Evolutionary Perspectives From Development of Mesodermal Components in the Lamprey

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Lampreys, a jawless vertebrate species, lack not only jaws but also several other organs, including ventral migratory muscles shared by gnathostomes. In the lamprey embryo, the mesoderm consists primarily of unsegmented head mesoderm, segmented somites, and yet uncharacterized lateral plate mesoderm, as in gnathostomes. Although the adult lamprey possesses segmented myotomes in the head, the head mesoderm of this animal is primarily unsegmented, similar to that in gnathostomes. In the trunk, the large part of lamprey somites is destined to form myotomes, and the *Pax3/7* gene expression domain in the lateral part of somites is suggested to represent a dermomyotome homologue. Lamprey myotomes are not segregated by a horizontal myoseptum, which has been regarded as consistent with the apparent absence of a migratory population of hypaxial muscles shared by gnathostomes. However, recent analysis suggests that lampreys have established the gene regulatory cascade necessary for the ventrally migrating myoblasts, which functions in part during the development of the primordial hypobranchial muscle. There have also been new insights on the developmental cascade of lamprey cartilages, in which the Sox family of transcription factors plays major roles, as in gnathostomes. Thus, mesoderm development in lampreys may represent the ancestral state of gene regulatory mechanisms required for the evolution of the complex and diverse body plan of gnathostomes. *Developmental Dynamics 236:2410–2420, 2007.* © 2007 Wiley-Liss, Inc.

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INTRODUCTION: FROM INVERTEBRATES TO VERTEBRATES

Complexity and morphological diversity of the mesodermal tissues of vertebrates have attracted the attention of biologists for centuries. Embryonic mesoderm provides the precursors for muscle, skeleton, and visceral organs. In the late 19th century, discovery of the persistent notochord and segmented paraxial mesoderm of the cephalochordate amphioxus and urochordate ascidians illuminated these animals, which had long been believed to be members of mollusks, as the putative missing link between invertebrates and vertebrates (Kowalevsky, 1866, 1871; Lankester, 1877). Presently, Cephalochordata, Urochordata, and Vertebrata are placed as subphyla of the phylum Chordata, in which the overall organization of embryonic tissues (dorsal hollow nerve cord, ventral digestive tract, axial notochord, and bilateral paraxial mesoderm) is largely conserved. In contrast, the echinoderms and hemichordates are sister groups of the chordates and they lack the notochord and paraxial mesoderm. Thus, the basic mesodermal organization of vertebrates must have appeared first in the common ancestor of the chordates.

The history of chordate (protochordates plus vertebrates) evolution could be viewed as a gradual elaboration of the body plan. However, there have been several obstacles to a clearcut view of sequential timing of evolutionary changes. First, the body plan of protochordates is far simpler than that of vertebrates, and they lack many of the primordia for major ver-

tebrate organs throughout their lives. Second, the life cycles of many protochordate species include metamorphosis in which the overall body structure is completely reconstructed, and lineages and functions of many of the embryonic and adult tissue cannot be simply homologized with those of vertebrates (Hirano and Nishida, 1997; Mazet and Shimeld, 2005). Third, many of the extant chordate species seem to have accumulated nucleotide substitutions and gene duplications independently in their lineages (reviewed by Minguillón et al., 2002; Holland and Gibson-Brown, 2003). Thus, there is a growing consensus that the insights obtained from direct comparison of the expression domains of homologous developmental genes are not sufficiently straightforward to provide direct evidence for the molecular changes that have occurred in chordate evolution (Galliot, 2005; Ikuta and Saiga, 2005).

Most living vertebrates are gnathostomes equipped with jaws in the oral region. All other vertebrates belong to the agnathans, most of which are extinct. The only extant species of agnathans are lampreys and hagfishes, which together form a monophyletic group called "Cyclostomes" (Mallatt and Sullivan, 1998; Kuraku et al., 1999; Takezaki et al., 2003; for the phylogenetic relationships of agnathans, see Delarbre et al., 2000). Lampreys possess several features absent from protochordates. First, cartilaginous vertebrae surround the lamprey neural tube, although it does not become calcified. Second, in the head region, the pharyngeal cartilages are derived apparently from neural crest cells (Langille and Hall, 1988; also see Newth, 1951, 1956), an important cell population that does not exist in protochordates (but see Jeffery et al., 2004; Graham, 2004). Unlike amphioxus or ascidians, lampreys possess well-developed head structures in which the anterior domain of the neural tube differentiates as a brain. Recently, lamprey brains have been shown to have cellular and molecular characteristics similar to those of gnathostomes (Murakami et al., 2001, 2004, 2005; Osorio et al., 2005).

As for mesodermal configuration, lampreys possess different populations of mesoderm, which predominantly occupy the trunk or the head. For instance, trunk muscles differentiate directly from the somites, and the muscles in the pharynx appear to be derived from the unsegmented head mesoderm (Kusakabe et al., 2004; and see below).

Although lampreys resemble gnathostomes more than protochordates do, lampreys also lack several major organs found in gnathostomes. One such feature is the jaw. In gnathostomes, the jaws initially form as a dorsoventrally divided mandibular arch. The skeletal elements of the upper and lower jaws primarily derive from the cephalic neural crest in the mandibular arch and become associated with skeletal muscles that derive from the head mesodermal tissue. In contrast, the lamprey mouth forms a characteristic disc suitable for sucking, in which both the mandibular and the premandibular ectomesenchyme participate in formation (Shigetani et al., 2002, 2005; Kuratani et al., 2004). During embryogenesis, separate bilateral muscles develop in both the upper and the lower lips, innervated by branches of the trigeminal nerves (Song and Boord, 1993; Kuratani et al., 2004). However, no homologies of the trigeminal nerve branches seem to exist between lampreys and gnathostome (Kuratani et al., 2004). Lampreys also lack the true tongue of gnathostomes and the muscles associated with it (see below).

Lampreys also lack the paired fins that must have evolved into the tetrapod fore- and hindlimbs (for the presence of paired fins in fossil agnathans, see Janvier, 1996). Consistently, there are no pectoral or pelvic girdle elements and associated muscles. They also lack the myoblast population, which in gnathostomes originates from the somites and migrates through the adjacent lateral plate mesoderm to reach the limb bud where they differentiate into the mature muscle cells. The cucullaris muscles, another migratory muscle group, are also missing in the lamprey (reviewed by Kusakabe et al., 2005).

