Hox genes, neural crest cells and branchial arch patterning

Paul A Trainor* and Robb Krumlauf†

Proper craniofacial development requires the orchestrated integration of multiple specialized tissue interactions. Recent analyses suggest that craniofacial development is not dependent upon neural crest pre-programming as previously thought but is regulated by a more complex integration of cell and tissue interactions. In the absence of neural crest cells it is still possible to obtain normal arch patterning indicating that neural crest is not responsible for patterning all of arch development. The mesoderm, endoderm and surface ectoderm tissues play a role in the patterning of the branchial arches, and there is now strong evidence that Hoxa2 acts as a selector gene for the pathways that govern second arch structures.

Addresses
Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA
*e-mail: pat@stowers-institute.org
†e-mail: rek@stowers-institute.org

Current Opinion in Cell Biology 2001, **13**:698–705

0955-0674/01/$ – see front matter

Abbreviations

A–P	antero–posterior
ba	branchial arches
ncc	neural crest cell
OV	otic vesicle
r	rhombomere

Introduction

The classic models for craniofacial patterning argue that the morphogenetic fate and the *Hox* gene identity of the neural crest is pre-programmed carrying positional information acquired in the hindbrain to the peripheral nervous system and branchial arches. This is a very topical issue due to the high degree of interest in the development of the hindbrain and cranial neural crest and the roles they play in craniofacial patterning. Although the vertebrate head is composed principally of neural crest cells, it also relies on contributions from paraxial mesoderm, ectoderm and endoderm. In this review we discuss the recent analyses suggesting that craniofacial development is not dependent upon neural crest pre-programming but is regulated by a more complex integration of cell and tissue interactions.

Hindbrain segmentation and its influence on craniofacial development

The vertebrate head is a complex assemblage of the central and peripheral nervous systems, axial skeleton, musculature and connective tissues. Hence, proper craniofacial development requires the orchestrated integration of multiple specialized tissue interactions. How then do the facial structures form in the correct location with the appropriate shape and size? The patterning information could be intrinsic to each tissue precursor or alternatively, the program for patterning could depend upon interactions between the mesenchymal and epithelial tissues surrounding each cell type.

One key source of patterning information in the developing head is the vertebrate hindbrain, which exerts a profound influence on craniofacial morphogenesis in part through its ability to generate cranial neural crest. During early embryo development the hindbrain is transiently subdivided into seven contiguous segments called rhombomeres (r) [1]. Each rhombomere has a unique identity based on segment-restricted domains of *Hox* gene expression that are ordered and partially overlapping and gives rise to a well-defined region of the adult brain [2–4]. This segmental organization is critical for establishing the proper spatial organization of the cranial ganglia, branchiomotor nerves and pathways of cranial neural crest migration (Figure 1). The first subsets of neurons form in the even numbered rhombomeres [5]. The motor nerves that innervate the first three branchial arches (trigeminal, facio-acoustic, glossopharyngeal) arise in a two-segment periodicity [6,7]. Hindbrain-derived neural crest cells migrate in three segmental streams adjacent to r2, r4 and r6, which populate the first, second and third branchial arches respectively [8–11]. Hence hindbrain segmentation is a conserved strategy used by vertebrates for organizing the diverse craniofacial features.

The neural crest and pre-patterning model

The cranial neural crest is a pluripotent, mesenchymal population that plays a critical role in construction of the vertebrate head. Arising at the junction between the neural plate and surface ectoderm, cranial neural crest cells form nerve, ganglia, cartilage, bone and connective tissue. Many craniofacial malformations are therefore largely attributable to defects in the proliferation, migration or differentiation of this cell population. Transpositions of neural folds in a number of species [12–16] led to the concept that regional diversity in the vertebrate head was a consequence of patterning information provided by the neural crest. When presumptive first arch (mandibular) neural crest primordia were transplanted more posteriorly in the neural tube in place of presumptive second (hyoid) or third (visceral) arch neural crest, the transplanted neural crest cells migrated into the nearest arch but therein formed ectopic proximal first arch skeletal elements such as the quadrate and Meckel's cartilage [16]. Not only were these crest-derived structures inappropriate for their new location but the muscle cell types and attachments associated with the ectopic structures were also characteristic of a first arch pattern. This suggested that myogenic populations and other cell types receive spatial cues from the invading neural crest derived connective tissue. Molecular evidence supporting this scenario was provided

by the observation that the same domains of *Hox* gene expression that are restricted in the hindbrain were emulated in the ganglia and branchial arches, reflecting the origins of the neural crest cells contributing to these tissues [17,18].

Collectively, these pivotal studies led to speculation that the spatial organization of cranial structures was determined by the neural crest and that the pattern was irreversibly set before the neural crest emigrates from the neural tube. Under this pre-patterning model, positional information including *Hox* genes was carried passively from the hindbrain to peripheral tissues and branchial arches by the neural crest, where it was elaborated to form the characteristic head structures.

