

# Head segmentation in vertebrates

Shigeru Kuratani<sup>1,\*</sup> and Thomas Schilling<sup>†</sup>

\*Laboratory for Evolutionary Morphology, Center for Developmental Biology, RIKEN, 2-2-3 Minatojima-minami, Chuo, Kobe, Hyogo 650-0047, JAPAN; <sup>†</sup>Developmental and Cell Biology, University of California, Irvine, Irvine, CA 92697-2300

**Synopsis** Classic theories of vertebrate head segmentation clearly exemplify the idealistic nature of comparative embryology prior to the 20th century. Comparative embryology aimed at recognizing the basic, primary structure that is shared by all vertebrates, either as an archetype or an ancestral developmental pattern. Modern evolutionary developmental (Evo-Devo) studies are also based on comparison, and therefore have a tendency to reduce complex embryonic anatomy into overly simplified patterns. Here again, a basic segmental plan for the head has been sought among chordates. We convened a symposium that brought together leading researchers dealing with this problem, in a number of different evolutionary and developmental contexts. Here we give an overview of the outcome and the status of the field in this modern era of Evo-Devo. We emphasize the fact that the head segmentation problem is not fully resolved, and we discuss new directions in the search for hints for a way out of this maze.

The study of segmentation is comparable to the study of the Apocalypse. That way leads to madness.

A. S. Romer (cited by Thomson 1993)

## What are head segments?

The segmental plan of the vertebrate head is often illustrated in the introduction or conclusion to textbooks on vertebrate morphology (Goodrich 1930; de Beer 1937; Neal and Rand 1946; Romer 1966; Portmann 1969; Romer and Parsons 1977; Kardong 1998). This reflects the central importance that the idea of head segmentation has had in attempts to understand the various and complicated shapes of vertebrate heads or skulls. In fact, the idea of head segmentation was among the earliest theories in animal morphology (the German term “Morphologie” was coined by the great poet Goethe, one of the first advocates of “vertebral” theories of the skull. See Northcutt—this issue). The concept of segmentation (metamerism—reiteration of structure along the body axis), was considered together with the idea of transformation (metamorphosis—in which one metamere could transform into the likeness of another).

A second wave of research in head segmentation emerged in comparative embryology at the beginning of the 20th century. This renewed interest was strongly motivated by the discovery of mesodermal

cysts in the heads of shark embryos as well as evidence that mesodermal segments extend to the anterior end of invertebrate chordate relatives, such as amphioxus. This led to a scheme of mesodermal segments in the vertebrate head that was likened to an array of somites. However, more recent studies have challenged this view.

A third wave of research in head segmentation has emerged out of contemporary evolutionary developmental (Evo-Devo) biology stemming from major new insights from paleontology, systematics, experimental embryology, and molecular genetics. Molecular developmental biology, in particular, has shown various segmental patterns of gene expression and cell lineage restrictions in the hindbrain and pharyngeal arches of vertebrate embryos (though not in the mesoderm). A clear segmental logic underlies the pattern formation of cranial peripheral nerves and musculoskeletal systems. Positional values within these systems are specified by the combined functions of Hox genes. This so-called Hox code is thought to be a universal trait of development in all metazoans (Slack et al. 1993).

A trend in modern Evo-Devo is to try and unify different doctrines. Therefore, we have asked the question—where has the concept of head segments gone? Have we graduated beyond the realm of idealistic morphology? Has the classic segmental view been reconciled with more recent results from

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<sup>1</sup>E-mail: saizo@cdb.riken.jp

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molecular embryology? Is it totally meaningless? Are segmentalists still alive? We organized a symposium entitled “Vertebrate head segmentation in a modern Evo-Devo context”, on January 3, 2008, at the Annual Meeting of the Society of Integrative and Comparative Biology held in San Antonio, Texas USA. As an introduction to these proceedings, we first give an historical overview of major head segmentation theories (summarized in much greater detail by R. G. Northcutt, this issue), to illuminate the major problems. We then summarize relevant achievements in studies of head segmentation that have accompanied the rise of Evo-Devo. One take-home message of the symposium is that the problem of head segmentation remains an unresolved and exciting area of study.

