CHAPTER 1

Neural Crest Cells and the Community of Plan for Craniofacial Development: Historical Debates and Current Perspectives

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Abstract

After their initial discovery in the mid 1800s, neural crest cells transitioned from the category of renegade intra-embryonic wanderers to achieve rebel status, provoked especially by the outrageous claim that they participate in skeletogenesis, an embryonic event theretofore reserved exclusively for mesoderm. Much of the 20th century found neural crest cells increasingly viewed as a unique population set apart from other embryonic populations and more often treated as orphans rather than fully embraced by mainstream developmental biology. Now frequently touted as a fourth germ layer, the neural crest has become a fundamental character for distinguishing craniates from other metazoans, and has radically redefined perceptions about the organization and evolution of the vertebrate jaws and head. In this chapter we provide an historical overview of four main research areas in which the neural crest have incited fervent discord among workers past and present. Specifically, we describe how discussions surrounding the neural crest threatened the germ layer theory, upended traditional schemes of vertebrate head organization, challenged assumptions about morphological conservation and homology, and redefined concepts on mechanisms of craniofacial patterning. In each case we frame these debates in the context of recent data on the developmental fate and roles of the neural crest.

Introduction

> *"The biological science of the last half-century is honourably distinguished from that of preceding epochs, by the constantly increasing prominence of the idea, that a community of plan is discernable amidst the manifold diversities of organic structure."*
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> —T.H. Huxley, 1858[1]

During the past 125 years, the neural crest has featured prominently as a provocateur for many great debates in vertebrate biology. Initially described by His in 1868 as a novel longitudinal band of cells dorsal to the spinal cord, this mesenchymal population was subsequently named the neural crest by Marshall in 1879[2] and immediately piqued the interest of embryologists and morphologists for a variety of reasons. In particular, the neural crest appeared at an embryonic stage that was surprisingly later than what had been observed for other progenitor populations, they displayed an unusually high degree of motility and dispersion throughout

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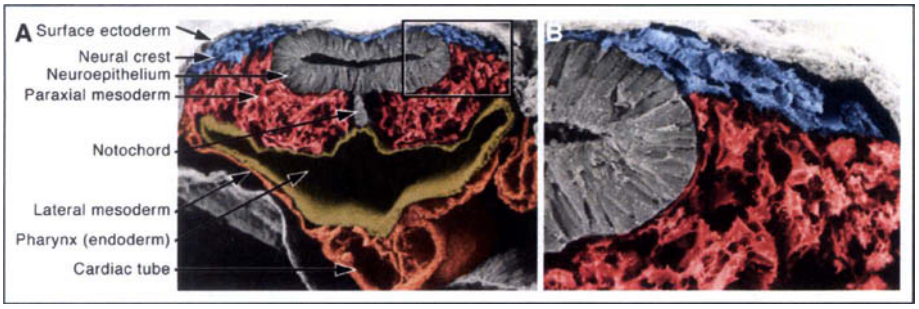


Figure 1. Embryonic tissues of the head. A) Pseudocolored scanning electron micrographs showing the major embryonic progenitor tissues of the head in a transverse section cut at the level of the caudal midbrain from a stage 10- (9-somite) chick embryo. B) At higher magnification (inset box), neural crest cells can be observed emigrating from their origin along the dorsal midline and are partially overlying paraxial mesoderm. These neural crest cells will continue their translocations until they fully underlie the pharynx. Modified from reference 173; original micrographs by K. Reiss.

the body, and they seemed to diversify into a range of cell types that was unexpected given their ancestry from neural ectoderm. These striking attributes, as well as those that became apparent from subsequent experimental analyses, sparked controversy on at least four intellectual fronts.

First, discovery of the origins and derivatives of the neural crest challenged fundamental notions about the basic building blocks of vertebrate embryos, especially the prevailing germ layer doctrine, which imparted exclusive and unique potentials to ectoderm, mesoderm, and endoderm (Fig. 1). Second, data regarding the underlying developmental organization of the neural crest necessitated that paradigmatic evolutionary scenarios for the plan of the vertebrate head be rejected, revamped, or reinvented. Third, analyses of the embryonic distribution and contributions of cranial neural crest cells defied concepts on the conservation of morphological boundaries across vertebrate taxa, and revealed problems in assigning musculoskeletal homologies. Fourth, experiments designed to examine the mechanistic potential of the neural crest created conflicting interpretations on the sources of patterning information that underlie craniofacial morphogenesis.

In this chapter we render the histories for each of the above controversies in light of current perspectives on the fate of cranial neural crest cells across many diverse species. Our goal is to provide resolution to past debates surrounding the neural crest by conveying an expanded, though far from complete, contemporary portrayal of the communal roles that these cells play during craniofacial development.

Primary Germ Layers and Neural Crest Cell Lineages

> *"Each of these three layers hurries toward its goal; although each is not yet independent enough to indicate what it truly is; it still needs the help of its sister travelers, and therefore, although already designated for different ends, all three influence each other collectively until each has reached an appropriate level."*
>
> —C. Pander, 1817[3]

The above insightful description is based on observations of early chick embryo development, and laid the foundation for the precept that many animals establish three stacked or concentric germinal layers from which all intra- and extra-embryonic structures subsequently arise.[4] During the ensuing decades of the 19th century, an apparently equivalent trilaminar organization was found among many diverse taxa.[5,6] However, driven by a post-Darwinian fervor to integrate evolutionary and developmental processes, the model itself became much more rigid and in fact restrictive, projecting the requirement that each germ layer represent an exclusive and autonomous source of particular cell types.

Observational data consistent with this notion were available for some ectodermal and endodermal populations. Mesodermal populations, however, with their frequent transformations between epithelial and mesenchymal states, were generally less well defined but nonetheless were assumed to follow equivalent rules. Further constraints upon the model were imposed by an unbridled zeal to unify developmental and evolutionary events across a wide spectrum of animal groups. Often, putative similarities of germ layers, which were considered shared among many taxa, were given primacy over features found only in a few species.

Within this dogmatic intellectual climate, extant during the last decade of the 19th century, several investigators including Kastschenko, Goronowitsch, and Platt described a dual origin of head mesenchyme.[2,7-9] Exploiting intrinsic cytological distinctions between mesodermal and ectodermal cells in the shark, *Acanthias*, and the mud puppy *Necturus*, Platt[10,11] thoroughly documented movements of neural crest cells into the pharyngeal region and their subsequent differentiation as cartilage and odontoblasts. A few vertebrate biologists welcomed her discovery, which seemed to unite features common to all jawed vertebrates. However, the apparent violation of the germ layer doctrine was soundly rejected by a majority of the scientific community, and some confirmatory findings such as those of Watson (1911) in marsupials,[12] were denied publication for several decades.[2,13]

While convincing evidence for neural crest contributions to the pharyngeal skeleton accumulated from many descriptive and experimental studies primarily in amphibian species,[8] the full extent to which neural crest cells participate in mid-facial as well as jaw and pharyngeal development did not emerge until stable cell labeling methods became available, first in avian species[14-18] and more recently in mice[19-25] and frogs.[26,27] Along with cartilage, a broad assortment of dense and loose connective tissues, smooth muscles, and secretory cells were added to the catalog of cranial neural crest derivatives.

In addition to the extensive array of sensory and autonomic neuronal and supportive cells, and also peripheral pigment cells, the diversity of cell types formed by the neural crest rivals that of mesoderm, prompting some to elevate the neural crest to germ layer status.[28] This comes, ironically, at a time when support for the autonomy of germ layers in vertebrate development is waning.[29] Processes leading to the emergence of mature cell phenotypes are progressive, with each stage building upon prior modifications. For some lineages, important programmatic events commence concomitant with or shortly following the formation of germ layers. For others, commitment seems to be initiated earlier such as delineation of early angioblasts,[30] which occurs in epiblast populations, with germ layers subsequently serving as convenient staging arenas.

An often-asked question is why does cephalic paraxial mesoderm not form the complete assembly of connective tissue lineages within the head as it does in the trunk? With few exceptions, such as transient preotic epithelial condensations found in the frog, *Xenopus*, head mesoderm likely lost the ability to form somites before the emergence of amphibians as well as in most fish groups. Suggestions that a loss of epithelialization may have stripped head mesoderm of certain connective tissue competencies are negated by lineage mapping studies, which demonstrate that head paraxial mesoderm normally forms the same diversity of connective tissues as do somites and neural crest cells.[31,32] Therefore, the answer does not appear related to restricted ability to form certain cell types.

The germ layer doctrine was tissue-centric, focusing on emerging cell phenotypes, and in this context the redundancy between ectodermally-derived neural crest cells and paraxial mesoderm remains perplexing. However, consideration of another developmental parameter, *morphogenesis*, provides a very different perspective. In this process, the history of mesenchymal populations in large part determines their ability to respond collectively to inductive stimuli. As discussed below, many of the skeletogenic signals impinging upon the neural crest and paraxial mesoderm are similar, but the spatial organization of their responses is dissimilar. Thus, the formation of an iterative, multi-element pharyngeal musculoskeletal system, which encompasses a number of adaptations characterizing the rise of gnathostomes (i.e., jawed vertebrates),[33,34] was apparently not within the programming competence of paraxial (or lateral) mesodermal populations.

Neural Crest and the Cranial Bauplan

"At the present day, the very questions regarding the composition of the skull, which were mooted and discussed so long ago by the ablest anatomists of the time, are still unsettled."

—T.H. Huxley, 1858[1]

Almost 150 years ago, Huxley delivered a pivotal lecture to the Royal Society of London in which he woefully made the above observation. Despite much subsequent attention and countless advances in scientific methods of analysis, in many regards Huxley's remark still applies to current disagreement about the basic plan of the vertebrate head. Generally, there are two perspectives, each of which incorporate data on the cranial neural crest to bolster their arguments. The first school of thought views the head as a modified extension of the trunk and emphasizes the segmented nature of its components. The second regards much of the head as a novel appendage to the body and focuses on lack of correspondence among its many parts, especially those derived from the neural crest.

Early segmental theories on origins of the vertebrate head were developed with the transcendental trappings of philosophical anatomy and idealistic morphology. During the 18th and 19th centuries, patterns of repetition and unity of form were believed to underlie the organization of all structures within the body. Correspondingly, the skull was seen as being constructed by a series of discrete units homologous to vertebrae. Goethe is credited with first proposing in 1790 that the skull is composed of several vertebrae, and his idea gained widespread acceptance and elaboration by leading zoologists such as Oken, Spix, Bojanus, Dumerfl, Blainville, Geoffroy, and Owen.[6] However, the theory was not without dissenters. For example, Cuvier argued in 1837 that similarity between the skull and vertebrae could only be found in the most caudal regions of the head, and even this, he believed, was due to equivalent functional requirements and not a unity of plan.[35] Likewise, works of Agassiz and Remak were critical of the theory on embryological grounds.[36]

Opposition gained momentum when Huxley (1858) attacked the extremes to which the vertebral theory had been taken.[1] He argued, "it is no more true that the adult skull is a modified vertebral column, than it would be to affirm that the vertebral column is a modified skull" (p.433). Huxley had made detailed developmental dissections among all major classes of vertebrates and observed that the basic organization of the skull is inherently different from that of the spinal column in both pattern and process of ossification. Though he concluded that vertebral organization does not extend across the entire skull, Huxley did concede that the occipital bones around the notochord may have been derived from vertebrae, and that a segmental plan, albeit different from that of the trunk, could exist in the head.