Cranial neural crest plasticity

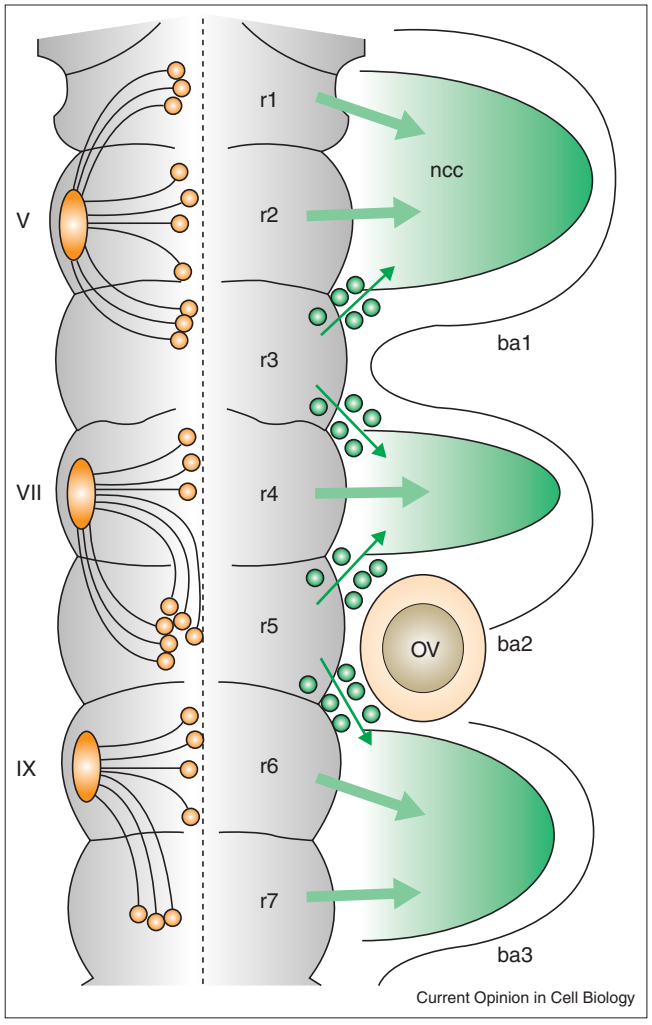
The neural crest pre-patterning model predicts that experimental alterations to the spatial organization of the hindbrain should result in a re-organization of the patterns of *Hox* gene expression and neural crest migration, and ultimately craniofacial abnormalities. Owing to the ease of tissue manipulation, the chick embryo has been the primary species for testing this hypothesis via rhombomere transplantations, rotations and ablations. These analyses have yielded conflicting results regarding the degree of autonomy of *Hox* gene expression [19]. Recently there have been significant advances in our understanding of these developmental issues, which have arisen principally from the development of new techniques for transposing cells within the hindbrains of mouse [20••] and zebrafish [21••] embryos.

In the mouse, cells from r3, r4 or r5 were heterotopically grafted into r2 [20••]. The majority of the transplanted cells remained as a cohort and maintained their *Hox* gene antero–posterior (A–P) identity (Figure 2). A few transplanted cells, however, became separated from the primary graft and dispersed becoming intermingled with the neighboring populations. These cells displayed plasticity as they failed to maintain their appropriate *Hox* gene expression patterns and consequently altered their identity in their new location. This implies that single or dispersed rhombomere cells lack the neighboring signals necessary to reinforce their identity. Hence they respond and adapt to their new surrounding environment by altering gene expression [20••].

Further evidence for neural plasticity and an influence of cell community effects has been provided through the formidable task of transplanting single rhombomere cells in zebrafish [21••]. The transposition of single hindbrain cells from r2 into r6 or vice versa resulted in a complete switch in *Hox* gene expression (Figure 2). This was accompanied by changes in cell fate, which was now characteristic of the new location of the transplanted cells. This degree of plasticity is dependent upon the timing and size of the transplant. At later stages when morphological boundaries are well established, rhombomere cells are more likely to be irreversibly committed and maintain their *Hox* gene expression characteristics. This implies that cells in the neural tube progressively lose responsiveness to the environmental signals that specify their segmental identities. Together the mouse and fish studies show that cell-community effects and their associated signals are important in maintaining the axial identity of an individual cell in the hindbrain.

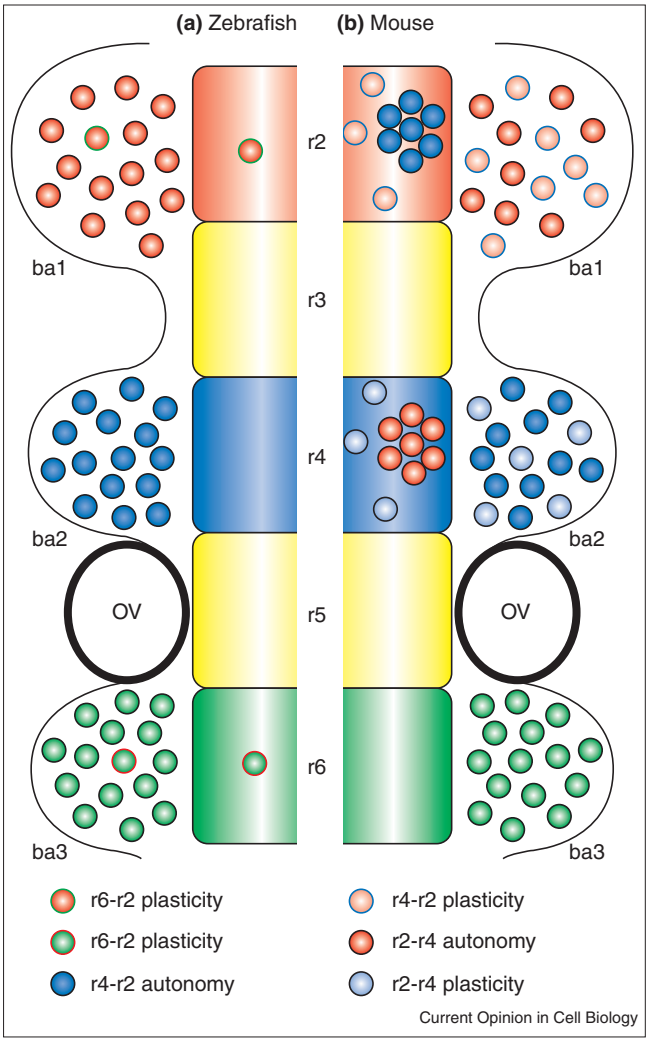
These grafting experiments also revealed the absence of pre-programming in the character or fate of cranial neural crest cells. In heterotopic transpositions of cells within the mouse and zebrafish hindbrains, graft-derived neural crest