In this review, we will first describe the characteristics of lampreys as an animal that retains the ancestral head–trunk organization of vertebrates, an issue that has attracted the attention of comparative anatomists for more than 100 years. Second, we will focus on the patterning of somitic muscles and discuss the evolution of the complex skeletal muscle morphology of vertebrates. Recent progress of analyses on gene expression patterns in lamprey embryos will also be described briefly, in which insights for the evolution of embryonic compartments of somites (myotome, dermomyotome, and sclerotome) will be discussed.

LAMPREY AS AN INTERMEDIATE CREATURE? HEAD MESODERM AND THE QUESTION OF HEAD SEGMENTATION

The developmental patterns of the lamprey mesoderm have been described several times, primarily based on histological analyses. Because of the potentially critical position of this animal for interpreting the vertebrate phylogenetic tree, morphological patterns of the lamprey mesoderm have been occasionally misunderstood. Namely, there has been a facile tendency to view lamprey as an intermediate form linking cephalochordates and gnathostomes, and the mesoderm of the embryonic lamprey head has been illustrated as if it were segmented along the anteroposterior axis as seen in amphioxus (Fig. 1; Koltzoff, 1901; Damas, 1944; reviewed by Gee, 1996, and Kuratani, 2003). These segments were assigned to hypothetical head segments and were given names based on the head cavities (see below) found in elasmobranch embryos. Thus, so-called premandibular, mandibular, and hyoid mesodermal segments were recognized and were designated as head somites (Damas, 1944; Neal, 1897; Koltzoff, 1901). Veit (1939) even suggested that the rostral mesodermal segments of the lamprey often show left–right asymmetry at early stages, as is encountered in the developmental pattern of cephalochordates (reviewed by Kuratani et al., 1999).

This prejudice about the lamprey head partly stemmed from interpretations of the adult and larval anatomy of the lamprey head, i.e., there are preotically located myotomes, both
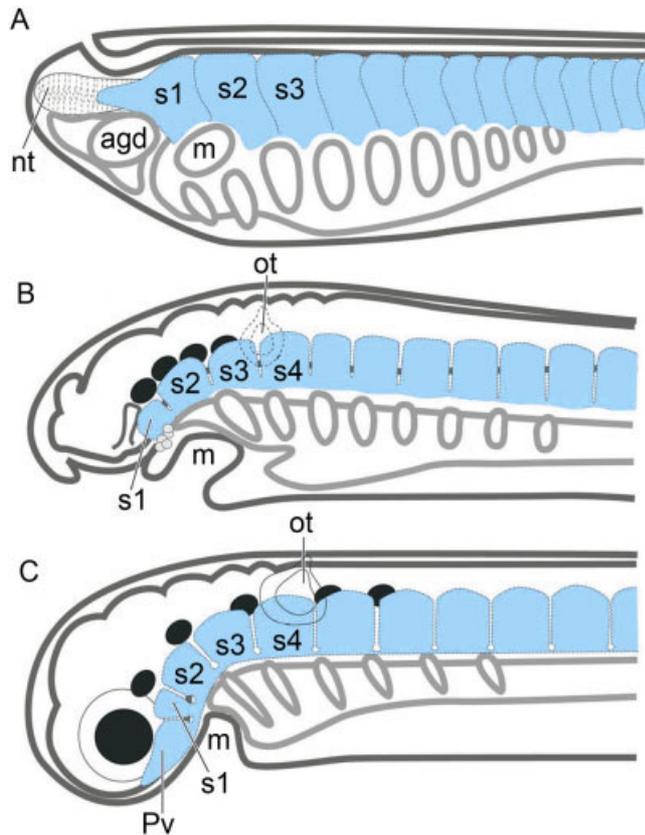
Fig. 1. Head segments in classic comparative embryology. **A–C:** Embryo and larvae of amphioxus (A), lamprey (ammocoete larva, B), and shark (C) are illustrated to represent evolutionary stages of hypothetical "cephalic somites" that do not exist in real vertebrates. In this scenario, head cavities in the shark and head mesodermal segments in the lamprey (s1–s4, shown in blue) are regarded as homologous with the rostral myotomes of amphioxus. Small black circles indicate the location of cephalic neural ganglions. Modified from Neal and Rand (1946). ot, otic pit; m, mouth; agd, anterior gut diverticulum; pv, Platt's vesicle.
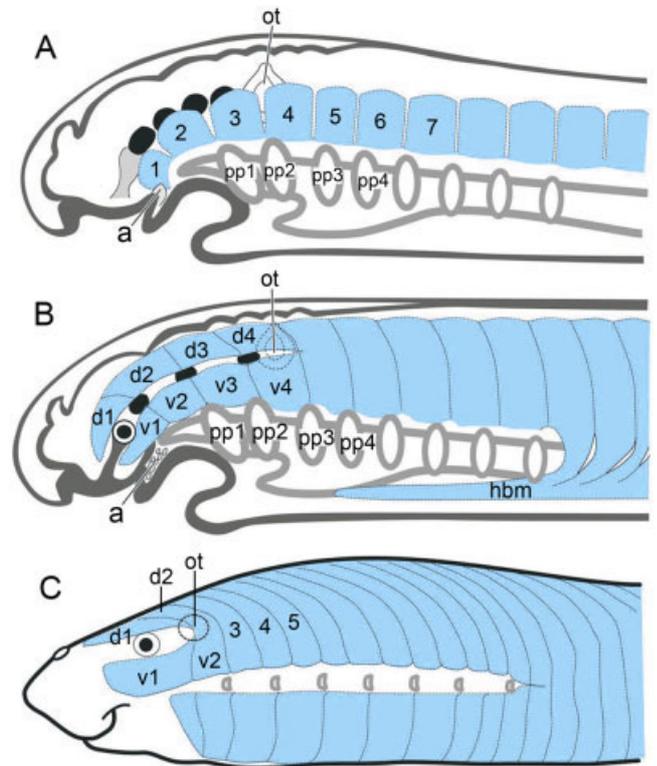

Fig. 2.