By allowing for these possibilities, Huxley actuated two opposing viewpoints that were to follow for nearly a century. One viewpoint emphasized the incorporation of vertebrae solely into caudal regions of the head. Workers including Gegenbaur, Stöhr, Rosenberg, and Sagemehl divided the skull into rostral nonvertebral and caudal vertebral portions.[36,37] Most often the boundary was placed at the level of the notochord tip, suggesting that important distinctions were to be made between chordal and prechordal regions of the head.[38] This put the basisphenoid within the caudal, chordal domain, but generated considerable uncertainty about the positions of remaining sphenoid elements in different species. Other researchers emphasized the presence in some taxa of a cranio-occipital joint, which aligned with the exit of the vagus nerve from the skull. This observation led to theories stressing prespinal and spinal portions of the head. In 1875, Fürbringer attempted to integrate these views by proposing that changes in the prespinal part of the skull underwent successive steps throughout the history of gnathostomes.[39] He categorized the prespinal head of different taxa as a palaeocranium (e.g., cyclostomes), a protometameric neocranium (e.g., elasmobranchs and Amphibia), and an auximetameric neocranium (e.g., Amniota). More than half a century later workers such as Augier used similar ideas to explain the progressive enlargement of rostral portions of the head during vertebrate evolution.[31]

The other, and more widely accepted viewpoint that followed Huxley's lecture championed the concept of segmentation as manifest in neural and peripheral musculoskeletal structures. These ideas gained particular prominence due in part to the 1875 works of Dohrn and Semper, who proposed an annelid origin for vertebrates.[6] Additionally, Balfour's seminal descriptions in 1877 of a series of mesodermal "head cavities" in cartilaginous fishes provided unassailable evidence for a set of iterative structures that he believed were distinct from gill structures yet established serial relations with pharyngeal clefts and cranial nerves.[40] Further research on the arrangements of cephalic neuromeres (i.e., segmental swellings in the brain), cranial nerves, and pharyngeal arches in other organisms seemed to support the theory that the preoccipital part of the head in vertebrate embryos was essentially organized as a series of repeated units.[10,41-43]

After the turn of the century most workers agreed with the sentiments of Goodrich (1930) "that the head region of the Craniate is truly segmented, that it is composed of a number of segments essentially similar to those of the trunk, and that segmentation originally extended to the anterior end of the body" (p.213).[39] Each head segment contained a somite or equivalent population of mesoderm that formed initially autonomous sclerotomic, myotomic, and dermatomic structures; both dorsal (sensory) and ventral (motor) cranial nerve roots; a discrete endodermal outpocketing; and the musculoskeletal and aortic components of an oropharyngeal arch (Fig. 2; also see Box 1 for discussion of arch terminology). According to de Beer (1937), once these "facts" had been assembled, the race was on to determine precisely "how many segments of the body are involved in the formation of the skull" (p. 15).[36] Indeed, some subsequent metameric models of craniate head organization included additional rostral segments,[44-46] stretching "the creative imagination of most readers to force all anatomical structures into a rigid segmental framework" (p. 133).[47] Nonetheless, due primarily to the preeminence of workers such as Goodrich and de Beer,[36,48,49] as well as their students, the metameric scheme of head segmentation became standard in 20th century comparative anatomy textbooks.[50]

Despite what seemed to be solid conceptual grounds for an archetypal plan of head organization, there were some nonconformists who noted problems with segmental interpretations of both post-otic and preotic regions of the head. The contributions of the somites to the post-otic area seemed to vary among vertebrates. Moreover, evidence from studies of neural crest distribution suggested that patterns of segmentation found in the pharyngeal arches and nervous system did not correspond to that present in occipital somites.[51] This objection was recently confirmed by fate mapping studies (see ref. 159). Along similar lines, several workers opposed plans of segmentation that encompassed all regions of the head. For example, Neal (1918), who was highly skeptical of single organism-based schemes, was the first to note the apparent inverse phylogenetic relationship between overt segmentation in the brain and in head mesoderm.[52] Epithelial mesodermal segments extending rostral to the ear are prominent in many fishes, occasional in amphibians, and lacking in amniotes, whereas the opposite appeared true of hindbrain rhombomeres. Neal argued that rhombomeres (Fig. 3) evolved in conjunction with pharyngeal segmentation and not mesomeric segmentation, particularly since nerve nuclei traverse rhombomeric divisions and correlate with the pharyngeal arches, clefts, and pouches instead of cephalic somites.

Kingsbury and Adelmann (1924) also raised concerns that the segmentation model was driving interpretations, which were often based on scant and purely descriptive biological observations.[53] Kingsbury (1926) maintained that, "if the head is really segmental in its composition, each segment must at least embody a neuromere, a nerve, and a mesodermal somite. Even the most adequate plan of segmentation, such as that of Goodrich, fails fully to meet the requirements" (p. 84).[54] In his work, Kingsbury emphasized the inconsistent and generally inadequate descriptions of head mesoderm and noted the failure by morphologists to link together pharyngeal arches, head somites, and the unsegmented arrangements of several cranial nerves.

Neural crest cells, by arising from a dorsal tissue and moving to form ventral structures, were a constant source of aggravation equally to the most ardent supporters as well as vociferous opponents of segmentation. For example, by the time Goodrich wrote his classic tome in

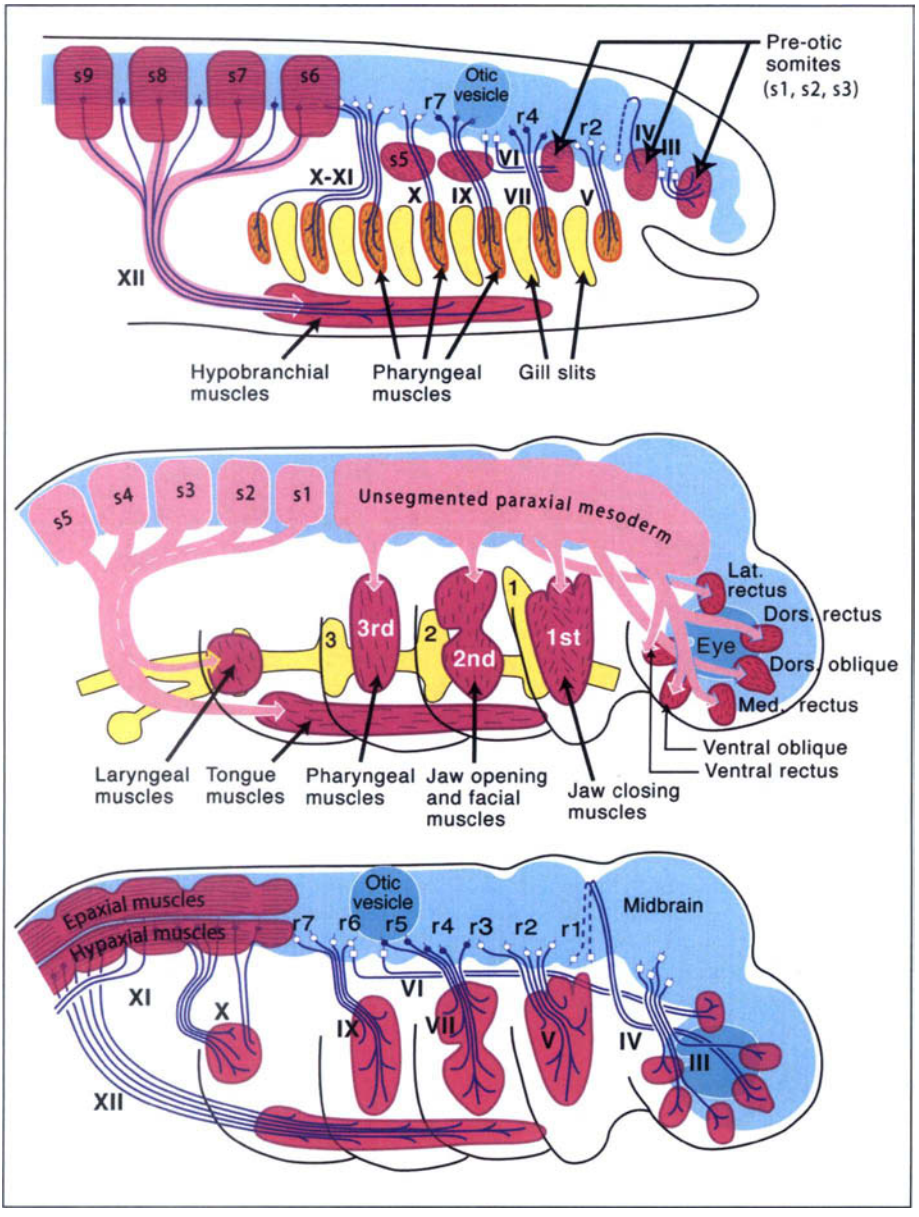


Figure 2. The vertebrate cranial Bauplan. Schematic representations of the classic segmental model for neuromuscular organization of the vertebrate head (top), as well as a contemporary scheme based on recent mapping analyses (middle and lower). In the segmental model, pharyngeal muscles arise from lateral mesoderm between gill slits. Paraxial mesoderm forms somites along the full length of the head, with three preotic somites forming extra-ocular muscles. Additional somites are found adjacent to the otic vesicle, but their homologues—if any—in extant vertebrates are unknown. In the middle sketch, the movements of myogenic myoblasts from paraxial mesoderm into the periphery are illustrated, and the lower drawing adds the distribution of cranial somatic motor nerves. Based on a variety of sources including Goodrich (1918), Noden (1991), and Northcutt (1993).[49,84,174]

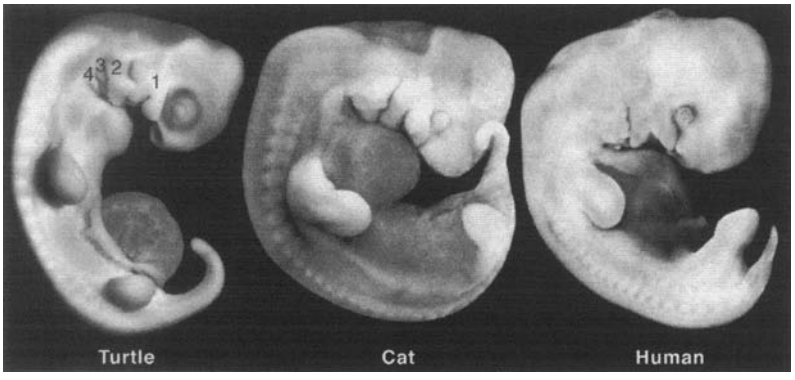
Box 1. Who's the real arch?