### **The (NC) and head segmentation—a new head?**

One of the main reasons for the revival in interest in head segmentation has come from studies on the NC, especially experiments with chicken/quail chimeras during the 1980s (Le Douarin 1982; Noden 1983, 1988; also see Couly et al. 1993; see Hanken and Hall 1993). These provided indisputable evidence for the NC origin of craniofacial (branchiomeric) skeletal elements—an idea first put forth by Julia Platt (1893) a century ago (reviewed by Hall and Hörstadius 1988). A chimeric cell-labelling strategy also showed that the cranium contains both NC and mesoderm-derived portions, roughly corresponding to the division into neuro and viscerocrania, respectively (Le Lievre 1974, 1978; Couly et al. 1993; Noden 1983, 1988) and this has been confirmed recently in mice (ref). Thus, the skull is derived from different germ layers along the anterior-posterior axis, NC (ectoderm) in the anterior skull and mesoderm further posteriorly. The boundary between these two portions lies dorsally at the sagittal suture, and ventrally somewhere near the hypophysial foramen, corresponding to the rostral end of the notochord. The mesodermally derived skull (like somites that form the vertebrae) requires signals emanating from the notochord to differentiate into cartilage, whereas the NC-derived skeletogenic mesenchyme (ectomesenchyme) does not (Couly et al. 1993), further suggesting that head and trunk segments develop via distinct mechanisms.

Although research on vertebrate head development and the role of NC in the latter half of the 20th century was not focused exclusively on the segmental architecture of the cranium, it indirectly confirmed many of the segmental schemes of comparative

embryologists like Goodrich (1930), de Beer (1937), Balfour (1878), and van Wijhe (1882). These “segmentalists” rarely took ectomesenchyme into consideration in their formulation because they viewed the vertebrate body as a series of mesodermal segments—the serial homologues of somites (for further discussion of segmentalist theory, see articles by R. G. Northcutt and by S. Kuratani—this issue).

The concept of a “New Head” by Gans and Northcutt (1983) (Northcutt and Gans 1983) was born out of an attempt to reconcile classical theories of head segmentation with experimental embryology. These authors proposed that the NC and epidermal placodes, which give rise to skeletal elements and cranial nerve ganglia, were unique inventions in vertebrates that accompanied the evolution of a highly specialized skull and sense organs. This concept is still a central one in modern vertebrate Evo-Devo (NC and placodes are generally regarded as “synapomorphies” that define vertebrates or craniates). The New Head hypothesis acknowledges the difficulty of incorporating a NC origin for the skull into the classical views of the segmentalists, and argues against the idea that the vertebrate head is simply built through modifications of the same processes that pattern segments in the trunk. However, there is still room for argument here, since the pharyngeal arch segments of the skull are derived from partially iterated “streams” of migrating NC cells which, in turn, correspond to segmental bulges (rhombomeres) of the hindbrain (see below). In addition, the NC-derived neurocranium that surrounds the forebrain (the prechordal cranium of Couly et al. 1993) incorporates the trabeculae that segmentalists once considered to be an anterior head segment (or premandibular arch). As discussed extensively at our symposium, the idea of a “premandibular arch” may be wrong, at least developmentally, but any theory of head segmentation needs to consider the mass of ectomesenchyme that exists anterior to the mandibular arch.

Recent advances in cell labelling techniques have enabled embryologists to perform cell lineage studies of putative head segments in nonavian model systems. These have revealed curious differences in the contributions of mesoderm and NC cells to the skull in different species—morphologically identical (homologous) skeletal elements are NC-derived in one animal, but mesodermally derived in another. This problem first became apparent for the origins of dermal bones in the cranial vault (Le Lievre 1974, 1978; Noden 1983, 1988; Couly et al. 1993), and it now seems likely that skulls of different species each have their own, unique boundaries between

mesodermal and NC domains (Jiang et al. 2002). The unity of experimental embryology and comparative morphology, which once seemed beautifully simple, has now become much more complicated. We can no longer assume the NC-origin of dermal bones simply because they belong to the exoskeleton. Furthermore, the segmental specification of the early streams of cranial NC does not seem to correspond necessarily to the segmental anatomy of the adult skull (see also J Hanken—this issue).