Fig. 2. Head mesodermal segments in developing lampreys viewed in classic concepts. Paraxial mesodermal elements are colored blue. **A:** Early ammocoete. **B:** Middle stage larva. **C:** Fully grown ammocoete with differentiated preotic myotomes that was equated with the head mesodermal subdivision. The differentiated supra- and infraoptic myotomes in C (d1, d2, V1, and V2) are identified as derivatives of cephalic mesodermal segments, but they really arise from postotic somites and secondarily move rostrally (Kuratani et al., 1999). Also see Figure 4B. Modified from Neal (1914). a, adenohypophysis; ot, otic pit; hbm, hypobranchial muscle; pp1–pp4, pharyngeal pouches.

Fig. 3. Expression of muscle actin gene *LjMA2* (A,B) and the myosin heavy chain gene *LjMyHC2* (C) during development, detected by whole mount in situ hybridization. **A:** A stage 25 embryo. *LjMA2* is expressed in somitic muscles, heart, and in muscles derived from head mesoderm. **B:** At stage 28, strong *LjMA2* expression is detected in the ventral region of the somites, and in many branchial muscles such as velar muscle (vm). **C:** A stage 30 embryo expressing the *LjMyHC2* gene only in muscles of somitic origin, including the hypobranchial muscle. bm, branchial muscles; hbm, hypobranchial muscle; iom, infraoptic muscle; llm, lower lip muscle; m, mouth; som, supraoptic muscle; ulm, upper lip muscle; vm, velar muscle.
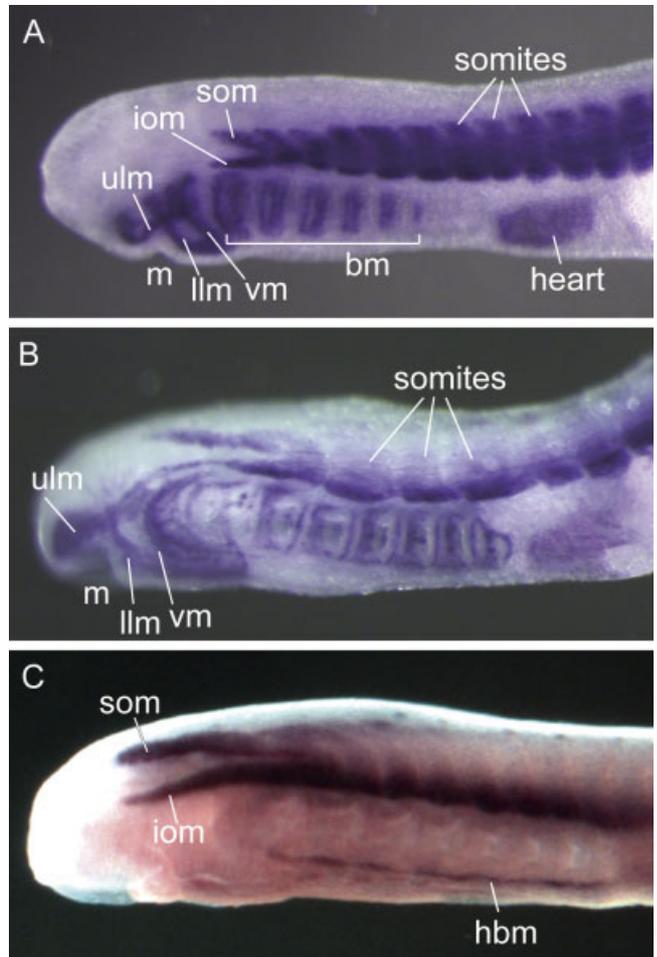

Fig. 3.

dorsal and ventral to the eye (Fig. 2; supraoptic and infraoptic myotomes). Neal (1897) first believed that they represented the differentiated preotic head mesodermal segments and depicted them as if they had served as the anlagen for the extrinsic ocular muscles of this animal. There are also segmental muscle blocks beneath the branchial pores of ammocoetes (Fig. 2B,C; hypobranchial muscle; discussed below). All these muscles together give the impression that the lamprey body is covered entirely by segmented muscle blocks along the anteroposterior axis, as in amphioxus (for resemblance between lampreys and amphioxus, see Holland et al., 1993, 1997). Neal revised this figure in his later writings and showed that the hypobranchial muscle is segmented in register with the branchial arches, not with the myotomes (Neal, 1914).

In the past decade, the patterns of muscle-related gene expression and immunoreactivities of muscle-related antigens (Kuratani et al., 1997, 1999; Kusakabe et al., 2004; Noden and Francis-West, 2006; Fig. 3) showed that the preotic muscle blocks arise postotically and only secondarily do they shift rostrally into the preotic region. In other words, the myotomes in the lamprey originate from the true somites that only develop posterior to the otic placode, the situation found in gnathostomes (Figs. 3, 4C; Kuratani et al., 1999). It was simultaneously revealed that these lamprey postotic somites share a similar timetable of muscle differentiation to the somites of many gnathostome species, where body muscles differentiate much earlier than muscle in the head.

ONTOGENY OF THE LAMPREY HEAD MESODERM AND HEAD SEGMENTATION

Using the scanning electron microscope, mesodermal development was recently reexamined by Kuratani et al. (1999; also see Horigome et al., 1999, for mesenchymal morphology). According to this study, the lamprey mesoderm appears to follow a pattern very similar to that of gnathostome embryos. At stage 19, the early pri-

mordium of lamprey mesoderm lies lateral to the notochord (Fig. 5A,B), and segmentation of the somites proceeds in anterior to posterior direction (Kuratani et al., 1999). The left and right rows of early somites do not match precisely. In other words, the boundaries of somites deviate from each other across the midline (Fig. 5C). However, this apparent left–right asymmetry is not similar to that in amphioxus, in which the asymmetry of myotomes is clearly directional (Hatschek, 1881); in *L. japonicum*, the patterns of bilaterally asymmetric orientations of somites fluctuate between individuals, and there was no clear preference as to which side of the embryo has more anterior myotomal boundaries than the other. The morphology of bilateral somites are equilibrated and symmetrized later in development during the growth of the body (Kuratani et al., 1999).