Historically, numerous terms have been used to describe the same serially arranged pairs of swellings that appear along the sides of the head in most vertebrates. This superfluity of jargon has caused much confusion and consternation. At some stage in its development, each swelling contains progenitors of cartilage and bone, skeletal and smooth muscle, an aortic arch, a pharyngeal pouch, and a cranial motor nerve. Conventionally in jawed vertebrates (i.e., gnathostomes), the first arch is named "mandibular" and the second arch is named "hyoid".

Branchial Arch (*G. brankhia*, "gills")
Associated with the aquatic respiration of fish and amphibians (anamniotes). Implies a scenario of evolutionary homology to the gills when used in amniotes since amniotes do not possess gills. This term is probably not appropriate for describing the mandibular arch (i.e., the jaws) of gnathostomes. Other related terms: branchial cleft or groove, which refer to indentations on the surface epithelium that separate each successive swelling.

Visceral Arch (*L. viscus*, "internal organ")
Pertaining to a viscus or an organ inside the vertebrate body. Conveys functional and developmental biases particularly with regard to early theories, now proven inaccurate, that the muscles associated with the visceral arch skeleton are un-striated and derived from either endoderm or lateral plate mesoderm. These muscles are in fact striated and derived from paraxial mesoderm like all other skeletal (i.e., voluntary) muscles. Related term: viscerocranium, which refers to all structures associated with the jaws and gills and their homologues.

Pharyngeal Arch (*G. pharunx*, "windpipe" or "throat")
Related to the region between the oral cavity and esophagus of all vertebrates. Has both anatomical and embryological connotations being specifically associated with the endodermally-derived pharynx. Could accurately encompass the mandibular arch, which includes the ectodermally-derived stomodeum, if amended as "oropharyngeal arches," *which is what we prefer.* Related terms: pharyngeal cleft, pharyngeal pouch.



Arch terminology. Snapping turtle, cat, and human embryos in lateral view at comparable stages. Numbers indicate oropharyngeal arches. Turtle embryo courtesy of D. Packard and mammalian embryos from the Cornell Embryology Collection.

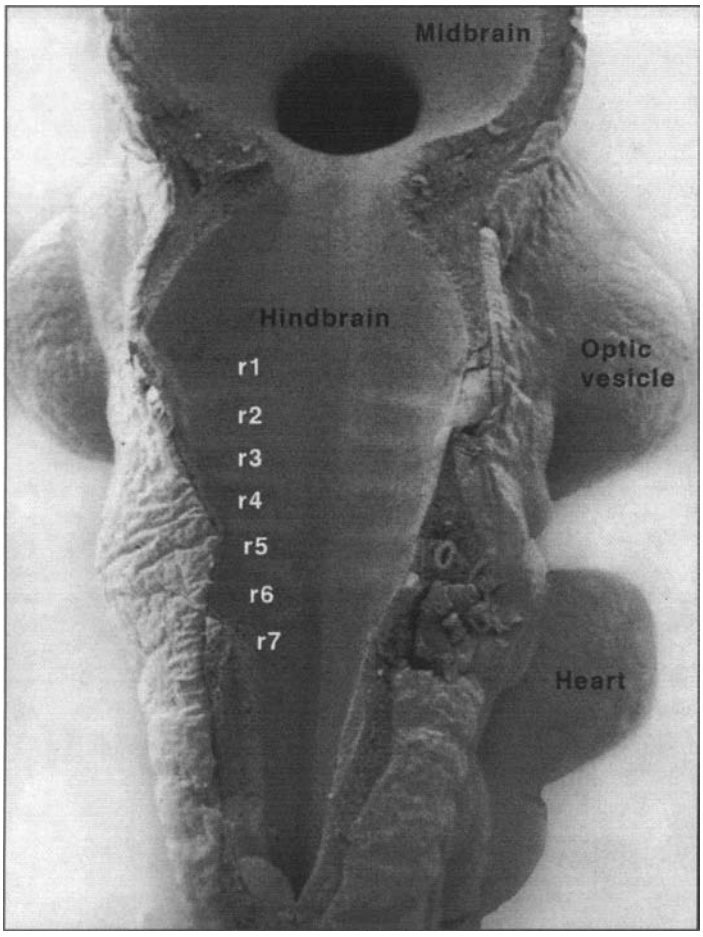


Figure 3. Hindbrain segmentation. SEM, dorsal view of a ferret embryo at embryonic day 14 in which the roof of the hind- and mid-brain regions was removed prior to processing. The segmental organization of the hindbrain is structurally evident by the presence of transverse inter-rhombomeric sulci. Successive rhombomeres are indicated as r1, r2, r3, etc. Courtesy of C. Wahl.

1930, there were numerous studies on the contributions of neural crest to the viscerocranium[2,55,56] and yet he ignored these and argued unequivocally that the visceral arches "are derived from splanchnic mesoblast" (p. 396).[39] Even de Beer (1937), a student of Goodrich, reluctantly accepted the true role of the neural crest in the head and stated, "it is difficult to resist the conclusion that the cartilage of the...visceral arches is derived from the neural crest, strange as it may seem" (p. 476).[13] Notwithstanding this admission, de Beer relegated the neural crest to subordinate status throughout his definitive work on the vertebrate head[9] and did not publish his own study on their skeletal contributions until a decade later.[7]

Towards the end of his illustrious career, Romer attempted to meld these divergent views of cranial organization via his "dual animal" hypothesis.[13] Herein he emphasized the independence of the oropharyngeal and axial regions during vertebrate ontogeny and phylogeny. Romer argued that rather than being fully segmented, the vertebrate head is organized into two functionally and structurally distinct compartments, one essentially external and "somatic" and the

other internal and "visceral," analogous to structural relations evident in thoracic and abdominal regions. Romer based these divisions on muscle types (striated versus smooth), skeletal origins (mesodermal versus neural crest), and nerve components. Of particular importance to Romer's argument was the ancient skeleton that supports the gill apparatus in vertebrate relatives such as Amphioxus, tunicates, and acorn worms, and that phylogenetically predates the skeletal elements of the somatic animal.

In rejecting previous theories of segmentation Romer stated, "it may happen by chance that during development some one gill bar and its musculature may lie below some specific myotome and its derived musculature. But there is no a priori reason to think that the two segmental systems—one basically mesodermal and related to the "somatic" animal, the other basically endodermal, "visceral" in origin-have any necessary relationship to one another" (p. 141).[13] Romer believed his major contribution was to rectify older theories of metamerism with a more accurate picture of vertebrate evolution, beginning with an entirely visceral, sessile, and nonsegmented ancestor. Embryology did not weigh prominently in his model.

Such an emphasis on the role of evolutionary adaptations in shaping the vertebrate head was expanded upon by Gans and Northcutt (1983).[57,58] Noting fundamental differences between vertebrates and protochordates, specifically roles played by cells derived from the neural crest and ectodermal placodes, Gans and Northcutt incorporated the well established fact that all skeletal and connective tissues located rostral to the notochord tip and also lateral and ventral to the pharynx were of neural crest rather than mesodermal origin. They concluded that the vertebrate head "may be conceived as an addition to the existing body of protochordates," and as such "does not represent a modified portion of the existing trunk" (p. 272).[57] Likewise, they rejected classic schemes of segmentation and asserted that the preotic region of the vertebrate head "is intrinsically unsegmented" (p. 271).[57] Yet, a shortcoming of the "new head" theory, and other hypotheses that seemed to invalidate traditional views of segmentation, was that most of the conclusions were based largely on classical descriptive studies. Gans and Northcutt did not incorporate contemporaneous experimental data, which included detailed fate maps of neural crest and paraxial mesoderm populations, as well as revelations about the mechanistic roles that each of these mesenchymal populations play during craniofacial development. More recently, Northcutt (2005) has revisited his "new head" hypothesis and thoroughly addressed these issues.[59]

Two discoveries about cranial paraxial mesoderm forced additional reconsideration of traditional schemes of head organization. The first came from Meier and his collaborators, who found a nascent pattern within paraxial mesoderm in chick,[60,61] mouse,[62,63] snapping turtle,[64,65] shark, newt[66] and medaka.[67] In their work they describe an iterative series of concentrically arranged mesenchymal cells on the dorsal (superficial) and ventral surfaces of paraxial mesoderm, first evident at the neural plate stage and remaining during the stages when neural crest cells move onto the surface of paraxial mesoderm. These domains, which Meier called somitomeres, number seven in amniotes and fish, and fewer in amphibians.[68]

Meier's observations on cranial somitomeres engendered euphoria among the segmentalists, for at long last a key component of metamerism was found. However, to this day, the existence and especially the developmental significance of somitomeres remain controversial.[69,70] Subsequent efforts to observe somitomeres have been unsuccessful,[71,72] and discrepancies in somitomeric boundaries and numbers have emerged depending on the manner in which overlying ectoderm and extracellular matrix are removed.[73] Also, cell labeling experiments using fluorescent dyes indicate that unlike somites, somitomeres lack segmental identity, are not units of lineage restriction, and do not form compartments of cells with discrete spatial properties.[74] Moreover, the cryptic demarcations between somitomeres do not correspond to any identifiable boundary separating individual myogenic or skeletogenic precursor populations.[32]

Once neural crest cells embark upon their translocations to the oropharyngeal region, they establish close contacts with underlying myogenic cells residing in paraxial mesoderm, and these two populations remain in registration during the formation of the arches.[70,75] Anderson

and Meier suggested that the somitomeric pattern might provide specific guidance cues for emigrating neural crest cells.[76] However, neural crest lesion experiments indicate that interactions within neural crest populations may be more essential for establishing the pathways of cell movement.[77] In contrast, somites provide both impediments and conduits for the passage of neural crest cells within and over their surface.[78,79] Moreover, replacing head mesoderm with somites creates a local barricade to the dispersal of cranial neural crest.[80] Thus, the relevance of somitomeres to the developmental organization of the vertebrate head remains uncertain.

A second significant contribution that caused a reconsideration of previous schemes of cranial organization was the discovery that paraxial mesoderm is the exclusive source of all skeletal muscles in the amniote head.[81,82] Myogenic precursors secondarily move into corresponding oropharyngeal arches in concert with neural crest cells, and also in registration with the appropriate cranial motor nerves. This fact, along with extended analyses of the contributions by cranial neural crest cells to skeletal and other connective tissues, led to a formal revision of vertebrate craniofacial organization, in which paraxial mesoderm of the head was portrayed no differently than that of the trunk with respect to the generation of types of cells.[75,83,84] Such ideas focused attention upon the existence of an interface between neural crest and paraxial mesoderm, and suggested that the location of this boundary might be a constant and defining landmark among vertebrate taxa.

Topology and Homology along the Neural Crest-Mesoderm Interface

> *"Sometimes, however, the nonconcordance of morphological relations present problems of special difficulty, for the answer in such cases does not appear to be as simple as mere nonhomology of the structure in question with other structures."*
>
> —G.R. de Beer, 1937[36]

Gans and Northcutt (1983)[57] and others[31,85] postulated that the boundary between neural crest and mesodermal mesenchyme represents a fundamental division between the rostral "new" head and caudal "old" head of vertebrates. Such a hypothesis evokes several questions. Is the location of this interface constant at different stages of development and in various species? Is this interface a permanent barrier that maintains complete separation between the two mesenchymal populations? And is there evidence for cooperation across this interface?

