

# Evolutionary Developmental Biology and Vertebrate Head Segmentation: A perspective from developmental constraint

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**Summary:** The question of vertebrate head segmentation has become one of the central issues in Evolutionary Developmental Biology. Beginning as a theory based in comparative anatomy, a segmental theory of the head has been adopted and further developed by comparative embryologists. With the use of molecular and cellular biology, and in particular analyses of the *Hox* gene complex, the question has been addressed at new levels, but it remains unresolved. In this review, vertebrate head segmentation is reevaluated, by introducing findings from experimental embryology and evolutionary biology. Developmental biology has shown that pattern is generated through hierarchically organized and causally linked series of events. The question of head segmentation can be viewed as a question of generative constraint, that is whether segmentation in the head is imposed by underlying segmental patterns, as it is in the trunk. In this respect, amphioxus appears to be segmented along the entire anteroposterior axis, with myotomes and peripheral nerves repeating with the same rhythm (somitomerism). Similarly, in the vertebrate trunk, the segmental patterns shared by myotomes, peripheral nerves and vertebrae are derived from the somites. However, in the head of vertebrates there is no such mesodermal pattern, although neuromerism and branchiomerism do indicate the presence of constraints derived from rhombomeres and pharyngeal pouches, respectively. These data fit better the concept of dual metamerism of the vertebrate body proposed by Romer (1972), than the traditional head cavity-based segmental model by Goodrich (1930).

## Introduction

– *Quelle bizarre époque! reprit Dotal, en le reconduisant. C'est juste au moment où le positivisme bat son plein, que le mysticisme s'éveille et que les folies de l'occulte commencent.*

– *Mais il en a toujours été ainsi; les queues de siècle se ressemblent. Toutes vacillent et sont troubles. Alors que le matérialisme sévit, la magie se lève. Ce phénomène reparait, tous les cent ans.*

J.-K. HUYSMANS – “Là-bas”

The question whether the vertebrate head is segmented or not was the origin of morphology itself. The term “Morphologie” was coined by the German poet, Goethe, who first tried to see serially arranged vertebrae in the dried skull of a young mammal (reviewed by Gaupp 1898). For Goethe, the vertebrate body was composed of serially arranged vertebrae (metamerism), each modified according to its position (metamorphosis) (Figure 1). In those days, the question of head segmentation belonged to comparative osteology, in which anatomists assumed various numbers of

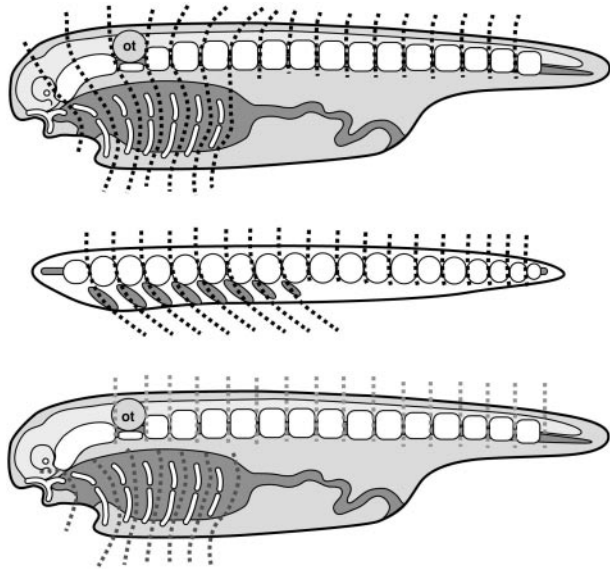


Fig. 1. Recognizing vertebrate head segmentation. Top: segmentalists view the vertebrate head as a serial array of segments equivalent to vertebrae or somites. In this extreme explanation of somitomerism, pharyngeal arches are reiterating with the same intervals as somites. Middle: simplified morphology of amphioxus. Head segmentation theory equates vertebrates with amphioxus as animals possessing a common basic morphological plan. Bottom: As an example of a non-segmental view of the vertebrate head, branchiomerism and somitomerism are recognized as different developmental programs or morphological patterns in the head and trunk part, respectively. ot, otocyst.

vertebrae in the skull of various vertebrate species (reviewed by de Beer 1937). For Goethe, to count the head vertebrae was to search for the “archetype” (*Urtyp*) which he believed was the manifestation of the *Urkraft* (internal force) that defines the basic architecture from which any animal form could be derived (*Ableitung*). As far as the occipital region of the gnathostome cranium is concerned, Goethe was correct, since the occipital bone is a somite derivative, similar to more caudal vertebrae (Couly et al. 1993). This does represent a trunk, or postotic part of the body, secondarily incorporated into the skull (reviewed by de Beer 1937). The real problem concerns the presence of vertebrae homologues in the preotic region of the head.

