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[REVIEW]

Evolution of the Vertebrate Cranium: Viewed from Hagfish Developmental Studies

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Our knowledge of vertebrate cranium evolution has relied largely on the study of gnathostomes. Recent evolutionary and developmental studies of cyclostomes have shed new light on the history of the vertebrate skull. The recent ability to obtain embryos of the hagfish, *Eptatretus burgeri*, has enabled new studies which have suggested an embryonic morphological pattern (the "cyclostome pattern") of craniofacial development. This pattern is shared by cyclostomes, but not by modern jawed vertebrates. Because this pattern of embryonic head development is thought to be present in some stem gnathostomes (ostracoderms), it is possible that the cyclostome pattern represents the vertebrate ancestral pattern. The study of cyclostomes may thus lead to an understanding of the most ancestral basis of craniofacial development. In this review, we summarize the development of the hagfish chondrocranium in light of the cyclostome pattern, present an updated comparison of the cyclostome chondrocranium, and discuss several aspects of the evolution and development of the vertebrate skull.

Key words: agnathans, cranium, cyclostomes, embryo, evolution, hagfish

INTRODUCTION

The vertebrate cranium is characterized by its complicated and functionally sophisticated morphological pattern, and the cranium per se represents one of the major traits that define the vertebrate body plan. The developmental and evolutionary origins of the cranium have, therefore, long been intriguing questions in comparative morphology and evolutionary developmental biology. To date, our understanding of the vertebrate skull has relied mainly on the comparative morphology and development of gnathostome skulls based on numerous descriptive and experimental studies (Parker and Bettany, 1877; Goodrich, 1930; de Beer, 1937; Hanken and Hall, 1993).

Classical comparative morphology sought to provide an archetypical model of the vertebrate skull, from which all the variety among vertebrate skulls was derived. In this model, the cranium was divided into several components or modules: the dorsally located neurocranium which encases the brain, and a ventral moiety, or the viscerocranium that supports the pharynx. Of these, the neurocranium often included sensory capsules for head-specific sensory organs (eyes, nose, and inner ear), which are often regarded as

modules more or less independent from the rest of the neurocranium. These major cranial components are formed of endoskeletal elements that arise primarily from cartilage during development. In addition to these, the exoskeletal elements form an outer shield called the dermatocranium (de Beer, 1937; Romer and Parsons, 1977; Portmann, 1976; Hirasawa and Kuratani, 2015).

Regarding the ancestral type of the cranium, the vertebrate head was once assumed to be segmented along the anteroposterior axis, in a manner akin to that of vertebrae in the trunk (reviewed by Goodrich, 1930; Sewertzoff, 1931; de Beer, 1937; Jollie, 1977). Near the end of 19th century, this traditional concept was largely substantiated by elasmobranch embryology, in which somite-like coeloms do arise in the preotic region of the head (Gee, 1996; reviewed by Kuratani and Adachi, 2016). These coelomic cavities, or head cavities, were thus homologized with somites (Balfour, 1877; van Wijhe, 1882; reviewed by Wedin, 1949; Kuratani, 2003, 2008b, 2015; Adachi and Kuratani, 2012; Adachi et al., 2012).

The cyclostome cranium, on the other hand, contributed little to the morphological concept of the vertebrate head or skull in traditional comparative morphology. This was due in part to the difficulty of histological observation of lamprey embryos and the inaccessibility of embryos of hagfish, another group of cyclostomes (reviewed by Ota and Kuratani, 2006, 2008; Ota et al., 2007; Kuratani and Ota,

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2008; also see Dean, 1899). In addition, the adult hagfish cranium was often believed to be either highly modified from its ancestral form, or to represent a state more ancestral than the rest of vertebrates (reviewed by Janvier, 1996). However, it is now clear that cyclostomes constitute a monophyletic group, with gnathostomes as an outgroup (Mallatt and Sullivan, 1998; Kuraku et al., 1999, 2008, 2009; Delarbre et al., 2002; Takezaki et al., 2003; Mallatt and Winchell, 2007), and they may have acquired derived features that gnathostomes have not. Hagfish embryos have become available since 2007 in RIKEN, Japan (Ota and Kuratani, 2007, 2008; Ota et al., 2007), and this, in conjunction with the advancement of molecular-level developmental studies on the lamprey, has greatly increased our understanding of cranial evolution in vertebrates. Thus, the goal of the present review is to summarize hagfish cranial development, and to propose an updated scenario to explain the development and evolution of the vertebrate cranium.