In most species the interface between neural crest and mesodermal mesenchyme is cytologically cryptic, identifiable only when a lineage-specific label is utilized. In avian embryos, the interface is located at the mesencephalic-prosencephalic junction, beside the adenohypophyseal diverticulum (anterior pituitary), and extends caudally along the dorsolateral margins of the pharynx and pharyngeal pouches to the laryngotracheal diverticulum (Fig. 4). This boundary separates dorsal and caudal mesodermal mesenchyme from rostral and ventral neural crest-derived populations. The location of the interface in lampreys, zebrafish, several amphibians, birds, and mammals (mice) has been defined through cell-lineage analyses using vital dyes, radioactive and fluorescent labels, interspecific transplantations, or reporter gene constructs.[2,9,32,34,83,86,87] According to Thorogood (1993), "the broad distribution of the mesenchyme from the two lineages is surprisingly regular and consistent" (p. 113).[88] However, most analyses have been limited to early developmental stages, and in far fewer species has the location of the interface been defined at later stages, when all skeletal elements are in place. This is especially problematic for vertebrates that undergo metamorphosis.

Detailed mapping experiments using quail-chick transplantations have identified neural crest cells as the exclusive source of skeletal and other connective tissues in the midfacial, oral, and pharyngeal regions of the head.[16-18,31,81,82,89,90] For the most part, these mapping studies reveal that each skeletal element originates fully on either side of the interface. More recently, transgenic mice carrying reporter constructs activated in neural crest or mesodermal progenitors have been used to provide a robust examination of skeletal origins in mammals.[21,23,24,91] Again, most elements fall into the categories predicted based on homologies between birds and mammals.

One region of the skull for which there has been much contention is the calvaria. In mice the interface between skeletogenic neural crest and mesodermal populations corresponds roughly to the site of the coronal suture, between the frontal and parietal bones.[21,25,92] Some neural crest cells do move further caudally where they participate in suture formation but do not contribute directly to the intramembranous ossification associated with the parietal bones.[21] The interface appears to be located more caudally in post-metamorphic *Xenopus*, between the large frontoparietal and occipital bones.[26,27]

Transplantation approaches in avians have produced conflicting results. Some investigators found the boundary within the frontal bone, at the junction of the supraorbital (rostral) and calvarial (caudal) parts of this bone.[17,18,89,90] These regions arise from separate ossification centers that fuse together in most avian species. Other investigators concluded that the boundary for neural crest contributions to the roof of the avian skull was located further caudally, at the junction between parietal and occipital bones.[31] While these contradictory findings could have arisen for a variety of reasons,[70] a more recent study using a replication-incompetent retrovirus, which contains a stable reporter construct,[93] has confirmed that the interface in chick embryos is located within the frontal bone, with the parietal being derived exclusively from mesoderm.[32] Thus, in both birds and mammals, the calvaria is of dual mesenchymal origin.

The apparent difference in the precise location of the neural crest-mesoderm interface along the roofing bones of chick and mouse is problematical and prompts several questions. Has the location of the interface indeed shifted during the evolution of one or both of these species, and, if so, what was the location in the common ancestor of birds and mammals? Or, alternatively, has the site of the interface remained constant while specific patterns of fusion or separation of ossification centers changed? Overriding both of these questions is the issue of whether or not the site of the interface is at all pertinent to subsequent patterns of ossification.

In the absence of mapping data from multiple species, assessing the evolutionary stability of the interface between neural crest and mesoderm is filled with uncertainty. The common tetrapod ancestor had a nearly solid roof within which putative frontal and parietal bones are recognized.[94-96] But also present are prefrontal, postfrontal, postparietal, and additional more caudal elements. The phylogenetic fates of these roofing bones have been disputed for decades as they have undergone variable amounts of loss, reduction, expansion, and/or fusion. Most of these changes appear in association with the large openings that evolved in the temporal region of the skull.[97] In the fossil record, archosaurs (diapsids) underwent a reduction of the postfrontal and of the postparietal in their skulls, and of the dermal roof.[98,99] Birds eventually lost the postfrontal. In the mammal-like reptiles (synapsids), the skulls contain a greatly enlarged lateral fenestra that is accompanied by reduction in the size of the calvaria.[100,101] Mammals subsequently lost their postfrontal and reexpanded their frontal and parietal bones.

While considerable attention has been given to identifying homologies among bones in the oropharyngeal arches[102,103] and in the floor and lateral wall of the braincase,[38,104-112] comparable verifications are not available for roofing elements.[29,46,101,113] Comparative analyses of the locations and regulatory mechanisms associated with mammalian sutures are valuable,[114] especially because of their significance in the genesis of craniosynostoses,[115] but they are not informative regarding ancestral patterns. Given the complexities in following the evolution of specific roofing elements, the nomenclature used to label bones in the avian and mammalian calvariae are based on proposed rather than proven homologies. In the chick, the frontal-parietal junction overlies the otic capsule, whereas in mice this boundary occurs over the orbit. Thus, on both embryological (i.e., dual mesenchymal origin), and topographical (i.e., anatomical) grounds, labeling this element in the chick as the "frontoparietal" bone, and calling the parietal a postparietal or interparietal, may be more apropos. If this is the case, then the interface between neural crest and mesoderm can be considered constant among these avian and mammalian species.

Mechanisms that pattern the skull roof, like those operating in the pharyngeal arch skeleton, involve multiple hierarchical and reciprocal interactions whereby individual tissues participate

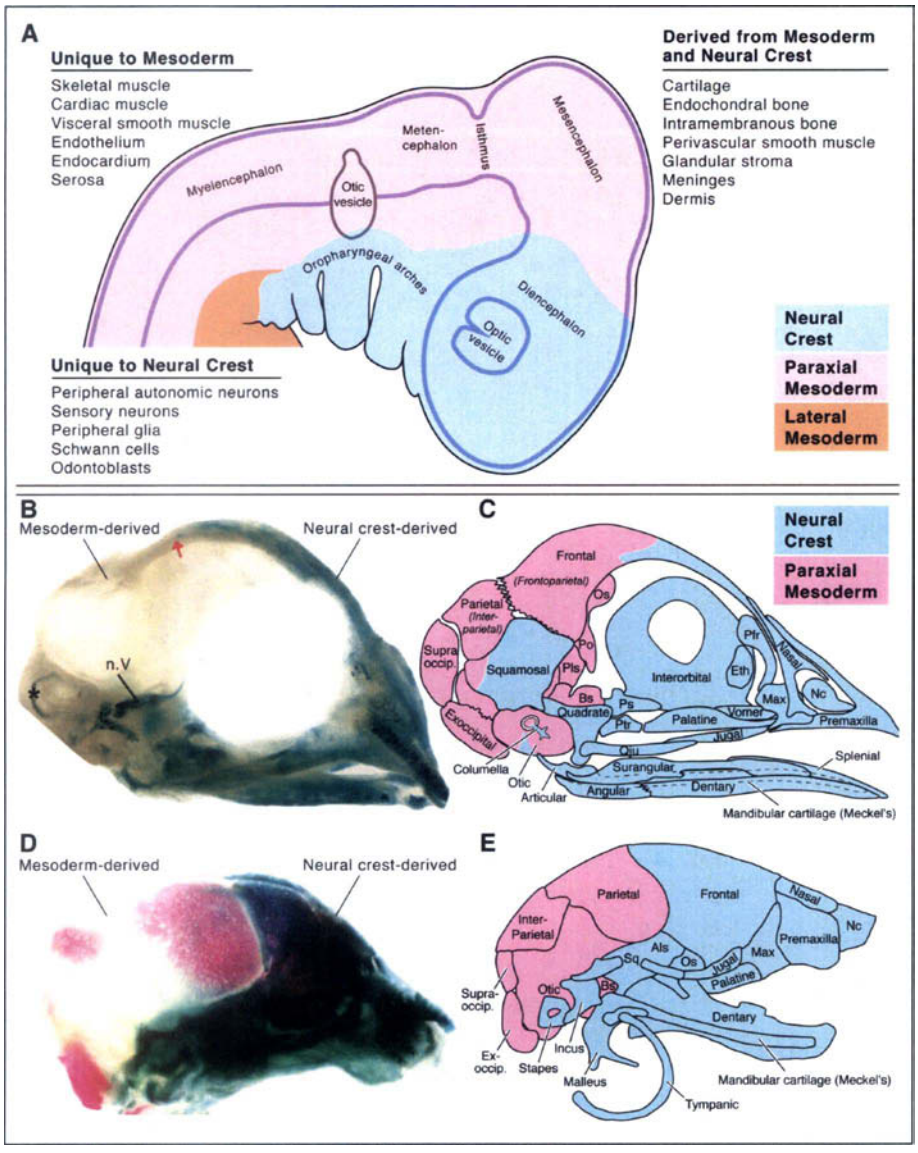


Figure 4. Neural crest-mesoderm boundary in the head. A) The location of the neural crest-mesoderm interface is shown at a stage following the initial translocation of neural crest cells but before the secondary movements associated with muscle morphogenesis. Listed are tissue types derived in amniotes from either neural crest or mesoderm exclusively, or from both of these mesenchymal populations. B) The extent and boundaries of neural crest contributions to the avian skull are shown in a bisected head from a 14-day chick embryo whose neural plate, including neural crest precursors, and surface ectoderm were washed with a replication-incompetent retrovirus containing the *LacZ* (β galactosidase) reporter gene. Note the complete labeling (blue stain) of frontonasal, maxillary, mandibular, and other pharyngeal arch skeletal structures in addition to sensory ganglia such as the trigeminal (n.V). The red arrow points to the site of the neural crest-mesoderm boundary between rostral and calvarial parts of the frontal bone. Asterisk (*) denotes labeled cells within a semicircular duct, which is derived from the otic placode. Figure legend continued on next page.

Figure 4, continued. C) Schematic showing the contributions of mesodermal and neural crest mesenchyme to the cartilages and bones in the avian head skeleton based on data from quail-chick chimeras and retroviral labeling. D) Neural crest-derived tissues in a *Wnt1-Cre/R26R* transgenic mouse embryo appear blue following X-Gal staining. Bones are also stained with Alizarin Red. Image courtesy of G. Morriss-Kay. E) Schematic of neural crest contributions to the mouse head skeleton as extrapolated from transgenic data. Redrawn from reference 175. Abbreviations are as follows: Als = Alisphenoid; Bs = Basisphenoid; Eth = Ethmoid; Nc = Nasal capsule; Os = Orbitosphenoid; Ps = Parasphenoid; Pls = Pleurosphenoid; Po = Postorbital; Prf = Prefrontal; Ptr = Pterygoid; Qju = Quadratojugal; Sq = Squamosal; Supraoccip = Supraoccipital; n.V = Trigeminal Nerve.

according to their unique history and responsiveness.[70,113] In oropharyngeal regions, neural crest populations bring essential pattern generating properties based on their sites of origin,[8,89] whereas in the frontonasal region neural crest populations appear more dependent upon signals emanating from surrounding tissues for their morphogenesis.[116-120] The meager data available relative to the roof of the skull suggest that the brain plays an important inductive role.[121-123] If skeletal patterning of the calvaria is indeed established through the actions of extrinsic signals, then evolutionary changes in the distribution of neural crest and mesodermal mesenchymal populations lateral and dorsal to the brain may be irrelevant.