The vertebral theory of the skull was denied on embryological grounds in the Croonian Lecture by Huxley (1858), but comparative embryology took over the question of head segmentation and pushed it back to earlier developmental stages. For this smooth transition from idealistic morphology to evolutionary embryology, the research project conducted by Gegenbaur and Haeckel in Jena with no doubt played an important role (Nyhart, 1995). By anatomical examination of vertebrates, especially focused on the peripheral nervous system and its innervation patterns, Gegenbaur laid a foundation for the morphological framework of comparative embryology with the concept of serial homology, which he called Homodynamie (Gegenbaur, 1898; also see Mitgutsch, in this issue). Thereafter, embryonic patterns were thought to reflect the basic developmental plan shared by various vertebrate species, like a morphological archetype. Here again, the number of mesodermal segments was sought and counted

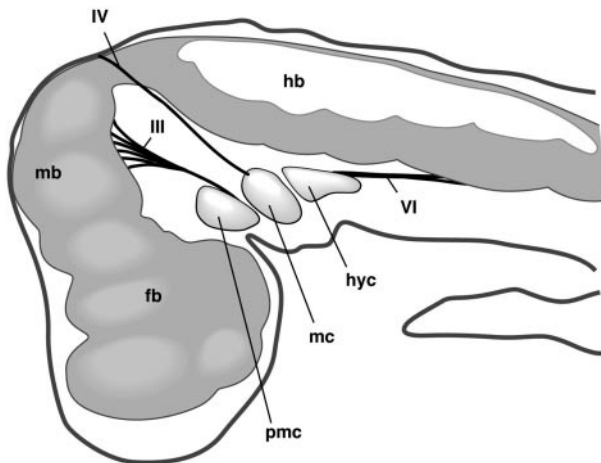


Fig. 2. Head cavities. Three pairs of mesodermal epithelial vesicles appear in the elasmobranch pharyngula. Note that three cranial nerves (cranial nerves III, IV, and VI) are innervating premandibular (pmc), mandibular (mc) and hyoid cavities (hyc), respectively. fb, forebrain; hb, hindbrain; hyc, hyoid cavity; mb, midbrain; mc, mandibular cavity; pmc, premandibular cavity; III, IV, and VI, cranial nerves. Redrawn from Neal and Rand (1942).

just as cephalic vertebrae had been a century before. Of the vertebrate species examined, most popular were the elasmobranch embryos that develop head cavities, overt mesodermal epithelial vesicles or the anlagen of extrinsic eye muscles (Figure 2; Balfour 1878; van Wihje 1882; reviewed by Goodrich 1930; de Beer 1937). Because these cavities occur in repeating units, their discovery initiated a second round of the quest for head segmentation.

The focus of debate was the identification of the serial homologues, that is, the assignment of each head structure to a corresponding mesodermal segment, and the determination of the number of these segments found in the head (Balfour 1878; van Wihje 1882; Platt 1891; Killian 1891; Rabl 1892; Dohrn 1890; Ziegler 1908; Koltzoff 1899; Hoffmann 1897; Sewertzoff 1899; Neal 1898; reviewed by de Beer 1937). Those scientists who tried to see the vertebrate skull as a serial array of mesodermal metameres can be collectively called “segmentalists,” and they shared the same structural view of morphology as Goethe (Fig. 1). Other scientists saw neuromeres as indicators of the head segments, (Locy 1895; Hill 1900), but neuromeres were often thought to arise from the presence of mesodermal segmentation (see Neal 1918).

Such a mesoderm-biased formulation of the segmentation of the vertebrate head implied the morphological plan of a possible ancestor: the vertebrate head composed of mesodermal segments was compared to the head of amphioxus, where segmentation clearly extends to the anterior end of the body (Neal 1918; Neal and Rand 1942). However, counting of head segments was not always constant, raising doubts about the presence of a common plan, and thereby repeating the confusion of the first round of this problem (Balfour 1878; van Wihje 1882; Platt 1894; Oppel 1890; Gornowitsch 1892; Marshall 1878, 1881; Ahlborn 1884). In the beginning of the twentieth century, the debate was summarized by Goodrich (1918, 1930), who also used elasmobranch embryos, and put forward the conclusion of the second round as a famous scheme (Figure 3). De Beer’s famous textbook also advocated the same basic idea (de Beer 1937). For a historical treatment of the embryonic version of head segmentation, see Singer (1989), Gee (1993) and Bowler (1996).

Whether the approaches were in comparative morphology or in embryology, most of the theories for head segmentation before Goodrich (1930) exhibited one common view for morphology, the shared plan for all the vertebrate species (Fig. 1; Owen 1848; Gegenbaur 1888). In the context of evolutionary biology, the search for head segmentation, particularly the attempts to equate vertebrates and amphioxus, could be taken as the search for deep homology, or a symplesiomorphic developmental pattern in the chordate head. Based on the archetype, this line of research could often be integrated to the phylogeny of chordates in modern zoology, as seen in Jollie (1977), Bjerring (1977), Jarvik (1980) and Jefferies (1986).

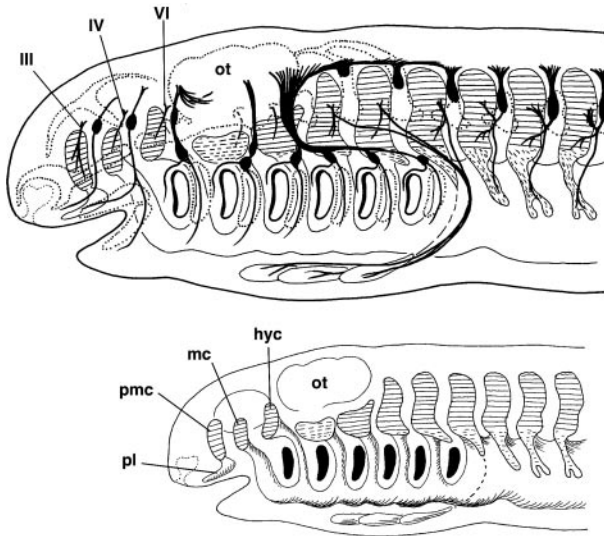


Fig. 3. Goodrich's conclusion. Top: Goodrich's scheme of head segmentation based on elasmobranch embryos. This complicated scheme shows the chondrocranium as well as the peripheral nervous system. Bottom: By removing the cartilage and peripheral nerves, it becomes clear that Goodrich's scheme is based on mesodermal segmentation. Head cavities found in elasmobranch embryos are equated with somites in the trunk. hyc, hyoid cavity; mc, mandibular cavity; ot, otocyst; pl, Platt's vesicle; pmc, premandibular cavity; III, IV, and VI, cranial nerves. Redrawn from Goodrich (1930).

