

Review

Evolutionary developmental studies of cyclostomes and the origin of the vertebrate neck

Shigeru Kuratani*

Evolutionary Morphology Research Group, Center for Developmental Biology, RIKEN, Kobe, Japan

Because they lack some gnathostome-specific traits, cyclostomes have often been regarded as representing an intermediate state linking non-vertebrate chordates and gnathostomes. To understand the evolutionary origins of the jaw and paired fins, lamprey embryos and larvae have been used as comparative models. The lack of the jaw–neck region is a conspicuous feature specific to cyclostomes; however, the absence of these features has been largely neglected both in evolutionary developmental studies and in the field of classical comparative embryology. This review seeks to develop a possible evolutionary scenario of the vertebrate neck muscles by taking the cucullaris (trapezius) muscle as the focus. By combining the comparative embryology of lampreys and gnathostomes, and considering the molecular-level developmental mechanism of skeletal muscle differentiation, this review argues that the establishment of the vertebrate neck deserves to be called an evolutionary novelty based on the remodeling of mesenchymal components between the cranium and the shoulder girdle, which involves both mesodermal and neural crest cell lineages.

Key words: body plan, cyclostomes, evolution, muscle development, neural crest.

Introduction

As a basal group of vertebrates, cyclostomes possess several interesting and important features dissimilar to those of gnathostomes, or jawed vertebrates. In this review, “cyclostomes” refers to extant jawless vertebrates comprising lampreys and hagfish, which are assumed to form a monophyletic group based on recent molecular phylogenetic analyses (reviewed by Kuratani & Ota 2008). There are roughly two different kinds of cyclostome-specific traits, namely, those only cyclostomes have acquired in their own lineages and which are therefore absent from gnathostomes, and those absent specifically from cyclostomes because evolution of these traits occurred only in gnathostomes. The former features correspond to synapomorphies (shared derived characteristics) that define cyclostomes, and the latter to synapomorphies of gnathostomes.

Most intriguing issues associated with evolutionary developmental studies using cyclostomes aim at understanding the origins of gnathostome-specific traits such as biting jaws, cucullaris muscles, division of epaxial and hypaxial systems in the trunk muscles, paired nostrils, three semicircular canals, a real (internalized) hypobranchial muscle system, the sympathetic trunk, and paired fins with their associated muscles and skeletal features. These character states in cyclostomes (i.e. absence of the features mentioned above) are regarded as plesiomorphic (primitive) and were lost specifically from gnathostomes; thus, cyclostomes are expected to provide us with hints about which developmental mechanisms have been newly added to the ancestor of gnathostomes. Importantly, the presence of these features has been regarded as a general vertebrate trait. According to the gnathostome model proposed by animal-based developmental biologists, cyclostomes are expected to be an intermediate stage of evolution along the lineage from tunicates leading toward the gnathostomes. However, cyclostomes are not grouped according to the loss of these characteristics, and they have their own derived characteristics such as a mandibular arch-derived “tongue” and velum, which have complicated and sophisticated morphological components (Yalden 1985). With this in mind,

*Author to whom all correspondence should be addressed.
Email: saizo@cdb.riken.jp

Received 19 November 2007; revised 19 November 2007;
accepted 20 November 2007.

© 2008 The Author

Journal compilation © 2008 Japanese Society of
Developmental Biologists

cyclostomes can serve as an ideal organism for comparison to gain insights into the origin of some organ systems because non-vertebrate chordates such as amphioxus and tunicates are too distantly related to compare. This review discusses the evolutionary origins of one of the gnathostome-specific traits from the modern developmental and comparative embryological perspectives. The origin of jaws and mono- to diplophorous transition have been discussed elsewhere (Kuratani & Ota 2007; Kuratani *et al.* 2001; Shigetani *et al.* 2005; Kuratani & Ota 2007).

Cucullaris muscles and the vertebrate neck

The evolutionary origin of the cucullaris muscle has not been discussed for a long time, except for an indirect mention in the context of the origin of the vertebrate neck by Matsuoka *et al.* (2005). In the evolutionary developmental sense, the vertebrate neck can be defined as the domain of the cucullaris and hypobranchial muscle systems attached to either the branchial arch skeleton or the pectoral girdle, along with the contribution of cephalic crest cells (i.e. circumpharyngeal crest cells; Kuratani 1997), which produce connective tissue (Fig. 1C). The term cucullaris muscle is a general name referring to a gnathostome-specific muscle that is a homologue of sternocleidomastoid and trapezius muscles in mammals. The latter two muscles are derived evolutionarily from a single muscle that split into two parts during amniote evolution (Lubosch 1938).

