CHAPTER 15

Evolution of the Neural Crest

Alejandro Barrallo-Gimeno and M. Angela Nieto*

Abstract

The recent advances in studies of the neural crest in vertebrates and the analysis of basal chordates using molecular and embryological approaches have demonstrated that at least part of the genetic programs and the cellular behavior were in place in nonvertebrate chordates before the neural crest evolved. Nevertheless, both the missing aspects and the close similarities found could explain why basal chordates lack a bona fide neural crest population, even though some migratory neurons and pigment cells have been recently identified in ascidians and amphioxus.

Introduction: Was There Anything Like This Before?

The most interesting aspect of the neural crest, besides its amazing multipotency, is the pivotal role it has played in the evolution of vertebrates.[1] The neural crest is a vertebrate characteristic and indeed without it, the vertebrate head would look quite different. Together with the ectodermal placodes, the neural crest was crucial to the formation of paired sense organs and the transition towards a more active life style with complex behaviors. It is considered to be so important, that the neural crest has been termed the fourth germ layer. In this sense, the neural crest together with the ectoderm, endoderm and mesoderm would make the vertebrates quadroblastic animals.[2]

As a vertebrate innovation, the neural crest is considered as one of the important steps in the evolution of Chordates and in evolutionary terms, it can define how this Phylum developed. The Chordates are animals with a bilateral symmetry, a notochord (a hollow tube with support functions) and a dorsal tubular central nervous system. The most primitive Chordates, the ascidians (Urochordata = most basal chordates) regress in adulthood to the sessile filtering form having existed as a free-swimming larva (tadpole) that contains an axial notochord and a dorsal neural tube. The amphioxus or lancelets (Cephalochordata = chordates with head) preserve the notochord in the adult as an endoskeletal support, and have a regionalized pharyngeal endoderm. With respect to the basal vertebrates, the hagfish (Myxinoidea) develop a cranium that surrounds their primitive brain, and the lampreys (Agnatha = no jaws) have a vertebral column to support the body and protect the spinal cord. More importantly, they have a truly segmented neural crest, but no jaws. Finally, the jawed vertebrates (Gnatosthomata = mouth with jaws: fishes, reptiles, birds and mammals) depict all of the evolutionary novelties that gave rise to the vertebrate head.

It is important to establish when the neural crest arose in animal evolution and how this cell population acquired the capacity to migrate and form such a wide variety of structures, the two key characters of the neural crest cells. Did a latent neural crest exist in the more primitive chordates? It is certainly difficult to assume that the neural crest just suddenly appeared. Indeed, evidence is now accumulating to suggest that nonvertebrate chordates do develop a precursor

*Corresponding Author: M. Angela Nieto—Instituto de Neurociencias de Alicante CSIC-UMH, Apartado 18, Sant Joan d' Alacant, 03550 Spain. Email: anieto@umh.es

Neural Crest Induction and Differentiation, edited by Jean-Pierre Saint-Jeannet.

neural crest population. Essentially, gene expression patterns and the identification of some cells with a migratory behavior indicate that part of the genetic and cellular programs related to neural crest development were definitely in place. However, as discussed by Stone and Hall,[3] they may still be considered as provocative observations awaiting hierarchical developmental evidence, not only at the cellular level but also at the tissue level. In relation to this, we will discuss here the evidence that has arisen in the last few years that may define different steps in the development and appearance of the neural crest.

Neural Crest and the Neural Tube: The Only Way Is Dorsal

Since the neural crest arises from the dorsalmost part of the neural tube, whether the dorso-ventral patterning of the neural tube is already in place in basal chordates has been examined. As a result, it seems that prototypes of the dorsal and ventral genes are indeed expressed similarly in amphioxus and ascidians. With respect to the ventral neural tube, both signaling molecule sonic hedgehog and transcription factor HNF3 expressions are conserved between vertebrates and amphioxus,[4,5] as is also the case for the ascidian HNF3beta ortholog.[6]

Similarly, the orthologs of genes expressed in the dorsal neural tube in vertebrates are conserved in basal chordates. With respect to the *Pax* family of transcription factors, a single *Pax3/7* gene in amphioxus and ascidians corresponds to the vertebrate *Pax3* and *Pax7* genes. Not only is *Pax3/7* expressed in the dorsal part of the neural tube in both *Ciona* and amphioxus,[7,8] but over-expression of Pax3/7 in ascidia leads to dorsalization.[9] Mutations in *Pax3* and *Pax7* in mice and humans have been related to neural crest defects,[10,11] highlighting the relevance of the appropriate dorsoventral patterning of the neural tube for neural crest development.