### **Somitomeres—a segmentalists' dream?**

Around the same time that experimental embryology in avian systems was most productive in the context of vertebrate head development, researchers examining embryonic head morphology with the scanning electron microscope introduced the idea of cephalic “somitomeres”. By removing the surface ectoderm from the head, they reported that the paraxial mesoderm was organized into pseudosegmental units resembling incompletely differentiated somites, which they called somitomeres (Meier 1979; Jacobson 1988, 1993; Jacobson and Meier 1984). Since then, however, there has been no clear evidence for somitomeres. It remains unclear how they relate to the epithelial cysts in the head mesoderm, that have been reported both in shark and chick embryos (Jacob and Jacob 1993; Jacob et al. 1986; also see Northcutt and Kuratani—this issue). Thus, despite lengthy discussion of the subject at our symposium, we still have no definite answer as to the existence or significance of somitomeres.

As discussed by the proponents of somitomere theory, the idea stemmed, at least in part, from the classic vertebral theories of the skull espoused by Goethe (1824) and Oken (1807). Such a simple scheme was very attractive for subsequent fate mapping studies of the head mesoderm (Tam and Trainor 1994; Trainor and Tam 1995; Noden 1988; Couly et al. 1992), which may help explain why the somitomere idea survived despite no further evidence from molecular- or cell lineage-level studies (Freund et al. 1996; Jouve et al. 2002). As discussed at the symposium, another reason for the persistence of the idea may have been that the concept of a somitomere was ill-defined, ambiguous, and latent (only incompletely segmented and nonepithelialized). Models for mesodermal segmentation may have also failed to perish entirely simply because developmental biologists' attention was drawn to another set of much more clearly segmented structures in the hindbrain and NC, with clear expression of genes that function in a segmental manner, namely the Hox genes.

### **Rhombomeres, Hox genes, and transformation**

Beginning in the early 1990s, a new trend in studies of head segmentation emerged surrounding the rhombomeres of the hindbrain and the functions of Hox genes. Segmental bulges along the anterior-posterior axis of the neural tube had been described much earlier (von Baer 1828), but their significance remained unclear. Lumsden and Keynes (1989) showed that these bulges reflect an underlying neuronal organization, namely of motor neurons that contribute to specific cranial nerves. Through these nerves, motor neurons within two adjacent rhombomeres innervate muscles within one pharyngeal arch, thereby linking neuromeric and branchiomic segmentation. A further link came when it was shown that the Hox genes are expressed in a nested fashion along the neuraxis, with their anterior expression boundaries corresponding to boundaries within rhombomeres and pharyngeal arch NC (the so-called cephalic “Hox code”; Hunt et al. 1991). This provided the first molecular developmental evidence for segments within at least a subset of cranial tissues. It also once again suggested a link between the processes that pattern trunk and head segments since the Hox code extends into the trunk region with a similar nested pattern of expression.

The Hox code provides positional values that determine the identity of each segmental unit. Thus a shift in the Hox code shifts these positional values and can lead to shifts in the morphological identities of segments. For example, the small lipophilic signaling molecule, all-*trans* retinoic acid (RA), activates Hox expression and when applied ectopically this can lead to posteriorization—manifest as transformations of vertebral shape (reviewed by Kessel 1992), as well as the neuronal and skeletal identities of rhombomeres and pharyngeal arches, respectively, in the head (Rijli et al. 1993; Gendron-McGuire et al. 1993). RA is thought to form an anteriorly declining gradient across the hindbrain field in the early embryo that reflects its sites of synthesis and degradation, and may be quite distinct from its roles in patterning trunk segments (see T Schilling—this issue). A-P patterning involving RA signaling has been conserved among deuterostomes and, like the Hox code, appears to have been co-opted for roles in both head and trunk segments.