While most skeletal structures appear to form on either side of the interface, the cartilaginous otic capsule and several parts of the sphenoid complex incorporate skeletogenic cells from both the neural crest and paraxial mesoderm, in chick[17,18,31,81,82] and mouse.[21,23,24] These exceptions to the principle of segregation likely represent situations where ancestral neural crest-derived pharyngeal arch elements abut mesodermal neurocranial tissues. As occurred with the transformation of the jaw joint and "release" of elements for adaptation as middle ear ossicles, these former pharyngeal chondro-competent cells apparently became recruited into nearby cartilages.

Attempts to shift the location of the neural crest-mesoderm interface experimentally have generated mixed results. Schneider (1999) transplanted quail neural crest cells into locations normally occupied by head mesoderm, and produced cartilages in the lateral sphenoid skeleton and otic capsule that were morphologically indistinguishable from elements normally generated by mesoderm.[108] The implication from these experiments is that neural crest cells can respond to the same cues that both promote skeletogenesis and enable proper patterning in mesoderm. Yet, similar grafts of cranial neural crest populations into trunk mesoderm produce only small foci of cartilage within vertebrae.[124] To date, attempts to replace neural crest-derived skeletal elements with mesoderm have been unsuccessful.[80] Other clues come from in vitro experiments by Chiakulus (1957) and Fyfe and Hall (1979).[125,126] These workers artificially mixed populations of neural crest and mesodermal cells, and showed that cartilages derived from mesodermal mesenchyme fused together readily, as did all cartilages derived from neural crest mesenchyme. However, attempts to join cartilages of neural crest and mesodermal origins were unsuccessful, suggesting that the maintenance of these sharp boundaries during normal development may depend in part upon differences in cell cohesivity.

The rule of exclusivity that occurs on either side of the neural crest-mesoderm interface seems to apply only to connective tissues. Both angioblast and myoblast populations generated within paraxial mesoderm will cross over to the other side. Angioblasts are highly invasive, moving omni-directionally in order to ensure that all parts of the embryo are populated.[127-130] Indeed, these cells penetrate neural crest populations en route to oropharyngeal and periocular locations, allowing the assembly of aortic arches immediately upon the completion of neural crest dispersal. An interesting observation in this context is that human fetal hemangiomas often appear to be constrained by the boundary between neural crest and mesoderm along the temporal region of the head.[131]

Additional disruptions of the interface occur whenever individual muscles leave their original arch of origin. This is especially evident each time pharyngeal arch muscles establish attachments with mesodermal skeletal structures. In most cases the sites of attachment are formed by neural

crest cells that moved along with the myogenic cells from their original arch.[90] Perhaps the most extreme example of this is the trapezius muscle, which is one of several muscles that receives innervation from cranial nerve XI, yet is not associated with head skeletal structures. Lineage tracing analyses in transgenic mice reveal that neural crest cells from a caudal pharyngeal arch travel with these myoblasts and form tendinous and skeletal cells within the spine of the scapula.[23] This excursion seemingly recapitulates movements established ancestrally, when parts of the pectoral girdle abutted caudal portions of the skull.

The interface between neural crest and mesoderm is, not unexpectedly, a site at which signals affecting the differentiation and morphogenesis of both populations are exchanged. Prior to the arrival of neural crest cells, paraxial mesoderm cells are unable to progress along the muscle differentiation pathway due to the actions of inhibitory factors, especially BMPs and WNTs, released from overlying surface ectoderm. Neural crest cells physically separate these two tissues, but more importantly they release the BMP antagonists NOGGIN and GREMLIN plus the WNT antagonist FRZB, thereby allowing myogenesis to proceed.[132]

At later stages the morphogenesis of head muscles is dependent upon positional cues provided by neural crest cells.[87] Manipulation of pharyngeal arch neural crest populations, either by transplantation[89] or genetic alteration[133,134] results in changes in the orientation and attachments of pharyngeal arch muscles. Moreover, even trunk mesodermal cells grafted to the head will move within neural crest-populated areas and subsequently form normal pharyngeal arch or extra-ocular muscles.[135] These results demonstrate the integrated relationship between neural crest and mesoderm populations, physically separated but in close communication across the interface. Moreover, they show that embryonic muscle anatomy is largely a reflection of the patterning imposed by oropharyngeal and periocular neural crest populations.

Neural Crest and the Origins of Craniofacial Pattern

> *"Not even experimental methods have so far been able to unravel the fate of all cells, or to solve all of the problems regarding the role of the neural crest in determination processes. One explanation of the many contradictory statements made seems to be that many investigators have not attacked the problems on sufficiently broad lines...Further investigations with a variety of methods and giving due consideration to possible sources of error will certainly give us a deeper insight into the many problems offered by the developing neural crest."*
>
> —S. Hörstadius, 1950[8]

As evidenced by the above passage from Hörstadius' 1950 monograph on the neural crest, attempts to understand cell differentiation and tissue patterning have historically been volatile and erratic. Cell differentiation has consistently been more accessible using reductionist approaches but often at the expense of information on populations as a whole, whereas tissue patterning has typically been mired in phenomenology that has lacked robust mechanistic analysis. This uneasy relationship is reflected in the preceding overview of debates regarding the roles of neural crest cells in craniofacial development. While much research has provided data on locations and lineages, the most unsettled issues remain those involving multicellular organization and tissue patterning.

With regard to patterning, generally two perspectives have emerged, each reflecting biases prevalent at the time they were proposed. The first imbues cranial neural crest cells with the ability to execute spatial patterning based on properties that they acquire prior to emigrating from the neuroepithelium. The second emphasizes ongoing interactions between neural crest cells and surrounding tissues that are necessary for both the differentiation and morphogenesis of oropharyngeal arch musculoskeletal structures, with the implication that premigratory neural crest cells are relatively naïve. While this conceptual dichotomy has made for lively debates, it is tempered by recently emerging evidence that regulatory processes underlying craniofacial development involve progenitor populations acting in neither fully autonomous nor wholly dependent manners. Rather, each progenitor population carries circumstantial capabilities and restrictions based on its embryonic history. While there may be hierarchical inequities that

drive the outcome of interactions among the various players, proper histogenesis and morphogenesis require communal relationships. Such a phenomenon was revealed by Schotte and Spemann in the early 1930s following transplantations of mouth-forming tissues between frogs and newts.[136-138] In these experiments, local signals directed the regional character of the outcome, but intrinsic constraints imposed species-specific features.

Similarly in the early 1950s, Hörstadius provided remarkable insights into the issues of induction and determination.[8] In his experiments, amphibian neural folds containing neural crest precursors were transplanted from one axial level of the head to another. The resultant larvae developed with abnormal jaw and branchial cartilaginous skeletons. However, these were not random dysmorphologies. Rather, the skeletal structures produced by progeny of grafted neural crest cells closely resembled those they would have formed in their original location. These pioneering experiments defined the importance of prior history in the execution of tissue assembly.

Several decades later, Noden[89,139] demonstrated the same in avian embryos, with the advantages that better cell marking tools were available using the quail-chick chimeric system[15] and embryos could be reared until nearly all bones of the head were ossified. Neural crest progenitors transplanted from one site along the midbrain-hindbrain axis to a different site dispersed appropriate to their new location,[139] but their subsequent patterns of morphogenesis were inappropriate. Transplanting neural crest precursors from midbrain (future mandibular arch) to the hindbrain (future hyoid arch) levels produced embryos with an additional jaw skeleton in the location normally occupied by hyoid elements.[89]

These findings, fully consistent with those of Hörstadius, have been used to support the argument that neural crest cells are "prepatterned" prior to their migration. However, results of these transplantations and several subsequent studies indicate that spatial programming involving cranial neural crest cells is a much more complex process. Among Noden's transplants were many in which donor cells would normally have formed maxillary or frontonasal structures. Yet, the outcome in all these was strikingly similar: formation of a mandibular arch skeleton (squamosal-quadrate-pterygoid-proximal mandible). Clearly then, neural crest cells are bringing a response bias to each peripheral location, but one that is not consistently based on the precise site of their origin.

Additionally complicating the interpretation of Noden's data was the observation that some cells derived from these neural crest transplants adopted patterns of skeletogenesis appropriate for their new locations. In each case these cells were near the perimeter of areas occupied by grafted cells. Critical analysis of controls indicated that cooperativity among neural crest populations in adjacent arches is normal; individual skeletal elements such as the basihyoid cartilage and articular bone are formed by progenitor populations that arise at different axial levels.[32,89,90]

Much work has been done to explore the interactions through which neural crest cells acquire their patterning biases. For example, Le Douarin and coworkers found that contacts with neuroepithelium during the emigration process are important.[140] Not surprisingly, signals involved in establishing regional identities within the developing brain also affect prospective neural crest cells. Neuroepithelial cells at the midbrain-hindbrain boundary (isthmus) produce Fibroblast Growth Factor 8 (FGF8), which activates in neighboring cells a cascade of gene activities that collectively are necessary to specify the formation of the cerebellum, caudally, and the colliculi, rostrally.[141,142] Trainor and his colleagues[143] recently demonstrated that this isthmic signaling center also provides positional cues to nearby neural crest progenitors, which could explain how many of the neural crest progenitor populations grafted by Noden and Hörstadius acquired mandibular arch patterning information.

Some of the antecedents to early patterning of neural crest populations have been revealed through analyses of *Hox* genes. Unique combinations of these regulatory genes are expressed among different hindbrain rhombomeres and their associated neural crest cells.[144] The rostral limit of *Hox* gene expression is rhombomere 3, thus neural crest cells that populate the mandibular arch arise in a *Hox*-free zone and do not activate members of this regulatory

network. In transgenic mice partially lacking expression of the hyoid arch-specific *Hox* gene code (i.e., *Hoxa2* mutants), neural crest cells form mandibular skeletal structures in place of hyoid arch elements,[145,146] which is similar to the transplantation-induced phenotypes (with the caveat that these skeletal arrays had reversed rostro-caudal orientation). In converse experiments, forcing expression of *Hoxa2* in mandibular arch neural crest precursors results in the formation of hyoid rather than mandibular skeletal structures.[147,148] Interestingly, expression of *Hoxa2* is downregulated by FGF8, which when expressed ectopically in the hindbrain similarly disrupts development of hyoid arch structures.[149]

A large number of studies have identified signals emanating from tissues encountered by neural crest cells en route to or within the oropharyngeal arches. Interactions with paraxial mesoderm,[144] pharyngeal endoderm,[150-152] and both neural and surface ectoderm[88,153] all modify the execution of prior specifications and are necessary to drive histogenesis and morphogenesis of skeletal tissues. In some cases, paraxial mesoderm acts as an intermediary agent in this signaling.[154] These interactions occur on the way to as well as at the terminal sites of neural crest cell differentiation. Extirpation and transplantation experiments revealed that both pharyngeal endoderm and surface ectoderm provide important skeletogenic signals.[2,150,155] Signals from pharyngeal endoderm, especially FGFs and Sonic Hedgehog, are positive regulators of chondrogenesis in pharyngeal neural crest cells.[156-158] These studies strongly suggest a broader role for epithelia such as pharyngeal endoderm during arch morphogenesis.[151] Graham (2004)[159] has proposed that this deep epithelium is a dominant source of oropharyngeal arch patterning information.