Figure 1



Segmental organization of the hindbrain, motor nerves and pathways of cranial neural crest migration. The hindbrain is divided into seven segments or rhombomeres (r1–r7). The branchiomotor nerves collect axons from cell bodies (orange balls) located in multiple segments but they exit the hindbrain only from the even numbered segments (orange ovals) to innervate their peripheral targets. Large numbers of neural crest cells migrate laterally from r1, r2, r4, r6 and r7 (bold green arrows) into the branchial arches (ba). However, r3 and r5 generate smaller numbers of migrating cells (small green balls) that migrate rostrally and caudally (thin green arrows) to join the stream arising from even-numbered segments. ncc, neural crest cell; OV, otic vesicle; V, trigeminal motor nerve; VII, facial motor nerve; IX, glossopharyngeal motor nerve. This figure is adapted from Figure 1a [19].

Figure 2



Plasticity in the A–P cell fates of transposed hindbrain and cranial neural crest cells. **(a)** In zebrafish, single r2 cells moved into r6 and adopt an r6 character (green balls with red outline). Similarly single r6 cells grafted into r2 adopt an r2 character (red balls with green outline). This indicates plasticity in fates depending upon the environment. **(b)** In mouse experiments small groups of r4 cells grafted into r2 maintain their original r4 identity (dark blue balls) if they remain as a group. However, if they disperse and mix with other cells in r2, they lose their r4 character (light red balls in r2). This shows that signals or community effects from neighboring cells can regulate cell fates. Neural crest cells derived from this graft of r4 cells (light red balls in ba1) always lose their r4 or ba2 identity. Reciprocal transplants of r2 cells into r4 display similar cell community and plasticity effects. An r2 character is maintained if the cells remain in a group (red balls) and is lost if they disperse in r4 (light blue balls). Ncc from this graft (light blue balls) that migrate into ba2 do not express ba1 markers. At the bottom of the diagram the shaded balls indicate where evidence of autonomy or plasticity is observed in grafted cells. Note in the hindbrain, plasticity is observed in single dispersed cells and autonomy in cell groups or clusters. This figure is adapted from Figure 2 [19].

cells migrated into the nearest branchial arch without any evidence of pathfinding or re-routing to their original axial level (Figure 2). Plasticity in *Hox* gene expression in mouse neural crest cells was evident by the complete downregulation of *Hoxb1*, *Hoxb2*, and *Hoxa2* in these cells [20••]. In zebrafish, experimental embryos raised to larval stages revealed that the transplanted cells differentiated and contributed to pharyngeal cartilages appropriate to their new A–P location [21••]. Therefore these results show that the A–P character of cranial neural crest cells are neither fixed nor passively transferred from the hindbrain to the branchial arches. Such experiments reveal a surprising degree of plasticity in cranial neural crest cells, inconsistent with the pre-patterning model. Instead, since transposed neural crest can be reprogrammed it appears crest cells rely on distinctive cues in the branchial arch environment through which they migrate to elaborate their proper regional identity. Furthermore, the size of the cell community is functionally important suggesting that a far more complex balance of genetic and cellular interactions are involved in hindbrain and neural crest patterning that previously thought.

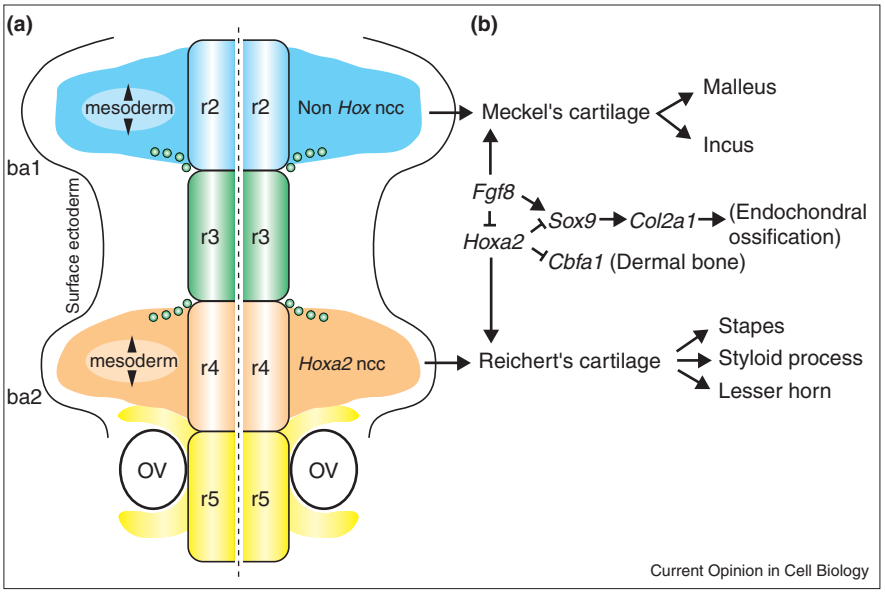
Pharyngeal patterning in the absence of neural crest

The pre-patterning model argues that branchial arch identity is determined by the neural crest. In contrast, the neural crest plasticity described above implies that branchial arch patterning arises due to interactions between the arch components and the neural crest. This raises the question of what happens to the identity of the branchial arches in the absence of contributing neural crest cells.

