Amphioxus and the evolution of head segmentation

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Synopsis Whether or not the vertebrate head is fundamentally segmented has been controversial for over 150 years. Beginning in the late 19th century, segmentalist theories proposed that the vertebrate head evolved from an amphioxus-like ancestor in which mesodermal somites extended the full length of the body with remnants of segmentation persisting as the mesodermal head cavities of sharks and lampreys. Antisegmentalists generally argued either that the vertebrate ancestors never had any mesodermal segmentation anteriorly or that they lost it before the origin of the vertebrates; in either case, the earliest vertebrates had an unsegmented head and the embryonic cranial mesoderm of vertebrates is at best pseudo-segmented, evolving independently of any pre-vertebrate segmental pattern. Recent morphologic studies have generally confirmed the accuracy of the major classical studies of head development in lampreys and sharks, yet disagree with their theoretical conclusions regarding the evolution of head segmentation. Studies of developmental genes in amphioxus and vertebrates, which have demonstrated conservation of the mechanisms of anterior–posterior patterning in the two groups, have shed new light on this controversy. Most pertinently, some homologs of genes expressed in the anterior amphioxus somites, which form as outpocketings of the gut, are also expressed in the walls of the head cavities of lampreys, which form similarly, and in their major derivatives (the velar muscles) as well as in the eye and jaw muscles of bony gnathostomes, which derive from unsegmented head mesoderm. These muscles share gene expression with the corresponding muscles of the shark, which derive from the walls of head cavities that form, not as outpocketings of the gut, but as secondary cavities within solid blocks of tissue. While molecular data that can be compared across all the relevant taxa remain limited, they are consistent with an evolutionary scenario in which the cranial paraxial mesoderm of the lamprey and shark evolved from the anterior somites of an amphioxus-like ancestor. Although, bony vertebrates have lost the mesodermal head segments present in the shark and lamprey, their remnants persist in the muscles of the eye and jaw.

Segmentalist/antisegmentalist controversies

The idea that the vertebrate head is fundamentally segmented like the trunk began with Goethe and Oken, who argued that the skull bones were modified vertebrae (reviewed by Olsson et al. 2005). Subsequently, Huxley (1857–1859) proposed that, although the skull was not segmented, there were still head segments as evidenced by the cranial nerves and branchial arches. The next change in emphasis was initiated by Balfour (1876), who, based on his discovery of serially repeated head cavities in shark embryos, concluded that the head mesoderm is organized into segments comparable to the somites more posteriorly in the body. Finally, Locy (1894) and others proposed that serially repeated dilations in the neuroepithelium of the developing brain were the earliest and best evidence for head segmentation.

By the early part of the 20th century, the prevailing view of vertebrate head organization was that segmental units of mesoderm alternated with segmental gill slits. The strongest proponents of a segmentally-organized head in vertebrates (Goodrich 1930) held that the mesodermal head somites, branchial arches and cranial nerves were in register with one another (except perhaps for a few secondary losses). In the aggregate, such views have been called “segmentalist,” although there was considerable variability in the degree of head segmentation proposed from one author to the next.

In general, segmentalists agreed that vertebrates evolved from an invertebrate ancestor in which several tissues were segmented all the way to the anterior end of the body. Some went so far as to propose homologies between the mesodermal head segments of vertebrates and coelomic structures of extant amphioxus, which they considered to be a...
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ported discovery of segmented mesodermal units
interpretation. Second has been the pur-
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and paraxial mesoderm (Noden and Trainor 2005); this coopera-
tion, although of great interest, certainly introduces
discussion of cranial motor innervation. Second, it has been the
portrayal of segmented mesodermal units
termed head somitomeres in developing vertebrates; in contrast, Smith and Newth (1917)
suggested that the first pair of muscular somites of amphioxus correspond to the mandibular somites of
vertebrates.

Opposition to segmentalism arose late in the nine-
teenth century and continues to this day (Froriep
McMurrich 1912; Romer 1972; Kuratani et al. 1999; Olsson et al. 2005). Although such
authors have been grouped under the heading of
antisegmentalist, they represent a considerable
diversity of opinions (reviewed by Delsman 1922). Features that unite antisegmentalist are: (1) their
emphasis that mesoderm is the only relevant tissue in
paragraphs of cells, (2) their belief that
the mesoderm is fundamentally unsegmented in the
head of all vertebrate embryos, and often (3) their
proposals that vertebrates evolved from an inverte-
brate ancestor rather like a tunicate tadpole larva or a
calcichordate (Minelli 2000).

In recent decades, three developments have added
new levels of complexity to the old segmentalist/
antisegmentalist argument. First, head mesenchyme
of cranial neural crest origin is now known to
interact with the paraxial mesoderm during mor-
phogenesis (Noden and Trainor 2005); this coopera-
tion, although of great interest, certainly introduces
difficulties at the levels of both experimental tech-
nique and interpretation. Second has been the
portrayal of segmented mesodermal units
termed head somitomeres in developing vertebrates
(Meier 1979). Although, such structures have some-
times been invoked as supporting the segmentalist
point of view, their significance (and even their very
existence) is still being debated (Pourquier 2000; Noden and Trainor 2005). Third, studies of develop-
mental gene expression in amphibious and verte-
brates have added another dimension to comparisons
between amphibious and vertebrates (Holland et al.
Kusakabe and Kuratani 2007).