Several transcription factors of the Msx family are expressed in the dorsal neural tube and the neural crest, where they are crucial for craniofacial development.[12] Likewise, the single *AmphiMsx* gene is expressed in the lateral neural plate and later restricted to the dorsal part of the neural tube.[13] *Msx-a*, one of the two *Msx* genes found in ascidians, is expressed in the ectoderm and mesoderm at sites that are undergoing morphogenetic movements, such as the neural plate as it folds to form the neural tube.[14] The second, *Msx-b*, is expressed in the neural tube.[15]

Several members of the Zic family of transcription factors are involved in vertebrate neural development, and Zic2 seems to retain the cells at the border of the neural plate in an undifferentiated state, preventing them from differentiating into dorsal neurons. In this way, the development of alternative dorsal fates, such as neural crest, is favored.[16] Furthermore, other family members have been more directly implicated in neural crest development.[17,18] It is therefore noteworthy that, a Zic ortholog in amphioxus is also expressed at the neural plate border during early neurulation stages.[19]

In summary, the transcription factors involved in the dorsalization of the neural plate/neural tube in vertebrates have representatives in the nonvertebrate chordates, indicating that the dorsoventral patterning is conserved and established before the divergence of the vertebrate lineage.

Regarding the signaling pathways that induce dorsalization and neural crest formation in vertebrates, it is worth mentioning that triggered by the bone morphogenetic proteins (BMPs). A gradient of BMP activity has been described in *Xenopus* and zebrafish that influences cell fate.[20,21] In areas with intense BMP activity epidermis forms, whereas low levels of activity are permissive for neural development and in the intermediate regions, the neural crest forms. In amniotes, BMPs are clearly expressed in the nonneural ectoderm from where they can influence the development of the neural crest. Amphioxus has a single BMP2/4 ortholog that is also expressed in the nonneural ectoderm[22] and it could therefore play a role in determining the dorsal part of the neural tube.

Interestingly, one of the functions of BMP4 later in development highlights the evolutionary importance of the neural crest. Transplanting neural crest cells from duck into quail embryos and viceversa, resulted in the formation of a characteristic duck beak in a quail embryo and viceversa.[23] Hence, the morphology of the beak depends on the neural crest, this being one of the best examples of evolutionary adaptation to functional diversity in different birds. Indeed, differences

in the levels of BMP4 seem to be responsible for different beak morphologies, not only between chick and duck,[24] but also between closely related species such as the Galapagos finches.[25]

Cell Migration: A Whole Body to Populate

One of the key characteristics of the neural crest cells is its ability to migrate and populate all parts of the embryo. Once specified, they undergo an epithelium to mesenchyme transition (EMT) and migrate as individual cells. To study the origin of the neural crest in evolution, the basal chordate embryos have been scrutinized to search for migratory cells that might resemble a primitive version of the vertebrate crest.

In the amphioxus embryo, nonneural ectoderm cells at the neural plate border migrate towards the midline to cover the neural plate.[26] Unlike the true neural crest, these cells migrate as a continuous sheet rather than as individual cells because they do not undergo EMT. Interestingly, the single *Dlx* representative in amphioxus, *AmphiDll*, is expressed in those migrating ectodermal cells[26] while in vertebrates *Dlx* genes are expressed in the neural crest after the onset of migration. As discussed by Holland et al,[26] it is possible that the ancestor of craniates neurulated as amphioxus does, and that the migratory epidermal cells became integrated into the dorsal part of the neural tube in the transition to vertebrate neurulation. Thus, these cells may be the precursors of the neural crest. The acquisition of the ability to migrate as individual cells would have subsequently been acquired to permit delamination from the neural tube. If this were true, at least part of the neural crest would have a nonneural origin. In relation to this, James Weston has recently proposed a provocative theory that suggests that the nonneural ectoderm adjacent to the neural plate could produce cells that after undergoing EMT, would migrate to give rise to the head ectomesenchyme (cartilage and bone) which is generally believed to derive from the neural crest.[27] The proposal is that the crest would have a neural and a nonneural component. Interestingly, the Dlx-positive neural crest cells give rise to ectomesenchymal derivatives in the vertebrate head.[28] Thus, if the "nonneural" crest exists, the *Dll*-positive cells of amphioxus could be the precursors.

In addition to the movements of the nonneural ectodermal cells to cover the neural plate in amphioxus, it has recently been reported that some cells from the anterior part of the neural tube in the ascidian embryo have migratory capacities characteristic of neural crest cells.[29] These migratory cells were identified in *Ecteinascidia turbinata*, an ascidia species whose embryos are especially large, therefore facilitating cell labeling and tracing. Labeled cells from the anterior neural tube could be seen to migrate individually between the dorsal mesoderm and the epidermis. Most of these cells differentiated into pigmented cells and expressed neural crest specific markers such as HNK1 and the transcription factor Zic. This is possibly the best evidence that these cells are neural-crest-like in the sense discussed by Stone and Hall.[3] The cells are located at the appropriate place, express genes characteristic of vertebrate neural crest, and they delaminate and migrate individually generating one of the cell types known to be derived from the neural crest, pigment cells. From this finding, one may speculate that in a further step, other cells that arise in the neural tube could also migrate and differentiate into different cells types, such as peripheral neurons and glia.