This issue was investigated in chick embryos through rhombomere ablations [22••] and in mouse embryos by genetic manipulation of Hoxa1 and Hoxb1, which are required for the generation of neural crest cells in r4 [23••]. In both types of analyses, despite the absence of neural crest cells, the second branchial arch still develops and is properly regionalized. The branchial arch expression patterns of Bmp7 in the posterior endoderm, Fgf8 in the anterior surface ectoderm, Pax1 in the pharyngeal pouch endoderm and Shh in the endoderm were all normal and unchanged. In addition there was no evidence for excessive cell death or loss of proliferation in the arch epithelium, which suggests that the neural crest cells are not the source of any indispensable branchial arch mitogenic or survival signal [23••]. These results clearly demonstrate that the branchial arches are not dependent upon the neural crest for their formation, nor for their anterior–posterior and proximo–distal regionalization.

These findings are consistent with the evolutionary history of the branchial arches and neural crest. Pharyngeal segmentation is characteristic of the phylum chordata, whereas neural crest cells are exclusively a craniate (vertebrates plus hagfish) characteristic [24]. This suggests branchial arch segmentation occurred before the evolutionary origin of the cranial neural crest. Therefore, it might be expected that the branchial arches are not dependent on the cranial neural crest for their development and identity. Further support for this idea comes from analyses of *Pax* gene

Figure 3



The influence of mesoderm and ectoderm on axial identity of neural crest cells and the role of *Hoxa2* in regulating second arch morphogenesis. **(a)** Head mesoderms play a role in maintaining the proper domains of *Hox* expression in migrating cranial neural crest cells (red and blue ovals with black arrowheads), while the surface ectoderm plays a role in patterning the branchial arches (black arrows). **(b)** The skeletal derivatives arising from ba1 and ba2 are indicated. *Hoxa2*, which is expressed in crest migrating into ba2 but not ba1, plays a key role as a selector gene that imposes the unique identity of second arch structures. In its absence second arch derivatives are transformed to a first arch fate and ectopic expression of *Hoxa2* transforms first arch structures to a second arch identity. The arrows and bars at the far right indicate the emerging pathways by which *Hoxa2* acts to regulate arch morphogenesis.

expression in *Amphioxus* (the nearest extant vertebrate relative), which show that the pharyngeal pouches are still regionalized despite the absence of neural crest cells [25]. Thus the mechanism for generating pharyngeal pouches predates the evolution of the vertebrate head.

Patterning roles for the mesoderm, endoderm and ectoderm in branchial arch development

Since normal branchial arch development can occur independently of a contribution from the neural crest, perhaps the branchial arches rely on the paraxial mesoderm, endoderm and surface ectoderm tissues for their patterning information.

The mesoderm

The cranial mesoderm forms the predominantly myogenic cores of each branchial arch, which are enveloped by migrating neural crest cells [26–28]. Until recently, the cranial mesoderm was not thought to play a patterning role during craniofacial development. However, it has now been shown that the cranial mesoderm provides maintenance signals for regulating the identity of second branchial arch neural crest cells [20••]. If the second arch neural crest is transplanted alone into the first arch, it downregulates its expression of *Hoxb1*. In contrast, if the neural crest is transplanted in combination with second arch mesoderm, then *Hoxb1* expression is maintained. The cranial mesoderm therefore provides maintenance signals that elaborate the program of *Hox* expression, but cannot initiate *Hox* gene expression in neural crest cells [20••] (Figure 3). This is consistent with the fact that the fate of the cranial mesoderm is primarily myogenic and the musculature is inextricably linked to neural crest derived skeletal and connective tissue patterning. Therefore, one of the roles of the cranial mesoderm may be in maintaining an A–P register between these different primordial tissues, which is essential for subsequent craniofacial morphogenesis [28].

The endoderm

The neurogenic placodes (dorsolateral and epibranchial) form in characteristic positions in all vertebrates suggesting that conserved localized inductive interactions underlie their formation [29]. The epibranchial placodes develop near the branchial clefts in close proximity to the cranial neural crest and the pharyngeal endoderm. Analyses of the nature of the signals, which underlie epibranchial placode formation, have found that the epibranchial placodes do not require cranial neural crest cells for their induction [30]. Rather, it is the pharyngeal endoderm that is the source of the BMP7-inducing signal. The endoderm has also been shown to be responsible for promoting the formation of branchial arch components in amphibians by directing neural crest cells towards a chondrogenic fate [31]. Therefore, the endoderm plays a major role in establishing and patterning the branchial arches.