Derived from some rostral somites (McKenzie 1962; Noden 1983) and innervated by cervical spinal nerves, the cucullaris is primarily a somitic trunk muscle, as are typical skeletal muscles (see Kuratani 2004). However, this muscle is innervated by the accessorius nerve (the 11th cranial nerve, which is also missing in cyclostomes) that appears as a branch of the vagus nerve, and this muscle is often mistakenly interpreted as a branchial arch muscle (see Kuratani 2004). Regardless of whether the cucullaris is branchiomeric or somitic in nature, it always occupies a characteristic place in the gnathostome body plan, and it is usually easy to identify (Fig. 1C; Lubosch, 1938; Kuratani 1997; also see below). Similar to the hypobranchial muscles such as the tongue and infrahyoid (extrinsic laryngeal) muscles in amniotes, the cucullaris muscle in the mouse possesses connective tissues (tendon and fascia) derived from cephalic crest cells but not from the mesoderm (Matsuoka *et al.* 2005; but see also Noden 1983). Such a feature is found normally in extrinsic eye muscles or pharyngeal arch muscles that are derived from the unsegmented head mesoderm. Moreover, the crest

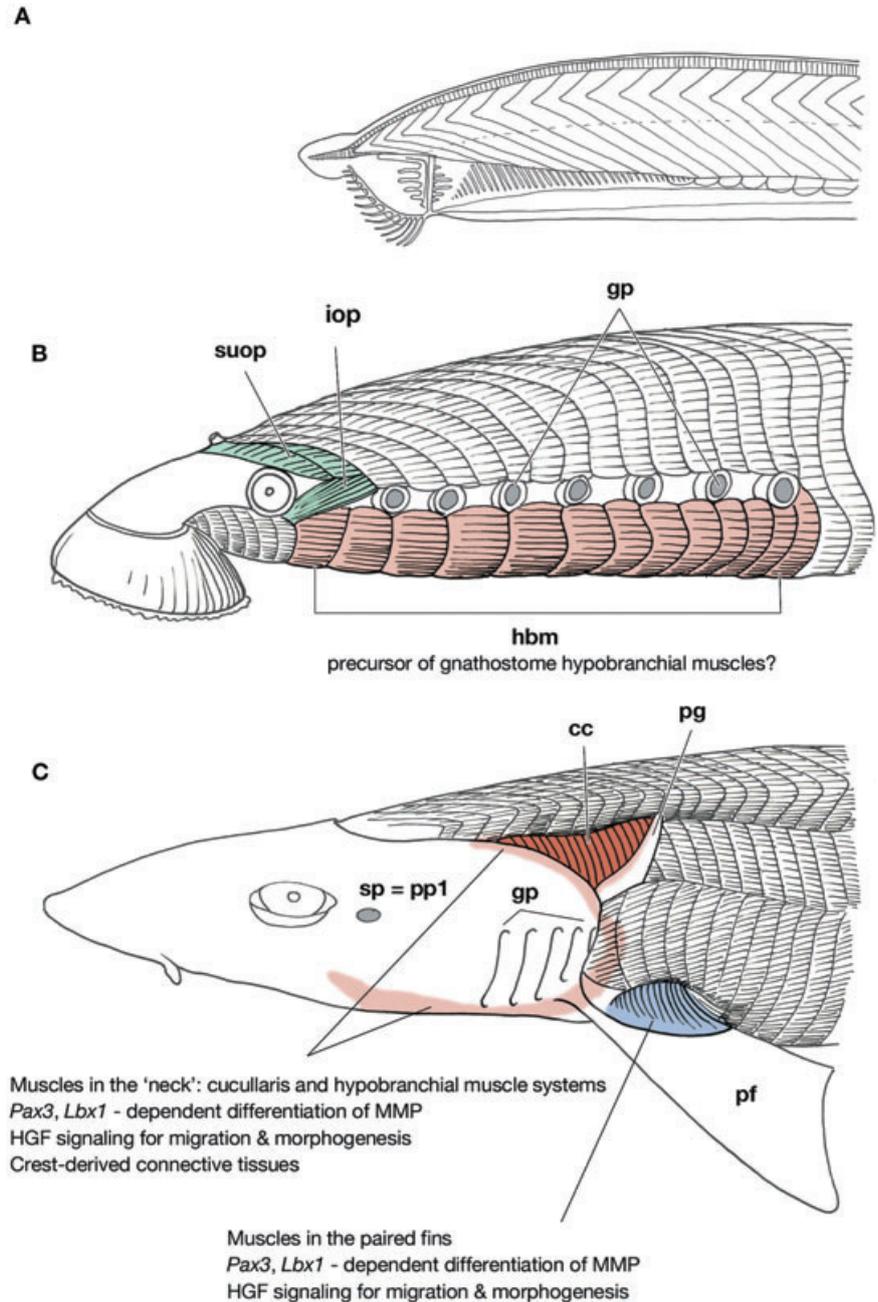
cells appear to play a role in the patterning of the cucullaris and hypobranchial muscles during development (Noden 1983, 1988; Köntges & Lumsden 1996; Evans & Noden 2006; reviewed by Noden & Francis-West 2006).