Another example of individual ectodermal cells migrating through the mesenchyme comes from amphioxus.[30] Cells that migrate dorsolaterally from the ventral side of the embryo fit with the dorsolateral shift in the expression of the single amphioxus ortholog of the vertebrate *Trk* genes, the neurotrophin tyrosine kinase receptors. Trks are related to the sensory functions of structures derived from the neural crest and placodes in vertebrates.[31]

Members of the Snail-family of transcription factors are among the earliest genes expressed in the prospective neural crest at the neural plate border where they are essential for triggering the EMT.[32] In amphioxus and ascidia, *snail* genes are also expressed along the edges of the neural plate,[6,33] although, *snail*-expressing cells have not been seen to delaminate and migrate. It would be very interesting to determine whether the migratory cells recently described in ascidia express the *snail* ortholog.[29] Even considering the exciting discovery of these cells, the

production of migratory cells in nonvertebrate chordates is minimal when compared to that in vertebrates. Since *Snail* genes are crucial for triggering the EMT, they could represent the link between neural crest determination and migration. This raises the intriguing question of why so many *snail*-expressing cells do not delaminate and migrate in basal chordates. It could be that Snail needs partners to be able to fully induce the migratory phenotype and that these can only be recruited in vertebrates. The recruitment of downstream targets could also have occurred only in the vertebrate lineage. Alternatively, it is possible that the environment is not permissive even if *snail*-expressing cells in amphioxus could migrate. In addition, there is still no evidence that snail is active in the neural tube of ascidian and amphioxus embryos. The possibility that the Snail protein is not translated or is maintained inactive by post-translational mechanisms still cannot be excluded. To try to understand why the snail-expressing cells (or the majority of them) are retained in the neural tube, it would be extremely interesting to analyze whether ectopic expression of ascidian, amphioxus or vertebrate *Snail* genes could induce migratory behavior in amphioxus or ascidian embryos.

Consolidation of the Neural Crest Population: The More, the Merrier

As we have just mentioned, it seems possible that cells can migrate away from the neural tube in basal chordates. However, as far as we know, migration appears to be quite limited in terms of the number of cells. Thus, in order to develop a proper neural crest population, a further step had to be acquired during evolution: the capacity to produce migratory cells in significant numbers. In this sense, it has been shown that Sox2 expression in the neural plate is incompatible with neural crest formation in vertebrates, and that it is downregulated at the neural plate borders.[34] The only representative of the B sub-family of *Sox* genes in amphioxus is *sox 1/2/3* and it is not excluded from the neural plate borders.[35] As discussed by Meulemans and Bronner-Fraser,[37] the repression of *sox2* at this location may have been a necessary evolutionary step to permit the neural crest to form.

In addition to establishing permissive conditions, a mechanism to consolidate the potential population of neural crest cells is also necessary. Such a process could be fulfilled by *Id3*, a member of the helix-loop-helix inhibitors. Knock-down experiments in *Xenopus* revealed that Id3 is essential for the survival and cell cycle progression of neural crest progenitors at the neural plate border.[37] Moreover, forced expression of Id3 in migratory neural crest apparently maintains them in a progenitor state.[38] Although it remains unclear whether Id3 plays a role in fate determination in the prospective neural crest territory, the proposed functions of Id3 would contribute to the segregation of the neural crest from other dorsal derivatives and its consolidation as an undifferentiated precursor population. In relation to this, it is interesting to note that the single amphioxus *Id* gene is not expressed in the neural plate.[39] This is compatible with the idea that its expression in the dorsal neural tube could have helped to consolidate and expand the scarce population of migratory cells that constitute the evolutionary predecessor of the neural crest.

To Hox or not to Hox

The *Hox* genes provide some of the main influences that pattern the anteroposterior axis of the animal body from flies to humans. Their particular genomic organization in clusters has fascinated biologists since its discovery. Within each *Hox* cluster, the rostral limit of expression for each gene is directly related to its position in the cluster i.e.: those located towards the 3′ end of the cluster are expressed more anteriorly.[40,41] This property is known as colinearity and has important implications for the patterning of the hindbrain and the neural crest that streams out of the rhombomeres.[42,43] This colinear expression of *Hox* genes is also observed in the neural tube of amphioxus,[44] and interestingly, a regulatory region of the most "anterior" amphioxus *Hox* gene is able to drive expression in the migratory neural crest of transgenic mice and chicken embryos.[45] This finding implies that amphioxus has the regulatory machinery to express genes in the neural crest were it to develop and thus, that they just lack the final crest-inducing factor that appeared in the next step of evolution which shaped vertebrates.