The rostralmost part of the early lamprey mesoderm is unsegmented and is continuous rostrally and medially with the prechordal plate, the embryonic structure arising rostral to the notochord (Fig. 5D). There is no clear boundary between this medial plate and the lateral mesodermal bulge that later forms the mandibular mesoderm. It is the protrusion of the rostral endoderm, or the first pharyngeal pouch, that delineates the mandibular mesoderm and the more posteriorly located hyoid mesoderm. The hyoid mesoderm is further delineated posteriorly (as opposed to the more posteriorly located mesodermal region called "somite 0"), by the developing otic pit as well as the second pharyngeal pouch (Fig. 4A). Thus, these mesodermal elements are not segmented by any clear boundaries, but merely "regionalized" by embryonic structures such as noted above, unlike true somites that undergo regular process of segmentation proceeding from anterior to posterior.

The overt premandibular mesoderm becomes apparent only after the rostral end of the notochord is defined by a clear boundary at stage 21 (Fig. 5D,E). Even at this stage, the prechordal mesoderm takes the shape of an undifferentiated unpaired mass of cells (Fig. 5D). This cell mass gives rise to a pair of bars rostral to the mandibular mesoderm (Fig. 5F). This

premandibular mesoderm may possibly represent a real segment, for it is delineated by a clear boundary and its appearance does not seem to depend on any embryonic environment as seen in more posterior head mesodermal regions (Fig. 5F). In this connection, it may be worth mentioning that, in chicken embryos, only two rounds of oscillation of gene expression, like those observed in the segmentation of somites (*hairy* and *lunatic fringe*; Palmeirim et al., 1997; McGrew et al., 1998), have been detected in the cephalic mesoderm, one in the prechordal plate (the source of the premandibular mesoderm) and the other for the rest of the entire mesoderm (Jouve et al., 2002). Thus, the vertebrate cephalic mesoderm may be entirely different from the somites, or at most it may correspond to two mesodermal segments, one small and another large, that partly share the same molecular segmentation mechanism as the somites.

Although the lamprey head mesoderm first appears as an enterocoelic structure as in embryos of amphioxus, this type of mesodermal formation should not be confused with the so-called "head cavities" in the elasmobranch embryos. The latter structures are only characterized by their appearance as coelomic spaces lined by thin epithelium in the loose head mesenchyme, and possibly giving rise to primordia for extrinsic ocular muscles (see next paragraph). Typical head cavities are found in elasmobranch pharyngula, and there are three pairs of cavities consisting of premandibular, mandibular, and hyoid cavities (Fig. 1C). The premandibular cavity is the most commonly observed (even in mammals). In amniote embryos, the head cavities appear very similar to blood vessels (Jacob et al., 1986). By counting the recognized cavities that match the above definition, it becomes apparent that so-called head cavities are absent from the lamprey. Thus, head cavities represent a gnathostome synapomorphy that is gradually lost from posterior to anterior toward the crown groups like sauropsids (reviewed by Kuratani, 2003).

Although Koltzoff has illustrated the extrinsic ocular muscles in the developing lamprey as differentiating from the head mesodermal compo-

nents, this has not been clarified by cell-lineage analyses. Recent finding by Shimeld (Boorman and Shimeld, 2002) that *Pitx1* expression in the lamprey prechordal mesoderm continues into the periocular mesenchyme in older larvae may reflect the prechordal mesoderm origin of the muscles, at least in part, as is the case with gnathostome embryos (see Jacob et al., 1986; Noden et al., 1999).

LAMPREY HYPOBRANCHIAL MUSCLE AND THE EVOLUTION OF MIGRATORY HYPAXIAL MUSCLES

As we mentioned above, lampreys possess several groups of head muscles with somitic origin—the supra- and infraoptic muscles, and the hypobranchial muscles (Figs. 3, 4B). Among them, the hypobranchial muscle is of particular interest with respect to its developmental origin and the morphological similarity to the amniote tongue muscle precursors (called the hypoglossal cord; Hammond, 1965; Hazelton, 1970; O'Rahily and Müller, 1984; Kusakabe and Kuratani, 2005). Consistently, like the gnathostome tongue muscles, the lamprey hypobranchial muscle is innervated by the hypoglossal nerve (Kuratani et al., 1997), although it is one of the gill-supporting muscles and does not actually contribute to the oral apparatus called the "tongue" of lampreys (the cyclostome tongues are of mandibular arch derivatives innervated by the trigeminal nerves; Yalden, 1985).

Briefly, the precursors for the lamprey hypobranchial muscle originate in the ventrolateral edges of the anterior of somites, and migrate first ventrally and posteriorly around the posterior pharyngeal arch, and then proceed anteriorly along the ventral aspect of the pharyngeal arches (Kusakabe and Kuratani, 2005; also see Neal, 1897; and Kuratani et al., 1997, 1998). These precursors express a member of myogenic regulatory factor genes encoding basic helix–loop–helix (bHLH) protein family (Kusakabe, Kuraku and Kuratani, unpublished), but do not express contractile protein genes until they reach the ventrolateral part

of the pharyngeal region where they undergo differentiation (Fig. 3C). This developmental behavior of hypobranchial myoblasts closely resembles that of gna-

thostome hypoglossal muscles, which include those of the tongue and larynx (more appropriately, hypohyoideal) muscles, and also possibly the mamma-
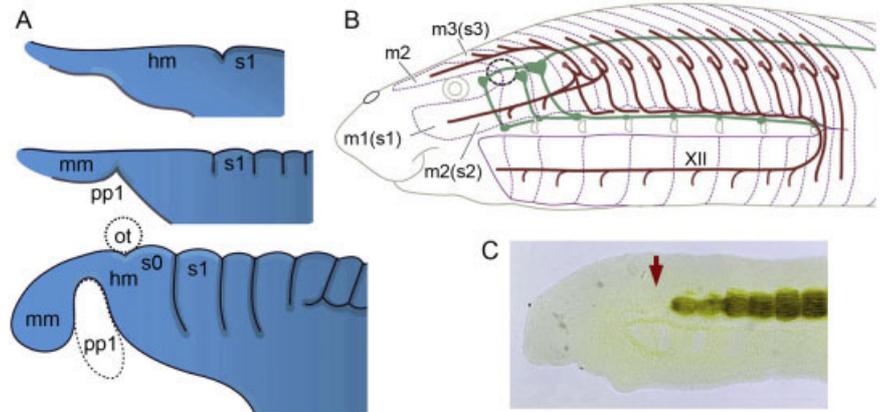

