary primordia (forming the upper jaw) and the proximal mandibular primordia only express Dlx-1 and -2. The rest of the mandibular mesenchyme expresses these genes and also Dlx-5 and -6. Additionally, the distal-most mesenchyme expresses Dlx-7 and -3. In mice mutant for both Dlx-5 and -6, several mandibular primordia skeletal elements are missing and in their place skeletal elements characteristic of the maxillary primordia form (Depew et al., 2002). In these mutants, most of the mandibular mesenchyme only expresses Dlx-1 and -2, thus these cells behave as if they were maxillary. Thus, the patterning of the pharyngeal arches is a consensual process in which patterning cues emanating from the endoderm, or other pharyngeal tissues, are differentially interpreted by the crest cells of each of the arches, and this process is dependent upon the transcription factors they express.

IMPORTANCE OF THE STREAMING OF THE CRANIAL NEURAL CREST

The demonstration that it is the endoderm that is the prime mover in organising the development of the pharyngeal arches diminishes the idea that the key role of crest streaming is for the patterning the arches, segregating the first stream carrying cues for the morphogenesis of the jaw from the second for the hyoid arch. This idea has become even less attractive with the finding that lamprey embryos also display three obvious crest streams, even though these animals lack a jaw and hyoid (Horigome et al., 1999). We have demonstrated recently that the streaming of cranial neural crest, in fact, is necessary for a completely different role: the organisation of the afferent innervation of the hindbrain.

The majority of the neurons of the cranial sensory ganglia derive from neurogenic placodes; focal thickenings of the embryonic ectoderm that generate neuronal cells (Graham and Begbie, 2000). The neurogenic placodes form in stereotypical positions in all vertebrates. The trigeminal placodes emerge opposite the anterior hindbrain, the otic placodes at the middle hindbrain level, and the epibranchial placodes—geniculate, petrosal, and nodose—close to the tips of the clefts between the pharyngeal arches. The neuronal cells that are formed within these placodes then move internally to the site of ganglion formation wherein they differentiate and send out axons toward their central and peripheral targets. Importantly, these ganglia innervate the hindbrain at specific axial levels (Lumsden and Keynes, 1989). The ax-

ons of the trigeminal ganglion enter the hindbrain at rhombomere 2, those of the geniculate and vestibuloaccoustic, which is derived from the otic placode, at rhombomere 4, and the axons of the petrosal and the nodose ganglia at rhombomeres 6 and 7. Rhombomeres 3 and 5 do not receive any afferent innervation. There is, thus, a clear relationship between the rhombomeres that receive sensory inputs and those that generate the crest streams (Fig. 3).

The neural crest streams that extend from the hindbrain into the periphery are generated in advance of the production of any of the placodal neuronal cells. Importantly, the neuronal cells that delaminate from the placodes migrate internally along the neural crest streams (Begbie and Graham, 2001). For example, the cells of the geniculate placode, which is associated with the second arch, move inward along the hyoid crest stream (Fig. 3). If the hindbrain is ablated before the production of the neural crest, the cells leaving the placode enter a crest-free environment and do not migrate internally. Instead, they form disconnected subectodermal ganglia with aberrant projections that do not reach the hindbrain and often extend toward other ganglia (Begbie and Graham, 2001). It could be argued that this failure in the migration of the placodal cells is due to the absence of the neural tube and not specifically the neural crest. However, if the hindbrain is removed after the production of the crest, the placodal cells do move inward and establish appropriate projections (Begbie and Graham, 2001). Thus, the presence of a neural crest stream is essential for the projection of sensory axons to the correct location in the developing hindbrain.