More recently, a resurgence of interest in the problem of head segmentation has been tightly linked with the advance of molecular genetics. The question of head segmentation in this modern context was inspired by the discovery of the *Hox* code, an ordered array of homeobox-containing genes thought to determine rostrocaudal identity through their combinatorial presence. *Hox* genes are expressed along the anteroposterior axis of the embryo, especially in somites and in branchial arches and within the neural tube, thereby their gene expression patterns coincide with the boundaries of segmental units (neuromeres, pharyngeal arches and somites). Indeed, in the trunk these genes have been shown to specify a particular segmental unit at each axial level (Hunt and Krumlauf 1991; Hunt et al. 1991; reviewed by McGinnis and Krumlauf 1992). In the vertebrate head, however, homeotic transformations, the phenotype that results from altering the *Hox* complement of a particular segment, have only been found in the pharyngeal arches and the hindbrain segments (Rijli et al. 1993; Gendron-Maguire et al. 1993; Grammatopoulos et al. 2000; Pasqualetti et al. 2000), not in any structures reminiscent of head vertebrae.

The *Hox* genes themselves were originally discovered in the fruit fly, *Drosophila melanogaster*. After the identification of their homologues and the observation of their expression patterns in various metazoans with an anterior-posterior axis and left-right symmetry (Bilateria), the evolutionary sig-

nificance of the *Hox* code was soon recognized (Slack et al. 1993). The *Hox* code was thought to represent the most basic and ancient patterning program in animals, and this set of expression patterns was called the “zootype.” Curiously, modern molecular genetics often sounds like transcendental morphology when referring to conserved patterns of gene expression and the body plan. However, the developmental program also evolves. An excellent example of work that incorporates this evolutionary phylogenetic approach can be found in Hughes and Kaufman (2002) who analyzed the changes in the function and regulation of *Hox* genes in arthropods. Unfortunately, *Hox* gene evolution in chordates is still at a relatively ambiguous stage of elucidation when compared to arthropods.

Since the discovery of the *Hox* genes, a number of other developmental regulatory genes have been found in discrete domains in embryos, driving us to interpret the morphological architecture in the developmental program. The waves of re-ignited interest in the head segmentation problem has always arisen at the turn of centuries (*fin de siècle*). It may somehow attract scientists’ attention when established scientific fields come to certain points of their maturity. Without doubt, the old problem of head segmentation, or “Kopfproblem,” has found a new forum of debate in modern biology. In this review, I will discuss how we can deal with this old and new problem now. By introducing concepts obtained by experimental embryology and evolutionary biology, I will emphasize the meaning of this question, and suggest an answer in the context of Evolutionary Developmental Biology.

## The phylotype as a developmental constraint

The generalized vertebrate body plan can be characterized embryologically by the presence of somites in the trunk, two pairs of fin- or limb buds, pharyngeal arches in the head, a segmented dorsal neural tube, and so forth. In addition, as the fourth germ layer specific to vertebrates (Hall 1998), neural crest-derived cells contribute to various tissues and organs (reviewed by Le Douarin, 1982). The crest cells show distinctive patterns of distribution in the head and in the trunk (see Kuratani 1997). Cephalic crest cell populations do not show the clear metameric pattern seen in the trunk, where the cells are segmentally associated with somites. Unlike in amphioxus, no typical somites develop in the vertebrate head.

The above embryonic patterns are shared by a variety of gnathostomes (Hertwig 1906), and are considered to play a fundamental roles in subsequent developmental processes. For developmental burdens derived from later stages (Riedl 1978), these structures should be preserved, resulting in the conserved embryonic morphology through stabilizing selection. Such a pattern is also called the “phylotype,” the constrained developmental pat-

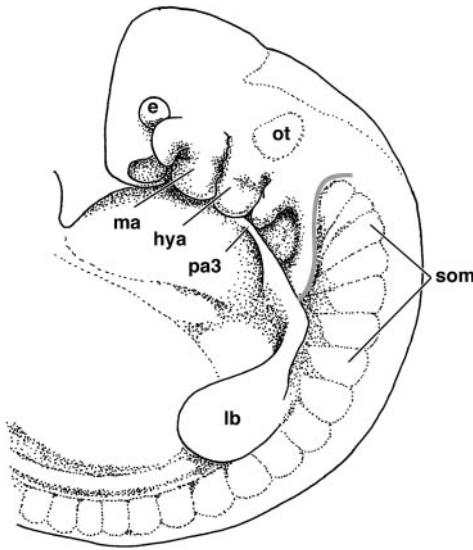


Fig. 4. Mammalian pharyngula. At an organogenetic stage, vertebrate embryos share a common morphological pattern. Pharyngeal arches are specific to the head, and somites appear postotically in the trunk. Dissociation of branchiomeres and somitomeres is emphasized by a gray line in this drawing. e, eye;hya, hyoid arch; lb, limb bud; ma, mandibular arch; ot, otocyst; pa3, pharyngeal arch 3; som, somites. Redrawn from Froriep (1885).

tern seen at the organogenetic stage when a number of global cell-cell- and tissue interactions are functioning, possibly established through internal selection (Figure 4; Sander 1983; reviewed by Raff 1996). It is thus important to realize that the embryonic pattern, the topographic arrangement of developmental units and cell populations, reflects the underlying mechanisms that generate the adult morphology.