Fig. 4. Mesodermal development in the lamprey head—schematically illustrated. **A:** Regionalization of the head mesoderm. The head mesoderm in the lamprey is not segmented, but secondarily (epigenetically) regionalized by embryonic structures such as otic pit (ot) and pharyngeal pouches. hm, hyoid mesoderm; mm, mandibular mesoderm; pp1, the first pharyngeal pouch; s0, a postotic, incomplete somite that lacks an overt rostral boundary, s1, the first somite. **B:** Rostral myotomes of the fully grown ammocoete larva. Myotomes are correctly labeled (compare with Fig. 2C). The first and second myotomes (m1–2) contribute to the first infraoptic myotome. Red lines indicate the spinal nerves. Green lines indicate the cranial and lateral line nerves. m1–m3, myotomes; XII, hypoglossal nerve. **C:** Stage 25 lamprey embryo stained with CH-1 monoclonal antibody that recognizes the tropomyosin. All the myotomes (starting from myotome 1) are developing posterior to the otic vesicle indicated by a red arrow.
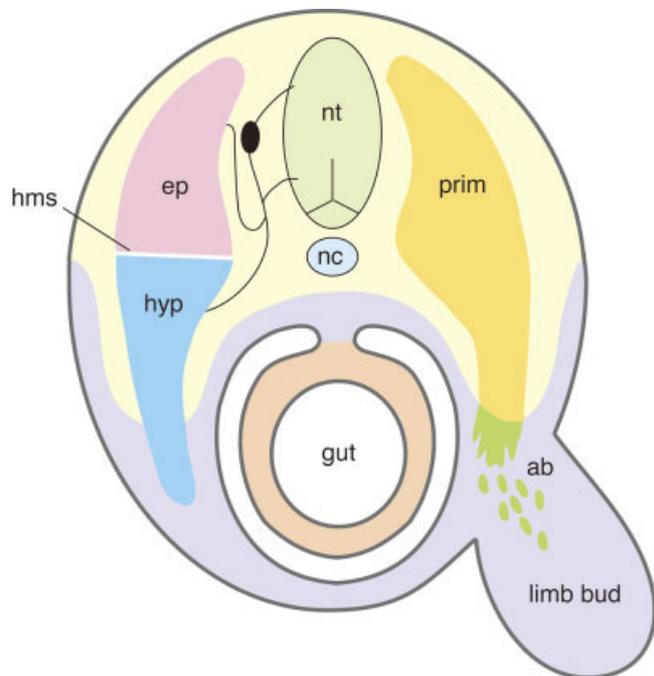

Fig. 6. Schematic representation comparing mesoderm patterning based on two different concepts, epaxial/hypaxial (left half) and primaxial/abaxial (right half) distinctions. The gnathostome myotomes are divided into the epaxial (pink) and hypaxial (blue) regions, separated by the horizontal myoseptum (hms). The epaxial domain is surrounded solely by the primaxial dermis and connective tissue (pale yellow). The hypaxial domain (blue) contains cells that develop in two different environments: primaxial and abaxial (lavender) dermis and connective tissue. Abaxial myotomes (green) undergo deepithelialization to release the migratory population of myoblasts. See text for details. ep, epaxial myotome; hyp, hypaxial myotome; nc, notochord; nt, neural tube.
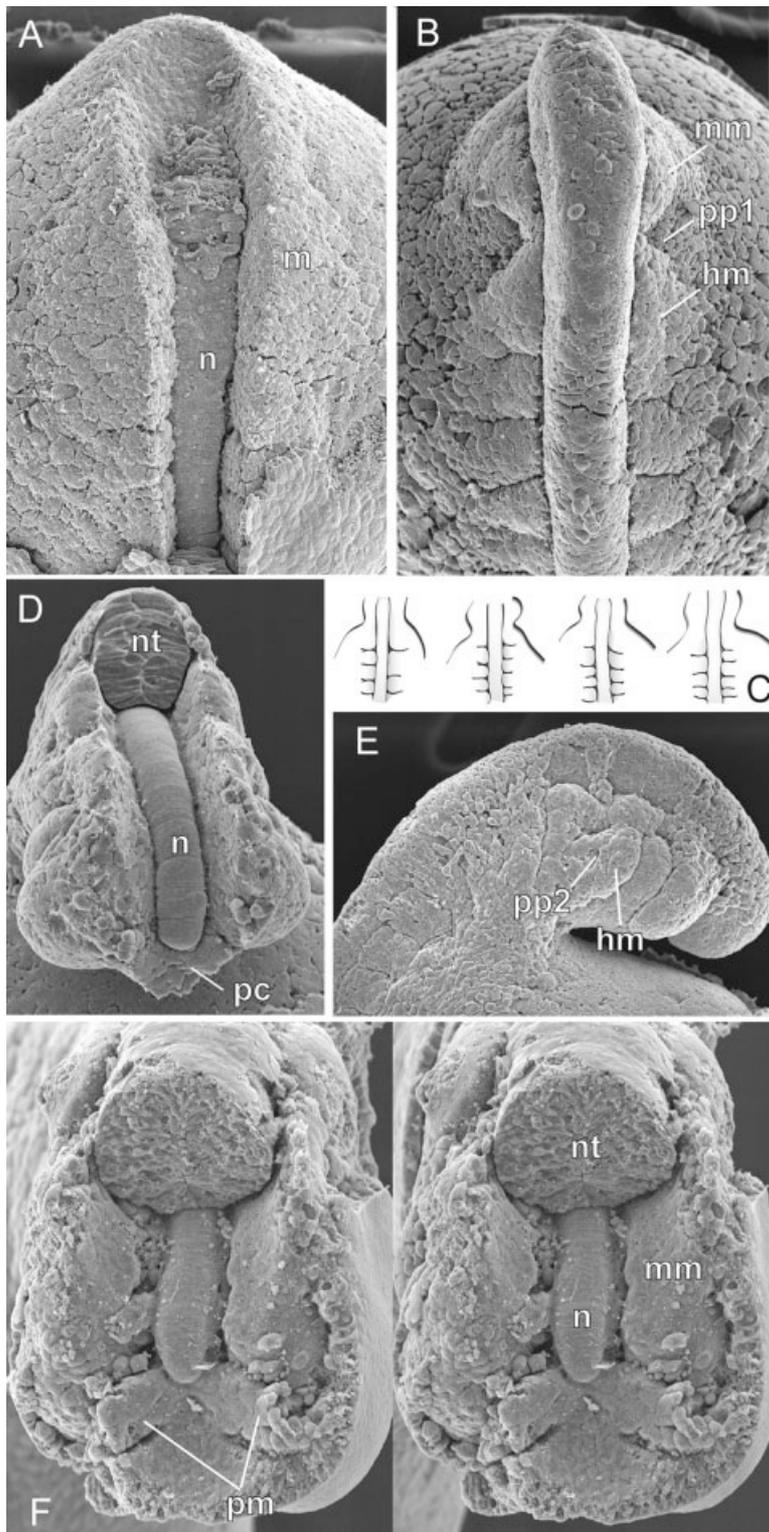
Fig. 5. Development of the lamprey head mesoderm observed by scanning electron microscopy. **A:** A stage 19.5 (Tahara, 1988) embryo of *Lethenteron japonicum* with ectoderm and neural tube removed. Unsegmented head mesoderm (m) is seen on both sides of the notochord (n). **B:** A stage 20 embryo after the removal of the surface ectoderm. Somites are clearly segmented posteriorly, but the mandibular mesoderm (mm) is only partially regionalized by protruding pharyngeal pouch (pp1). **C:** Various types of somite segmentation. **D:** A stage 21 embryo to show the prechordal plate (pc). **E:** Lateral view of the stage 21 embryo. The second pharyngeal pouch (pp2) is protruding to demarcate the hyoid mesoderm (hm). **F:** Stereoscopic photograph of stage 24 embryo to show the premandibular mesoderm (pm) differentiated from the previous prechordal plate. Photos by N. Horigome. nt, neural tube.