This integrative role for the neural crest further clarifies the phenotypic alterations seen in several mutant mice in which one or more of the epibranchial ganglia fail to connect to the hindbrain, even though none of the genes in question are expressed in the epibranchial ganglia: Sox-10 (Britsch et al., 2001), COUP-TFI (Qiu et al., 1997b), ErbB4 (Golding et al., 2000), CRKL (Guris et al., 2001), AP-2 (Zhang et al., 1996). It can now

be appreciated that these defects are likely to be a secondary consequence of alteration in the behaviour of the neuroglial neural crest cells. Further insights into this process can be gained from another mouse mutant in which β-catenin is mutated at the sites of wnt-1 expression and which, thus, lacks β-catenin in its migratory crest cells (Brault et al., 2001). In this animal, the neural crest cells migrate normally but are subsequently eliminated by means of apoptosis, and again the epibranchial ganglia fail to connect to the hindbrain. Thus, the epibranchial neuronal cells are dependent upon the crest cells themselves, rather than some common earlier pathway that is used by both the crest and the placodal neuronal cells.

This evidence suggests that the prime function of the cranial crest streaming is to organise the afferent innervation of the hindbrain. The real clue to this comes with the fact that crest streaming occurs in lampreys, which lack a jaw but have basically the same relationship between their cranial sensory ganglia and the hindbrain as all other vertebrates. That is not to say, however, that with the evolution of the gnathostomes, existing crest streaming has been co-opted to help pattern the jaws and jaw-support, and indeed to help organise the constrained pattern of skeletomuscular connectivity in the head. Rather, it is likely that these functions of crest streaming are built upon a more ancestral function of helping to organise the sensory innervation of the hindbrain.

CRANIAL NEURAL CREST HAS SKELETOGENIC POTENTIAL

Besides any morphogenetic role, the major difference between the cranial and the trunk neural crest lies in the fact that the cranial crest generates ectomesenchymal derivatives: bone, cartilage, dentine, muscle, and connective tissue. Indeed, fate maps of the cranial crest constructed in chick have suggested that the majority of the head skeleton is crest derived (Noden, 1988; Couly et al., 1993). These elements include dermal bones of the skull and all of the cartilages and bones

of the oro-pharyngeal region. Furthermore, it has also been shown that the cranial crest generates the connective tissues of the muscles, the dermis of the head, and the smooth muscle cells associated with the feathers and the aortic arches (Le Douarin and Kalcheim, 1999). Similar fate maps of the chick trunk crest have suggested that these cells do not generate bone and cartilage. Although studies in zebrafish and *Xenopus* have shown that trunk neural crest cells do contribute to fin connective tissue (Collazo et al., 1993; Smith et al., 1994), these studies did not find trunk crest forming skeletal derivatives.

The different fates of the cranial and the trunk neural crest cells were also thought to represent differences in potential. Thus, while cranial crest can form all ectomesenchymal derivatives, trunk crest is only capable of forming connective tissues and smooth muscle, but not cartilage or bone. The supporting evidence for this comes from both in vitro and in vivo studies. Clonal analysis of cranial crest cells found that these would generate cartilage in addition to neurons, glia, and melanocytes when cultured in rich medium on a 3T3 feeder layer, which offered support for a range of differentiation options (Baroffio et al., 1991). Yet, trunk crest cultured under the same conditions were only ever observed to produce, neurons, glia, and melanocytes (Baroffio et al., 1988). Correspondingly, it was found that when trunk neural tube was grafted into the head, the neural crest cells produced by this graft never contributed to cranial skeletal elements (Nakamura and Ayer-le Lievre, 1982). Rather, these transposed cells tended to stay close to the neural tube and differentiate as neurons and Schwann cells. It was found that these cells could form connective tissue and muscle, but they were never found to contribute to cranial skeletal elements (Nakamura and Ayer-le Lievre, 1982).

Although these experimental approaches yielded results that were consistent with trunk neural crest cells lacking the potential to generate skeletal derivatives, they did not directly test its ability to do so. There-