The most typical and probably the most ancestral pattern of the cucullaris muscle can be found in chondrichthyes (see Tanaka 1988; reviewed by Ribbing 1938). This muscle assumes a triangular shape and spans from the pectoral girdle to the cranium at the junction of the head and trunk. In the amniote embryo, the cucullaris anlage is found as a triangular primordium located at the junction of the pharynx and trunk regions (Keibel & Mall 1910; see also Kuratani 1997). In other words, in these muscles, the muscle fibers arise from the rostral-most part of the trunk element (rostral-most somites), and the material for their connective tissues arises from the caudal-most cephalic crest cells. This embryonic region thus corresponds to the head-trunk interface (Kuratani 1997), which does not exist in non-vertebrate chordates (Fig. 1A). The search for equivalent muscles or an anatomical place for this muscle development in cyclostomes is a critical issue for understanding early gnathostome evolution.

There appear to be no signs of cucullaris muscles in the hagfish or in the lamprey (Fig. 1B; Marinelli & Strenger 1954; reviewed by Kusakabe & Kuratani 2005, 2007). Cyclostomes appear to possess similar hypobranchial muscles distributed in the pharyngeal wall, which are not internalized to be located in the oropharyngeal floor. These muscles are innervated by some spinal nerves (Kuratani *et al.* 1997; Kusakabe & Kuratani 2007) and may represent a precursor of hypobranchial muscles. Thus, apart from the absence of the jaw and paired fin-related muscles, loss of the cucullaris muscles is one of the most conspicuous anatomical features that differentiate cyclostomes from gnathostomes. Instead, cyclostomes possess somite-derived muscles in the head, which is reminiscent of amphioxus. However, the resemblance is superficial, and these muscles represent, at least in the lamprey, typical trunk muscles that originate in the postotic region, as in gnathostomes, and which moved secondarily into the preotic domain to cover the head (Kuratani *et al.* 1999). The latter behavior of myotomes is not encountered in the gnathostome developmental process.

The above-noted preotic myotomes were once regarded as the evolutionary precursor of extrinsic ocular muscles in gnathostomes. However, this view is no longer held (Neal 1897, 1914), because the extrinsic eye muscles are present also in the lamprey, and histologically, they appear to be derived from the unsegmented head mesoderm rostral to the otic

Fig. 1. Comparison of somite-derived muscles among chordates and establishment of the neck in gnathostomes. (A) Amphioxus. Segmented myotomes are present along the entire axis. (B) Lamprey. Somitic muscles are restricted primarily in the postotic levels. In the lamprey, however, some rostral myotomes move rostrally to cover the head as supraoptic (suop) and infraoptic muscles (iop). Light red coloring indicates the hypobranchial muscle of the lamprey (hbm), which morphologically resembles the gnathostome hypobranchial muscles lying ventral to the gill pores (gp). These colored muscles have been specified specifically in cyclostomes. (C) In the shark anatomy, which represents the basic gnathostome body plan, rostral myotomes form both hypobranchial muscles and cucullaris muscles (cc). The latter arise as an element to bind the neurocranium, pharynx, and pectoral girdle (pg). Similar to the hypobranchial system, the cucullaris also possesses connective tissues derived from the neural crest, which represents a major component of the vertebrate "neck" according to the definition of Matsuoka *et al.* (2005). The anlage of these two types of muscles is called migratory muscle precursors (MMP), which share the same molecular-level developmental program that involves functions of Pax3, Lbx1, HGF, and c-met, as well as the contribution of neural crest for their connective tissue. In gnathostomes, paired fins (pf) have also appeared, with muscles developing as MMP, but with no contribution from the neural crest, which is the restricted property found only between the cranium and the anterior edge of the pectoral girdle. sp=pp1, spiracle or the first pharyngeal pouch.