The ectoderm

Similar to the neuroepithelium, it has been suggested that the ectoderm is regionalized into territories, called ectomeres, that contribute to specific regions of the branchial arches [32]. Currently, there is no evidence to support the idea that each ectomere represents a functional developmental unit. In contrast, however, there is evidence that the surface ectoderm plays a major role in the induction of odontogenesis during branchial arch development [33]. The oral ectoderm of the first branchial arch directly regulates the patterning of the underlying neural crest mesenchyme into teeth and the ability to respond to these instructive or inducing signals is not confined to first arch neural crest cells [34]. Fgf8, which is expressed in the

anterior surface ectoderm of the first arch, is essential for determining the polarity of the branchial arch [35]. Hence, the surface ectoderm plays an important role in patterning the branchial arch derivatives (Figure 3).

Independent molecular regulation of *Hoxa2* in the hindbrain and neural crest

The neural plasticity described above correlates with molecular analyses that have identified distinct regulatory elements controlling *Hox* gene expression in different tissues such as the hindbrain and neural crest. *Hoxa2* is expressed in the hindbrain anteriorly up to the r1/2 boundary and in cranial neural crest cells that migrate into the second branchial arch [36]. Transgenic regulatory analyses of *Hoxa2* have revealed that multiple cis-acting elements are required independently for hindbrain-specific and neural crest-specific activity [37–39]. In r3 and r5, *Hoxa2* expression is directly regulated by the transcription factor *Krox20*. In contrast, *Hoxa2* expression in second branchial arch neural crest cells is tightly controlled by a number of elements, one of which binds to *AP-2* family members. Mutation or deletion of this *AP-2* site in the *Hoxa2* enhancer abrogates expression in cranial neural crest cells but not in the hindbrain. These findings clearly demonstrate that at the molecular level, *Hoxa2* is independently regulated in rhombomeres and neural crest cells. This provides a mechanism for how neural crest cells can respond to the environment through which they migrate independently of the neural tube.

The role of *Hoxa2* in branchial arch patterning

During craniofacial development, neural crest cells migrate into the branchial arches to form the skeletogenic elements [16,40]. In mammals, neural crest of the first arch form Meckel's cartilage, while neural crest of the second arch form Reichert's cartilage (Figure 3). The proximal region of Meckel's cartilage develops into two of the middle ear bones, the malleus and the incus. Reichert's cartilage forms the stapes (third bone of the middle ear), the styloid process of the temporal bone, the lesser horn and part of the hyoid bone [41]. Both endochondral and intramembranous dermal ossification occur during the first branchial arch differentiation, whereas in the second arch only endochondral ossification takes place.

The targeted inactivation of *Hoxa2* results in lethality at birth and homeotic transformations of elements derived from the second arch neural crest into proximal first arch derivatives, including a partial duplication of Meckel's cartilage and ossification centers of the middle ear bones [42,43]. In these mutants, ectopic intramembranous ossification (dermal bone formation) takes place in the second arch. Therefore, *Hoxa2* is essential for proper patterning of structures derived from the neural crest in the second branchial arch, as it inhibits intramembranous ossification and allows only endochondral ossification to occur. Interestingly, only the mesenchymal and not the neurogenic derivatives of the second branchial arch are transformed in *Hoxa2*-null mutants. The fact that r4 is unaffected in these mutants suggests that the primary role of *Hoxa2* is in the neural crest and is independent of the neural tube [42,43]. This provides further support for the idea that neural crest cells are not pre-specified before their migration from the neural tube. Further analyses in *Hoxa2*-mutant mice involving retinoic acid response show that the segmental identities of the hindbrain and its derived neural crest are not linked and can be altered independently [44]. This suggests that *Hoxa2* acts as a positive selector gene in neural crest and branchial arch morphogenesis.

If this is true, then ectopic expression of *Hoxa2* in the first arch should result in the development of second arch structures replacing those of the first arch [43,45]. This has been confirmed by recent studies in chick [46••] and *Xenopus* [47••] embryos that have overexpressed *Hoxa2* in the first branchial arches. In both cases overexpression of *Hoxa2* resulted in a transformation of first arch structures, such as Meckel's cartilage and the quadrate, into second arch elements. The duplicated elements are fused to the original elements in a manner similar to that seen in the *Hoxa2* knockout mutant. This confirms the role of *Hoxa2* as a selector gene specifying second arch fate. In addition, these studies imply that the neural crest is not pre-patterned before its emigration from the neural tube but that it needs to read cues from the arch environment. When first arch crest, before its emigration from the neural tube, is targeted with *Hoxa2*, these neural crest cells are unable to develop into second arch elements (Figure 3). The upregulation of second arch specific genes in the first arch and the homeotic transformation of cartilage elements only occur after global expression of *Hoxa2* in the neural crest and surrounding tissues during formation of the first arch [46••,47••]. These results argue that although neural crest cells are born with some patterning information or identity, the elaboration of their developmental program is achieved through integration with signals from the surrounding tissue environments in which they migrate.