The focus of the present review is on the head
mesoderm in amphibious and vertebrates. Although
tunicates are now considered to be the sister group
of vertebrates (Bourlat et al. 2007), they are not
relevant for the arguments concerning the evolution
of head segmentation since they have apparently lost
muscular somites. The mesenchyme of the larval
trunk gives rise in part to the adult muscles, which
have no clear counterparts in either amphioxus or
vertebrates, while the central nervous system (CNS)
of the adult is reduced to a ganglion. We give special
attention to the head cavities and their enclosing
mesodermal layer in the developing heads of basal
vertebrates. We then consider the relevance of recent
developmental genetic data for the segmentalist/
antisegmentalist debate and for evolutionary schemes
deriving vertebrates from an invertebrate chordate
ancestor.

**Early evidence for head segmentation**

in shark embryos

Balfour (1875, 1876, 1877), as noted above, was the
first to use mesodermal structures as indications of
vertebrate head segmentation. The mesodermal cells
comprising each segment enclosed a central space,
which Balfour termed a “head cavity.” For shark
embryos, Balfour (1876) described three bilateral
pairs of head cavities (premandibular, mandibular,
and hyoid) in the preotic region of the head and
five (branchial) head cavities in the postotic region.
Balfour maintained that the three preotic head
cavities initially formed within the head mesoderm
by schizocoely, which opened up a single cavity con-
ected posteriorly with the lumen of the embryonic
gut—a connection that was never confirmed by later
workers. Subsequently, the single head cavity became
subdivided into three. He considered each of the
eight head segments to be equivalent to the muscle-
plate portion of a trunk somite with each in line
with a branch of a cranial nerve and with a visceral
arch (Balfour 1877). This was the germ of the idea
that segmentation of the head involved all of the
germ layers and that segmentation of the CNS
was essentially in register with segmentation of the
head mesoderm and pharynx. The likely fate of the
mesodermal cells associated with the preotic head
cavities was to differentiate into head muscles asso-
ciated with the jaws and the eye.

Marshall (1881) followed up Balfour’s studies by
demonstrating the origin of the lateral rectus muscle
from the hyoid mesodermal segment and proposed
that the abducens and facial nerves form the ventral
and dorsal roots, respectively, of a segmental nerve
to the hyoid region. The final and truly critical
contribution to the task of assembling mesodermal
segments and cranial nerves into a consistent theory
of head segmentation was provided by Van Wijhe
(1882). He refined Marshall’s suggested division of
the head cavities into somitic and visceral portions
and provided a relatively complete analysis of
the origins of the extraocular and branchial
arch muscles. Most importantly, he rationalized the system of dorsal and ventral nerve pairs so that each mesodermal segment was matched by a dorsal ganglionated nerve that contained the motor fibers to the branchial muscle plate and a ventral non-ganglionated nerve that provided the motor innervation to the dorsal, somitic portion. By refining the definition of a head cavity to include only the dorsal cranial mesoderm adjacent to the notochord, Van Wijhe established the view that head cavities are continuous with, but distinct from, the more ventrally located branchial muscle plates. The dorsal mesoderm which gives rise to the head cavities was proposed to be serially homologous to the epimere of the trunk from which somites derive, while the ventrolateral cranial mesoderm that lies in the branchial arches was serially homologous to the undivided hypomere (lateral plate mesoderm) of the trunk, the inner, splanchnopleural layer of which gives rise to the smooth musculature of the gut wall. Thus, the head cavities and trunk somites are members of a continuous somatic segmental series and the branchial muscles are specialized cranial visceral muscles. Van Wijhe’s scheme not only provided evidence that vertebrates had evolved from an amphioxus-like ancestor that was segmented all the way to its anterior end, but it also served as one of the theoretical bases for interpreting branchial nerves as “special” visceral nerves in the analysis by Gaskell, Strong, and others of the components of the cranial nerves.

Over the subsequent few decades, a host of studies examined the development of cranial mesodermal and neural structures in elasmobranchs. Studies on the morphogenesis of head cavities and the origins of eye and branchial muscles (Platt 1891a, 1891b; Edgeworth 1902; Lamb 1902) were complemented with studies of neuromeric and cranial nerve development (Neal 1898; Dohrn 1901), the goals of which were to establish a phyletogenetic theory for the origins of a segmental plan of the vertebrate head along the general lines proposed by Van Wijhe. These studies provided descriptions of the development of muscles and nerves that, except for a few disputed points, remain among the best and most valuable data on vertebrate craniogenesis. According to several authors, the innervation of the eye muscles reflected their inheritance from a segmented ancestor like amphioxus. For example, (Neal 1918) found that the third cranial nerve innervated four eye muscles (the rectus superior, rectus anterior, and rectus inferior and the inferior oblique) that derive from the premandibular head cavity, while the fourth and sixth cranial nerves innervated two eye muscles (the obliquus superior and rectus externus) deriving from the mandibular and hyoid cavities. He argued that these eye muscles are remnants of lateral trunk muscles that extended to the anterior tip of an ancestral vertebrate similar to amphioxus (Fig. 1). The mandibular head cavity of the shark not only gives rise to eye muscles (Marshall 1881), it also gives rise to muscles of the jaw (Edgeworth 1902) which are innervated by the fifth (trigeminal) nerve.

The production of serially repeated blocks of mesoderm that then hollow out by schizocoely is widely accepted, although antisegmentalists deny that these units qualify as segments. For antisegmentalists, the shark head mesoderm only appears to be segmented because it has been divided into serially repeating units by the active morphogenetic movements by neighboring tissues like the pharyngeal pouches. The most influential study combating the Balfour–Van Wijhe theory was that by Froriep (1902) on development of the electric ray, Torpedo, in which he concluded that early somitic segments extended as far forward as the first pharyngeal pouch but that the pro-otic head cavities arose from more rostral sources during the rapid growth of the head in subsequent stages.