vesicle (Koltzoff 1901), although this remains to be shown by cell-labeling experiments. A molecular developmental study has implied a prechordal mesodermal origin of the muscles, as in gnathostomes (Boorman & Shimeld 2002).

Taken together, the evidence suggests that rostral myotomes of lampreys (or possibly cyclostomes as a whole) are not necessarily simple or ancestral, but these myotomes may have experienced different types of morphogenesis between cyclostomes and

gnathostomes (Fig. 2). As suggested by Matsuoka *et al.* (2005), the evolution of cucullaris may have been concomitant with that of the pectoral girdle and fins, which secondarily define the domain of the neck as the gnathostome synapomorphy (Fig. 2). Although gnathostomes and lampreys share the basic distribution pattern of cephalic crest cells at early developmental stages, lamprey larvae do not possess any clear landmarks to limit the caudal distribution of cephalic crest cells (Horigome *et al.* 1999).

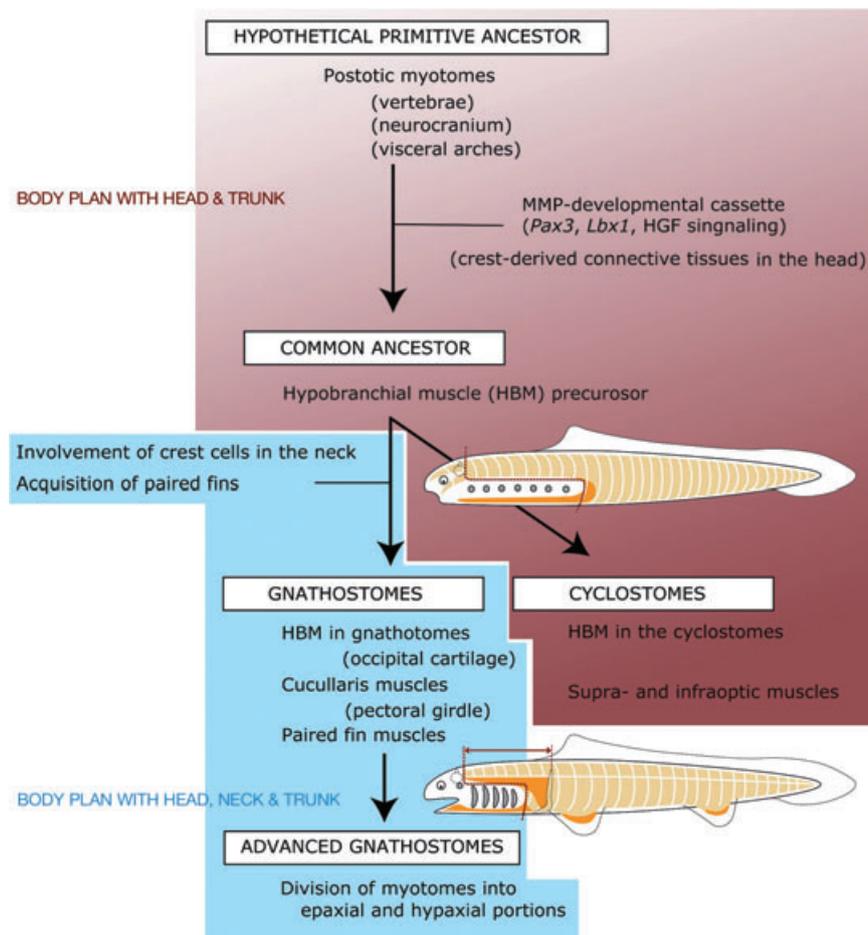


Fig. 2. Evolutionary scenario of vertebrate skeletal muscles in the context of evolutionary reorganization of the mesenchymal system to establish the “neck” as a new domain in the vertebrate body plan. A hypothetical primitive cyclostome and gnathostome fish are illustrated schematically with migratory muscle precursors (MMP) colored orange. The neck in gnathostomes is indicated between the true head and shoulder girdle (double-sided arrow). In this scheme, epaxial and hypaxial domains of the myotome are assumed to have been acquired after the establishment of the “neck” and paired fins in primitive gnathostomes because the dorso-ventral division does not appear to be a prerequisite for hypobranchial or cucullaris muscles, or even paired fin muscles, in development. Another reason that justifies this assumption is that a primitive form of the hypobranchial muscle is also present in the lamprey, in which myotomes are not dorso-ventrally segmented (see Kusakabe & Kuratani 2005). Hypothetical apomorphic changes in the developmental program are plotted on the evolutionary pathway to show how these changes could have served as the background for newly acquired patterns such as cucullaris muscles and gnathostome-type hypobranchial muscles. New skeletal elements associated with the newly acquired muscle types are shown in parentheses. These muscle and skeletal elements may be functionally and developmentally coupled to each other as a module.

Developmentally, fin (and limb) muscles, cucullaris, and hypobranchial muscles partly share the same pathway of differentiation. That is, the myoblasts derived from the ventral part of specific sets of myotomes (i.e. the origins of the muscles mentioned above) require expression of *Pax3*, which upregulates *Lbx1*, and this allows muscle precursors to migrate further (Gross *et al.* 2000; reviewed by Dietrich 1999; Birchmeier & Brohmann 2000). Hepatocyte growth factor (HGF, scatter factor)/c-met signaling also functions in the migratory pathways and morphogenesis of myoblasts (Neyt *et al.* 2000; Haines *et al.* 2004). These migratory muscle precursors (MMP; Alvares *et al.* 2003) differ from the cells that form other skeletal muscles in the adult anatomy because they originate