Rhombomeres are closely associated with the migration patterns of cranial NC cells. There are three major streams of these NC cells, which in all species appear adjacent to and are primarily populated by NC emerging from “even-numbered” rhombomeres

(i.e. r2, r4, and r6) (Lumsden et al. 1991; Kuratani 1991; Kuratani and Eichele 1993; Graham et al. 1993, 1994). These streams migrate ventrally to populate the frontonasal mesenchyme and pharyngeal arches, whereby rhombomeric segmentation again appears to form a prepatter for NC cell migration (Köntges and Lumsden 1996). However, interestingly, the Hox code in the pharyngeal NC appears to be regulated independently of Hox expression in rhombomeres (see, for example, Prince and Lumsden 1994; Hunt et al. 1998; Trainor et al. 2002). Rhombomeres, NC cells, and the Hox code are thus recognized as conserved elements of a basic system of A-P patterning and segmentation of the posterior part of the vertebrate head (including the hindbrain and pharyngeal arches) in developmental biology. More anterior regions, such as the forebrain, do not express Hox genes and exhibit cellular organization and gene expression patterns that are much harder to interpret in a segmental manner. Posteriorly, mesodermal segmentation into somites, which dictates patterning of the spinal cord and NC migration, is under the control of a molecular segmentation clock (cycling waves of growth factor signaling and transcriptional activity) that does not appear to function in segmentation of the cranial region (Palmeirim et al. 1997; Jouve et al. 2002; Aulehla and Pourquie 2006; Riedel-Kruse et al. 2007). Taken together, these results largely support the New Head hypothesis, despite a common role for Hox genes in A-P patterning, the head is no longer recognized to be segmented in a similar way to the trunk.

### Evo-Devo and head segmentation

In the early days of Evo-Devo, the task was to compare. Various animal embryos were compared at the level of morphology and gene expression patterns to identify shared versus distinct patterning programs in evolution. One striking case of a shared, and therefore evolutionarily conserved, program was the Hox code, found in all metazoan embryos with an A-P axis (bilaterians). A shared Hox code was recognized as a synapomorphy of these animals and termed the “zootype” (Slack et al. 1993). It was also suggested that ancestral bilateria must have possessed a certain repertoire of regulatory genes, including Hox genes, to develop three germ layers and a metazoan-style body plan, in which a Hox-dependent mechanism of positional specification was a prerequisite.

The origin of the segment-based body plan, however, still remains enigmatic. Homologous genes function in the segmentation process in

apparently nonhomologous segments in distantly-related organisms. For example, the Notch signaling pathway is functional both in vertebrate somitogenesis and segment formation in some arthropods (Palmeirim et al. 1997; Schoppmeier and Damen 2005). This issue has become even more enigmatic since recent molecular evolutionary studies have suggested that the so-called protostomes consist of lophotrochozoans (annelids, molluscs etc) and ecdysozoans (arthropods, nematodes etc) (Aguinaldo et al. 1997). In traditional comparative zoology, annelids were long regarded as ancestral to arthropods, with a similar segmental organization, but distinct from that of chordates. However, with the new phylogenetic organization segmentation of the body axis appears to have evolved in all three major clades of metazoans. It is therefore easy to assume that segments originated in the common ancestor of bilaterians (see Carroll et al. 2001, for example). For this to be true, however, one must simultaneously hypothesize secondary losses of segments in a tremendous number of animal phyla. Alternatively, segmentation along the A-P axis may be a much more dynamic and varied process than previously assumed. With this in mind, it becomes easier to accept that the Hox code was recruited independently in various types of segments and tissues for their positional specification during evolution. This is exactly what appears to have happened between the vertebrate head and trunk.

Another major area of Evo-Devo research has compared embryos of vertebrate model animals (mainly those of amniotes) with those of nonvertebrate chordates such as amphioxus and tunicates, as well as agnathans (lampreys and hagfishes) as representatives of the basal lineages of vertebrates. Among these, studies of regulatory gene expression patterns (including some in hemichordates) indicate that positional specification within neural tissue, at least, is largely shared among deuterostomes, and among chordates the neural tube develops along a similar ground plan. Detailed histological studies of tunicate and amphioxus nervous systems also provided supporting anatomical evidence for these conclusions (see Lacalli in this issue).