In contrast to the oropharyngeal arches, in which the positional history of neural crest cells is an essential feature of their morphogenesis, the site of origin of frontonasal neural crest populations is not a major morphogenetic influence.[89] Here, local signals emanating from the forebrain and facial ectoderm are essential to patterning of the region.[117,119,120,160,161] This does not in any way lessen the importance of genetic-based responses within the mesenchyme for establishing pattern since other research indicates that differential domains of gene expression in the facial mesenchyme correlate with species-specific variations in the size and shape of beaks among various bird embryos including Darwin's finches.[162,163]

In fact, the extent to which neural crest cells bring essential intrinsic biases to their local developmental environment has been made most apparent by exploiting species-specific differences in surgically-created chimeric embryos. Classical neural crest transplant experiments involving salamanders and frogs showed that patterning the jaws and teeth is largely driven by genetic-based response properties within neural crest populations.[164,165] Similar tactics have been taken with divergent species of birds.[166-170] Schneider and coworkers grafted neural crest progenitors between quail and duck embryos in order to capitalize on three unique features that set these species of birds apart. Quail cells can be detected by using a ubiquitous nuclear marker not present in the duck. Also, quail and duck embryos have morphologically distinct beak and head feather patterns, which permit assays of donor and host contributions to both differentiation and morphogenesis.[171] Finally, quail and duck embryos develop at different rates. Changes to the timing of tissue interactions can therefore be assessed.

These quail-duck transplants revealed that neural crest cells provide species-specific information for patterning the beak and the cranial feathers. When transplanted into duck embryos, quail neural crest cells gave rise to beaks and feathers like those found in quail, specifically by establishing the pattern of their own derivatives as well as those of the host. Reciprocal transplantations of duck neural crest into quail hosts produced analogous alterations. Molecular analyses demonstrated that neural crest mesenchyme is able to bring about species-specific morphology by dominating the initial interactions with overlying epithelium, and in particular by regulating the mesenchymal and epithelial expression of genes known to affect patterning of the beak and feathers.[168,170] Both transcription factors and secreted molecules exhibited temporal shifts in the initiation of their expression consistent with differences in maturation rates between donor and host cells, providing evidence that quail donor neural crest cells created

quail-like morphology on duck hosts by maintaining their own molecular programs and by altering patterns of gene expression in nonneural crest derived host tissues.

What becomes evident after a survey of experimental data on the neural crest and their neighbors is that a continuous and dynamic dialogue exists among multiple embryonic tissues, which in turn mediates craniofacial morphogenesis. Changes to this dialogue on the molecular or cellular level can alter three-dimensional pattern in astounding ways. At least initially, cranial neural crest cells have the potential to form any other structure within their portfolio of derivatives, both histogenetically and morphogenetically. This seems especially true for the most rostral populations, which when rotated 180° to transpose frontonasal and mandibular neural crest progenitors, can still produce normal facial and jaw skeletons.[89] Also confirming the high degree of plasticity and responsiveness inherent within neural crest mesenchyme are loss-of-function experiments in certain murine Dlx genes, which transform the mandibular primordia into the maxillary primordia.[172] Likewise, neural crest cells can be reprogrammed in avian embryos exposed to retinoic acid and the BMP antagonist Noggin, which changes the maxillary primordia into a frontonasal process.[160] Taken together, such results reveal that neural crest cells contain within them intrinsic programmatic modules, which can be activated in a site-specific manner via cues from adjacent tissues and signaling centers.

Conclusion

> *"What role these cells play in the formation of later tissues I do not know, nor do I know what becomes of the "lost" portions of the neural crest which lie between the spinal ganglia, but it has become evident that the whole question of the nature of "mesoderm" in Vertebrates needs revision founded on fact rather than theory."*
>
> —J.B. Platt, 1893[55]

Observational and experimental data on the neural crest gathered during the last two centuries have ignited many heated deliberations about the developmental programs of vertebrates. The discovery of neural crest cells deposed what Hall (1999) called "entrenched notions of germ-layer specificity and the germ-layer theory, a theory that placed a straightjacket around embryology and evolution for almost a century" (p. v).[2] With the luxury of a modern retrospective, the neural crest now seems to be not at all recalcitrant but rather quite a unifying force in the community of plan for craniofacial morphogenesis. Despite the intentions of the original theory, each of the germ layers does not exist in isolation but instead coexists inclusively with neural crest mesenchyme mediating many of their reciprocal interactions. As numerous recent molecular and cellular analyses reveal, the cranial neural crest especially, can be seen as a microcosm for the inherent capabilities and functionalities of ectoderm, mesoderm, and endoderm, by moving so deftly between a totality of histological states and morphological conditions.

In a similar sense, the neural crest serves to coalesce disparate concepts on the basic organization and patterning of the vertebrate head. Through their various movements and dispersions, cranial neural crest cells integrate the community of plan among neural ectoderm, paraxial mesoderm, pharyngeal endoderm, and superficial ectoderm. Patterning of craniofacial connective tissues and all of those structures dependent upon them results from a consortium of influences, with no constituent fully naïve nor totally autocratic. While developmental origins may be critical for guiding oropharyngeal neural crest, patterning of frontonasal and calvarial populations appears predominantly driven by local epithelial-mesenchymal signaling interactions. Many of the molecules that mediate these tissue interactions and regulate differentiation and patterning of the various head regions continue to be elucidated, and tools for experimentally creating quantitative changes to the timing or intensity of signal-response networks are becoming more readily available. Such advances will likely enable direct assaults on heretofore-intractable problems of craniofacial morphogenesis, particularly in mid- and lower facial areas where on the one hand, some of the greatest evolutionary variations have occurred (e.g., quail versus duck or pug versus borzoi), and, on the other hand, minor disruptions can often have the most severe consequences in the form of birth defects that negatively affect human health.

References

1. Huxley TH. On the theory of the vertebrate skull. Proc R Soc Lond 1858; 9:381-457.
2. Hall BK. The Neural Crest in Development and Evolution. New York, Berlin: Springer, 1999.
3. Churchill FB. The rise of classical descriptive embryology. Dev Biol (NY 1985) 1991; 7:1-29.
4. Gilbert SF. A Conceptual History of Modern Embryology. New York: Plenum, 1991.
5. Huxley TH. Lectures on the Elements of Comparative Anatomy. London: J Churchill and Sons, 1864.
6. Russell ES. Form and Function: A Contribution to the History of Animal Morphology. London: John Murray Publishers Ltd., 1916.
7. de Beer GR. The differentiation of neural crest cells into visceral cartilages and odontoblasts in amblystoma, and a reexamination of the germ-layer theory. Proc R Soc Lond B Biol Sci 1947; 134(876):377-398.
8. Hörstadius S. The Neural Crest; Its Properties and Derivatives in the Light of Experimental Research. London, New York: Oxford University Press, 1950.
9. Hall BK, Hörstadius S. The Neural Crest. London: Oxford University Press, 1988.
10. Platt JB. A contribution to the morphology of the vertebrate head, based on a study of Acanthias vulgaris. J Morphol 1891; 5:78-112.
11. Platt J. The development of the cartilaginous skull and of the branchial and hypoglossal musculature in Necturus. Morphologisches Jahrbuch 1898; 25:377-467.
12. Hill JP, Watson KM. The early development of the brain in marsupials; preliminary communication. J Anat 1958; 92(4):493-497.
13. Romer AS. The vertebrate as dual animal-somatic and visceral. In: Dobzhansky T, Hecht MK, Steere WC, eds. Evolutionary Biology, Vol 6. New York: Appleton-Century-Crofts, 1972:121-156.
14. Johnston MC. A radioautographic study of the migration and fate of cranial neural crest cells in the chick embryo. Anat Rec 1966; 156(2):143-155.
15. Le Douarin NM. A biological cell labelling technique and its use in experimental embryology. Dev Biol 1973; 30:217-222.
16. Le Lièvre CS, Le Douarin NM. Mesenchymal derivatives of the neural crest: Analysis of chimaeric quail and chick embryos. J Embryol Exp Morphol 1975; 34(1):125-154.
17. Le Lièvre CS. Participation of neural crest-derived cells in the genesis of the skull in birds. J Embryol Exp Morphol 1978; 47:17-37.
18. Noden DM. The control of avian cephalic neural crest cytodifferentiation. I. Skeletal and connective tissues. Dev Biol 1978; 67(2):296-312.
19. Osumi-Yamashita N, Ninomiya Y, Doi H et al. The contribution of both forebrain and midbrain crest cells to the mesenchyme in the frontonasal mass of mouse embryos. Dev Biol 1994; 164(2):409-419.
20. Trainor PA, Tam PP. Cranial paraxial mesoderm and neural crest cells of the mouse embryo: Codistribution in the craniofacial mesenchyme but distinct segregation in branchial arches. Development 1995; 121(8):2569-2582.
21. Jiang X, Iseki S, Maxson RE et al. Tissue origins and interactions in the mammalian skull vault. Dev Biol 2002; 241(1):106-116.
22. Chai Y, Jiang X, Ito Y et al. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 2000; 127(8):1671-1679.
23. Matsuoka T, Ahlberg PE, Kessaris N et al. Neural crest origins of the neck and shoulder. Nature 2005; 436(7049):347-355.
24. O'Gorman S. Second branchial arch lineages of the middle ear of wild-type and Hoxa2 mutant mice. Dev Dyn 2005; 234:124-131.
25. Yu HM, Liu B, Chiu SY et al. Development of a unique system for spatiotemporal and lineage-specific gene expression in mice. Proc Natl Acad Sci USA 2005; 102(24):8615-8620.
26. Gross JB, Hanken J. Cranial neural crest contributes to the bony skull vault in adult Xenopus laevis: Insights from cell labeling studies. J Exp Zoolog B Mol Dev Evol 2005; 304(2):169-176.
27. Hanken J, Gross JB. Evolution of cranial development and the role of neural crest: Insights from amphibians. J Anat 2005; 207(5):437-446.
28. Hall BK. The neural crest as a fourth germ layer and vertebrates as quadroblastic not triploblastic. Evol Dev 2000; 2(1):3-5.
29. Kuratani S. Craniofacial development and the evolution of the vertebrates: The old problems on a new background. Zoolog Sci 2005; 22(1):1-19.
30. Kurz H, Gartner T, Eggli PS et al. First blood vessels in the avian neural tube are formed by a combination of dorsal angioblast immigration and ventral sprouting of endothelial cells. Dev Biol 1996; 173(1):133-147.