Inroads have been made into the precise mechanisms by which *Hoxa2* influences the morphogenesis of second arch elements [41] (Figure 3). During normal development, *Hoxa2* is widely expressed in the second arch mesenchyme, but it is excluded from the chondrogenic condensations in the core of the arches. In the absence of *Hoxa2*, ectopic chondrogenesis coincides with an expansion of *Sox9* expression into the normal *Hoxa2* expression domain where *Sox9* is not normally expressed. *Sox9* is a direct regulator of the cartilage-specific gene, *Col2a1* [48,49], and using a transgenic approach it has been shown that changes in *Sox9* expression are indeed responsible for the ectopic cartilaginous elements found in the second arch of *Hoxa2* mutants. This is supported by misexpression of *Sox9* in the second arch, which produces a phenotype resembling that of the *Hoxa2* mutants [48]. Therefore, *Hoxa2* acts very early in the chondrogenic pathway and is upstream of *Sox9*. In addition, *Cbaf1*, an activator of

osteoblast differentiation, is upregulated in the second branchial arches of *Hoxa2* mutant embryos suggesting that the prevention of *Cbfa1* induction might mediate *Hoxa2* inhibition of dermal (intramembranous) bone formation during second arch development [41] (Figure 3).

Resolving the issues of neural crest plasticity versus pre-patterning and skeletal duplications

The analyses detailed above provide a wealth of evidence supporting the idea that cranial neural crest cells are not pre-specified, but are in fact plastic or flexible and are able to respond to signals from the environment in which they migrate. How then do we reconcile these findings with the skeletal duplications observed by Noden [16] in posterior transplantations of presumptive first arch neural crest? Two things are often ignored from this study. Firstly, in addition to forming duplicated first arch structures, the transplanted neural crest also contributed to the development of normal second arch skeletal elements including the paraglossals and basihyoid, which make up part of the tongue skeleton. This actually provides evidence for neural crest plasticity. Secondly, when presumptive frontonasal neural crest was grafted posteriorly in place of second arch neural crest, duplicated first arch skeletal elements (quadrate and proximal region of Meckel's cartilage) also developed. Similar to the transplanted first arch crest, the frontonasal crest also contributed to second arch specific structures such as the paraglossals and basihyoid, providing further evidence in support of neural crest plasticity [16]. It's important to point out that the same skeletal structures were being formed by grafted neural crest cells regardless of their origin.

What links these two transplantations together is the probable inclusion of the isthmus in each graft. Being an easily recognizable neuromeric constriction or landmark, the isthmus was used as the anterior or posterior limit of the tissue to be grafted [16,50,51]. Recently it was demonstrated in chick embryos, that the isthmus is able to block *Hoxa2* expression in r1 via an FGF8-mediated signaling mechanism [52••]. Our recent experiments have revealed that posterior transplantations of the isthmus/r1 in place of r4 blocks *Hoxa2* expression in the second branchial arch and neural crest [53]. In the absence of *Hoxa2* expression, these grafted chick embryos develop duplicated first arch skeletal structures including the quadrate and proximal portion of Meckel's cartilage. Therefore, the inclusion of the isthmus in the transpositions and the ability of *Fgf8* to suppression of *Hoxa2* in the second arch and neural crest accounts mechanistically for the development of duplicated first arch structures in ectopic posterior locations. Hence, rather than providing evidence for pre-patterning, Noden's experiments [16] highlight the effects of local signaling centers such as the isthmus in A–P patterning and regulation of *Hox* gene expression by FGFs. Together with recent evidence from mouse, chick and zebrafish transplantation studies, this argues as a general principle that cranial neural crest cells are not pre-specified or irreversibly committed before their emigration from the neural tube. Rather, that neural crest patterning is based on plasticity and the ability of neural crest cells to respond to environmental signals and interactions with the tissues through which they migrate [19].

Conclusions

Cranial evolution is considered fundamental to the origin of vertebrates and in evolutionary terms the vertebrate head is a relatively new structure [54]. This review details the multiple levels of regulation and the diverse tissue interactions that are involved in generating the characteristic craniofacial features. The hindbrain clearly has a profound impact on craniofacial development and consequently the potential for generating substantially distinct cranial phenotypes by minor changes of the primordial pattern is one probable reason for the successful radiation of vertebrates into new environments. The potential for generating distinct cranial phenotypes during evolution through alterations in the interactions or signals in the primordial pattern could facilitate diversity. A rigid pre-patterning model in which programs for head morphogenesis were set in the hindbrain would offer restricted opportunities for diversifying head structures. Therefore, understanding the genetic programs and tissue interactions that direct cell patterning will be critical in future studies for elucidating the establishment of the primordial pattern and its evolution during craniofacial morphogenesis in craniates.

Acknowledgements

The authors would like to thank Paul Kulesa, Scott Fraser, Marianne Bronner-Fraser and David Wilkinson for valuable discussions and access to data from their ongoing studies. This work was supported in part by an HFSPO Collaborative Research Grant (RG0146/2000-B) to R Krumlauf as the principal co-coordinator.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Vaage S: **The segmentation of the primitive neural tube in chick embryos (*Gallus domesticus*).** *Adv Anat Embryol Cell Biol* 1969, **41**:1-88.

2. Lumsden A, Krumlauf R: **Patterning the vertebrate neuraxis.** *Science* 1996, **274**:1109-1115.

3. Marín F, Puelles L: **Morphological fate of rhombomeres in quail/chick chimeras: a segmental analysis of hindbrain nuclei.** *Eur J Neurosci* 1995, **7**:1714-1738.

4. Wintgate R, Lumsden A: **Persistence of rhombomeric organisation in the postsegmental avian hindbrain.** *Development* 1996, **122**:2143-2152.

5. Sechrist J, Bronner-Fraser M: **Birth and differentiation of reticular neurons in the chick hindbrain: ontogeny of the first neuronal population.** *Neuron* 1991, **7**:947-963.