**Organization of the head in lamprey embryos**

Stimulated by the work of Balfour and others on the head cavities of the shark, several authors undertook the study of lampreys to determine if these jawless vertebrates represented an intermediate condition between the shark and amphioxus (Figs. 1 and 2). The first extensive study was by Koltzoff (1902), who argued that segmentation of the mesoderm in lampreys and sharks was comparable. He was followed by (Neal 1918) and (Damas 1944). Neal not only agreed with Koltzoff concerning the homology of segmentation in the shark and lamprey, he reasoned that the mesodermal head segments of sharks and lampreys could well correspond to the first three muscular somites of amphioxus and, thus, that the “history of the eye-muscles may seem to be the history of the transformation of the first three myotomes of an Amphioxus-like ancestor into the definitive six eye-muscles of man.” The argument that the three head segments of lampreys and sharks were evolutionarily related to the anterior somites of amphioxus received further support from the results of Damas (1944) that, like the anterior somites of amphioxus, the lamprey head segments form enterocoelically and their lumens are continuous with that of the embryonic gut (Fig. 2C). He, thus, described a series of head somites in the lamprey that formed an
unbroken series with those derived from the tailbud (Fig. 2C). Moreover, Damas found that the walls of the mandibular head cavity gave rise to the muscles of the mouth and the velum, and, that like the jaw muscles of gnathostomes, these muscles are innervated by the trigeminal nerve (Lindstrom 1949). Taken together, these data point to an evolutionary scenario in which the three anteriormost somites of an amphioxus-like ancestor gave rise to the velar muscles and eye muscles of the lamprey, and to the eye muscles and some of the jaw muscles of both sharks and bony gnathostomes, even though the last have lost clear homologs of the head cavities. More recently, Jollie (1977), Forey and Janvier (1993), and Holland (1996) considered the possibility that the ancestral vertebrate may have been amphioxus-like and that the lamprey velum and gnathostome jaws might be evolutionarily related to the anterior somites of this ancestor.

In recent years, the case for mesodermal segmentation in the vertebrate head seemed to be supported by the discovery of transient condensations of cells, the so-called head somitomeres, anterior to the true somites in several gnathostomes, which were proposed to be remnants of head somites (Meier 1979; Tam and Trainor 1994). However, several studies could not confirm the existence of somitomeres, which are only visible by scanning electron microscopy (reviewed by Noden and Trainor 2005). Whether they exist or not, the absence of somite boundaries would not rule out the evolution of mesoderm in this region of the head from the anterior somites in an amphioxus like ancestor. Segmentation may simply have been lost, as it apparently has been in otherwise conserved cranial mesoderm that contributes to musculoskeletal structures in the otic region.

Current arguments against a fundamental segmentation of the mesoderm of the vertebrate head

Although arguments against segmentation of the head mesoderm have often invoked a tunicate- or calcichordate-like ancestor for the vertebrates, and have generally regarded amphioxus as a highly specialized survivor of an animal ancestral to vertebrates (Romer 1972), more recent views have taken into consideration the current phylogenetic position of amphioxus as basal in the chordates with tunicates as the sister group of vertebrates (Bourlat et al. 2006). For example, Kusakabe and Kuratani (2007) proposed that the vertebrate ancestor was segmented like amphioxus, but segmentation of the head was subsequently lost. This scenario admits that the head...
Fig 2. Development of head segments in the lamprey. (A) Ammocoete of *Lethenteron japonicum* showing seven gill slits. Anterior to the left in (A), (C), (D), and (E). (B) Serial cross-sections through the head of a lamprey. The more anterior section, on the left, shows the first somites or premandibular head cavities (s1) forming enterocoelically as outpocketings of the archenteron (arrows). The more posterior section passes through the second somite or mandibular head cavity (s2) also formed from outpocketings of the embryonic gut (arrows). (C) Longitudinal section of an early lamprey embryo showing that the prechordal plate, from which the premandibular head cavity develops, the mandibular and hyoid head cavities (s2 and s3), and the more posterior somites (s4–s6). (D) Anterior end of an early embryo of *L. japonicum* showing expression of *en grailed* at the midbrain/hindbrain boundary (MHB) and in the precursors of the lower lip muscles (*velothyroideus*) developing from the wall of the mandibular head cavity. (E) Longitudinal section through a slightly older lamprey embryo showing expression of *en grailed* in the upper lip mesoderm, the wall of the mandibular head cavity (arrow), and at the MHB. (F) Cross-section through the head of a lamprey embryo showing expression of *Engrailed* in the walls of the mandibular head cavities (arrows). (G–I) Expression of *Tbx1* in the lamprey *Lamproptera fluviatilis*. (G) Anterior end of a lamprey embryo at the same stage as in (E) showing expression of *Tbx1* in the upper lip mesenchyme, the walls of the mandibular head cavity, and in the mesenchyme of the pharyngeal arches. (H) and (I) Frontal and cross-section through H–H and I–I, respectively, in (G) showing expression of *Tbx1* in the mandibular mesoderm (mm). da, dorsal aorta; m, mouth; ph, pharynx; end, endoderm; va, ventral aorta. (A) Modified from Holland et al. (2004). (B) after Koltzoff (1902). (C) from (D—F) from Holland et al. (1993). (G–I) from Sauka-Spengler et al. (2002).
cavities of the lamprey arise enterocoelically like the anterior somites of amphioxus, but maintains that the head cavities of lampreys are not related to those of elasmobranchs, which are proposed to be a gnathostome synapomorphy that was gradually lost during gnathostome evolution. Thus, although the ancestral vertebrate inherited a single head cavity from an amphioxus-like ancestor, segmentation into three anterior–posteriorly arranged blocks of mesoderm was not intrinsic, but resulted from the active movements of neighboring tissues during the formation of the pharyngeal pouches. Arguing from this point of view, Kuratani et al. (1999) concluded that the head mesoderm of the earliest vertebrates and that of basal extant vertebrates was fundamentally unsegmented.