Another peculiarity of *Hox* genes is that none of them are expressed in the most anterior stream of the hindbrain neural crest, which will contribute to the first branchial arch. In relation to this, it is interesting to note that Hoxa2 overexpression in neural crest cells that migrate to the first arch causes a transformation of the skeletal elements into a mirror image of those in the second arch.[46,47] Furthermore, in *Hoxa2* mutants, the skeletal elements of the second arch mimic those of the first arch.[48,49] These data indicate that the development of the structures derived from the first arch is not compatible with the presence of Hoxa2 and by extension, possibly with the expression of *Hox* genes. To investigate this suggestion further, the expression of *Hox* genes has been analyzed in lampreys, vertebrates without jaws (agnathans). In these vertebrates, a group *Hox6* gene is expressed in the first branchial arch,[50] which is compatible with the idea that its presence prevented the formation of the jaw. However, more recently the first pharyngeal arch of a different species of lamprey appeared not to express any *Hox* gene somewhat complicating this matter.[51] Hence, while the absence of Hox genes is compatible with the lack of jaws, it cannot be assumed that the presence of a *Hox* gene in the first arch is responsible for the lack of a mandible in lampreys. Nevertheless, we can still conclude that jaws are generally present when no *Hox* genes are expressed and thus, the absence of Hox may be a prerequisite for jaw formation, perhaps generating a permissive state.

Evolution's Toolbox I. Playing with Genes and Genomes: Two Is Better Than One

We cannot leave the topic of the Hox genes without talking about how evolution has affected the genome, these genes providing us with one of the clearest examples. One of the events that evolution has employed to produce diversity is the existence of massive gene or whole genome duplications.[52,53] In particular, it has been proposed that the evolutionary leap represented by the appearance of the craniates was aided by two whole genome duplications.[54] In support of this idea, many gene families composed of several members in vertebrates are represented in basal chordates by a single gene.[55] The *Hox* genes have undergone tandem gene duplications and have also been subjected to whole genome duplications. Among the most basal chordates, the tunicate *Hox* set is only partially clustered[56,57] and amphioxus has only one single *Hox* complex.[58] Vertebrates have four *Hox* clusters[59] and some ray-finned fish, like zebrafish, contain seven *hox* clusters.[60] This latter finding is in agreement with the extra-duplication proposed to have occurred in the teleost lineage.[61]

One of the current models that supports the genome duplication theory as a way to increase complexity and enhance evolution is the duplication-degeneration-complementation model (DDC).[62] This model explains the high rate of gene duplication and preservation. Right after the duplication event, the two duplicates are identical, yet since the native function can be fulfilled by only one of the copies, the other duplicate is left to mutate freely. Indeed, a rapid divergence of the duplicates may lead to the acquisition of a new function that conveys an adaptive advantage (neofunctionalization), while the other is forced to preserve the ancestral function. For pleiotropic genes, the partitioning of ancestral functions between copies (subfunctionalization) can also lead to positive selection and preservation of the duplicates.

The extra-duplication events believed to have occurred in the teleost lineage[61] enable the DDC model to be verified by comparing the expression patterns of the genes duplicated in zebrafish with that of the single-copy genes in other vertebrates. With respect to the neural crest, the duplications of the *snail1* and *sox9* genes represent good examples. The territories of expression of zebrafish *snail1a* and *snail1b* together (previously known as *snail1* and *snail2*, respectively)[63,64] are equivalent to those in which *Snail1* (previously *Snail*) expressed in the mouse. Of the two, only *snail1b* is expressed in the premigratory neural crest, a site of prominent *Snail1* expression in mammals. Thus, *snail1* has suffered subfunctionalization, which is compatible with our unpublished data showing that the knock-down of *snail1b*, but not of *snail1a*, leads to defects in neural crest development. Likewise, *sox9b* expression in the premigratory neural crest

is higher than that of *sox9a*.[65] *Sox9* is expressed very early during neural crest development in vertebrates and seems to be upstream of Snail genes in the network that regulates this process.[66] Within the framework of subfunctionalization, loss of *sox9b* but not *sox9a* function leads to defects in the neural crest that imply *snail1b* downregulation.[65]

Evolution's Toolbox II. Cooption: Take Genes from Your Neighbor Cells

In the context of the DDC model, neofunctionalization is exemplified by what has been called gene cooption, i.e.: when genes used in a particular tissue at a particular developmental stage, or for a particular process in the adult, are recruited to perform a new function. Cooption is a very valuable evolutionary tool and since the neural crest embodies an evolutionary novelty with significant implications for the formation of the vertebrate head, it could not escape from coopting genes already important in other tissues. The neural crest arises at the border between the neural plate and nonneural ectoderm, where complex networks of transcription factors interact throughout the processes from specification to differentiation. Furthermore, tissue interactions are also fundamental for neural crest formation since its development relies on signals from the ectoderm and the underlying mesoderm. Good examples of cooption can be seen by analyzing in nonvertebrate chordates several of the transcription factors involved in crest development in gnathostomes.[36]

The expression of the *Id* genes in mesoderm and endoderm is conserved between amphioxus and vertebrates. However, the expression in the dorsal neural tube is only observed in vertebrates,[39] compatible with *Id* being coopted to participate in the development of the nervous system. Indeed, as mentioned above, *Id3* seems to play an important role in consolidating the population of neural crest precursors in the vertebrate dorsal neural tube.[37,38] Hence, the lack of *Id* expression in the dorsal neural tube of nonvertebrate chordates may contribute to the absence of a consolidated neural crest population.