6. Lumsden A, Keynes R: **Segmental patterns of neuronal development in the chick hindbrain.** *Nature* 1989, **337**:424-428.

7. Clarke JD, Lumsden A: **Segmental repetition of neuronal phenotype sets in the chick embryo hindbrain.** *Development* 1993, **118**:151-162.

8. Lumsden A, Sprawson N, Graham A: **Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo.** *Development* 1991, **113**:1281-1291.

9. Sechrist J, Serbedzija GN, Scherson T, Fraser SE, Bronner-Fraser M: **Segmental migration of the hindbrain neural crest does not arise from its segmental generation.** *Development* 1993, **118**:691-703.

10. Serbedzija G, Fraser S, Bronner-Fraser M: **Vital dye analysis of cranial neural crest cell migration in the mouse embryo.** *Development* 1992, **116**:297-307.

11. Couly G, LeDouarin N: **The fate map of cephalic neural primordium at the presomitic to the 3-somite stage in the avian embryo.** *Development* 1988, **103**:101-113.

12. Andres G: **Induction and development of cranial structures in the newt, arising from toad ectoderm (Epidermis and derivatives of the neural crest).** *Rev Suisse Zool* 1946, **53**:502-510.

13. Andres G: **Investigations of triton and bombinator chimeras.** *Genetics* 1949, **24**:387-534.

14. Wagner G: **The importance of neural crest in amphibian cranio-facial patterning.** *Rev Suisse Zool* 1949, **56**:519-620.

15. Wagner G: **Examinations of bombinator-triton chimeras.** *Rouxs Arch EntwMech Org* 1959, **151**:136-158.

16. Noden D: **The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues.** *Dev Biol* 1983, **96**:144-165.

17. Hunt P, Whiting J, Muchamore I, Marshall H, Krumlauf R: **Homeobox genes and models for patterning the hindbrain and branchial arches.** *Development* 1991, **112**:187-196.

18. Hunt P, Gulisano M, Cook M, Sham M, Faiella A, Wilkinson D, Boncinelli E, Krumlauf R: **A distinct *Hox* code for the branchial region of the head.** *Nature* 1991, **353**:861-864.

19. Trainor P, Krumlauf R: **Patterning the cranial neural crest: Hindbrain segmentation and *Hox* gene pasticity.** *Nat Rev Neurosci* 2000, **1**:116-124.

20. •• Trainor P, Krumlauf R: **Plasticity in mouse neural crest cells reveals a new patterning role for cranial mesoderm.** *Nat Cell Biol* 2000, **2**:96-102.

This paper, along with [21••], documents by cell grafting in mouse and fish embryos the plasticity of hindbrain and cranial neural crest cells as transposed cells can switch cell fates. They reveal the importance of cell–cell interactions and signals from the branchial arch environment in regulating the programs that pattern hindbrain and craniofacial morphogenesis.

21. •• Schilling T: **Plasticity of zebrafish Hox expression in the hindbrain and cranial neural crest hindbrain.** *Dev Biol* 2001, **231**:201-216.

See annotation [20••].

22. •• Veitch E, Begbie J, Schilling TF, Smith MM, Graham A: **Pharyngeal arch patterning in the absence of neural crest.** *Curr Biol* 1999, **9**:1481-1484.

An important question concerns the degree to which patterning of branchial arch tissues depends upon interactions or signals from neural crest. This study in chick embryos shows that by surgically ablating crest it is still possible to obtain normal arch patterning. In conjunction with [23••] this indicates that neural crest is not responsible for patterning all of arch development.

23. •• Gavalas A, Trainor P, Ariza-McNaughton L, Krumlauf R: **Synergy between *Hoxa1* and *Hoxb1*: the relationship between arch patterning and the generation of cranial neural crest.** *Development* 2001, **128**:in press.

Genetic mutants specifically lacking both *Hoxa1* and *Hoxb1* only in the neural tube have uncovered a new role for *Hox* genes in the regulating the generation of neural crest and with [22••] indicate that formation of placodes, pouches and arch patterning can occur in the absence of cranial neural crest cells that migrate into the arches.

24. Schaeffer B: **Deuterostome monophyly and phylogeny.** *Evol Biol* 1987, **21**:179-234.

25. Holland PWH, Garcia-Fernandez J: ***Hox* genes and chordate evolution.** *Dev Biol* 1996, **173**:382-395.

26. Noden D: **Patterning of avian craniofacial muscles.** *Dev Biol* 1986, **116**:347-356.

27. Noden D: **Interactions between cephalic neural crest and mesodermal populations**. In *Developmental and Evolutionary Aspects of the Neural Crest*. Edited by Maderson PFA. London: John Wiley; 1987:89-119.

28. Trainor PA, Tam PPL: **Cranial paraxial mesoderm and neural crest of the mouse embryo-codistribution in the craniofacial mesenchyme but distinct segregation in the branchial arches.** *Development* 1995, **121**:2569-2582.

29. Baker CV, Bronner-Fraser M: **Vertebrate cranial placodes I. Embryonic induction.** *Dev Biol* 2001, **232**:1-61.

30. Begbie J, Brunet JF, Rubenstein JL, Graham A: **Induction of the epibranchial placodes.** *Development* 1999, **126**:895-902.

31. Epperlein HH: **The ectomesenchymal-endodermal interaction-system (EEIS) of *Triturus alpestris* in tissue culture. I. Observations on attachment, migration and differentiation of neural crest cells.** *Differentiation* 1974, **2**:151-168.