lian diaphragm as well (Keibel and Mall, 1910).

Another piece of evidence is that the lamprey *Pax3/7* gene, the putative homologue for the *Pax3* and *Pax7* genes in gnathostomes, is expressed in the migrating hypobranchial muscles of late pharyngula and larvae (Kusakabe and Kuratani, 2005). In amniotes, *Pax3* is expressed in the migrating precursor cells of the hypoglossal muscles and is required for development of these muscles (reviewed in Birchmeier and Brohmann, 2000). These facts suggest an evolutionary scenario, in which the hypobranchial muscle acquired in the jawless ancestor of the vertebrates might have evolved into the gnathostome tongue muscles.

The gnathostome tongue muscles are included in the "migratory hypaxial muscles," which develop under the regulation by *Pax3*, *Lbx1*, *c-Met*, and *HGF* genes (Dietrich, 1999). Other muscles of this category are the limb muscles, trapezius muscles, and the mammalian diaphragm. The presence of the lamprey hypobranchial muscles, which undergo similar developmental processes with those of tongue muscles, strongly suggests that the lampreys might have possessed the prototype of the developmental mechanism for "migratory hypaxial muscles," although they lack the counterpart for gnathostome paired fin/limb muscles (see below).

DEVELOPMENT OF THE LAMPREY TRUNK MUSCLES AND EVOLUTION OF THE DERMOMYOTOME

Putative homology of lamprey hypobranchial muscles to a member of gnathostome hypaxial muscles is intriguing in the light of the organization of lamprey myotomes, which, in the first place, contains no separate epaxial and hypaxial domains (Kusakabe and Kuratani, 2005). Lampreys do not possess a horizontal myoseptum, which in teleosts segregate these two portions of the myotomes (Fig. 6). All the lamprey trunk skeletal muscles are innervated only by the ventral roots of spinal nerves, whereas gnathostome epaxial and hypaxial myotomes receive independent innervation from dorsal and ventral rami of the spinal

nerves, respectively (Fig. 6). During evolution, separation of epaxial and hypaxial domains is supposed to have occurred after the agnathan/gnathostome divergence, because this categorization is firmly shared by gnathostomes, but not found in protochordates and lampreys.

Another potential similarity of lamprey and protochordate muscles is found in the lamellar organization of myofibers (Peachy, 1961; Nakao, 1976; Kusakabe and Kuratani, 2005). It had also been known that only a single type of skeletal muscle fibers exist in embryonic and larval lampreys (Nakao, 1976), in contrast to zebrafish in which the superficial layer of slow fibers and the medial fast fibers can be distinguished by the specific expression of myosin heavy chain genes (Devoto et al., 1996).

In zebrafish myogenesis, formation of the horizontal myoseptum and the establishment of separate slow/fast domains are tightly linked, and both require regulation by the notochordal hedgehog (Hh) signal (Zeller et al., 2002; Ochi and Westerfield, 2007). Because lamprey myotomes lack both of these morphological features, one attractive hypothesis would be that the lampreys might lack the developmental cascade for myotome patterning, in which Hh signal from the notochord plays the key role. Although the details of the Hh cascade in lampreys remains to be clarified (Uchida et al., 2003; Osorio et al., 2005), the differential expression of contractile protein genes, such as that of *LjMA2* localized in the adaxial region, might represent the ancestral state of vertebrate myotomes (Kusakabe et al., 2004; Kusakabe and Kuratani, 2005).

Comparison of the early phase of myotome formation also speaks to the evolution of these structures. In amniotes, myotomal cells are deposited from the dorsomedial and ventrolateral lips of the *Pax3*-positive epithelial dermomyotome (Ordahl and Le Douarin, 1992; Dietrich, 1999; Gross et al., 2004). These cells start expressing muscle regulatory factors and join continuously with the myotomes, causing muscle growth. This finding is in a clear contrast with the situation in anamniotes, such as teleosts, amphibians and lampreys (Scaal and Wiegreffe, 2006). In these animals,

most of the somitic cells seem to be specified as future myofibers and constitute myotomes as soon as the somites form from the presomitic mesoderm (for comparative embryology of myotomes, see Maurer, 1906). In other words, anamniote skeletal muscles appear to develop without passing through the epithelial dermomyotomal phase.