The phylotypic patterns described above are also seen in the lamprey, an agnathan species. The ammocoete larva of the lamprey used to be regarded as an intermediate animal linking amphioxus and gnathostome heads because of the presence of preotic myotomes (Neal 1918). However, histological observation has revealed that these rostral somites are nothing but secondarily shifted postotic myotomes and that all the typical myotomes develop postotically in this animal also (Kuratani et al. 1999). Furthermore, the lamprey has rhombomeres, segmental bulges in the hindbrain, with conserved topographic relationships with the branchiomeric nerve roots (Horigome et al. 1999; reviewed by Kuratani et al. 2001). Thus, the lamprey does not provide an intermediate form to derive the vertebrate phylotype, nor are the head segments clearly visible in agnathans, as usually expected.

### Pattern generation through developmental constraint

What is the meaning of metamerism? It does not simply mean the compartmentalization or epithelialization of tissues or the restriction of cell lineages associated with these phenomena, as is the case in somitogenesis or during the formation of rhombomeres (Fraser et al. 1990; Figdor and Stern 1993).

It also implies a higher order segmentation, one that is superimposed by primarily segmented tissues. For example, although pharyngeal pouches, or pharyngeal arches formed by the pouches, also show metamerism patterns, they do not arise as typical compartments. Due to the hierarchically organized epigenetic interactions of tissues, the presence of developmental units like pouches and somites exert morphogenetic influences on the patterning of other structures. What we know as branchiomerism (reiterating patterns of organs associated with pharyngeal arches; Figure 1) includes not only the pharyngeal arch-, or pouch-derivatives, but also the structures secondarily, and epigenetically patterned by the presence of pharyngeal arches (see below). Various structures with metamerism patterns can arise as non-metameric tissues, upon which the metamerism pattern is only secondarily imposed. Not only the metamerism segmentation itself, but its epigenetic interaction with other tissues can result in a metamerism morphology.

For secondarily imposed metamerism patterns, we can apply the concept of generative constraint (reviewed by Wagner 1994). This concept, in the present context, can be defined as the specific morphogenetic patterning effect imposed onto a tissue that does not possess its own morphological patterns *per se*. Since all the developmental processes are causally linked, every pattern can be generated as the constraint of surrounding structures to some extent. However, by limiting the types of generated patterns, it becomes possible to restrict this concept to certain morphological aspect of development such as somitomerism or branchiomerism patterns. Specifically, generative constraints can be recognized when the epigenetic interaction of embryonic tissues biases the following morphogenesis to limited patterns based on inevitable topographic relationships.

For example, trunk neural crest cells, the origin of spinal dorsal root ganglia, have no primary segmental pattern by themselves but the cells are generated and emigrate ubiquitously along the entire length of the neuraxis. A number of studies have shown that the segmental patterns of the trunk neural crest cells is imposed by the somites (Lehmann 1927; Detwiler 1934; Keynes and Stern 1984). Detwiler (1934) found that the number of spinal nerve roots perfectly matched the number of somites even after the experimental addition or removal of somites. Thus the metamerism pattern of the spinal nerves depends on the arrangement of the preexisting somites. Similar experiments have also been performed in chick embryos. In this animal, dorsal root ganglia can only arise in the rostral half of each somite where trunk neural crest cells are allowed to populate (Figure 5; Tosney 1982; Bronner-Fraser 1986; Teillet et al. 1987; Rickmann et al. 1985). Ventral motor neurons also arise ubiquitously along the neuraxis lateral to the floor plate, but are only allowed to sprout from the neural tube at the level corresponding to the rostral half of the somite (Keynes and Stern 1984). Finally, myelomeres, the neuromeres in the spinal cord, are also generated by the presence of somites (Lim et al. 1987, 1991).



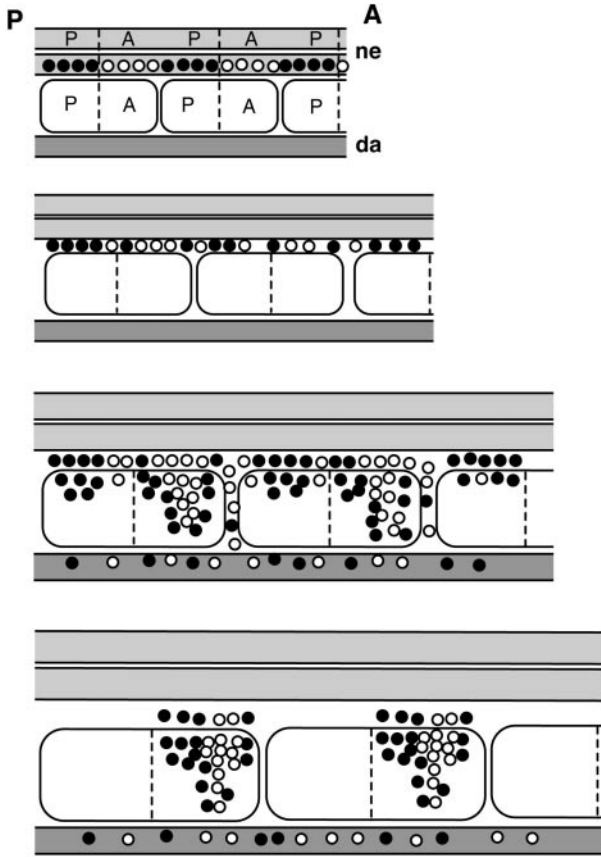


Fig. 5. Generative constraint and crest cell distribution. Trunk neural crest cells in the chick embryo have ubiquitous origins along the neuraxis (ne). During migration, however, the crest cells are only allowed to migrate and populate in the rostral half of a somite, thus resulting in the segmental distribution pattern of crest cells and eventually, a metamereric arrangement of dorsal root ganglia. da, dorsal aorta. Anterior (A) is to the right. Redrawn from Le Douarin and Dupin (1992).