Evolution of the craniofacial pattern in vertebrates

As was first pointed out by Haeckel (1874), the most conspicuous difference between the jawed and jawless vertebrates is not the presence or absence of the biting jaw, but the number of nostrils: jawed vertebrates have two nostrils (diplorhiny), whereas the other vertebrates possess only a single median nostril (monorhiny) (Janvier, 1996). This idea is consistent with evolutionary changes in the distribution of the craniofacial ectomesenchyme as well as the ectodermal placodes, including the stomodeum, during transition from the jawless to jawed condition (Shigetani et al., 2002; Kuratani, 2005, 2012; Oisi et al., 2013a; for development of the cyclostome placodes, see also McCauley and Bronner-Fraser, 2003, 2003; Uchida et al., 2003; Modrell et al., 2014). Importantly, diplorhiny is linked to the positional shift of the adenohypophysis to open in the oral cavity (Kuratani, 2005), and the premandibular ectomesenchyme that formed the dorsal part of the oral apparatus of the cyclostomes now provides the prechordal part of the neurocranium (the rostral half of the neurocranium is exclusively of neural crest-origin in modern gnathostomes; Couly et al., 1993). In addition, the position of the mouth opening shifted slightly caudally prior to jaw acquisition (Shigetani et al., 2002, 2005). Thus, the placodal evolution is concomitant with the evolution of the ectomesenchyme, the source of the major part of the gnathostome cranium.

In the above scenario, the mono- to diplorhiny shift in the acquisition of the jaw is consistent with the fossil evidence (Kuratani, 2005, 2012; Gai et al., 2011; Dupret et al., 2014). Cyclostomes as well as some of stem gnathostomes, often called ostracoderms, were devoid of jaws and often had only one nostril (Janvier, 1996). Curiously, galeaspids, one of the advanced lineages of ostracoderms, is reported to have possessed bilaterally separated nasal sacs and a posteriorly separated adenohypophysis opening to the oral cavity, not to the nasal duct, possibly exhibiting a transitional state to jawed vertebrates (Gai et al., 2011). However, this explanation is not entirely consistent with the phylogeny of stem gnathostomes. Namely, the nasohypophysial pattern of galeaspids may resemble that of jawed vertebrates, many other characters including cellular bone, paired fins, epicercal tail, shoulder girdle, braincase anatomy, suggest that

osteostracans, not galeaspids, are more closely related to jawed vertebrates. The transition from 'ostracoderms' to jawed vertebrates may be more complicated than the above scenario.

The evolutionary polarity of the nostril number is directed from one to two, and the stem gnathostomes had a cyclostome-like pattern of craniofacial morphology. Therefore, it seems likely that the primitive craniofacial pattern of vertebrates could be sought in cyclostome developmental patterns. Our recent finding of a cyclostome pattern would be relevant to this issue (Oisi et al., 2013a). Namely, embryos of hagfish and lampreys pass through a stage during which morphological patterns of the embryonic head look quite like each other, indicating the presence of the conserved developmental stage that defines cyclostome morphology (Oisi et al., 2013a). At that stage of development, both lamprey and hagfish embryos show two distinct processes, the anterior and posterior processes, in the premandibular domain, rostral and caudal to the median placode, the nasohypophyseal plate (Fig. 1).

Of the above two processes, the rostral process forms the posterior wall of the nasohypophyseal opening (generally called the nostril or external nares) in the lamprey, whereas in the hagfish the equivalent process forms the roof of the anteroposteriorly elongated nasohypophyseal duct (Fig. 1). This difference is consistent with the direction of the nostril, which opens dorsally in the lamprey and rostrally in the hagfish (Fig. 1).

In lamprey development, the posterior process grows dorsally and rostrally to differentiate into the upper lip, thereby pushing the nostril towards the dorsal aspect of the head (Fig. 1, left). The transformation process has been well documented by several authors (Sewertzoff, 1931; Strahan, 1960; Heintz, 1963; Kuratani et al., 2001; reviewed by Goodrich, 1909). Developmental modification of the posterior process in the hagfish clearly illustrates the morphological uniqueness of the hagfish head, as typically shown in the formation of the oronasal septum (Fig. 1, right; Oisi et al., 2013a). This anlage grows anteriorly to make a septum dividing the oronasal cavity dorsoventrally, as well as the ventral margin of the nostril rostrally, and the posterior root of this process later disappears, thereby making the nasohypophyseal duct and pharynx confluent with each other (Oisi et al., 2013a). By this penetration of nasopharyngeal passage, the hagfish head acquires a unique anatomical topography, not directly comparable to that of the lamprey.