Fig. 4. Trunk neural crest has skeletogenic potential. Neural crest fates are restricted along the anteroposterior axis of the embryo. Crest-derived parasympathetic (green) and sensory neurons (blue) are differentially distributed along the whole embryo, while sympathetic neurons (grey) are restricted to the trunk and ectomesenchyme (red) to the cranial region. Other fates not shown, such as cardiac and enteric crest, are similarly restricted. However, all neural crest has the potential to make the full range of possible crest derivatives, indicating that fate decisions are driven by the environment that crest migrates through and differentiates in.
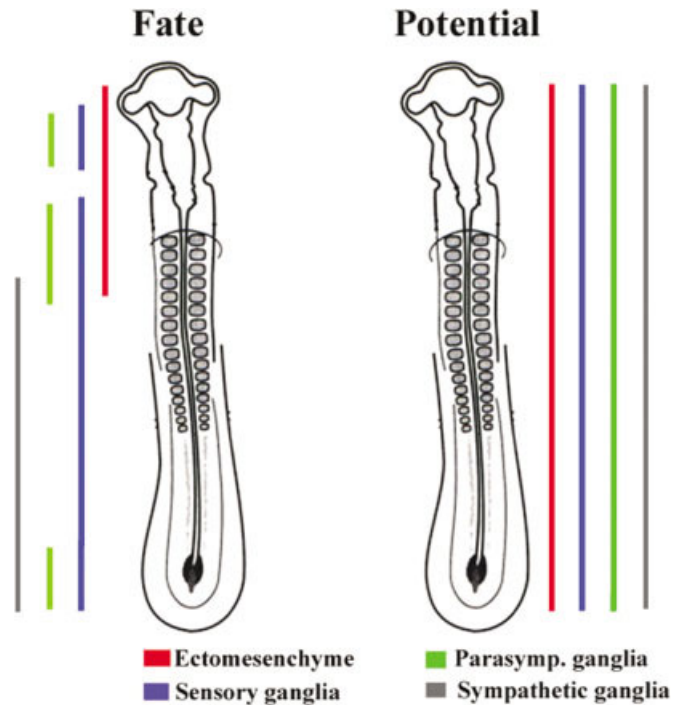

Fig. 5. Evolution of the trunk skeleton. Fossil evidence shows that early vertebrates, such as the Heterostracan, possessed extensive postcranial dermal exoskeleton (red), likely derived from the trunk neural crest. Trunk exoskeleton became much reduced with the appearance of the somite-derived endoskeleton. The zebrafish has a predominantly endoskeletal trunk support but possesses a remnant of exoskeleton in the dermal fin rays, which is also likely derived from the trunk neural crest. Higher vertebrates, such as the chick, have an exclusively endoskeletal trunk support derived solely from the sclerotome of the somite.
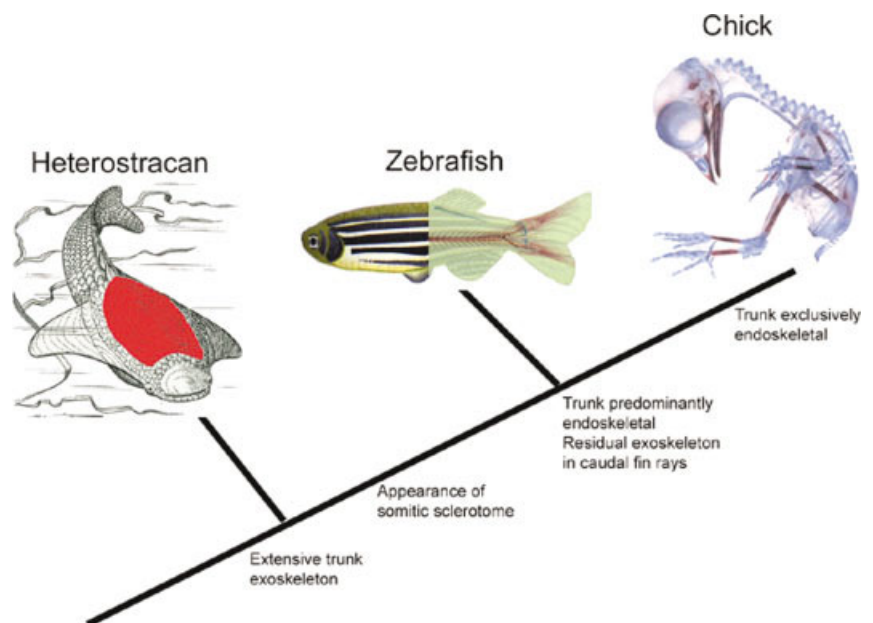
fore, we explicitly assessed the skeletogenic potential of trunk crest by culturing both chick cranial and trunk crest cells in media commonly used for growing bone and cartilage cells (McGonnell and Graham, 2002). As expected, bone and cartilage differentiation occurred in cultures of cranial crest. Importantly, this was also found to be true of trunk crest cultures, which under these conditions generated both chon-