In the context of vertebrate head segmentation, developmental studies of amphioxus are particularly important. In this animal, the mesoderm is segmented all the way to the anterior tip of the head. For this reason, amphioxus was thought to represent the vertebrate archetype by some comparative embryologists (Goodrich 1930; Neal and Rand 1946). Taking the expression of Hox genes as reference points, as well as *Engrailed* expression and an enterocoelic

mode of growth as the defining features of mesodermal segments, Linda Holland and her colleagues (see Holland and Gilland in this issue) have compared rostral segments of amphioxus with hypothetical head segments in vertebrates—such as typically described in some elasmobranch embryos as well as classical studies of lamprey embryos that were heavily influenced by elasmobranch embryology. Similar comparisons were also made by Peter Holland (2000) with reference to Goodrich's hypothesis. These arguments have been challenged by Kuratani and colleagues (see Kuratani in this issue), mainly based on observations of embryos of the Japanese lamprey, *Lethenteron japonicum*, in which mesodermal segments are absent from the anterior cranial region, similar to amniote embryos (Kuratani et al. 1999; Kuratani 2003). The question of somitomeres echoes in this controversy.

### Conclusion and perspectives

By the beginning of the 20th century, comparative morphology and embryology were thought to have a clear idea of the nature of the vertebrate ancestor. By idealizing animal body plans, mainly based on shared embryonic traits, the comparative embryologists searched for archetypes, which was thought to facilitate further comparisons among higher taxa. Among these, the head was regarded to be crucial since it represented the most complex part in the body of many bilaterians (e.g. arthropods or vertebrates) and was thought to have required an integration of many evolutionarily relevant developmental processes to achieve its complex structure. Naturally, the arguments surrounding such a complex and varied structure often confused ancestral patterns with an idealistic archetype.

Segmental views of the vertebrate head can be characterized by their unity of pattern. Like Richard Owen saw every part of the vertebrate skeleton as a modified vertebra (see Owen 1848), segmentalists assigned all anatomical elements to either a derivative of the somites, or a derivative of tissues that become segmented in response to the same mechanisms that give rise to somites. By the beginning of the 20th century, however, such a unity became increasingly unrealistic; some studies focused only on the nervous system, others only on specific stages of embryos. A typical example is the fact that neuromeres were the central conceptual elements for morphologically oriented neurologists (Johnston 1905), whereas they were largely neglected by those focusing on mesodermal segments (Goodrich 1930). It was not until Jarvik and his colleagues put forth

their segmental scheme as late as the 1980s that the unity of segments was first illustrated.

A similar dissociation characterizes current Evo-Devo studies of the vertebrate head. Despite being based on precise molecular markers or cell lineage analyzes, the search for a unified segmental scheme for the head continues to be more or less idealistic. Developmental biologists have become more aware of how developmental processes and the roles of developmental regulatory genes can change during evolution (Hall 1998). It is no longer so surprising that the mesoderm and neural tube might have dissociated programs of segmental patterning. The question remains, however, what the vertebrate ancestor's head would have looked like, and what segmental developmental program patterned the head of vertebrate ancestors that probably looked somewhat like tunicate larvae or amphioxus. To resolve this long-standing question, the *Kopfprobleme*, it now seems necessary to revisit the old comparative embryology of the head with new approaches and concepts in a modern Evo-Devo context.

### References

- Aguinaldo AM, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA, Lake JA. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 387:489–493.
- Aulehla A, Pourquie O. 2006. On periodicity and directionality of somitogenesis. *Anat Embryol* 211 (1 Suppl): S3–S8. doi:10.1007/s00429-006-0124-y.
- Balfour FM. 1878. The development of the elasmobranchial fishes. *J Anat Physiol* 11:405–706.
- Bjerring HC. 1977. A contribution to structural analysis of the head of craniate animals. *Zool Script* 6:127–183.
- Carroll SB, Greiner JK, Weatherbee SD. 2001. From DNA to diversity. London: Blackwell.
- Couly GF, Colty PM, Le Douarin NM. 1992. The developmental fate of the cephalic mesoderm in quail-chick chimeras. *Development* 114:1–15.
- Couly GF, Coltey PM, Le Douarin NM. 1993. The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. *Development* 117:409–429.
- De Beer GR. 1937. The Development of the Vertebrate Skull. London: Oxford University Press.
- Freund R, Dörfler D, Popp W, Wachtler F. 1996. The metameric pattern of the head mesoderm – does it exist? *Anat Embryol* 193:73–80.
- Gans C, Northcutt RG. 1983. Neural crest and the origin of vertebrates: a new head. *Science* 220:268–274.
- Gendron-Maguire M, Mallo M, Zhang M, Gridley T. 1993. *Hoxa-2* mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. *Cell* 75:1317–1331.