Each part of the head in cyclostomes can be developmentally reduced to the identical set of craniofacial primordia; namely, the anterior and posterior processes and the pharyngeal arches, enabling the homologization of anatomical components in both animals (Fig. 2). In particular, it has been shown that the trigeminal nerve branching and innervation patterns precisely coincide with each other in lamprey and hagfish; however, this not comparable to those in gnathostomes (Lindstrom, 1949; Oisi et al., 2013a; Higashiyama and Kuratani, 2014). Thus, the craniofacial morphotype shared by the modern cyclostomes cannot be applied to the patterns in jawed vertebrates. This suggests that an evolutionary event overrode the ancestral developmental constraints before the establishment of the morphotype of the jawed vertebrate head. As documented in several textbooks,
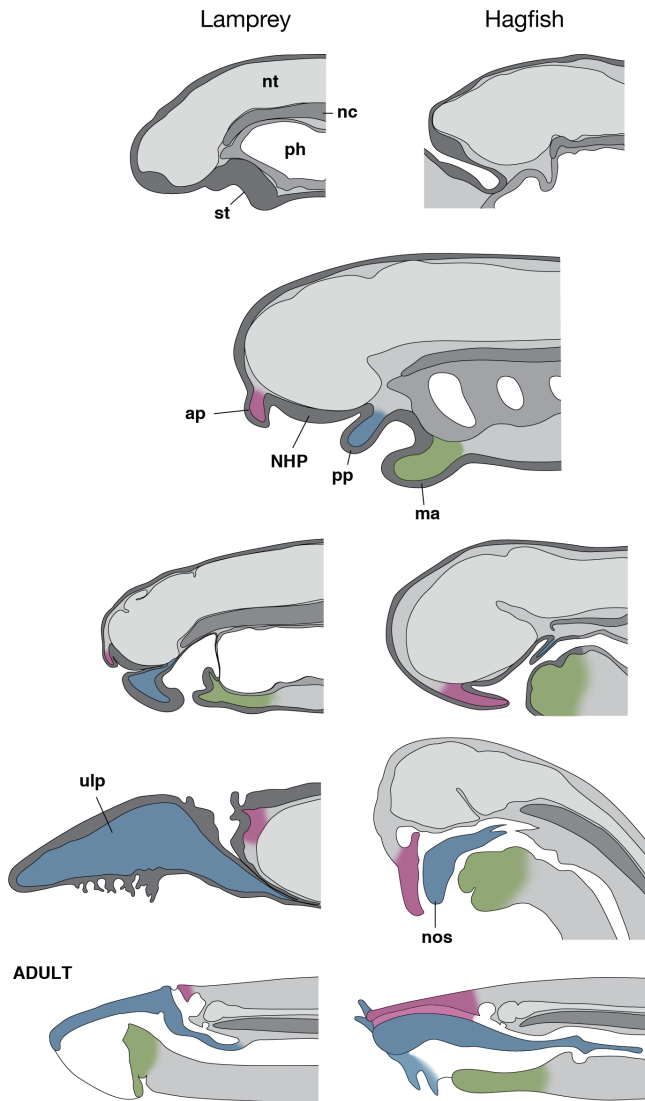
Fig. 1. Developmental pathways of lamprey and hagfish craniofacial patterns. The cyclostome pattern (second from the top) is schematically illustrated. This pattern appears after the establishment of the neural crest-derived ectomesenchyme.

Fig. 2. Cyclostome chondrocranium. Updated version of Fig. 10 in Oisi et al. (2013b). Homologies of cartilaginous elements are shown by different colors based on embryonic origins and morphology. The cornual plate-homologs appear to belong either to the mandibular or hyoid arches.

the crown gnathostome morphotype can be formulated by the derivatives of frontonasal process (= medial nasal prominence), lateral nasal prominence, maxillary process, and mandibular process. Of these, the maxillary and mandibular processes can be equated to the cyclostome mandibular arch, which may be dorsoventrally specified, but not articulated to form a jaw (see below). However, medial and lateral nasal prominences in jawed vertebrates cannot be directly compared to the anterior process in the cyclostome embryos, if they occupy a similar position in the head primordium (Oisi et al., 2013a). The cyclostome posterior process is rather similar to the anlage of trabecular cartilage in the cyclostomes, the homology of which will be further discussed below.