drocytes and osteocytes. The ability of trunk crest to contribute to cranial skeletal elements was also tested in vivo. As described, previous studies had found that grafted trunk crest cells did not contribute to skeletal elements, yet this of course could have resulted from the failure of these cells to migrate from the transposed piece of neural tissue to the sites of skeletal differentiation. To circumvent this potential difficulty, we

grafted trunk neural crest cells directly into the facial primordia (McGonnell and Graham, 2002). These cells dispersed, and as expected formed neurons, Schwann cells, and melanocytes; however, they also contributed to the forming skeletal elements, such as Meckel's cartilage. Another much older study has also shown that mammalian trunk neural crest cells can participate in tooth formation when recombined

with oral ectoderm (Lumsden, 1988). Thus, trunk crest cells do have skeletogenic potential.

In the past, the generation of different derivatives by the neural crest of distinct axial levels was thought not to reflect a lack of potential, but to be a consequence of environmental cues restricting these fates. The only exception to this scenario was thought to apply to skeletogenic potential, which was believed to be intrinsic to the cranial crest (Le Douarin and Kalcheim, 1999). Our results demonstrate that, in fact, there is no difference in potential between the cranial and the trunk neural crest; both can generate the full repertoire of crest derivatives (Fig. 4). Thus, with regard to potential, there is nothing special about the cranial crest.

During normal development the skeletogenic potential of trunk crest must be suppressed. However, the skeletogenic potential of the trunk crest was likely realized in early vertebrates (Fig. 5). There are many fossil fish that display extensive postcranial exoskeletal coverings of dermal bone and dentine, two cells types that derive from neural crest cells (Maisey, 1988; Smith and Hall, 1990; Donoghue and Sansom, 2002). The skeletogenic potential of trunk crest is also expressed, albeit to a limited extent, in some extant vertebrates. A study in zebrafish has suggested that the lepidotrichia, the distal mineralised portions of the fins rays, are neural crest derived (Smith et al., 1994). Thus, it would seem that, during vertebrate evolution, the suppression in the skeletogenic potential of the trunk crest mirrors the reduction in the extent of the trunk exoskeleton. Contrastingly, during this process, the endoskeleton has emerged to be the major axial skeletal support. This has undoubtedly involved an enhancement of the role of the somites, and in particular of the sclerotome. It is likely, therefore, that the environmental cues that suppress trunk neural crest skeletal differentiation, in amniotes, are either expressed in or related to the formation of the sclerotome.

It is also important to appreciate that, from an evolutionary perspective, the separation of the neural crest into cranial versus truncal populations is likely to be a derived feature, with what we term cranial crest representing the ancestral state. Indeed, in many ways it's not that the development of the head is distinct because of the role played by the crest, but rather it's the trunk that differs, because in this region of the embryo, the mesoderm has become dominant.

CONCLUDING REMARKS

Five years ago, many people, ourselves included, would have argued strongly that the cranial neural crest played a pivotal role in the development of the vertebrate head. The cranial crest generates the head skeleton, and these cells were believed to coordinate the developed of this region. Now, however, it is apparent that this view must be reassessed. The ability of the cranial crest to generate skeletal derivatives is not unique to this axial level, but is shared by all crest cells. Rather, as with all other crest potentials, skeletal potential is restricted by environmental cues. Furthermore, it is now clear that the crest does not play the key role in patterning the head and that, for the pharyngeal arches, this patterning is fulfilled by the pharyngeal endoderm. However, it is now evident that the streaming of the cranial neural crest is fundamental to the organisation of the afferent innervation of the hindbrain. Thus, today, our views of the significance of the cranial neural crest have changed.

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