from and insert into skeletal elements that are not derived from the same sets of somites (sclerotomes) the muscles themselves have derived from. These specific muscles always attach to an entirely different type of skeleton. For example, occipital somite-derived tongue muscles attach to the hyoid arch (neural crest)-derived skeleton, or more typically, the myoblasts of the mammalian diaphragm travel a long distance in development (Jinguji & Takisawa 1983).

Although no data have been reported so far for gene expression of *Lbx1*, *HGF*, or *c-met*, in the lamprey it appears very likely that the evolutionary origin of cucullaris muscles is associated with establishment of the neck and simultaneous development of the pectoral fins and girdles. In this context, *Pax3/7*

expression has been observed in lamprey larvae, especially at high levels in the hypobranchial muscle (Kusakabe & Kuratani 2005; reviewed by Kusakabe & Kuratani 2007). Thus, in the common ancestor of vertebrates, before the dichotomy of gnathostomes and cyclostomes, it is possible that part of the MMP-developing program involving expression of *Pax3/7* was already established (Fig. 2). The gnathostome-specific morphogenesis in the “neck” region would be involved in the remodeling of the mesenchymal components between the neurocranium and the pectoral girdle by specific “moving” of the myoblasts. In this sense, it will be intriguing to observe the ectomesenchymal contribution to the connective tissues of some rostral muscles in the lamprey. It should be noted that it is only the gnathostomes that have assimilated some of the rostral vertebrae as the occipital bone as a part of the neurocranium (see Couly *et al.* 1993 for development of the occipital bone; for its evolution, see de Beer 1937).

From the above discussion, it becomes clear that several items may be associated with the formation of the vertebrate cucullaris muscle and that these are interconnected (or evolutionarily and developmentally coupled) to each other under the context of the evolution and development of the “neck” in gnathostomes. These items are:

1. Appearance of the cucullaris muscle (disappearance of infra- and supraoptic muscles in cyclostomes?)
2. Appearance of the accessorius nerve.
3. Appearance of the pectoral girdle and fins (or origin of paired fins per se?)
4. Appearance of the hypobranchial muscle system.
5. Appearance of the hypoglossal nerve (including some specific peripheral nerves associated with this muscle system such as cervical ansa or the phrenic nerve in mammals).
6. Assimilation of occipital vertebrae into the cranium.