Recently, molecular evidence on anamniote early myogenesis has been accumulated. We found that the lamprey *Pax3/7* gene is expressed in a thin layer of cells covering the lateral surface of the somites in early larval stages (Kusakabe and Kuratani, 2005). This cell layer had been discovered by Nakao (1976) using electron microscopy as "a single layer of flattened cells" located laterally to the myotomes. A similar cell layer expressing *Pax3* and/or *Pax7* has been discovered in zebrafish ("external cells"; Devoto et al., 2006), pearlfish ("dermomyotome"; Steinbacher et al., 2006), and the African clawed frog *Xenopus laevis* ("dermomyotome"; Grimaldi et al., 2004; reviewed in Scaal and Wiegreffe, 2006). In these species, the cell layer superficial to the myotomes is speculated to be the source of myogenic cells that undergo stratified hyperplasia (Steinbacher et al., 2006). Correspondingly, in the case of zebrafish and pearlfish, hyperplasia occurs at the dorsal and ventral extremities of the myotomes, the regions adjacent to the edges of the external cell layer. Thus, it is plausible that this thin cell layer at the surface of the somites might represent a dermomyotome-like tissue conserved in all the major taxa of vertebrates (Scaal and Wiegreffe, 2006).

If these cell layers in which fish and lampreys are both homologous to the amniote dermomyotome, some dermis-related characteristics might be associated in addition to myogenic features. Indeed, external cells of the trout (Rescan et al., 2005) and the dermomyotome in *Xenopus* (Grimaldi et al., 2004) express the type I collagen gene (*Col1A1*), a major marker gene for the dermomyotome and dermis (van der Rest and Garone, 1991). Recently, the lateral region of lamprey somites was reported to express a collagen gene (*Col2A1a*; Zhang et al., 2006). However, this gene is grouped

with the type II subfamily of collagens with a high support value on the phylogenetic tree (Zhang et al., 2006). This gene is also expressed in the floor plate of the neural tube and the hypochord (Zhang et al., 2006), both of which are the major expression domains of type II collagen genes in gnathostomes. No type I collagen gene has been reported from lampreys. Thus, at present the expression pattern of collagen genes in lamprey somites does not clearly indicate the homology of this region to the gnathostome dermomyotome, and the origin of the lamprey dermis still remains controversial.

The cephalochordate amphioxus possesses a collagen-rich dermis. However, the embryonic origin of dermal tissue of this animal is not well understood, and amphioxus *Pax3/7* is expressed only in a small number of scattered paraxial muscle cells (Holland et al., 1999). The ascidian *Pax3/7* gene is not expressed in the mesoderm (Wada et al., 1997). The evolutionary origin of the dermomyotome does not seem to be traceable back beyond the agnathans, in which the molecular basis for the formation of dermomyotome may have been acquired.

DEVELOPMENTAL AND EVOLUTIONARY ORIGIN OF THE LAMPREY CARTILAGINOUS SKELETON

Most of the lamprey cartilages belong to the head, consisting of neurocranial elements around the brain and viscerocranial elements that support the oral disc, mouth cavity, branchial arches, and pericardium (Johnels, 1948; Hardisty, 1981). The contribution of the cephalic neural crest cells to the lamprey cranial cartilages has been studied extensively (Langille and Hall, 1988; McCauley and Bronner-Fraser, 2003). These cartilages are not calcified throughout life (Hardisty, 1981). The observation of fine ultrastructural components had led to the notion that there may be no collagen fibrils in lamprey cartilages, unlike all other vertebrate cartilages, which use type II collagen (Wright and Youson, 1983). Instead, an elastin-like molecule, lamprin, was discov-

ered as a major structural protein in lamprey cartilages (reviewed in Wright et al., 2001). Thus, it had long been hypothesized that the skeleton of the vertebrate ancestor would have lacked collagen in cartilages, and that collagenous and calcified bone is a synapomorphic feature of gnathostomes (Wright et al., 2001).

Recently, it was discovered that lampreys possess more than two type II collagen genes and at least one of them, *Col2A1a*, is expressed in the adult lamprey branchial cartilage, notochord, and notochordal sheath (Zhang et al., 2006). This gene and another collagen gene *Col2A1b* are both expressed in embryonic pharyngeal arches and in the medial part of the somites. Moreover, members of the *SoxE* family genes, the important transcriptional activators for neural crest cells forming cranial cartilages in gnathostomes, have been shown to be coexpressed with *Col2α1b* in lamprey cranial cartilages (McCauley and Bronner-Fraser, 2006; Zhang et al., 2006). When the function of *SoxE* is disrupted during development, the pharyngeal cartilages do not form (McCauley and Bronner-Fraser, 2006). The conservation in both developmental regulatory genes and structural protein genes, such as *Col2A1* and *Col2A1b*, suggests that similar genetic pathways regulate cartilage formation in lampreys and gnathostomes. These pathways might involve other transcription factor genes such as the *Hox*, *Pax*, and *Dlx* groups expressed in the cranial neural crest cells (Ogasawara et al., 2000; Neidert et al., 2001; Shigetani et al., 2002; Cohn, 2002; McCauley and Bronner-Fraser, 2003; Uchida et al., 2003; Takio et al., 2004). Taken together, the developmental mechanism generating cartilages strengthened by collagens may have been established in the common ancestor of vertebrates.

Although there are accumulating insights for development of lamprey cephalic cartilages, the origin of trunk axial cartilages remains unknown. The hypothetical "sclerotome" in the lamprey has been proposed as a somitic domain at the ventral edges of the early somites, which grows dorsally to surround the neural tube and extend into the dorsal fin fold (Zhang et al., 2006; Freitas et al., 2006).