Kusakabe and Kuratani (2007) also criticized the idea that there was any true mesoderm segmentation in the head of embryonic sharks. One of their arguments was that, because the lamprey head cavities initially form by enterocoely and those of the shark do not, the head cavities of lampreys are not homologous to those of sharks or to any additional mesodermal structures in the head of other gnathostome embryos. Kusakabe and Kuratani (2007) also argued against such a homology on the basis of developmental genetic evidence. All of the lamprey somites, including extensions sent from more posterior somites into the head region, express myosin heavy chain 2 (Kusakabe et al. 2004; Kusakabe and Kuratani 2007). However, this gene is not expressed in the muscles that derive from the head cavities, although both types of muscle express muscle actin. Therefore, Kusakabe and Kuratani (2007) argued that the muscular mesoderm anterior to the otic vesicle derives from anterior migration of cells from more posterior somites and is unrelated to the muscles deriving from the head cavities. They did not include cell-labeling experiments to demonstrate such cell migrations directly.

The genetic mechanism of segmentation is conserved in amphioxus and vertebrates

Originally, arguments between segmentalists and anti-segmentalists were based on results from the developmental anatomy and histology of agnathans and gnathostomes. However, in the last twenty years, data on genes and development have refocused attention on amphioxus as the best available proxy for the invertebrate chordate ancestor of the vertebrates. The first developmental genetic study of amphioxus embryos showed that the anterior limit of Hox3 expression is at the level of the boundary between somites 4 and 5, compared to the boundary between rhombomeres 4 and 5 in vertebrate embryos, indicating that the amphioxus CNS has a homolog of the vertebrate hindbrain (Holland et al. 1992). Soon thereafter, Gilland and Baker (1993) suggested that the vertebrate head is “directly homologous with the entire primary gastrula of an ancestral chordate.” However, because of the 520 million years separating amphioxus and vertebrates, they added a note of caution in equating a modern amphioxus with this hypothetical ancestor.

More recent data on gene expression in amphioxus and vertebrate embryos have confirmed that the genetic basis of A/P patterning and segmentation of the amphioxus embryo is shared with vertebrates. Based both on expression of genes, including Hox genes and detailed neuroanatomy [reviewed in Wicht and Lacalli (2005)], the gastrula is equivalent to the vertebrate head anterior to rhombomere 5, while the mid-neurula is equivalent to the entire vertebrate head. The trunk begins to form at the late neurula stage as somites bud off from the tail bud. Segmentation of the amphioxus embryo begins to be established during the gastrula stage by nested expression of anterior Hox genes and the related homeobox gene Gbx in both ectoderm and mesoderm (Fig. 3) (Schubert et al. 2005, 2006; Castro et al. 2006). Thus, the rostral limits of these expression domains precede and direct the disposition of structures along the A/P axis. The rostral limit of Gbx is anterior to that of Hox1, which is in turn, anterior to that of Hox3. The Hox genes also display temporal collinearity with Hox1 being turned on at the early gastrula before Hox3. Hox4 turns on at the late gastrula stage and Hox6 during the neurula stage.

Importantly, amphioxus Hox genes are expressed in all three germ layers, including the mesoderm (Schubert et al. 2005), although the relatively weak signal in the latter tissue was overlooked in several earlier studies of amphioxus. Among the germ layers, the anterior limits of expression of a given gene are not necessarily in register (Fig. 3). Expression of the above genes in the CNS and the somites is slightly in advance of that in the gut endoderm. Gbx has the most anterior limit, which at the neurula stage lies between the cerebral vesicle and hindbrain in the CNS and between the first and second somites in the mesoderm, which is slightly anterior to the limit in the CNS. The limit in the gut endoderm coincides with that in the CNS and is between the mouth, thought to be a modified gill slit, and the first gill slit. The anterior limit of Gbx in the amphioxus CNS marks the amphioxus equivalent of the vertebrate
midbrain/hindbrain boundary (MHB). Expression of the forebrain/midbrain marker *Otx* abuts that of *Gbx* at this boundary just as it does at the vertebrate MHB (Castro et al. 2006). However, homologs of genes that confer organizer properties on the vertebrate MHB (e.g., *en-grailed*, *Wnt1*, *fgf8*) are not expressed at the *Gbx/Otx* boundary in the amphioxus CNS, suggesting that it does not function as an organizer (Castro et al. 2006). Even so, both patterns of gene expression and detailed neuroanatomy support the homology of the amphioxus cerebral vesicle with the vertebrate diencephalon; a homolog of the vertebrate telencephalon is lacking (reviewed in Holland and Holland 1999; Wicht and Lacalli 2005). The photoreceptor at the anterior tip of the amphioxus CNS has been homologized to the paired eyes of vertebrates (Lacalli 1996).

The anterior limits of *Hox* gene expression in both amphioxus and vertebrates are set by levels of retinoic acid (RA) signaling during the gastrula stage (Fig. 4). RA is a natural morphogen derived from vitamin A, which binds to heterodimers of the RA receptor (RAR) and the retinoid X receptor (RXR) that in turn bind to RA response elements (RAREs) in the regulatory regions of target genes. Because RAR is autoregulated, a pulse of RA during half the gastrula stage has lasting effects on morphogenesis during the neurula stage (Escriva et al. 2002). Increased RA expands the domains of *Hox* and other genes anteriorly; reduced RA shifts them posteriorly (Schubert et al. 2004, 2005, 2006). Thus, in amphioxus, the positions of somite boundaries in the dorsolateral mesoderm and of the pharyngeal gill slits plus the position and numbers of motor neurons in the CNS and the positions and numbers of ectodermal sensory cells all begin to be established at the gastrula stage and are all regulated by RA via *Hox* genes.