Importantly, in the trunk no segmental pattern is primarily set up in the neuroectoderm. Movement of neurepithelial cell lineages is restricted at mid-somite levels, and removal of somites leads to obliteration of the compartments (Lim et al. 1991). Importantly, some comparative embryologists like Neal (1918) who focused on the neuromeres as indicator of vertebrate head metamerism, were aware that these trunk neurepithelial segments were secondarily generated by the presence of somites. Neal (1918) however inappropriately applied the same mechanism to rhombomeres or to even more rostral neuromeres in the brain that are not segmented by the constraint derived from the mesoderm (Källén 1954): the number of neuromeres and assumed number of head mesodermal segments (head cavities) never matched (reviewed by Neal and Rand 1942).

By invoking the concept of generative constraints, the metamerism of vertebrates can be viewed not only as the synthesis of primary repeating units such as somites and pharyngeal pouches and their derivatives, but also the patterns generated by the generative constraints by these units. From the above discussions, two categories can be discerned in the structures we recognize as units of trunk-specific metamerism, the somitomerism (somite-related metamerism; Figure 1). The first category is those somitomerical structures directly derived from somites, including vertebrae, myotomes, and dermatomes. The second category includes the structures under the generative constraint of somites, including myelomeres and spinal nerves.

### **Rhombomeres and pharyngeal pouches as generative constraints**

As seen in the generation of metameric patterns in the trunk, the potential metamerism of the head can be dealt with in a way similar to that for that trunk, i. e., by identification of generative constraints. Rhombomeres, for example, seem to cause generative constraints to some head structures. Unlike myelomeres that develop under the influence of somites, rhombomeres arise autonomously in the absence of head mesoderm (Källén 1956). When formed, even-numbered rhombomeres selectively adhere cephalic crest cells on their lateral aspects to create boundary caps of cranial nerve roots (Kuratani 1991; Kuratani and Eichele 1993; Niederländer and Lumsden 1996). Although crest cells are generated ubiquitously in the hind-brain, they are secondarily divided into three streams, being divided at levels of two odd-numbered rhombomeres, r3 and r5 (Sechrist et al. 1993; but also see Lumsden et al. 1991; Graham et al. 1993). Thus, the selective adhesion of crest cells onto even-numbered rhombomeres is a secondarily established pattern, not representing the localized origin of crest cells themselves. Sprouting of branchiomotor axons also depends on this pattern (Moody and Heaton 1983a, b, c). Rhombomere-specific cell adhesion molecules and cell-cell contact dependent signaling system are assumed to lie behind this selective adhesion (Nieto et al. 1992; Inoue et al. 1997; Smith et al. 1997; Garel et al. 2000; also see Kuratani 1991). It has also been shown that the rhombomeres actually serve as the generative constraint for cranial nerve roots. When an even-numbered rhombomere is transplanted to an ectopic site, it gives rise to an additional nerve root. On the contrary, odd-numbered rhombomeres are able to inhibit nerve root formation (Kuratani and Eichele 1993). Such an even-odd patterning by the rhombomeres seems to be restricted to the preotic region of the hind-brain since the postotic rhombomeres develop unsegmented nerve port on their lateral aspects to form the glossopharyngeal-vagal nerve complex (Kuratani and Eichele 1993). Rostral somites that overlap with the postotic

hindbrain impose the segmented pattern of hypoglossal nerve roots as seen in more caudal spinal nerves (see Shigetani et al. 1995 and Kuratani 1997).

The ventral part of the vertebrate head develops depending on the generative constraint derived from the pharyngeal pouches. Pharyngeal arches are the pharyngeal wall segmented by the pouches, including crest-derived ectomesenchyme and the mesoderm (reviewed by Graham et al. 2001). Epibranchial placodes are induced by the endodermal pouches as well (Begbie et al. 1999). Thus experimental elimination of the pharyngeal pouches leads not only to the absence of the pharyngeal arch and pouch-derivatives, but also to the loss of the inferior ganglia and the distal portion of the cranial nerves (Kuratani and Bockman 1992).

From the standpoint of generative constraints, the question of whether somitomerism and branchiomerism are or are not in register can be rephrased as the question: is there a common constraint that governs both the patterning of pharyngeal pouches and somites? There are no such developmental mechanisms described to date. Rather, there is ample circumstantial evidence to show that these two metameric systems develop independently from each other (Froriep 1885; de Beer 1922; Kingsbury 1926; Damas 1944; Kuratani et al. 1999; reviewed by Kuratani 1997). In this respect, the hagfish embryo also seems to belong to the same vertebrate morphogenetic pattern (Dean 1899; see Kuratani 1997).

The absence of somites in the preotic head is consistent with the presence of an alternative generative constraint of rhombomeres which function in the patterning of the cranial nerve roots. Actually, ectopically transplanted somites in the preotic head disturb the dorsolateral migration of crest cells, leading to inhibition of the cranial nerve root (Kuratani and Aizawa 1995). Thus, cranial and spinal nerves do not share the single generative constraint for nerve root patterning, and cannot be regarded as serial homologues. The origin of the constraint that patterns the cranial nerves is associated with the acquisition of rhombomeres, which are absent in the amphioxus. This constraint is a vertebrate-specific feature newly added after the divergence of vertebrates and other chordates (see below).