Of these items, 1, 3, and 4 may involve changes in the interactions between mesodermal and ectomesenchymal cells; 2 and 5 would involve evolution of global interactions between motor axons and substrate for axonogenesis at the anatomical level; and 6 implies the specification and morphological differentiation of vertebrae under the positional value provided by the gnathostome-specific Hox code. Importantly, all of these features are not always found as *de novo* traits, but their precursors may be found in cyclostome embryos and larvae in somewhat primitive forms such as partially shared expression patterns of *Hox* genes in the lamprey (Takio *et al.* 2004, 2007) or the lamprey-type hypobranchial muscle that never reaches the

oropharyngeal floor. The sequence and pathway by which these changes took place have yet to be elucidated. Similar to the acquisition of the jaw, which involved simultaneous changes in the nasal and mandibular arch regions, development of the vertebrate neck also implies that these changes cannot be understood as modules. Instead, they appear to have been linked together tightly both developmentally and evolutionarily, because it is almost impossible to imagine the evolution of the cucullaris muscle in the finless condition. The dissection of the developmental mechanisms to establish the new and old patterns as discussed above will allow us to imagine how the neck and shoulder came into existence in our own ancestor.

Acknowledgment

I thank Rie Kusakabe for critical reading of the manuscript and valuable discussion.

Conflict of Interest

No conflict of interest has been declared by S. Kuratani.

References

- Alvares, L. E., Schubert, F. R., Thorpe, C., *et al.* 2003. Intrinsic, *Hox*-dependent cues determine the fate of skeletal muscle precursors. *Dev. Cell* **5**, 379–390.
- de Beer, G. R. 1937. *The Development of the Vertebrate Skull*. Oxford University Press, London.
- Birchmeier, C. & Brohmann, H. 2000. Genes that control the development of migrating muscle precursor cells. *Curr. Opin. Cell Biol.* **12**, 725–730.
- Boorman, C. J. & Shimeld, S. M. 2002. Cloning and expression of a *Pitx* homeobox gene from the lamprey, a jawless vertebrate. *Dev. Genes Evol.* **212**, 349–353.
- Couly, G. F., Coltey, P. M. & Le Douarin, N. M. 1993. The triple origin of skull in higher vertebrates: a study in quail–chick chimeras. *Development* **117**, 409–429.
- Dietrich, S. 1999. Regulation of hypaxial muscle development. *Cell Tiss. Res.* **296**, 175–182.
- Evans, D. J. & Noden, D. M. 2006. Spatial relations between avian craniofacial neural crest and paraxial mesoderm cells. *Dev Dyn.* **235**, 1310–1325.
- Gross, M. K., Moran-Rivard, L., Velasquez, T., Nakatsu, M. N., Jagla, K. & Goulding, M. 2000. *Lbx1* is required for muscle precursor migration along a lateral pathway into the limb. *Development* **127**, 413–424.
- Haines, L., Neyt, C., Gautier, P. *et al.* 2004. Met and HGF signaling controls hypaxial muscle and lateral line development in the zebrafish. *Development* **131**, 4857–4869.
- Horigome, N., Myojin, M., Hirano, S., Ueki, T., Aizawa, S. & Kuratani, S. 1999. Development of cephalic neural crest cells in embryos of *Lampetra japonica*, with special reference to the evolution of the jaw. *Dev. Biol.* **207**, 287–308.
- Jinguiji, Y. & Takisawa, A. 1983. Development of the mouse diaphragm. *Okajimas Fol. Anat. Jpn.* **60**, 17–42.