- Goethe JW. 1824. Schädelgrüst aus sechs Wirbelknochen aufgebaut. Zur Morphologie, Band 2, Heft 2.
- Goodrich ES. 1930. Studies on the Structure and Development of Vertebrates. London: McMillan.
- Graham A, Francis-West P, Brickell P, Lumsden A. 1994. The signalling molecule BMP-4 mediates apoptosis in the rhombencephalic neural crest. *Nature* 372:684–686.
- Graham A, Heyman I, Lumsden A. 1993. Even-numbered rhombomeres control the apoptotic elimination of neural crest cells from odd-numbered rhombomeres in the chick hindbrain. *Development* 119:233–245.
- Hall BK. 1998. Evolutionary developmental biology. Second Edition. London: Chapman & Hall.
- Hall BK, Hörstadius S. 1998. The Neural Crest. New York: Oxford University Press.
- Hanken J, Hall BK, editors. 1993. The skull, vols. 1–3. Chicago (IL): University of Chicago Press.
- Holland PW. 2000. Embryonic development of heads, skeletons and amphioxus: Edwin S. Goodrich revisited. *Int. J. Devel Biol* 44:29–34.
- Hunt P, Clarke JD, Buxton P, Ferretti P, Thorogood P. 1998. Stability and plasticity of neural crest patterning and branchial arch Hox code after extensive cephalic crest rotation. *Dev Biol* 198:82–104.
- Hunt P, Krumlauf R. 1991. Deciphering the *Hox* code: clues to patterning branchial regions of the head. *Cell* 66:1075–1078.
- Jacob M, Wachtler F, Jacob HJ, Christ B. 1986. On the problem of metamerism in the head mesenchyme of chick embryos. In: Bellairs R, et al. editors. NATO ASI series. Somites in developing embryos. London: Plenum Press New York. p. 79–89.
- Jacob RSM, Jacob HJ. 1993. The avian prechordal head region: a morphological study. *J Anat* 183:75–89.
- Jacobson AG. 1988. Somitomeres: mesodermal segments of vertebrate embryos. *Development* (104 Suppl):209–220.
- Jacobson AG. 1993. Somitomeres: mesodermal segments of the head and trunk. In: J Hanken, BK Hall, editors. The skull, vol. 1. Chicago (IL): Chicago Press. p. 42–76.
- Jacobson AG, Meier S. 1984. Morphogenesis of the head of the newt: mesodermal segments, neuromeres, and distribution of neural crest. *Dev Biol* 106:181–193.
- Jarvik E. 1980. Basic structure and evolution of vertebrates, vol. 2. New York: Academic Press.
- Jiang X, Iseki S, Maxson RE, Sucov HM, Morriss-Kay GM. 2002. Tissue origins and interactions in the mammalian skull vault. *Dev Biol* 241:106–116.
- Johnston JB. 1905. The morphology of the vertebrate head from the viewpoint of the functional division of the nervous system. *J Comp Neurol* 15:175–275.
- Jouve C, Iimura T, Pourquie O. 2002. Onset of the segmentation clock in the chick embryo: evidence for oscillations in the somite precursors in the primitive streak. *Development* 129:1107–1117.
- Kardong KV. 1998. Vertebrates: comparative anatomy, function, evolution. Boston (MA): McGraw-Hill.
- Kessel M. 1992. Respecification of vertebral identities by retinoic acid. *Development* 115:487–501.
- Köntges G, Lumsden A. 1996. Phombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* 122:3229–3242.
- Kuratani S. 2003. Evolutionary developmental biology and vertebrate head segmentation: a perspective from developmental constraint. *Theory Biosci* 122:230–251.
- Kuratani S, Horigome N, Hirano S. 1999. Developmental morphology of the cephalic mesoderm and re-evaluation of segmental theories of the vertebrate head: Evidence from embryos of an agnathan vertebrate, *Lampetra japonica*. *Devel Biol* 210:381–400.
- Kuratani SC. 1991. Alternate expression of the HNK-1 epitope in rhombomeres of the chick embryo. *Dev Biol* 144:215–219.
- Kuratani SC, Eichele G. 1993. Rhombomere transplantation repatterns the segmental organization of cranial nerves and reveals autonomous expression of a homeodomain protein. *Development* 117:105–117.
- Le Douarin NM. 1982. The neural crest. Cambridge: Cambridge University Press.
- Le Lièvre CS. 1974. Rôle des cellules mesectodermiques issues des crêtes neurales céphaliques dans la formation des arcs branchiaux et du squelette viscéral. *J Embryol Exp Morphol* 31:453–577.
- Le Lièvre CS. 1978. Participation of neural crest-derived cells in the genesis of the skull in birds. *J Embryol Exp Morphol* 47:17–37.
- Lumsden A, Keynes R. 1989. Segmental patterns of neuronal development in the chick hindbrain. *Nature* 337:424–428.
- Lumsden A, Sprawson N, Graham A. 1991. Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. *Development* 113:1281–1291.
- Meier S. 1979. Development of the chick mesoblast. Formation of the embryonic axis and establishment of the metameric pattern. *Dev Biol* 73:25–45.
- Neal HV, Rand HW. 1946. Comparative anatomy. Philadelphia (PA): Blakiston.
- Noden DM. 1983. The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. *Dev Biol* 96:144–165.
- Noden DM. 1988. Interactions and fates of avian craniofacial mesenchyme. *Development* 103 (Suppl):121–140.
- Northcutt RG, Gans C. 1983. The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Quart Rev Biol* 58:1–28.
- Oken L. 1807. Über die Bedeutung der Schädelknochen. Bamberg (Germany): Göbhardt.
- Owen R. 1848. On the archetype and homologies of the vertebrate skeleton. London: J. Van Voorst.
- Palmeirim I, Henrique D, Ish-Horowicz D, Pourquie O. 1997. Avian *hairy* gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* 91:639–648.
- Platt JB. 1893. Ectodermic origin of the cartilages of the head. *Anat Anz* 8:506–509.