Amphioxus, which possesses a rostrally extending series of myotomes but no rhombomeres, has all the peripheral nerves arising from the intersomitic positions, even if their peripheral nerves contain elements functionally similar to branchiomic nerves in vertebrates (Fritsch and Northcutt 1993). We thus see that there is a different distribution of generative constraint along the neuraxis in these two animal groups. The vertebrate body plan is characterized by the presence of somite-derived constraint in the trunk and rhombomere- and pharyngeal pouch-derived constraints in the preotic head. The latter two patterns do not seem to originate from a single constraint either; neither the removal of hindbrain neural crest nor the transplantation of rhombomeres results in the alteration of the pattern of

arches (Guthrie et al. 1992; Kuratani and Eichele 1993; Veitch et al. 1999). It appears that the vertebrate-specific generative constraint in the preotic region is made possible not only by invention of rhombomeres, but that it is also permitted by the absence of a somite-derived generative constraint in this region. Whether such the a developmental pattern has been achieved by the modification of an amphioxus-like condition or not remains uncertain (see Kuratani et al., 1999 and Holland 2000).

Against the rule of dual metamerism caused by dual developmental constraints, there seems to be an ambiguity in the interface between the head and trunk of amniote embryos. Overlapping with some postotic pharyngeal arches, the rostralmost somites (the suprpharyngeal somites of Kuratani, 1997) behave differently from the more caudal ones, i. e., the rostral somites differentiates muscles innervated by the vagus and accessory nerves (Noden, 1983). How this discrepancy can be reconciled needs further investigation (also see the discussion about mesodermal fate mapping below).

## Segmentation in the head mesoderm

One of the central issues of head segmentation in a modern context concerns the presence of the cephalic somitomeres. The term somitomeres primarily refers to pseudosegmental blocks in the segmental plate, appearing before the somitogenesis. Based on observations using scanning electron microscopy, similar structures were also found in the chick embryonic head and were called cephalic somitomeres (Meier 1981; reviewed by Jacobson 1988, 1993). Meier, Jacobson and their colleagues described cephalic somitomeres in various vertebrate species (Anderson and Meier 1981; Meier and Tam 1982; Meier and Packard 1984), and related their findings to the segmentalists' view of head metamerism. However, morphological patterns of cephalic somitomeres are inconsistent among animal species: somitomeres with the same number do not always occupy identical positions in the head between species. Furthermore, the number of somitomeres in most of the observed species exceeds what had been assumed for elasmobranch embryos in classical comparative embryology (Goodrich 1930; also see Jacobson 1988, 1993).

In the past several years, there have been several lines of evidence presented to deny the existence of cephalic somitomeres. Freund and others (1996) plotted the positions of nuclei in the chick head mesoderm to analyze the contraction of the mesenchyme. They could not detect any periodic pattern comparable to what can be found in the segmental plate. We have also observed developmental patterns of the cephalic mesoderm in the lamprey and could not find any periodic pseudosegmental structures therein (Kuratani et al. 1999). In lampreys, each domain of the head meso-

derm is only regionalized secondarily by the surrounding structures such as the otic vesicle and pharyngeal pouches. The only possible segment is the premandibular mesoderm that develops directly from the prechordal plate. Finally, genes known to function in somitogenesis are not expressed in the expected pattern in the head mesoderm. In the chick segmental plate, cyclic expression of *hairy* gene is detected, and a new somite is added for each cycle (Palmeirim et al. 1997; Müller et al. 1996). In the head, only two cycles can be detected; one corresponds to the presumptive premandibular mesoderm, and the other to the rest of the cephalic mesoderm (Jouve et al. 2002), consistent with the observation in the lamprey. As far as the existence of an animal like amphioxus can imply that the vertebrate ancestor may have once possessed segmented head mesoderm, it would be difficult to entirely deny the possibility of the presence of cephalic somitomeres. In the context of the present review, however, it is more important to determine whether during vertebrate development there are any generative constraints that the hypothetical cephalic mesodermal segments could impose onto other tissues. As we have seen above, the pattern of structures recognized by comparative morphology as metamerism is not necessarily derived directly from segmented primordia. Only rhombomeres and pharyngeal pouches appear to serve as generators of repeating patterns in the vertebrate head. It is these generative constraints that can create anatomical pattern in the embryo. Whether somitomeres exist or not, vertebrate head mesoderm is apparently incapable of generating any metameric pattern in the head, due to its incapacity to exert any generative constraints on neighboring tissues.

## Head cavities

Even though somitomeres are apparently unreal, head cavities do exist in some gnathostome embryos. They are not only the sources of extrinsic eye muscles, but have a one to one topographical relationships with the eye-moving cranial nerves (cranial nerves III, IV, and VI). Head cavities were first described in shark embryos by Balfour (1878), and were re-defined by van Wijhe (1882) as the paraxial portion of the mesoderm. Although the developmental sequence of the head cavities still remains unclear, they cannot be equated with early enterocoelic mesoderm. Head cavities are overt epithelial coelomic vesicles embedded in the loose mesenchyme of the embryonic head (Figure 2).

Three pairs of head cavities are termed, from rostral to caudal, premandibular, mandibular and hyoid cavities. Of those, the mandibular cavity always arises between the ophthalmic and maxillomandibular branches of the trigeminal nerve. In addition to these, another pair of cavities is found in some elasmobranch species, and are called Platt's vesicles after the name

of the embryologist who first discovered them (Platt 1891; see Jarvik 1980 and Jefferies 1986 for their morphological consideration). This pair of cavities, however, most likely represent a portion or secondary growth of the premandibular cavity (see Jefferies 1986; also see Goodrich 1930 for its inclusion into the embryonic archetype).