31. Couly GF, Coltey PM, Douarin ML. The triple origin of skull in higher vertebrates: A study in quail-chick chimeras. Development 1993; 117:409-429.
32. Evans DJ, Noden DM. Spatial relations between avian craniofacial neural crest and paraxial mesoderm cells. Dev Dyn 2006; 235(3).
33. Gans C. The feeding mechanism of snakes and its possible evolution. Am Zool 1961; 1:217-227.
34. Gans C. Evolutionary origin of the vertebrate skull. In: Hanken J, Hall BK, eds. The Skull, Vol 2. Chicago: University of Chicago Press, 1993:1-35.
35. Appel TA. The Cuvier-Geoffroy Debate: French Biology in the Decade before Darwin. Oxford: Oxford University Press, 1987.
36. de Beer GR. The Development of the Vertebrate Skull. Chicago: University of Chicago Press, 1937.
37. Mitgutsch C. On Carl Gegenbaur's theory on head metamerism and the selection of taxa for comparisons. Theory Biosci 2003; 122:204-229.
38. Allis EP. Concerning the development of the prechordal portion of the vertebrate head. J Anat 1938; 72:584-607.
39. Goodrich ES. Studies on the Structure and Development of Vertebrates. Chicago: University of Chicago Press, 1930.
40. Balfour FM. On the development of elasmobranch fishes. Journal of Anatomy and Physiology 1877; 11(3):406-490.
41. Marshall AM. On the head cavities and associated nerves of elasmobranchs. Q J Microsc Sci 1881; 21:72-97.
42. Orr HB. Contribution to the Embryology of the Lizard. J Morphol 1887; 1:311-372.
43. Locy WA. Contribution to the structure and development of the Vertebrate head. J Morphol 1895; 11(3):497-594.
44. Bjerring HC. A contribution to the structural analysis of the head of craniate animals. Zoologica scripta 1977; 6:127-183.
45. Bjerring HC. Major anatomical steps toward craniotedness: A heterodox view based largely on embryological data. Journal of Vertebrate Paleontology 1984; 4(1):17-29.
46. Jarvik E. Basic Structure and Evolution of Vertebrates. London: Academic Press, 1980.
47. Northcutt RG. Ontogeny and phylogeny: A reevaluation of conceptual relationships and some applications. Brain Behav Evol 1990; 36:116-140.
48. Goodrich ES. Metameric segmentation and homology. Q J Microsc Sci 1913; 59:227-248.
49. Goodrich ES. On the development of the segments of the head in Scyllium. Q J Microsc Sci 1918; 63:1-30.
50. Jollie M. The vertebrate head-segmented or a single morphological structure? Journal of Vertebrate Paleontology 1984; 4(3):320-329.
51. Hill C. Developmental history of primary segments of the vertebrate head. Zoologische Jahrbücher 1900; 13:393-446.
52. Neal HV. Neuromeres and metameres. J Morphol 1918; 31(2):293-315.
53. Kingsbury BF, Adelmann HB. The morphological plan of the head. Q J Microsc Sci 1924; 68:239-285.
54. Kingsbury BF. Branchiomerism and the theory of head segmentation. Journal of Morphology and Physiology 1926; 42:83-109.
55. Platt JB. Ectodermic origin of the cartilages of the head. Anatomischer Anzeiger 1893; 8:506-509.
56. Stone LS. Further experiments on the extirpation and transplantation of mesectoderm in amblystoma punctatum. J Exp Zool 1926; 44:95-131.
57. Gans C, Northcutt RG. Neural crest and the origin of vertebrates: A new head. Science 1983; 220:268-274.
58. Northcutt RG, Gans C. The genesis of neural crest and epidermal placodes. Quarterly Review of Biology 1983; 58(1):1-27.
59. Northcutt RG. The new head hypothesis revisited. J Exp Zoolog B Mol Dev Evol 2005; 304B(4):274-297.
60. Meier S. Development of the chick embryo mesoblast. Formation of the embryonic and establishment of the metameric pattern. Dev Biol 1979; 73:24-45.
61. Meier S, Jacobson AG. Experimental studies of the origin and expression of metameric pattern in the chick embryo. J Exp Zool 1982; 219:217-232.
62. Meier S, Tam PP. Metameric pattern development in the embryonic axis of the mouse. I. Differentiation of the cranial segments. Differentiation 1982; 21(2):95-108.
63. Tam PP, Meier S, Jacobson AG. Differentiation of the metameric pattern in the embryonic axis of the mouse. II. Somitomeric organization of the presomitic mesoderm. Differentiation 1982; 21(2):109-122.

64. Meier S, Packard Jr DS. Morphogenesis of the cranial segments and distribution of neural crest in the embryos of the snapping turtle, Chelydra serpentina. Dev Biol 1984; 102(2):309-323.
65. Packard Jr DS, Meier S. Morphological and experimental studies of the somitomeric organization of the segmental plate in snapping turtle embryos. J Embryol Exp Morphol 1984; 84:35-48.
66. Jacobson AG, Meier S. Morphogenesis of the head of a newt: Mesodermal segments, neuromeres, and distribution of neural crest. Dev Biol 1984; 106(1):181-193.
67. Martindale MQ, Meier S, Jacobson AG. Mesodermal metamerism in the teleost, Oryzias latipes (the medaka). J Morphol 1987; 193(3):241-252.
68. Jacobson AG. Somitomeres: Mesodermal segments of the head and trunk. In: Hanken J, Hall BK, eds. The Skull, Vol 1. Chicago: University of Chicago Press, 1993:42-76.
69. Kuratani S, Horigome N, Hirano S. Developmental morphology of the head mesoderm and reevaluation of segmental theories of the vertebrate head: Evidence from embryos of an agnathan vertebrate, Lampetra japonica. Dev Biol 1999; 210(2):381-400.
70. Noden DM, Trainor PA. Relations and interactions between cranial mesoderm and neural crest populations. J Anat 2005; 207(5):575-601.
71. Wachtler F, Jacob M. Origin and development of the cranial skeletal muscles. Bibl Anat 1986; (29):24-46.
72. Freund R, Dorfler D, Popp W et al. The metameric pattern of the head mesoderm - Does it exist? Anat Embryol (Berl) 1996; 193(1):73-80.
73. Jacobson AG. Somitomeres: Mesodermal segments of vertebrate embryos. Development 1988; 104(Suppl):209-220.
74. Trainor PA, Tan SS, Tam PP. Cranial paraxial mesoderm: Regionalisation of cell fate and impact on craniofacial development in mouse embryos. Development 1994; 120(9):2397-2408.
75. Noden DM. Craniofacial development: New views on old problems. Anat Rec 1984; 208:1-13.
76. Anderson CB, Meier S. The influence of the metameric pattern in the mesoderm on migration of cranial neural crest cells in the chick embryo. Dev Biol 1981; 85:385-402.
77. Noden DM. The migration and cytodifferentiation of cranial neural crest cells. In: Pratt RM, ed. Current Research Trends in Prenatal Craniofacial Development. New York: Elsevier/North Holland, 1980:3-25.
78. Erickson CA, Reedy MV. Neural crest development: The interplay between morphogenesis and cell differentiation. Curr Top Dev Biol 1998; 40:177-209.
79. Bronner-Fraser M. Rostrocaudal differences within the somites confer segmental pattern to trunk neural crest migration. Curr Top Dev Biol 2000; 47:279-296.
80. Noden DM. Patterning of avian craniofacial muscles. Dev Biol 1986; 116:347-356.
81. Noden DM. The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. Am J Anat 1983; 168:257-276.
82. Couly GF, Coltey PM, Le Douarin NM. The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development 1992; 114(1):1-15.
83. Noden DM. Interactions and fates of avian craniofacial mesenchyme. Development 1988; 103:121-140.
84. Noden DM. Vertebrate craniofacial development: The relation between ontogenetic process and morphological outcome. Brain Behav Evol 1991; 38:190-225.
85. Hunt P, Whiting J, Nonchev S et al. The branchial Hox code and its implications for gene regulation, patterning of the nervous system and head evolution. Development 1991; (Suppl 2):63-77.
86. Le Douarin N, Kalcheim C. The Neural Crest. 2nd ed. Cambridge, New York: Cambridge University Press, 1999.
87. Francis-West PH, Robson L, Evans DJ. Craniofacial development: The tissue and molecular interactions that control development of the head. Adv Anat Embryol Cell Biol 2003; 169:III-VI, 1-138.
88. Thorogood P. Differentiation and morphogenesis of cranial skeletal tissues. In: Hanken J, Hall BK, eds. The Skull, Vol 1. Chicago: University of Chicago Press, 1993:112-152.
89. Noden DM. The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Dev Biol 1983; 96:144-165.
90. Köntges G, Lumsden A. Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. Development 1996; 122(10):3229-3242.
91. Gage PJ, Rhoades W, Prucka SK et al. Fate maps of neural crest and mesoderm in the mammalian eye. Invest Ophthalmol Vis Sci 2005; 46(11):4200-4208.
92. Morriss-Kay GM. Derivation of the mammalian skull vault. J Anat 2001; 199(Pt 1-2):143-151.
93. Mikawa T, Gourdie RG. Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of the epicardial organ. Dev Biol 1996; 174(2):221-232.
94. Romer AS. Vertebrate Paleontology. Chicago: The University of Chicago Press, 1933.