Comparison of the lamprey and hagfish crania

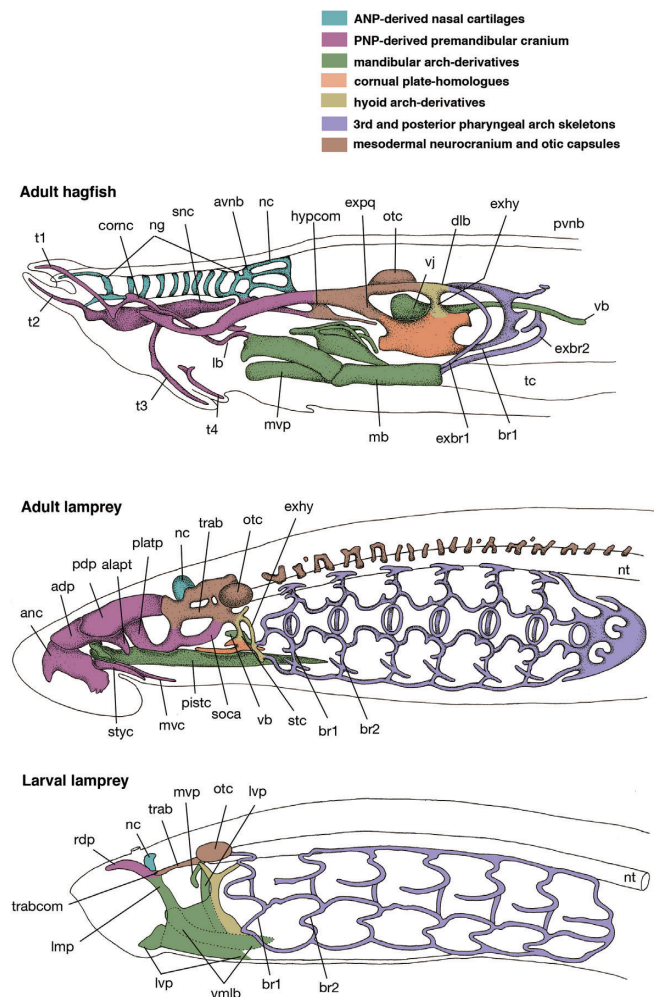Several studies attempted to compare cranial elements between the lamprey and hagfish (Marinelli and Strenger,

1954, 1956; Parker, 1883a, b; Holmgren and Stensiö, 1936; Neumayer, 1938; Holmgren, 1946; Heintz, 1963). However, homologization of cartilaginous elements has not always been as straightforward, and has been accomplished by topographical relationships of the elements and other anatomical structures. The only exception was the comparative morphology of the lingual apparatus, for which Yalden showed a very accurate one-to-one correspondence for skeletal and muscular elements (derived from the mandibular arch) between adult lampreys and hagfishes (Yalden, 1985).

Previously, we postulated a new method of comparison, based on a cyclostome-conserved pattern of craniofacial development, to compare systematically the whole cranial morphological patterns in the cyclostomes. Such a comparison reveals that lamprey and hagfish embryos both develop the same set of craniofacial processes, the anterior process, posterior process, and the mandibular arch, during the pharyngular stage (Oisi et al., 2013a, b). Thus, the posterior process-derivatives are comparable between lamprey and

hagfish embryos as a module (Figs. 1, 2). This schematization makes it evident that the anterior process-derivatives in hagfish and lamprey exhibit considerable differences. On the other hand, the posterior process-derivatives differentiate into rostral neurocranium and oral apparatus in both the animals, resembling the developmental role of the premandibular ectomesenchyme of jawed vertebrate embryos.

In our previous comparison, we proposed a module (craniofacial anlage)-level homologization in the cyclostome chondrocrania; however, during this procedure, we found an inaccurate identification regarding a cartilaginous element at the level of the hyoid arch (Oisi et al., 2013b; for morphological modules for homologization see Kuratani, 2009). As shown in Fig. 2, an updated version of the cranial comparison, there is a plate-like cartilage beneath the extrahyale in the adult hagfish chondrocranium. With respect to the position of other cartilaginous elements, as well as facial nerve branches, we found that a possible homologue of this cartilage, or the cornual plate of the hagfish, may be found below the extrahyale of the adult lamprey. Otherwise, it is not possible to homologize each viscerocranial element between the hagfish and lamprey.