Based on the above definition, lamprey embryos do not show head cavities in development (Kuratani et al. 1999). However in most gnathostome species some head cavities have been found. Premandibular and mandibular cavities are found in sturgeons (Kuratani et al. 2000). In amniotes, epithelial cysts have been described in association with the extrinsic eye muscle development, probably corresponding to the premandibular cavity (Adelmann 1925, 1927; Fraser 1915; reviewed by Brachet 1935; Wedin 1949; Jacob et al. 1984).

The developmental significance of head cavities is still unclear. Although they are the anlagen of the extrinsic eye muscles, these same muscles develop normally in animals that do not have head cavities from the part of the head mesoderm located in positions similar to head cavities (Noden 1983, 1988; Wachtler et al., 1984; Jacob et al., 1984; Wachtler and Jacob, 1986; Couly et al. 1992). As discussed previously (Wedin 1949; Kuratani and Horigome 2000; Kuratani et al. 2000), the head cavities may represent a synapomorphy for gnathostomes, but not for vertebrates or chordates. Although vertebrate ancestors may have possessed preotic mesodermal segments, it does not seem appropriate to assign to the head cavities the status as primary segmental units of the vertebrate head. Moreover, the head cavities in gnathostome embryos do not seem to impose any generative constraints that would yield metameric pattern for the head morphology. Aside from the nerves innervating the extrinsic ocular muscles, the only embryonic structure that shows a fixed topographic relationship with the cavities during embryogenesis is the trigeminal nerve, which bifurcates on anterior and posterior aspects of the mandibular cavity (Kuratani and Horigome 2000, Kuratani et al. 2000 and refs. therein). This relationship may result in elasmobranch-specific shift of the trigeminal nerve root from r2 to r3 (reviewed in Kuratani and Horigome 2000). This may be the obliteration of a primary head metamerism (rhombomerism), but it is not the generation of a primary segmental pattern of cranial nerves.

In relation to somitomeres, it may be of interest to note that mapping data of the chick head mesoderm based on chick/quail chimeras differ depending on developmental stages of the embryos employed (Noden 1983, 1988; Couly et al. 1992; Figure 6). When stage 10 embryos are used, the developmental fate of each part of the head mesoderm appears to correspond to Goodrich's scheme in terms of anteroposterior specification. Before that stage, the cephalic mesoderm does not seem to be allocated in the same topographical order as that of the head cavities or pharyngeal arches (Figure 6). It may be that the head mesoderm is secondarily regionalized ac-

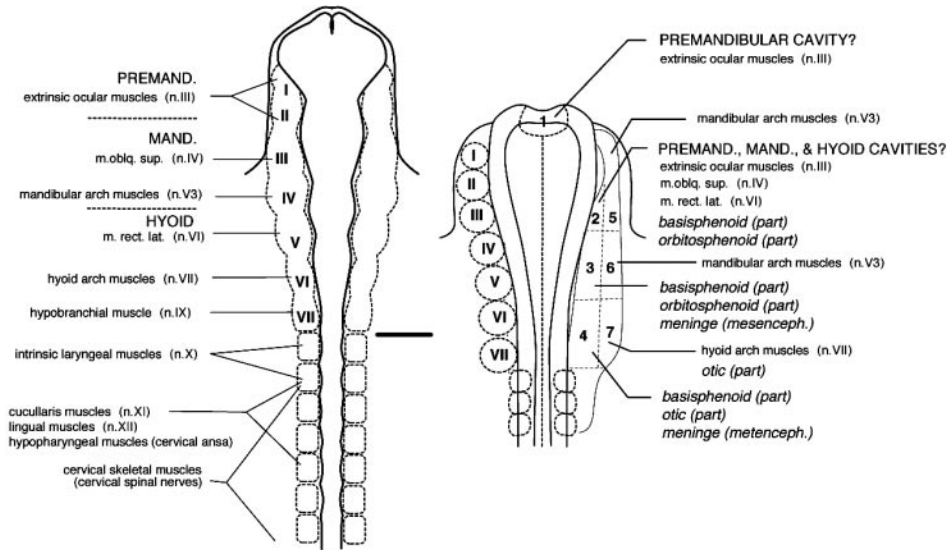


Fig. 6. Developmental fate of the chick head mesoderm. Left. Mesodermal mapping based on the stage 10 chick embryo by Noden (1988). The cephalic mesoderm appears to be specified anteroposteriorly as predicted by Goodrich's scheme; the rostral part differentiates into the premandibular cavity-derivative, the middle part into the mandibular cavity- and mandibular arch mesoderm-derivatives, and the posterior part into the hyoid cavity and hyoid arch mesoderm-derivatives of the shark embryo. Right: Similar mapping data based on stage 8 embryo by Couly et al. (1992). Although the mesoderm is mediolaterally specified in terms of histogenesis (the medial part differentiates into the chondrocranium), the anteroposterior specification of the mesoderm is not as clear as at stage 10 (left). Roman numerals I-VII indicate supposed somitomers. Based on Noden (1988) and Couly et al. (1992).

According to the pattern predicted by the phylotype towards the pharyngula. Whatever their developmental significance may be, the head cavities may also be components of the typical pharyngula morphology based on the presence of pharyngeal pouches and otocyst, not part of the primary segmentation of the paraxial mesoderm that arises together with axis formation.