- Keibel, F. & Mall, F. P. 1910. *Manual of Human Embryology*. JB Lippincott Co, Philadelphia.
- Koltzoff, N. K. 1901. Entwicklungsgeschichte des Kopfes von *Petromyzon planeri*. *Bull. Soc. Nat. Moscou* **15**, 259–289.
- Köntges, G. & Lumsden, A. 1996. Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* **122**, 3229–3242.
- Kuratani, S. 1997. Distribution of postotic crest cells in the chick embryo defines the trunk/head interface: embryological interpretation of crest cell distribution and evolution of the vertebrate head. *Anat. Embryol.* **195**, 1–13.
- Kuratani, S. 2004. [*Evolutionary Morphology – Bauplan and Embryonic Development of Vertebrates*.] University Press, Tokyo, Japan (in Japanese).
- Kuratani, S., Horigome, N. & Hirano, S. 1999. Developmental morphology of the cephalic mesoderm and re-evaluation of segmental theories of the vertebrate head: evidence from embryos of an agnathan vertebrate, *Lampetra japonica*. *Dev. Biol.* **210**, 381–400.
- Kuratani, S., Nobusada, Y., Horigome, N. & Shigetani, Y. 2001. Embryology of the lamprey and evolution of the vertebrate jaw: insights from molecular and developmental perspectives. *Phil. Trans. Roy. Soc.* **356**, 15–32.
- Kuratani, S. & Ota, G. K. 2008. The primitive versus derived traits in the developmental program of the vertebrate head: views from cyclostome developmental studies. *J. Exp. Zool. B Mol. Dev. Evol.* (forthcoming). [DOI 10.1002/jez.b.21190].
- Kuratani, S., Ueki, T., Aizawa, S. & Hirano, S. 1997. Peripheral development of the cranial nerves in a cyclostome, *Lampetra japonica*: morphological distribution of nerve branches and the vertebrate body plan. *J. Comp. Neurol.* **384**, 483–500.
- Kusakabe, R. & Kuratani, S. 2005. Evolution and developmental patterning of the vertebrate skeletal muscles: perspectives from the lamprey. *Dev. Dyn.* **234**, 824–834.
- Kusakabe, R. & Kuratani, S. 2007. Evolutionary perspectives from development of the mesodermal components in the lamprey. *Dev. Dyn.* **236**, 2410–2420.
- Lubosch, W. 1938. Muskeln des kopfes: viscerale muskulatur (Fortsetzung). In: *Handbuch der Vergleichenden Anatomie der Wirbeltiere*. V (eds Bolck, L. *et al.*), pp. 1011–1106. Urban & Schwarzenberg, Berlin.
- Marinelli, W. & Strenger, A. 1954. *Vergleichende Anatomie und Morphologie der Wirbeltiere*. Franz Deuticke, Vienna.
- Matsuoka, T., Ahlberg, P. E., Kassarlis, N. *et al.* 2005. Neural crest origins of the neck and shoulder. *Nature* **436**, 347–355.
- McKenzie, J. 1962. The development of the sternomastoid and trapezius muscles. *Cont. Embryol.* **37**, 121–129.
- Neal, H. V. 1897. The development of the hypoglossus musculature in *Petromyzon* and *Squalus*. *Anat. Anz.* **13**, 441–463.
- Neal, H. V. 1914. Morphology of the eye muscle nerves. *J. Morph.* **25**, 1–186.
- Neyt, C., Jagla, K., Thisse, C., Thisse, B., Haines, L. & Currie, P. D. 2000. Evolutionary origins of vertebrate appendicular muscle. *Nature* **408**, 82–86.
- Noden, D. M. 1983. The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. *Dev. Biol.* **96**, 144–165.
- Noden, D. M. 1988. Interactions and fates of avian craniofacial mesenchyme. *Development* **103** (Suppl.), 121–140.
- Noden, D. M. & Francis-West, P. 2006. The differentiation and morphogenesis of craniofacial muscles. *Dev. Dyn.* **235**, 1194–1218.
- Ribbing, L. 1938. Muskeln und Nerven der Extremitäten. In: *Handbuch der Vergleichenden Anatomie der Wirbeltiere*. V (eds Bolck, L. *et al.*), pp. 543–656. Urban & Schwarzenberg, Berlin.
- Shigetani, Y., Sugahara, F. & Kuratani, S. 2005. Evolutionary scenario of the vertebrate jaw: the heterotopy theory from the perspectives of comparative and molecular embryology. *Bioessays* **27**, 331–338.
- Takio, Y., Kuraku, S., Kusakabe, R. *et al.* 2007. *Hox* gene expression patterns in *Lethenteron japonicum* embryos insights into the evolution of the vertebrate *Hox* code. *Dev. Biol.* **308**, 606–620.
- Takio, Y., Pasqualetti, M., Kuraku, S., Hirano, S., Rijli, F. M. & Kuratani, S. 2004. Lamprey *Hox* genes and the evolution of jaws. *Nature* **429**, following 262.
- Tanaka, S. 1988. A macroscopical study of the trapezius muscle of sharks, with reference to the topographically related nerves and vein. *Anat. Anz.* **165**, 7–21.
- Yalden, D. W. 1985. Feeding mechanisms as evidence for cyclostome monophyly. *Zool. J. Linn. Soc.* **84**, 291–300.