Although the developing amphioxus CNS lacks rhombomeres and neuromeres, having only a slight anterior swelling called the cerebral vesicle, gene expression and neuroanatomy indicate that the amphioxus CNS is also segmented. As noted above, the nested expression patterns of *Hox* genes demonstrate that the region of the CNS just posterior to the cerebral vesicle is homologous to the

Fig. 3 The anterior limits of *Gbx* and *Hox* genes in amphioxus differ in the three germ layers. *Hox*2 expression is not shown as it is too weak to be detected in germ layers other than the CNS. *Gbx*, (A) Initial A/P patterning is established at the gastrula stage by *Gbx*, *Hox*1, and *Hox*3, which are expressed in nested patterns. (B) By the neurula stage, the anterior limit of *Gbx* in the paraxial mesoderm is between somites 1 and 2, while that in the CNS is between the cerebral vesicle (forebrain/midbrain) and the hindbrain. The anterior limit in the endoderm is congruent with that in the CNS. The anterior limit of *Hox*1 in the paraxial mesoderm is more anterior than that in the CNS, which is more anterior than that in the endoderm. The anterior limits of *Hox*3 in the CNS and mesoderm are congruent and anterior to that in the endoderm, while the anterior limit of *Hox*4 in the CNS is anterior to that in the mesoderm, which in turn is anterior to that in the endoderm. (C) By the early larval stage, the anterior limit of *Gbx* in the endoderm can be seen to coincide with the boundary between the mouth and first gill slit, while that of *Hox*1 is just posterior to the primordium of the third gill slit. The anterior limits of *Hox*3, *Hox*4, and *Hox*6 in the endoderm are far posterior.
vertebrate hindbrain. The anterior limits of Hox gene expression approximately delimit cryptic rhombomere boundaries. In addition, the dorsal compartment motor neurons, which innervate the first muscles to differentiate, those used in slow undulatory swimming, are segmentally arranged with a single pair in line with each of the anterior somites, except for somite one, which has two pairs (Lacalli and Kelly 1999) (Fig. 4). These motor neurons are almost exclusively marked by expression of the ERR gene and are probably homologous to the hindbrain motor neurons of vertebrates, which are also marked by expression of ERR genes (Bardet et al. 2005). Both the numbers and the positions of these motor neurons are regulated by RA signaling via Hox1 (Schubert et al. 2006). The amphioxus CNS lacks ventral roots; the paraxial muscles send muscle tails to synapse directly with motor neurons at the periphery of the CNS (Flood 1968). However, the amphioxus CNS does have dorsal roots, which probably receive sensory input and are serially arranged like the dorsal roots of vertebrates (Wicht and Lacalli 2005). The effects of RA and the knockdown of Hox expression on the position of the cranial nerves have not been determined in amphioxus.

The amphioxus endoderm is also regionalized with a series of pharyngeal gill slits. As in the CNS, RA regulates Hox expression and the anterior limit of Hox1 establishes the boundary between the first three gill slits and the more posterior ones (Schubert et al. 2005). Knockdown of Hox1 expression shifts this boundary posteriorly (Schubert et al. 2005). Gbx marks the boundary between the larval mouth and the first gill slit. At metamorphosis, an amphioxus larva has 9–11 gill slits, which are approximately in register with the somites. It is not known how the positions of the gill slits in excess of three are established.

A/P patterning of the vertebrate embryo is mediated by the same genetic mechanism as in amphioxus. In vertebrates, as in amphioxus, nested expression of Gbx and Hox genes in all three germ layers mediates A/P patterning. Similarly, as in amphioxus, the anterior limits of Hox genes are set by levels of RA signaling. For example Hoxb1 is expressed in a stripe in rhombomere 4 in vertebrates and has an anterior limit in the amphioxus CNS at the level of the boundary between somites 3 and 4 (Fig. 5), while Gbx2 has an anterior limit at the MHB in vertebrates and between the cerebral vesicle and hindbrain in amphioxus. As in amphioxus, the anterior limits of the expression domains of these genes in the somites and endoderm are not in register with those in the CNS. In the chick, Gbx2 is expressed in the pharyngeal endoderm and ectoderm adjacent to the posterior hindbrain and later in the six most posterior somites and the anterior segmental plate, as well as in the mesenchyme of all the branchial arches. Otx2 and Pax6 mark the forebrain and midbrain in both amphioxus and vertebrates. The anterior limits of Gbx2 and the Hox genes are more posterior in both the somites and in the endoderm than in the CNS (Fig. 5). Expression of these genes in the unsegmented head mesoderm has not been described, but that could be because expression is not strong and no one has looked carefully. In the lamprey, as in other vertebrates, GbxA is expressed in the hindbrain with an anterior limit at the MHB and Hox genes are expressed in nested patterns in the CNS (Takio et al. 2004, 2007). No data were presented on expression of GbxA in other germ layers.
Gene expression is conserved in the anterior somites of amphioxus and in the mesoderm of the vertebrate head