## Head segmentation and evolution

To ask whether the head is segmented or not, it is important to specify the context of the question. If it is a question as to the embryonic generative constraint that results in the segmental architecture in the anatomical plan of the embryonic or adult head, it must be asked whether there are segmental developmental units at the organogenetic period, namely during the phylotypic stage (Figure 4). In this sense, amphioxus is entirely segmented based on the arrangement of somites. In the vertebrate head, on the other hand, there is no segmental pattern imposed by any developmental units. Unlike in the trunk, the head mesoderm of vertebrates does not

impose any metameric pattern on the crest cell distribution or cranial nerve morphology. This developmental distinction is directly related to the distinct migration pathways of the crest cells between the trunk and the head. In the trunk, the dorsolateral pathway of the crest cells appears to be inhibited by glycoconjugates secreted by dermomyotomes (Oakley et al. 1994; also see Kuratani and Aizawa, 1995), whereas in the head, the head mesoderm is not epithelialized and allows a widespread dorsolateral cell migration. Because the crest cells contribute to a large part of vertebrate head structures, the head has to be embryologically evaluated very differently from the trunk. In terms of spatial distribution of generative constraints, the dual segmental body plan put forth by Romer (1972) appears most appropriate for the vertebrate body plan as well as the phylotype (Figure 7).

As to the evolutionary origin of vertebrate-specific generative constraints, we have to ask the question of the origin of unsegmented head mesoderm. In this connection, Neal and Rand (1942) pointed out the possibility that the segmental nature of the head mesoderm becomes obscured gradually and on the contrary neuromeres become clearer, as we move from the amphioxus through the lamprey and shark embryos towards the amniotes. Although this idea appropriately captures the transition of developmental constraint as the evolutionary motivation for changes in the head patterning, the lamprey does not represent an intermediate organism but belongs to the same phylotype of vertebrates (see above). Nor do the head cavities in the shark embryo represent primary segments of paraxial mesoderm, as noted above. Furthermore, the head cavities do not serve as a generative constraint to pattern the cranial nerves. The question is therefore when and how the condition of vertebrates originated.

There are several possibilities of how the vertebrate body plan was achieved from a hypothetical amphioxus-like condition. For example, some of the rostral somites in the conjectured ancestor may have lost their

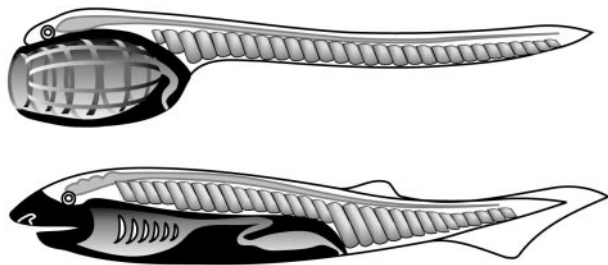


Fig. 7. Romer's vertebrate body as a dual metameric organism. Comparing a tunicate larva-like ancestor and the vertebrate body, Romer pointed out that there are two independent metameric patterns in vertebrates. Although the tunicate-origin of vertebrates has to be reexamined, his recognition of the dual nature of the vertebrate body is close to the conclusion of this review. Redrawn from Romer and Parsons (1977).



epithelial segmentation. If that were the case, it would be justified to compare specific parts of the head mesoderm with specific rostral somites in amphioxus, with the help of regulatory gene expression boundaries as markers (see Holland, 2000 and refs. therein). In this case, the origin of the vertebrate head would be partly a question of the evolution of the antero-posterior specification of the mesoderm. In this context, it may be of interest to note that the otic placode, which divides the head mesoderm and trunk somites, is induced by the specific axial level of mesoderm that secretes FGF19 (Ladher et al. 2000).

An alternative hypothesis that has been suggested for the origin of the vertebrate head is that a new part was added in the rostral part of the body axis; however this does not presently appear to be a viable hypothesis. The body axis of amphioxus and vertebrates seem to share the same front end. The rostral neural tube is comparable between the vertebrates and amphioxus in terms of several homeobox gene expression patterns (reviewed by Murakami et al. 2001) as well as the anatomical configuration (Lacalli et al. 1994): it appears to be the compartmentalization of neural tube into neuromeres that is missing in the amphioxus brain, not the forebrain itself (but also see Gans and Northcutt 1983). It is therefore highly improbable that the mesoderm alone has acquired a newly added front segment in the lineage of vertebrates. A third possible hypothesis would be, as implied by Jouve et al. (2002), that the second somite of the ancestral segmented animal has been enlarged and lost the epithelial nature. For this last scenario, it would have to be assumed that topographical relationships between the neural tube and mesodermal segments have shifted during evolution.

The question of head segmentation formerly was a debate about the morphological relation between amphioxus and vertebrates. It now has to be dealt with in terms of evolutionary developmental biology. When viewed as a problem of morphological patterning, it appears that vertebrate head mesoderm has no segmental pattern to impose on other structures. In this sense, the vertebrate head cannot be compared directly with the anterior end of amphioxus. Goodrich's segmental scheme may be specific to elasmobranch embryos, but it is inappropriate for describing vertebrates in general (Figure 3). The difference between an amphioxus-like ancestor and vertebrates has to be ascribed to changes in the developmental mechanisms that pattern head structures. As explained above, expanded distribution of cephalic crest cells and their topographical association with pharyngeal arches require the absence of cephalic somites in vertebrate embryos. What initiated the evolution of vertebrate body plan will continue to be an intriguing issue of Evolutionary Developmental Biology. Without a doubt, the achievement of the vertebrate head pattern is profoundly linked to the origin of neural crest cells, the vertebrate specific cell lineage, as well as to the embryonic environment that provides vertebrate-specific generative constraints.

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