- Portmann A. 1969. Einführung in die vergleichende Morphologie der Wirbeltiere. Basel (Switzerland): Schwabe & Co.
- Prince V, Lumsden A. 1994. *Hoxa-1* expression in normal and transposed rhombomeres: Independent regulation in the neural tube and neural crest. *Development* 120:911–923.
- Riedel-Kruse IH, Muller C, Oates AC. 2007. Synchrony dynamics during initiation, failure, and rescue of the segmentation clock. *Science* 317:1911–1915.
- Rijli FM, Mark M, Lakkaraju S, Dierich A, Dollé P, Chambon P. 1993. Homeotic transformation is generated in the rostral branchial region of the head by disruption of *Hoxa-2*, which acts as a selector gene. *Cell* 75:1333–1349.
- Romer AS. 1966. Vertebrate paleontology. Chicago (IL): Chicago University Press.
- Romer AS, Parsons TS. 1977. The vertebrate body. 5th edition. Philadelphia (PA): Saunders.
- Schoppmeier M, Damen WG. 2005. Suppressor of hairless and presenilin phenotypes imply involvement of canonical Notch-signalling in segmentation of the spider *Cupiennius salei*. *Dev Biol* 280:211–224.
- Slack JM, Holland PW, Graham CF. 1993. The zootype and the phylotypic stage. *Nature* 361:490–492.
- Tam PP, Trainor PA. 1994. Specification and segmentation of the paraxial mesoderm. *Anat Embryol* 189:275–305.
- Trainor PA, Ariza-McNaughton L, Krumlauf R. 2002. Role of the isthmus and FGFs in resolving the paradox of neural crest plasticity and prepatterning. *Science* 295:1288–1291.
- Trainor PA, Tam PP. 1995. Cranial paraxial mesoderm and neural crest cells of the mouse embryo; co-distribution in the craniofacial mesenchyme but distinct segregation in branchial arches. *Development* 121:2569–2582.
- van Wijhe JW. 1882. Über die mesodermsegmente und die entwicklung der nerven des Selachierkopfes. *Ver. Akad. Wiss. Amsterdam (the Netherlands): Groningen*. p. 1–50.
- von Baer KE. 1828. Entwicklungsgeschichte der Thiere: Beobachtung und Reflexion. Königsberg (Germany): Born Träger.