Basic composition of the neurocranium

Based on construction of chimeric avian embryos, Couly and others made a precise map of neural crest- and mesoderm-derived parts of the neurocranium, and defined chordal and prechordal portions, as anterior and posterior subdivisions of the neurocranium (Couly et al., 1993). In the mouse, a genetic approach produced a developmental map that is nearly identical to that of the avian cranium (McBratney-Owen et al., 2008). Thus, the chordal cranium is coextensive and associated with the notochord medially, and derived from the paraxial mesoderm, whereas the prechordal cranium arises in the region that is devoid of the notochord and differentiates from the neural crest-derived ectomesenchyme (see Wada et al., 2011 for development of trabecula in amniotes; for cyclostomes see Kuratani et al., 2004, 2013). Similar distinction of the neurocranium was recognized in classical comparative embryology. For example, Rathke (1839) was among the first to recognize the difference between the rostral part of the early embryonic neurocranium of jawed vertebrates, represented by trabecula, and more posterior parts of the cranium.

Thus, the boundary was positioned at the level where the hypophysis develops, and the early chondrocranium of jawed vertebrates consists of the trabecula that lies rostral to the hypophysis, and the parachordal posterior to it. This distinction corresponds to the difference between the vertebral and prevertebral regions postulated by Gegenbaur (1871, 1872). The vertebral region of the head is accompanied by the notochord, whereas the more rostrally located evertebral region is devoid of the notochord. This distinction should not be confused with another distinction of the neurocranium, namely the cephalic mesodermal part and somite-derived (occipital) part. Huxley (1858) first did not recognize this boundary. It was only after Froriep (1882, 1883, 1886, 1905a, b) and Stöhr (1881) that embryologists started to recognize a boundary between cephalic mesoderm and rostral somites (unsegmented prespinal portion and segmented spinal portion of the neurocranium) and that the latter forms the occipital part of the skull.

In the cyclostome cranium, a similar anteroposterior distinction of cranium has been recognized, although there is no occipital homologue in their crania. The key to identifying the boundary was the origin of the so called "trabecula" of the cyclostomes (in the sense of Sewertzoff, 1913; Langille and Hall, 1988; also see de Beer, 1937 for the hypothetical premandibular arch in the lamprey). As described above in connection with the heterotopic theory of jaw acquisition, in a functional sense the neurocranium is mostly formed by parachordals and their derivatives in the cyclostomes. Therefore, the prechordal (neuro)cranium is minor; the cyclostome premandibular ectomesenchyme is primarily employed for the formation of the oral apparatus (Fig. 3). Exceptionally, the hagfish nasal cartilage is expanded; however, it belongs to the sensory capsule.

Several studies have suggested that the trabecula in the lamprey does not represent the similarly named cartilage in jawed vertebrates, but rather a mesoderm-derived neurocranial part (parachordals) that has elongated rostrally to support the forebrain. In particular, the latter idea was substantiated by detailed observation of staged embryos (Johnels, 1948), as well as the experimental labeling of the head mesoderm (at the mandibular arch level) that differentiated into the trabecula (Kuratani et al., 2004; but also see Newth, 1956; Langille and Hall, 1988). The same is presumably also true for the hagfish chondrocranium (Oisi et al., 2013b). Importantly, a similar distribution pattern of mesodermal and neural crest-derived mesenchyme is expected in the hagfish embryo, which is suggested not only by the morphological
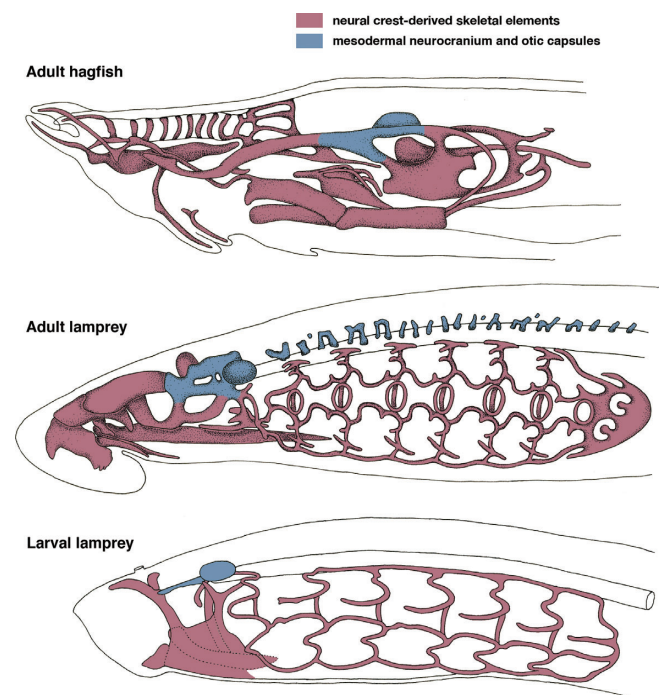