FoxD3 is one of the members of the wide fox (forkhead homeobox) gene family[67,68] that fulfills important functions in vertebrate neural crest development.[66,69-72] Both ascidians and amphioxus have a single *FoxD* gene whose expression is conserved in the mesoderm but not at the neural plate border.[73,74] Again, this is a case of cooption from the mesoderm that may have interesting implications in the process of neural crest development. Indeed, Cheung et al,[66] proposed that in the chick, FoxD3 participates in the alterations of cell-cell adhesion required for the neural crest to migrate.

Not only might cooption have been used to acquire the genes necessary during early steps of neural crest development, but it may also have been taken advantage of to obtain properties that may be beneficial at later stages. For instance, the AP-2alfa (tfpa2a) transcription factor is essential for the differentiation and survival of migratory neural crest cells, although it is expressed at the neural plate border from early stages.[75,76] The Amphioxus *AP2*, like the vertebrate *AP2* genes, is expressed in the nonneural ectoderm, but it is absent from the neural plate border.[77] Interestingly, AP2 family members are necessary for *Hoxa2* expression in the neural crest of mice,[78] and *AmphiAP2* and *AmphiHox2* expression overlaps in the preoral pit. In accordance with this, AP2 consensus binding sites have been found in the noncoding sequences of *AmphiHox2*.[77] Thus, the relationship between AP2 and Hox2 is conserved in amphioxus and the cooption of AP2 to play a role in the neural crest provided a new territory in which this gene network could interact.

Conclusions: So, What Now?

After reviewing some of the developmental and molecular aspects that might help to reveal the existence of neural crest precursors in nonvertebrate chordates, we can identify some of the factors involved that are either missing or misplaced in urochrodates and cephalochordates

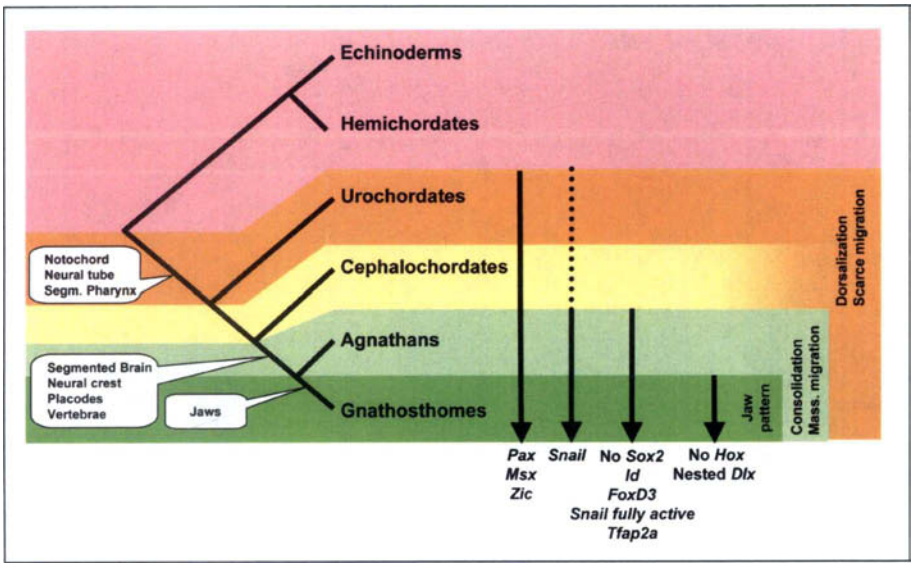

Figure 1. Scheme depicting the proposed staging towards the vertebrate state. Major features acquired in every evolutionary transition are indicated on the boxes on the left, while processes involved in neural crest formation are on the right and genes related to each process are cited below. In basal chordates, the dorsalization of the neural tube needed for the specification of the neural crest is already established, and the migration of some cells is observed; one *snail* gene is expressed at the appropriate location, but not linked to massive migratory behaviour (dotted line). The migratory neural crest population is consolidated in Agnathans. Gnathostomes add to this scenary the necessary machinery to pattern the jaw: absence of *Hox* expression in the first arch and nested expression of *Dlx* genes.

with respect to vertebrates, summarized in Figure 1. As such, the presence of Sox2 in the dorsal neural tube of basal chordates may have prevented the proper generation of the neural crest population and the expression of *Id* genes may be required to generate a solid neural crest population. The possibility of Snail being inactive or not fully active in the dorsal neural plate may be a further reason for cells not being able to migrate from the neural tube, and the absence of FoxD3 could prevent some of the necessary changes in cell-cell adhesion that favor migration. In the transition to the jawed vertebrates, the absence of *Hox* gene expression in the mandibular arch as a permissive state and the nested expression of *Dlx* genes allowed the formation and patterning of the jaw.[79]

Transgenic approaches in the mouse and ectopic expression studies in the chick have shown the ability of amphioxus sequences to drive expression in the vertebrate neural crest.[45] The possibility of microinjecting expression constructs in amphioxus[80] makes it extremely interesting to check whether the expression of the missing or misplaced genes could induce the formation of neural crest cells than might migrate from the neural tube and eventually differentiate.