32. Couly GF, Coltey PM, Le Douarin NM: **The triple origin of skull in higher vertebrates — a study in quail-chick chimeras.** *Development* 1993, **117**:409-429.

33. Lumsden AG: **Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ.** *Development* 1988, **103**:155-169.

34. Tucker AS, Sharpe PT: **Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place.** *J Dent Res* 1999, **78**:826-834.

35. Tucker AS, Yamada G, Grigoriou M, Pachnis V, Sharpe PT: **Fgf-8 determines rostral-caudal polarity in the first branchial arch.** *Development* 1999, **126**:51-61.

36. Prince V, Lumsden A: **Hoxa-2 expression in normal and transposed rhombomeres: independent regulation in the neural tube and neural crest.** *Development* 1994, **120**:911-923.

37. Nonchev S, Vesque C, Maconochie M, Seitanidou T, Ariza-McNaughton L, Frain M, Marshall H, Sham MH, Krumlauf R, Charnay P: **Segmental expression of Hoxa-2 in the hindbrain is directly regulated by Krox-20.** *Development* 1996, **122**:543-554.

38. Maconochie M, Krishnamurthy R, Nonchev S, Meier P, Manzanares M, Mitchell P, Krumlauf R: **Regulation of *Hoxa2* in cranial neural crest cells involves members of the *AP-2* family.** *Development* 1999, **126**:1483-1494.

39. Frasch M, Chen X, Lufkin T: **Evolutionary-conserved enhancers direct region-specific expression of the murine *Hoxa-1* and *Hoxa-2* loci in both mice and *Drosophila*.** *Development* 1995, **121**:957-974.

40. Noden DM: **Patterns and organization of craniofacial skeletogenic and myogenic mesenchyme: a perspective.** *Prog Clin Biol Res* 1982, **101**:167-203.

41. Kanzler B, Kuschert SJ, Liu YH, Mallo M: ***Hoxa2* restricts the chondrogenic domain and inhibits bone formation during development of the branchial area.** *Development* 1998, **125**:2587-2597.

42. Gendron-Maguire M, Mallo M, Zhang M, Gridley T: **Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest.** *Cell* 1993, **75**:1317-1331.

43. Rijli FM, Mark M, Lakkaraju S, Dierich A, Dolle P, Chambon P: **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.

44. Mallo M, Brandlin I: **Segmental identity can change independently in the hindbrain and rhombencephalic neural crest.** *Dev Dyn* 1997, **210**:146-156.

45. Rijli F, Gavalas A, Chambon P: **Segmentation and specification in the branchial region of the head: the role of *Hox* selector genes.** *Int J Dev Biol* 1998, **42**:393-401.

46. •• Grammatopoulos GA, Bell E, Toole L, Lumsden A, Tucker AS: **Homeotic transformation of branchial arch identity after Hoxa2 overexpression.** *Development* 2000, **127**:5355-5365.

See annotation [47••].

47. •• Pasqualetti M, Ori M, Nardi I, Rijli FM: **Ectopic Hoxa2 induction after neural crest migration results in homeosis of jaw elements in *Xenopus*.** *Development* 2000, **127**:5367-5378.

These papers [46••,47••] address the important question of how and where *Hox* genes act in regulating morphogenesis of the head skeletal derivatives. Ectopic expression of *Hoxa2* in both frog and fish embryos induces a transformation of first arch components to a second arch identity. This occurs only if the gene is expressed in the neural crest and not in the hindbrain, indicating that the patterning information is not set in the hindbrain and carried to the periphery. This provides strong evidence that *Hoxa2* acts as a selector gene for the pathways that govern second arch structures.

48. Bell DM, Leung KK, Wheatley SC, Ng LJ, Zhou S, Ling KW, Sham MH, Koopman P, Tam PP, Cheah KS: **SOX9 directly regulates the type-II collagen gene.** *Nat Genet* 1997, **16**:174-178.

49. Ng LJ, Wheatley S, Muscat GE, Conway-Campbell J, Bowles J, Wright E, Bell DM, Tam PP, Cheah KS, Koopman P: **SOX9 binds DNA, activates transcription, and coexpresses with type II collagen during chondrogenesis in the mouse.** *Dev Biol* 1997, **183**:108-121.

50. Noden D: **The control of avian cephalic neural crest cytodifferentiation I. skeletal and connective tissues.** *Dev Biol* 1978, **67**:296-312.

51. Noden D: **The control of avian cephalic neural crest cytodifferentiation II. neural tissues.** *Dev Biol* 1978, **67**:313-329.

52. •• Irving C, Mason I: **Signalling by fgf8 from the isthmus patterns the anterior hindbrain and establishes the anterior limit of *Hox* gene expression.** *Development* 2000, **127**:177-186.
This study illustrates how signals from the isthmus can influence patterning of the adjacent neural tube and suggests that Fgf signaling is an important component of the process that patterns the anterior hindbrain.

53. Gans C, Northcutt R: **Neural crest and the origin of vertebrates: a new head.** *Science* 1983, **220**:268-274.

54. Trainor P, Ariza-McNaughton L, Krumlauf R: **The isthmus and FGF signalling inhibit Hoxa2 expression in cranial neural crest and resolves the paradox of plasticity versus pre-patterning.** *Science* 2001, **293**, in press.