95. Romer AS. Osteology of the Reptiles. Chicago: University of Chicago Press, 1956.
96. Carroll RL. Vertebrate Paleontology and Evolution. New York: WH Freeman and Co., 1988.
97. Frazzetta TH. Adaptive problems and possibilities in the temporal fenestration of tetrapod skulls. J Mammal 1968; 125:145-158.
98. Elzanowski A, Galton PM. Braincase of Enaliornis, an early Cretaceous bird from England. Journal of Vertebrate Paleontology 1991; 11(1):90-107.
99. Zusi RL. Patterns of diversity in the avian skull. In: Hanken J, Hall BK, eds. The Skull, Vol 2. 1st ed. Chicago: University of Chicago Press, 1993:391-437.
100. Moore WJ. The Mammalian Skull, Vol 8. Cambridge: Cambridge University Press, 1981.
101. Novacek M. Patterns of diversity in the mammalian skull. In: Hanken J, Hall BK, eds. The Skull, Vol 2. Chicago: University of Chicago Press, 1993:438-545.
102. Striedter GF, Northcutt RG. Biological hierarchies and the concept of homology. Brain Behav Evol 1991; 38(4-5):177-189.
103. Jollie M. Segment theory and the homologizing of cranial bones. American Naturalist 1981; 118:785-802.
104. Broom R. On the mammalian presphenoid and mesethmoid bones. Proc Zool Soc Lond 1926; 17:257-264.
105. Kesteven HL. The osteogenesis of the base of the saurian cranium and a search for the parasphenoid bone. Proc Linn Soc N S W 1940; 65:447-467.
106. Kuhn H, Zeller U. The Cavum epitericum in monotremes and therian mammals. In: Kuhn H, Zeller U, eds. Morphogenesis of the Mammalian Skull, Vol 13. Hamburg: Verlag Paul Parey, 1987:51-70.
107. Smith KK, Schneider RA. Have gene knockouts caused evolutionary reversals in the mammalian first arch? BioEssays 1998; 20(3):245-255.
108. Schneider RA. Neural crest can form cartilages normally derived from mesoderm during development of the avian head skeleton. Dev Biol 1999; 208(2):441-455.
109. Presley R, Steel FLD. On the homology of the alisphenoid. J Anat 1976; 121:441-459.
110. Presley R. Alisphenoid equivalents in placentals, marsupials, monotremes and fossils. Nature 1981; 294(5842):668-670.
111. Maier W. Ala temporalis and alisphenoid in therian mammals. Vienna: Paper presented at: Trends in Vertebrate Morphology: Proceedings of the 2nd International Symposium on Vertebrate Morphology (1986), 1989.
112. Rieppel O. The homology of the laterosphenoid bone in snakes. Herpetologica 1976; 32:426-429.
113. Thompson KS. Segmentation, the adult skull, and the problem of homology. In: Hanken J, Hall BK, eds. The Skull, Vol 2. Chicago: University of Chicago Press, 1993:36-68.
114. Opperman LA. Cranial sutures as intramembranous bone growth sites. Dev Dyn 2000; 219(4):472-485.
115. Ridgway EB, Weiner HL. Skull deformities. Pediatr Clin North Am 2004; 51(2):359-387.
116. Schneider RA, Hu D, Helms JA. From head to toe: Conservation of molecular signals regulating limb and craniofacial morphogenesis. Cell Tissue Res 1999; 296(1):103-109.
117. Schneider RA, Hu D, Rubenstein JL et al. Local retinoid signaling coordinates forebrain and facial morphogenesis by maintaining FGF8 and SHH. Development 2001; 128(14):2755-2767.
118. Cordero DR, Schneider RA, Helms JA. Morphogenesis of the Face. In: Lin KY, Ogle RC, Jane JA, eds. Craniofacial Surgery: Science and Surgical Technique. Philadelphia: W B Saunders Company, 2002:75-83.
119. Hu D, Marcucio RS, Helms JA. A zone of frontonasal ectoderm regulates patterning and growth in the face. Development 2003; 130(9):1749-1758.
120. Cordero D, Marcucio R, Hu D et al. Temporal perturbations in sonic hedgehog signaling elicit the spectrum of holoprosencephaly phenotypes. J Clin Invest 2004; 114(4):485-494.
121. Schowing J. Influence inductrice de l'encéphale embryonnaire sur le développement du crâne chez le Poulet. J Embryol Exp Morphol 1968; 19(1):9-32.
122. Schowing J. Inductive influence of the embryonic brain on the development of the skull in the chicken. I. Influence of excision of the anterior nervous area on cranial development. J Embryol Exp Morphol 1968; 19(1):9-22.
123. Tyler MS. Development of the frontal bone and cranial meninges in the embryonic chick: An experimental study of tissue interactions. Anat Rec 1983; 206(1):61-70.
124. Le Douarin NM, Teillet MM. Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neurectodermal mesenchymal derivatives, using a biological cell marking technique. Dev Biol 1974; 41:162-184.
125. Chiakulas JJ. The specificity and differential fusion of cartilage derived from mesoderm and mesectoderm. J Exp Zool 1957; 136:287-300.

126. Fyfe DM, Hall BK. Lack of association between cartilages of different embryological origins when maintained in vitro. Am J Anat 1979; 154:485-495.
127. Noden DM. Embryonic origins and assembly of blood vessels. Am Rev Respir Dis 1989; 140(4):1097-1103.
128. Noden DM. Origins and assembly of avian embryonic blood vessels. Ann NY Acad Sci 1990; 588:236-249.
129. Noden DM. Cell movements and control of patterned tissue assembly during craniofacial development. J Craniofac Genet Dev Biol 1991; 11(4):192-213.
130. Ruberte J, Carretero A, Navarro M et al. Morphogenesis of blood vessels in the head muscles of avian embryo: Spatial, temporal, and VEGF expression analyses. Dev Dyn 2003; 227(4):470-483.
131. Haggstrom AN, Lammer EJ, Schneider RA et al. Patterns of infantile hemangiomas: New clues to hemangioma pathogenesis and embryonic facial development. Pediatrics 2006; in press.
132. Tzahor E, Kempf H, Mootoosamy RC et al. Antagonists of Wnt and BMP signaling promote the formation of vertebrate head muscle. Genes Dev 2003; 17(24):3087-3099.
133. Trainor PA, Krumlauf R. Hox genes, neural crest cells and branchial arch patterning. Curr Opin Cell Biol 2001; 13(6):698-705.
134. Santagati F, Minoux M, Ren SY et al. Temporal requirement of Hoxa2 in cranial neural crest skeletal morphogenesis. Development 2005; 132(22):4927-4936.
135. Borue X, Noden DM. Normal and aberrant craniofacial myogenesis by grafted trunk somitic and segmental plate mesoderm. Development 2004; 131(16):3967-3980.
136. Spemann H. Embryonic development and induction. New Haven: Yale University Press, 1938.
137. Olivera-Martinez I, Viallet JP, Michon F et al. The different steps of skin formation in vertebrates. Int J Dev Biol 2004; 48(2-3):107-115.
138. Kuroda H, Wessely O, De Robertis EM. Neural induction in Xenopus: Requirement for ectodermal and endomesodermal signals via Chordin, Noggin, beta-Catenin, and Cerberus. PLoS Biol 2004; 2(5):E92.
139. Noden DM. An analysis of the migratory behavior of avian cephalic neural crest cells. Dev Biol 1975; 42:106-130.
140. Couly G, Grapin-Botton A, Coltey P et al. Determination of the identity of the derivatives of the cephalic neural crest: Incompatibility between Hox gene expression and lower jaw development. Development 1998; 125(17):3445-3459.
141. Nakamura H, Katahira T, Matsunaga E et al. Isthmus organizer for midbrain and hindbrain development. Brain Res Brain Res Rev 2005; 49(2):120-126.
142. Alvarado-Mallart RM. The chick/quail transplantation model: Discovery of the isthmic organizer center. Brain Res Brain Res Rev 2005; 49(2):109-113.
143. Trainor PA, Ariza-McNaughton L, Krumlauf R. Role of the isthmus and FGFs in resolving the paradox of neural crest plasticity and prepatterning. Science 2002; 295(5558):1288-1291.
144. Trainor P, Krumlauf R. Plasticity in mouse neural crest cells reveals a new patterning role for cranial mesoderm. Nat Cell Biol 2000; 2(2):96-102.
145. 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(7):1317-1331.
146. 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(7):1333-1349.
147. Grammatopoulos GA, Bell E, Toole L et al. Homeotic transformation of branchial arch identity after Hoxa2 overexpression. Development 2000; 127(24):5355-5365.
148. Pasqualetti M, Ori M, Nardi I et al. Ectopic Hoxa2 induction after neural crest migration results in homeosis of jaw elements in Xenopus. Development 2000; 127(24):5367-5378.
149. Creuzet S, Couly G, Vincent C et al. Negative effect of Hox gene expression on the development of the neural crest-derived facial skeleton. Development 2002; 129(18):4301-4313.
150. Couly G, Creuzet S, Bennaceur S et al. Interactions between Hox-negative cephalic neural crest cells and the foregut endoderm in patterning the facial skeleton in the vertebrate head. Development 2002; 129(4):1061-1073.
151. Le Douarin NM, Creuzet S, Couly G et al. Neural crest cell plasticity and its limits. Development 2004; 131(19):4637-4650.
152. Cerny R, Meulemans D, Berger J et al. Combined intrinsic and extrinsic influences pattern cranial neural crest migration and pharyngeal arch morphogenesis in axolotl. Dev Biol 2004; 266(2):252-269.
153. Shigetani Y, Nobusada Y, Kuratani S. Ectodermally derived FGF8 defines the maxillomandibular region in the early chick embryo: Epithelial-mesenchymal interactions in the specification of the craniofacial ectomesenchyme. Dev Biol 2000; 228(1):73-85.

154. Ladher RK, Wright TJ, Moon AM et al. FGF8 initiates inner ear induction in chick and mouse. Genes Dev 2005; 19(5):603-613.
155. Helms JA, Schneider RA. Cranial skeletal biology. Nature 2003; 423(6937):326-331.
156. Crump JG, Maves L, Lawson ND et al. An essential role for Fgfs in endodermal pouch formation influences later craniofacial skeletal patterning. Development 2004; 131(22):5703-5716.
157. Walshe J, Mason I. Fgf signalling is required for formation of cartilage in the head. Dev Biol 2003; 264(2):522-536.
158. David NB, Saint-Etienne L, Tsang M et al. Requirement for endoderm and FGF3 in ventral head skeleton formation. Development 2002; 129(19):4457-4468.
159. Graham A, Begbie J, McGonnell I. Significance of the cranial neural crest. Dev Dyn 2004; 229(1):5-13.
160. Lee SH, Fu KK, Hui JN et al. Noggin and retinoic acid transform the identity of avian facial prominences. Nature 2001; 414(6866):909-912.
161. Song Y, Hui JN, Fu KK et al. Control of retinoic acid synthesis and FGF expression in the nasal pit is required to pattern the craniofacial skeleton. Dev Biol 2004; 276(2):313-329.
162. Abzhanov A, Protas M, Grant BR et al. Bmp4 and morphological variation of beaks in Darwin's finches. Science 2004; 305(5689):1462-1465.
163. Wu P, Jiang TX, Suksaweang S et al. Molecular shaping of the beak. Science 2004; 305(5689):1465-1466.
164. Andres G. Untersuchungen an Chimären von Triton und Bombinator. Genetica 1949; 24:387-534.
165. Wagner G. Untersuchungen an Bombinator-Triton-Chimaeren. Roux' Archiv für Entwicklungsmechanik der Organismen 1959; 151:136-158.
166. Sohal GS. Effects of reciprocal forebrain transplantation on motility and hatching in chick and duck embryos. Brain Res 1976; 113(1):35-43.
167. Yamashita T, Sohal GS. Development of smooth and skeletal muscle cells in the iris of the domestic duck, chick and quail. Cell Tissue Res 1986; 244(1):121-131.
168. Schneider RA, Helms JA. The cellular and molecular origins of beak morphology. Science 2003; 299(5606):565-568.
169. Tucker AS, Lumsden A. Neural crest cells provide species-specific patterning information in the developing branchial skeleton. Evol Dev 2004; 6(1):32-40.
170. Eames BF, Schneider RA. Quail-duck chimeras reveal spatiotemporal plasticity in molecular and histogenic programs of cranial feather development. Development 2005; 132(7):1499-1509.
171. Schneider RA. Developmental mechanisms facilitating the evolution of bills and quills. J Anat 2005; 207(5):563-573.
172. Depew MJ, Lufkin T, Rubenstein JL. Specification of jaw subdivisions by Dlx genes. Science 2002; 298(5592):381-385.
173. Noden DM, Francis-West KP. The differentiation and morphogenesis of craniofacial muscles. Dev Dyn 2006; in press.
174. Northcutt RG. A Reassessment of goodrich's model of cranial nerve phylogeny. Acta Anat 1993; 148:71-80.
175. Francis-West P, Ladher R, Barlow A et al. Signalling interactions during facial development. Mech Dev 1998; 75(1-2):3-28.