Acknowledgements

We are grateful to all members from M.A. Nieto's lab for encouraging discussions. Work in the lab is mainly supported by grants of the Spanish Ministry of Education and Science (DGICYT-BMC2002-0383 and MEC-BFU2004-02665) and the Local Government of Valencia (GV04B-292) to M.A.N.A. A. Barrallo-Gimeno is the recipient of a Contract under the Ramon y Cajal Programme (MEC).

References

1. Gans C, Northcutt RG. Neural crest and the origin of vertebrates - A new head. Science 1983; 220:268-274.
2. Hall BK. The neural crest as a fourth germ layer and vertebrates as quadroblastic not triploblastic. Evol Dev 2000; 2:3-5.
3. Stone JR, Hall BK. Latent homologues for the neural crest as an evolutionary novelty. Evol Dev 2004; 6:123-129.
4. Shimeld SM. Characterisation of amphioxus HNF-3 genes: Conserved Expression in the Notochord and Floor Plate. Dev Biol 1997; 183:74-85.
5. Shimeld SM. The evolution of the hedgehog gene family in chordates: Insights from amphioxus hedgehog. Devel Genes Evol 1999; 209:40-47.
6. Corbo J, Erives A, Di Gregorio A et al. Dorsoventral patterning of the vertebrate neural tube is conserved in a protochordate. Development 1997; 124:2335-2344.
7. Holland LZ, Schubert M, Kozmik Z et al. AmphiPax3/7, an amphioxus paired box gene: Insights into chordate myogenesis, neurogenesis, and the possible evolutionary precursor of definitive vertebrate neural crest. Evol Dev 1999; 1:153-165.
8. Wada H, Holland PW, Satoh N. Origin of patterning in neural tubes. Nature 1996; 384:123.
9. Wada H, Holland PWH, Sato S et al. Neural tube is partially dorsalized by overexpression ofHrPax-37: The ascidian homologue of Pax-3 and Pax-7. Dev Biol 1997; 187:240-252.
10. Conway SJ, Henderson DJ, Copp AJ. Pax3 is required for cardiac neural crest migration in the mouse: Evidence from the splotch (Sp2H) mutant. Development 1997; 124:505-514.
11. Mansouri A, Stoykova A, Torres M et al. Dysgenesis of cephalic neural crest derivatives in Pax7-/- mutant mice. Development 1996; 122:831-838.
12. Davidson D. The function and evolution of Msx genes: Pointers and paradoxes. Trends Genet 1995; 11:405-411.
13. Sharman AC, Shimeld SM, Holland PWH. An amphioxus Msx gene expressed predominantly in the dorsal neural tube. Devel Genes Evol 1999; 209:260-263.
14. Ma L, Swalla BJ, Zhou J et al. Expression of an Msx homeobox gene in ascidians: Insights into the archetypal chordate expression pattern. Dev Dyn 1996; 205:308-218.
15. Aniello F, Locascio A, Villani MG et al. Identification and developmental expression of Ci-msxb: A novel homologue of Drosophila msh gene in Ciona intestinalis. Mech Dev 1999; 88:123-126.
16. Brewster R, Lee J, Altaba AR. Gli/Zic factors pattern the neural plate by defining domains of cell differentiation. Nature 1998; 393:579-583.
17. Aruga J. The role of Zic genes in neural development. Mol Cell Neurosci 2004; 26:205-221.
18. Sato T, Sasai N, Sasai Y. Neural crest determination by coactivation of Pax3 and Zic1 genes in Xenopus ectoderm. Development 2005; 132:2355-2363.
19. Gostling NJ, Shimeld SM. Protochordate Zic genes define primitive somite compartments and highlight molecular changes underlying neural crest evolution. Evol Dev 2003; 5:136-144.
20. Marchant L, Linker C, Ruiz P et al. The inductive properties of mesoderm suggest that the neural crest cells are specified by a BMP gradient. Dev Biol 1998; 198:319-329.
21. Nguyen VH, Schmid B, Trout J et al. Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are stablished by a bmp2b/swirl pathway of genes. Dev Biol 1998; 1999:93-110.
22. Panopoulou GD, Clark MD, Holland LZ et al. AmphiBMP2/4, an amphioxus bone morphogenetic protein closely related to Drosophila decapentaplegic and vertebrate BMP2 and BMP4: Insights into evolution of dorsoventral axis specification. Dev Dyn 1998; 213:130-139.
23. Schneider RA, Helms JA. The cellular and molecular origins of beak morphology. Science 2003; 299:565-568.
24. Wu P, Jiang T-X, Suksaweang S et al. Molecular shaping of the beak. Science 2004; 305:1465-1466.
25. Abzhanov A, Protas M, Grant BR et al. Bmp4 and morphological variation of beaks in Darwin's finches. Science 2004; 305:1462-1465.
26. Holland N, Panganiban G, Henyey E et al. Sequence and developmental expression of AmphiDll, an amphioxus Distal-less gene transcribed in the ectoderm, epidermis and nervous system: Insights into evolution of craniate forebrain and neural crest. Development 1996; 122:2911-2920.
27. Weston JA, Yoshida H, Robinson V et al. Neural crest and the origin of ectomesenchyme: Neural fold heterogeneity suggests an alternative hypothesis. Dev Dyn 2004; 229:118-130.
28. Dolle P, Price M, Duboule D. Expression of the murine Dlx-1 homeobox gene during facial, ocular and limb development. Differentiation 1992; 49:93-99.
29. Jeffery WR, Strickler AG, Yamamoto Y. Migratory neural crest-like cells form body pigmentation in a urochordate embryo. Nature 2004; 431:696-699.