The anteriormost 8–10 somites of amphioxus form enterocoelically. That is, beginning at the late gastrula/early neural stage, they are pinched off in an anterior to posterior direction from grooves in the dorsolateral walls of the archenteron (Holland et al. 1997). The subsequent somites pinch off from the tailbud, similar to vertebrate somites except that in amphioxus bands of presomitic mesoderm are lacking (Schubert et al. 2001). It seems likely that nested expression of Gbx and Hox genes establishes the positions of the anterior somites, starting at the early gastrula stage. The future somite boundaries are already delimited in the mid- to late-gastrula by the expression of several genes in stripes in the paraxial mesoderm, including Tbx15/18/22, Six1/2, and Delta (Fig. 6) (Beaster-Jones et al. 2006; Kozmik et al. 2007; Rasmussen et al. 2007). Following the onset of expression of Tbx15/18/22, Six1/2, and Delta, but just before the tissue begins to segment, the engrailed gene turns on in the future posterior portion of each of the enterocoelically-formed somites (Holland et al. 1997). Tbx1 then turns on in the ventral portion of the somites (Mahadevan et al. 2004). Later, at the mid-neurula, Tbx1 is also expressed in the ventral outgrowths from the somites that form the ventral mesoderm (Fig. 6E). Expression of Tbx1 is not restricted to the mesoderm, but is also expressed in the pharyngeal endoderm around the future gill slits. Of relevance for the idea that the vertebrate head mesoderm evolved from the anterior somites of an amphioxus-like ancestor, neither Tbx1/10 nor engrailed is expressed in the more posterior somites that form from the tailbud (Fig. 3).

Gene expression in the head cavities of the lamprey and head muscles of gnathostomes supports the evolution of the head mesoderm from the anterior somites of an amphioxus-like ancestor

A superficial resemblance has often been observed between an amphioxus larva or adult and the ammocoete larva of the lamprey, which has a pharynx with eight gill slits, a notochord, a dorsal hollow nerve cord and a series of somites that extend anterior to the otic vesicle (Fig. 2). Like amphioxus, the ammocoete is a filter feeder and lacks jaws.

Hox genes are also expressed in the pharyngeal arches. Hox1w is expressed in all the somites in the trunk, but as expression is strongest in the more posterior somites, it is not clear just how far anteriorly expression extends (Takio et al. 2007). Hox1w is expressed in pharyngeal pouch 8, while Hox2 and Hox3 have anterior limits between pharyngeal pouches 2 and 3 and 3 and 4, respectively. Mesodermal expression of these genes in the lamprey has not been described. Expression of Gbx and of 3’ Hox genes has not been described in the shark.

Because the anterior limits of Gbx and Hox genes are not in register from one germ layer to the next in either amphioxus or vertebrates, the segments of each germ layer may not have been in register in the ancestral vertebrate. Moreover, even though the somites segment before the motor neurons differentiate and the motor neurons in turn differentiate before the gill slits, it cannot be concluded that one segmented structure directs segmentation of other structures. Their positions may simply be directed by the same global patterning genes with the blueprint being read at different times in the different germ layers. Nevertheless, correlations between the positions of segmentally arranged structures in the various germ layers may prove useful in discussions of the evolution of segmentation when more gene expression patterns are known.
However, lampreys have many features in common with other vertebrates that amphioxus lacks. These include paired eyes, definitive neural crest and a MHB that probably functions as an organizer (Holland et al. 1997).

In the lamprey, the engrailed gene is not only expressed at the MHB as it is in other vertebrates, it is expressed in the mesenchyme of the upper lip and in the wall of the mandibular cavity (Fig. 2D–F), suggesting an inheritance from an ancestor like amphioxus, in which engrailed is expressed in the posterior wall of each anterior somite (Holland et al. 1993). In addition, tbx1 is expressed in both the walls of the mandibular cavity and in the upper lip of the lamprey (Fig. 2G–I) (Sauka-Spengler et al. 2002). These results are in concordance with the idea that the lamprey head cavities are homologs of the anterior somites of amphioxus, which also express these genes. In gnathostomes, tbx1 is expressed in one of the eye muscles, the lateral rectus, and in muscles of branchial arch 1 and 2 (reviewed by Noden and Francis-West 2006), while engrailed is expressed in two of the jaw muscles, the levator arcus palatini and the dilator operculi (Hatta et al. 1990) (Fig. 7E–G). Interestingly, while expression of amphioxus engrailed suggests a role in segmentation of the anterior somites (Holland et al. 1997) and the muscle-specific enhancer appears to be conserved with vertebrate en-2 (Beaster-Jones et al. 2007), the jaw muscle precursors expressing en-2 in the mouse do not segment, and the gene appears to be involved in regulating muscle properties (Degenhardt and Sassoon 2001). This suggests that the function of a gene can change, even though the tissues in which it is expressed are evolutionarily conserved. In addition, Pitx genes are expressed in the anteriormost somite on the left side in amphioxus, and in the first branchial arch and extrinsic eye muscles in gnathostomes (Boorman and Shimeld 2002; Noden and Francis-West 2006). Together with morphologic data showing that in the lamprey and shark, eye muscles, velar muscles (lamprey), or jaw muscles (shark) derive from the head cavities, the molecular data, although still fragmentary, support the segmentationalist scenario wherein the anterior somites of an amphioxus-like ancestor gave rise to the head cavities.
of agnathans and sharks and to eye and jaw muscles of bony gnathostomes.

To further test this theory, expression of additional genes should be examined in amphioxus, lampreys, and vertebrates. These include Foxl2, which, as noted below is expressed in the mandibular head cavity of the shark (Fig. 7A–C) and later in one of the extrinsic eye muscles, and capsulin, which is expressed in the extrinsic eye muscles of gnathostomes, but not in the paraxial musculature (Dastjerdi et al. 2007) as well as Lbx1 and HGF [reviewed by Noden and Francis-West (2006)].