These cells express the *Parascleraxis* gene, the cognate gene for gnathostome *Paraxis/Scleraxis* (Freitas et al., 2006). Phylogenetic analysis of the deduced amino acid sequence of *Parascleraxis* placed this gene as the sister to the *Paraxis* (dermomyotome marker) and *Scleraxis* (sclerotome marker) clade of the bHLH transcription factor family (Freitas et al., 2006). However, the transcript of the lamprey cognate of *Pax1/9* genes, the markers for vertebrate sclerotomes, was reported to be absent in developing lamprey somites (Ogasawara et al., 2000). Additional information about the expression of markers for "lamprey sclerotomes" is required to clarify the molecular identity of the lamprey somitic region contributing to the trunk cartilage.

MEDIAN FINS OF THE LAMPREYS AND EVOLUTION OF LATERAL FINS

The complete absence of paired fins in the lampreys and hagfishes leads us to the question of whether agnathans lack the whole molecular cascades known to be important for the formation of paired appendages. Development of paired lateral limbs initiates with the formation of limb bud, which is established by the interaction between the lateral plate mesoderm-derived mesenchyme and overlying ectoderm. In lamprey embryos, however, no ectodermal structure similar to the amniote apical ectodermal ridge is observed in the trunk.

Because the earliest vertebrate fossils lack paired fins but possess median fins, it has been speculated that the developmental mechanisms for lateral fins might be first established in the median fins (Balfour, 1876; Zhang and Hou, 2004; Freitas et al., 2006). However, as mentioned above, the mesenchymal portion of the lamprey median fin buds express *Parascleraxis*, indicating its somitic origin, unlike the amniote limb bud mesenchyme, which derives from lateral plate mesoderm (Freitas et al., 2006). On the other hand, the expression of *Hox* and *Tbx* genes in the lamprey median fin buds suggests that the median fins of lampreys might develop under the similar regulatory cascades

to those for gnathostome lateral fin/limbs (Cohn et al., 1997; Freitas et al., 2006). These discoveries lead to the hypothesis that the primitive genetic cascade involving *Hox* and *Tbx* may have had a role in median fin development before the emergence of lateral fins. Later in evolution, this cascade may have become used in the paired fin buds, although consisting of mesenchymal cells of different developmental origins, somites, or lateral plate mesoderm (Freitas et al., 2006). Interestingly, several extinct agnathan species, such as the ostracoderm *Hemicyclaspis*, possessed a single pair of lateral fins adjacent to the pharynx. If these lateral fins were indeed homologous to the gnathostome pectoral fins, it is possible that these fossil agnathans possessed an embryonic fin bud containing mesenchyme originated from the lateral plate mesoderm. At this moment, however, the lateral plate mesoderm is one of the gnathostome traits, the evolutionary pathway of which is largely unknown.

PERSPECTIVES

Although lampreys have a long history of anatomical and developmental research (Hardisty, 1981), it was not until the past few decades that insights on gene expression and gene regulation during lamprey embryogenesis have accumulated. Many of the molecular data clearly show that this animal possesses the body plan of vertebrates, never showing any intermediate state between protochordates and vertebrates. For example, in the modern context, the problem of head segmentation refers to the search for plesiomorphic (chordate-like) characters in the vertebrate embryonic head. Meanwhile, vertebrate synapomorphy (evolutionarily new characters to define the vertebrates) stresses the absence of head mesodermal segments that divide the crest cell streams, which allows expanded ectomesenchymal cell populations to be more or less associated with pharyngeal arches and rhombomeres (Kuratani, 2003). Because the cephalic cell population should be the first paraxial mesoderm to be specified along the anteroposterior axis in many vertebrate species, comparison of initial expression of other regulatory genes will provide

more evidence for the evolution of head/trunk mesoderm in the lampreys and gnathostomes.

Recently, a new view for the categorization of gnathostome somitic cells, which might substitute for epaxial/hypaxial distinction, has been proposed by Burke and coworkers (Fig. 6; Nowicki et al., 2003; Burke and Nowicki, 2003). In this scheme, the vertebrate trunk is divided into primaxial and abaxial domains, depending on the environment in which the somite-derived cells undergo differentiation (Fig. 6, the right half). Primaxial derivatives include the intrinsic back muscles and the intercostal muscles that develop together with somite-derived connective tissues and dermis (in the somite-derived dermal elements) in a rather cell-autonomous way, whereas the abaxial derivatives differentiate under the local influences from the lateral plate mesoderm. In other words, the main constituents of the abaxial domain are the migratory population of hypaxial muscles, including those in tetrapod limbs. It is noteworthy that all these muscles are associated with skeletal elements and connective tissues derived from mesenchymal elements that are different from their own. Actually, also in the head and neck region, the trapezius (called cucullaris in nonmammals) and hypobranchial muscles, including the tongue and hypohyoideal muscles, develop under environmental influences from the nonsomitic-, neural crest-derived mesenchyme that also supplies the connective tissues and skeletal elements for these muscles (Noden, 1986; Matsuoka et al., 2005). Thus, the hypobranchial muscles also belong to the abaxial series in a wide sense, which forms in the distant mesenchymal environment.

From morphological and molecular evidences, the hypobranchial muscle (and possibly the supra- and infraoptic muscles) can be categorized as the sole putative abaxial elements in the lamprey (Figs. 3, 4B; summarized in Kusakabe and Kuratani, 2005). In both gnathostomes and lampreys, the hypobranchial muscles are located in the head/trunk interface, a peculiar developmental environment influenced by postotic crest cells (Kuratani, 1997). This environment eventually would have become the developmental field for the tetrapod neck (Matsuoka et al., 2005). In other words, the evolution of trunk mesodermal configuration (epaxial/hypaxial and/or primaxial/abaxial distinction) is coupled with evolution of the neck and shoulder (Matsuoka et al., 2005), which is hard to define in the lamprey.

The above discussion leads to the question of when and how the abaxial somitic elements evolved. Because protochordates do not possess skeletal muscle cells that undergo extensive migration during development, agnathans are the only living candidates that acquired the ancestral abaxial muscles. However, little is known about the development of the lateral plate mesoderm in lampreys, although its existence was proposed by lineage tracing analysis in the early 20th century (Weissenberg, 1934). Comparisons of the molecular nature of lateral plate mesoderm in agnathans and gnathostomes will provide important insights into the evolution of many vertebrate developmental traits, including those found in the head, neck, and shoulder regions.

ACKNOWLEDGMENTS

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