30. Benito-Gutierrez E, Nake C, Llovera M et al. The single AmphiTrk receptor highlights increased complexity of neurotrophin signalling in vertebrates and suggests an early role in developing sensory neuroepidermal cells. Development 2005; 132:2191-2202.
31. Martin-Zanca D, Barbacid M, Parada LF. Expression of the trk proto-oncogene is restricted to the sensory cranial and spinal ganglia of neural crest origin in mouse development. Genes Dev 1990; 4:683-694.
32. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol 2002; 3:155-166.
33. Langeland JA, Tomsa Jr JM, WRJ et al. An amphioxus snail gene: Expression in paraxial mesoderm and neural plate suggests a conserved role in patterning the chordate embryo. Devel Genes Evol 1998; 208:569-577.
34. Wakamatsu Y, Endo Y, Osumi N et al. Multiple roles of Sox2, an HMG-box transcription factor in avian neural crest development. Dev Dyn 2004; 229:74-67.
35. Holland LZ, Schubert M, Holland ND et al. Evolutionary conservation of the presumptive neural plate markers amphisox1/2/3 and amphineurogenin in the invertebrate chordate amphioxus. Dev Biol 2000; 226:18-33.
36. Meulemans D, Bronner-Fraser M. Gene-regulatory interactions in neural crest evolution and development. Dev Cell 2004; 7:291-299.
37. Kee Y, Bronner-Fraser M. To proliferate or to die: Role of Id3 in cell cycle progression and survival of neural crest progenitors. Genes Dev 2005; 19:744-755.
38. Light W, Vernon AE, Lasorella A et al. Xenopus Id3 is required downstream of Myc for the formation of multipotent neural crest progenitor cells. Development 2005; 132:1831-1841.
39. Meulemans D, McCauley D, Bronner-Fraser M. Id expression in amphioxus and lamprey highlights the role of gene cooption during neural crest evolution. Dev Biol 2003; 264:430-442.
40. Graham A, Papalopulu N, Krumlauf R. The murine and Drosophila homeobox gene complexes have common features of organization and expression. Cell 1989; 57:367-378.
41. Duboule D, Dolle P. The structural and functional organization of the murine HOX gene family resembles that of Drosophila homeotic genes. EMBO J 1989; 8:1497-1505.
42. Hunt P, Wilkinson D, Krumlauf R. Patterning the vertebrate head: Murine Hox 2 genes mark distinct subpopulations of premigratory and migrating cranial neural crest. Development 1991; 112:43-50.
43. Hunt P, Gulisano M, Cook M et al. A distinct Hox code for the branchial region of the vertebrate head. Nature 1991; 353:861-864.
44. Wada H, Garcia-Fernandez J, Holland PWH. Colinear and segmental expression of amphioxus Hox genes. Dev Biol 1999; 213:131-141.
45. Manzanares M, Wada H, Itasaki N et al. Conservation and elaboration of Hox gene regulation during evolution of the vertebrate head. Nature 2000; 408:854-857.
46. Pasqualetti M, Ori M, Nardi I et al. Ectopic Hox2a induction after neural crest migration results in homeosis of jaw elements in Xenopus. Development 2000; 127:5367-5378.
47. Grammatopoulos GA, Bell E, Toole L et al. Homeotic transformation of branchial arch identity after Hox2a overexpression. Development 2000; 127:5355-5365.
48. Gendron-Maguire M, Mallo M, Zhang M et al. Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. Cell 1993; 75:1317-1331.
49. Rijli FM, Mark M, Lakkaraju S et al. A homeotic transformation is generated in the rostral branchial region of the head by disruption of Hoxa-2, which acts as a selector gene. Cell 1993; 75:1333-1349.
50. Cohn MJ. Lamprey Hox genes and the origin of jaws. Nature 2002; 416:386-387.
51. Takio Y, Pasqualetti M, Kuraku S et al. Lamprey Hox genes and the evolution of jaws. Nature 2004; 429.
52. Ohno S. Evolution by gene duplication. New York: Spinger-Verlag, 1970.
53. Ohno S. Gene duplication and the uniqueness of vertebrate genomes circa 1970-1999. Semin Cell Dev Biol 1999; 10:517-522.
54. Holland PWH, García-Fernández J, Williams NA et al. Gene duplications and the origins of vertebrate development. Development 1994; (Suppl):125-133.
55. Leveugle M, Prat K, Popovici C et al. Phylogenetic analysis of ciona intestinalis gene superfamilies supports the hypothesis of successive gene expansions. J Mol Evol 2004; 58:168-181.
56. Seo H-C, Edvardsen RB, Maeland AD et al. Hox cluster disintegration with persistent anteroposterior order of expression in Oikopleura dioica. Nature 2004; 431:67-71.
57. Ikuta T, Yoshida N, Satoh N et al. Ciona intestinalis Hox gene cluster: Its dispersed structure and residual colinear expression in development. Proc Natl Acad Sci USA 2004; 101:15118-15123.