**Are the head cavities of lampreys and sharks homologous?**

The argument as to homology of the mesodermal segments of lampreys and sharks has chiefly been
Based on their mode of formation. As noted above, Balfour (1875, 1876, 1877) found that the head cavities of the shark initially form as solid blocks of mesoderm with a cavity subsequently opening up, at least in the mandibular segment. However, he thought that the cavity in this segment subsequently became connected with the larval gut. This supposition confused the issue of whether the head cavities of the shark formed by schizocoely or enterocoely. The formation of the shark head mesoderm has been carefully reexamined with scanning electron microscopy and tissue sections (Gilland 1992) (Fig. 8). The prechordal, mandibular, and hyoid head cavities and the glossopharyngeal mesoderm are visible anterior to the first somite by stage 11/12 in line with the somites. Cross sections through the head of an

**Fig. 8 Development of the head of the shark, *Squalus acanthias*. (A) and (B) Scanning electron micrographs in stage 13 (A) and stage 14 (B) embryos. The insets show lower magnification of the whole embryos. Anterior to the left. (A) At stage 13, the neural folds are apposed, but not fused. The dashed line on the neural tube shows the border between rhombomere (C) and myelomere 1. The small arrows indicate the ventral edge of the mesoderm. (B) The arrow with the asterisk above rhombomere 6 indicates the caudal edge of the expanded portion of the neuroepithelium. In the inset, the axial levels of a few later-forming body regions are indicated by the arrows at somites 5, 7, 17. These are the future cranio-vertebral joint (somite 5), the rostralmost portion of the pronephros and first pectoral fin muscle bud (somite 7) and the caudal most pectoral fin muscle bud (somite 17). The asterisk above point b indicates the dorsal tongue of the hyoid mesoderm. (C) and (D) Brightfield micrographs of the right side of transverse sections through the head of a stage 6 embryo showing formation of the rostral mesoderm from the mesendoderm lining the archenteron. The mesoderm does not appear to form by enterocoely. Instead, the mandibular head cavity opens up later within the mandibular mesoderm. The small arrows indicate the medial limit of putative endodermal cells. The plain arrows indicate the lateral edge of the mesodermal lamina. The larger arrows with asterisks indicate the junction of the archenteron wall with the vitelline hypoblast. In (D), small white arrows indicate the basal surface of the endodermal epithelium. A, b, c, i, borders between mesodermal divisions; En, pharyngeal endoderm; H, hyoid mesoderm; G, glossopharyngeal mesoderm; M, mesomere in (A); mesoderm in (B), and (C); MC, mandibular head cavity; NP, notochordal plate; P, prosomere; PC, premandibular head cavity; PE, rostral portion of the prechordal plate; PP, prechordal plate; s1, s2, first and second somites; SE, surface ectoderm. Rhombomeres in the hindbrain are numbered. Magnifications: (A), (B) 200×; insets 40×; (B), (C), 320×. All photographs from Gilland (1992).
embryo at stage 6 show that these head cavities do not form enterocoelically. By stage 8, a large cavity has opened in the mandibular head cavity, but none is visible in the prechordal or hyoid mesodermal segments (Fig. 8F).

The lack of enterocoely in the formation of shark head cavities led Kusakabe and Kuratani (2007) to propose that they are not homologous to lamprey head cavities. In our opinion, the common derivatives such as eye and velar/jaw muscles that develop from the head cavities of lampreys and sharks indicate that the differences between schizocoely and enterocoely are not adequate grounds for ruling out the homology. The anterior and posterior somites of amphioxus are considered to be homologs, and, indeed, except for a few genes such as engrailed and Tbx1, they express the same suite of genes (Holland et al. 1997; Mahadevan et al. 2004; Beaster-Jones et al. 2006). Relatively few studies of developmental genes that might address the question of the homologies of the lamprey and shark head cavities have been carried out in sharks.

Only a few genes with expression in the cranial mesoderm of shark embryos have been described so far. Emx2 is strongly expressed in rostral paraxial mesoderm from gastrula through pharyngula stages in a pattern that appears to precisely delimit the mandibular mesoderm (Derobert et al. 2002). Expression data for the amphioxus homolog is unfortunately not known (Minguillos and Garcia-Fernández 2003). Foxl2 is expressed in the walls of the mandibular head cavity of the shark as well as in the pharyngeal arches and in later development in the mesenchyme around the eye (Wotton et al. 2007). Expression of this gene has not been studied in the lamprey or amphioxus. However, it is expressed in the mammalian eyelid, while humans heterozygous for FoxL2 mutations lack the levator muscle of the upper eyelid (Dollfus et al. 2003).

**Does it matter that amphioxus has 8–10 enterocoelic somites and lampreys and sharks have but 3?**

It is possible to construct several evolutionary scenarios for the transition from the head of an amphioxus-like ancestor to a gnathostome. Figure 1 shows Neal’s comparison of amphioxus, lampreys, and sharks which he considered to represent stages in the evolutionary history of the eye muscles (Neal 1918). He did not consider the branchial muscles in this scenario. In Neal’s view, the somites of amphioxus, lampreys, and sharks are homologous, but during development of the shark, the first, second, and ventral half of the third persisted as eye muscles while the dorsal part of the third and the 4–6th somites degenerated. As outlined above, more recent data including patterns of gene expression, are consistent with the eye muscles, as well as certain jaw muscles, evolving from the anterior somites of an amphioxus-like ancestor. Data from the lamprey indicate that the mandibular head cavity gives rise to the velar muscles and the premandibular head cavity to those of the eye (Damas 1944), while in the shark, it is the jaw muscles that arise from the mandibular head cavity, while the eye muscles arise from all three head segments (Marshall 1881; Neal 1918). However, labeling of the mandibular mesoderm in the lamprey with vital dyes shows that some labeled cells migrate near the eye (Kuratani et al. 2004); whether any of these labeled cells develop into eye muscles is uncertain. Expression of genes expressed in gnathostome eye muscles could be informative in this regard. In early embryos of lampreys, tbx1 does not appear to be expressed anterior to the otic vesicle, but later expression as the eye develops has not been determined (Sauka-Spengler et al. 2002). Expression of other markers of gnathostome eye muscles has not been determined.