58. Garcia-Fernandez J, Holland PW. Archetypal organization of the amphioxus Hox gene cluster. Nature 1994; 370:563-566.
59. Garcia-Fernandez J. Hox, ParaHox, ProtoHox: Facts and guesses. Heredity 2005; 94:145-152.
60. Amores A, Force A, Yan YL et al. Zebrafish hox clusters and vertebrate genome evolution. Science 1998; 282:1711-1714.
61. Postlethwait JH, Yan LY, Gates MA et al. Vertebrate genome evolution and the zebrafish gene map. Nat Genet 1998; 18:345-349.
62. Force A, Lynch M, Pickett FB et al. Preservation of duplicate genes by complementary, degenerative mutations. Genetics 1999; 151:1531-1545.
63. Thisse C, Thisse B, Schilling TF et al. Structure of the zebrafish snail1 gene and its expression in wild-type, spadetail and no tail embryos. Development 1993; 119:1203-1215.
64. Thisse C, Thisse B, Postlethwait JH. Expression of snail2, a second member of the zebrafish snail family, in cephalic mesoderm and presumptive neural crest of wild-type and spadetail mutant embryos. Dev Biol 1995; 172:86-99.
65. Yan Y-L, Willoughby J, Liu D et al. A pair of Sox: Distinct and overlapping functions of zebrafish sox9 coorthologs in craniofacial and pectoral fin development. Development 2005; 132:1069-1083.
66. Cheung M, Chaboissier M-C, Mynett A et al. The transcriptional control of trunk neural crest induction, survival, and delamination. Dev Cell 2005; 8:179-192.
67. Kaestner KH, Knochel W, Martinez DE. Unified nomenclature for the winged helix/forkhead transcription factors. Genes Dev 2000; 14:142-146.
68. Lehmann OJ, Sowden JC, Carlsson P et al. Fox's in development and disease. Trends Genet 2003; 19:339-344.
69. Kos R, Reedy MV, Johnson RL et al. The winged-helix transcription factor FoxD3 is important for establishing the neural crest lineage and repressing melanogenesis in avian embryos. Development 2001; 128:1467-1479.
70. Sasai N, Mizuseki K, Sasai Y. Requirement of FoxD3-class signaling for neural crest determination in Xenopus. Development 2001; 128:2525-2536.
71. Dottori M, Gross MK, Labosky P et al. The winged-helix transcription factor Foxd3 suppresses interneuron differentiation and promotes neural crest cell fate. Development 2001; 128:4127-4138.
72. Dutton KA, Pauliny A, Lopes SS et al. Zebrafish colourless encodes sox10 and specifies nonectomesenchymal neural crest fates. Development 2001; 128:4113-4125.
73. Imai KS, Satoh N, Satou Y. An essential role of a FoxD gene in notochord induction in Ciona embryos. Development 2002; 129:3441-3453.
74. Yu JK, Holland ND, Holland LZ. An amphioxus winged helix/forkhead gene, AmphiFoxD: Insights into vertebrate neural crest evolution. Dev Dyn 2002; 225:289-297.
75. Knight RD, Nair S, Nelson SS et al. lockjaw encodes a zebrafish tfap2a required for early neural crest development. Development 2003; 130:5755-5768.
76. Barrallo-Gimeno A, Holzschuh J, Driever W et al. Neural crest survival and differentiation in zebrafish depends on mont blanc/tfap2a gene function. Development 2004; 131:1463-1477.
77. Meulemans D, Bronner-Fraser M. Amphioxus and lamprey AP-2 genes: Implications for neural crest evolution and migration patterns. Development 2002; 129:4953-4962.
78. Maconochie M, Krishnamurthy R, Nonchev S et al. Regulation of Hoxa2 in cranial neural crest cells involves members of the AP-2 family. Development 1999; 126:1483-1494.
79. Manzanares M, Nieto M. A celebration of the new head and a evaluation of the new mouth. Neuron 2003; 37:895-898.
80. Yu J-K, Holland ND, Holland LZ. Tissue-specific expression of FoxD reporter constructs in amphioxus embryos. Dev Biol 2004; 274:452-461.