Development of the head mesoderm of the other group of agnathans, the hagfish, has not been studied to any extent due to the dearth of hagfish embryos (Wicht and Northcutt 1995; Gorbman 1997). Hagfishes lack extrinsic eye muscles, but whether or not this lack is a derived feature of hagfish is uncertain. Certainly, if hagfish embryos become more available, a study of mesoderm development could be quite informative.

Even assuming a general homology between anterior somites of amphioxus and cranial mesoderm of vertebrates, the question remains whether there is a one-to-one correspondence between the somites, head cavities, and mesenchymal structures in various taxa. If there is such a one-to-one correspondence, then the three preotic and first five postotic mesodermal segments in agnathans should correspond to the enterocoelically-formed somites of amphioxus. Although the preotic mesoderm that forms the premandibular through hyoid segments in lampreys arises enterocoelically, the post-otic mesoderm does not (Damas 1944). Even so, differences in the mode of formation do not necessarily mean that the head cavities and somites of lampreys are not serial homologs. After all, the anterior and posterior somites of amphioxus are considered to be serial homologs even though they form by enterocoely and schizocoely, respectively. Therefore, except for tbx1 and engrailed, all of the
genes currently known to be expressed in the anterior somites of amphioxus are also expressed in the posterior ones (Beaster-Jones et al. 2006). The development of the jaw/velar muscles and the eye muscles from topographically similar cranial mesoderm in lampreys and gnathostomes suggests that the head somites/head cavities/mesenchymal masses in these animals are homologous in spite of variations in their mode of formation. It may be that delamination of mesoderm and subsequent formation of a central cavity by schizocoely is simply a variation on the theme of formation of head cavities by enterocoely. Moreover, there is as yet no evidence that segmentation of the head mesoderm in lampreys and sharks is imposed by the pharyngeal pouches as proposed by (Kusakabe and Kuratani 2007). A thorough study in these organisms of the developmental expression of homologs of genes involved in segmentation of the amphioxus mesoderm could be quite informative. Thus, in the lamprey, the first four postotic somites may be homologous to somites 4–7 of the amphioxus neurula. The three prootic segments may be homologs of the three anteriormost somites of the amphioxus neurula, with the caveat that the derivation of premandibular mesoderm from the prechordal plate may be unique to vertebrates. Bony gnathostomes have lost overt somitic patterning in cranial mesoderm, leaving remnants in the anlagen of eye and jaw muscles. Whether somitomeres exist or not as remnants of mesodermal head segments is irrelevant to arguments concerning the derivation of the eye and jaw muscles.

In conclusion, there are few novelties in the scheme depicted in Fig. 9 indicating that the anterior somites of an amphioxus-like vertebrate ancestor gave rise to the head cavities of agnathans (which became the developmental source of the velar and eye muscles) and to the head cavities (and jaw and eye muscles) of gnathostomes. Aspects of this scenario relating to the evolution of jaws were proposed by Jollie (1977); Forey and Janvier (1993); and Holland (1996), while theories of the evolution of the gnathostome eye muscles from the anterior somites of an amphioxus-like ancestor have a much longer history (Neal 1918). More recently, Gilland and Baker (1993) proposed that the vertebrate head was generated from the primary organizer and early gastrula of a hypothetical ancestor like the gastrula of amphioxus. More recent data from gene expression support this view (Yu et al. 2007). Although these data are limited, they are consistent with an evolutionary scenario in which the eye muscles in mammals are an evolutionary inheritance from the walls of ancestral vertebrate head cavities. Looking at expression of genes such as Foxl2 in the heads of sharks, lampreys, and amphioxus would be quite useful for shedding further light on the ongoing and still-contentious question of the evolutionary relationship between the head cavities of vertebrates and the somites of amphioxus.
Among their other attributes, somites can be seen as developmental packages for making certain types of muscle precursors, most basically, myotomal. With the increasing importance of non-myotomal eye and branchial muscles within vertebrate lineages, a corresponding reduction of somite formation occurred in the cranial mesoderm of the mandibular, hyoid, and otic regions. Conservation of topographic relationships between cranial mesoderm and the neural axis, as well as retention of expression of some genes expressed in anterior somites of amphioxus, suggest that suppression of somitogenic programs rather than creation of new anterior mesoderm was involved in the evolution of vertebrate head mesoderm from the anterior somites of an amphioxus-like chordate. Understanding the exact homologies between individual somites, head cavities, or somitomeres in amphioxus, lampreys, sharks, and bony gnathostomes may not be of critical importance to arguments of the evolution of head segmentation. The loss or gain of serially iterated structures has apparently occurred in the evolution of many groups, and it would not be surprising if amphioxus had gained some anterior somites and/or lampreys and sharks had lost some. However, additional data on morphology, cell fate, and gene expression could help clarify homologies between somites in amphioxus and the head mesoderm of vertebrates.

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References


Amphioxus and head segmentation