Fig. 3. Cell lineage origins of the cyclostome cranium. Based on the homologies of skeletal elements and developmental observations. As typically seen in the larval lamprey, rostral ectomesenchyme is mostly involved in the formation of the oral apparatus, and the rostral part of the neurocranium is provided by the rostrally elongated parachordal (blue).

patterns of development (identification of the premandibular mesoderm in cyclostome embryos, e.g., the rostralmost mesodermal element; Koltzoff, 1901; Wedin, 1949; Kuratani et al., 1999; Oisi et al., 2013a), but also by cephalic mesoderm-associated gene expression patterns (Oisi et al., 2013b). Notably, there is no direct evidence of the neural crest or mesodermal derivation of the cyclostome cranium, except for that obtained from primitive labeling experiments (Kuratani et al., 2004; for mesodermal origin of vestigial vertebral elements in hagfish see Ota et al., 2011, 2013, 2014; for extirpation of the cephalic neural crest see Newth, 1956; Langille and Hall, 1988).

With the identification of the lamprey trabecula, the position of prechordal/chordal boundary of the hagfish is found in the rostral end of the parachordal (Oisi et al., 2013b). This corresponds to the position of the original head mesoderm that is not segmented, as had been suggested previously (Koltzoff, 1901; Damas, 1944; reviewed by Kuratani et al., 1999; Kuratani, 2008b). The cartilage forming the otic capsule appears to be derived from the head mesoderm, but some crest cells may also contribute to its formation, as in jawed vertebrates (Noden, 1988; Couly et al., 1993). Rostral to the parachordals, all the cartilaginous elements in the hagfish derived from the above noted craniofacial anlagen (pharyngeal arches plus anterior and posterior processes) should be of crest origin. In the precartilaginous stage of the hagfish, the rostralmost mesodermal element can be seen in the prechordal plate. This structure, occupying a position identical to the premandibular mesoderm in the lamprey, produces a pair of dense chords of cells laterally, possibly representing a vestigial premandibular mesoderm (or cavity?) in hagfish (Horigome et al., 1999; Kuratani et al., 1999; Oisi et al., 2013a, b). Possessing only degenerate eyes, the hagfish does not develop extrinsic eye muscles, which presumably differentiate from the premandibular mesoderm (Koltzoff, 1901; Suzuki et al., 2016).

By extrapolating the data obtained from jawed vertebrates, the suggested cell lineage origins of the cyclostome cranium is shown in Fig. 3. It was once believed that a pharyngeal arch(es) rostral to the mandibular arch (premandibular arches) was secondarily incorporated in the formation of the prechordal neurocranium in gnathostomes, and thus the trabecula was assumed to represent the vestigial skeleton belonging to such arches (reviewed by de Beer, 1937). In the development of the cyclostome cranium, no evidence can be found to show the presence of the premandibular arch (also see Janvier, 1996 for ostracoderms). The transformed ectomesenchymal element in the agnathan to gnathostome transition appears to be the prechordal (premandibular) ectomesenchyme (Kuratani et al., 2013), forming the dorsal oral apparatus in agnathans. The parachordals are more extensive in cyclostomes and play roles like that of gnathostome trabecula as the floor of the forebrain.

The parachordals have long been recognized in the cyclostomes (Parker, 1883a, b; Neumayer, 1938; Holmgren, 1946). Their presence in the hagfish suggests that this cartilaginous element arose simultaneously with the vertebrae. Because both the vertebral elements and parachordals arise from paraxial mesoderm, possibly under the same notochord-derived signaling, these two mesodermal elements

may have evolved as an initial skeletal component surrounding the central nervous system, whereas the neural crest elements functioned exclusively as supporting tissue of the oro-pharyngeal system or the sensory organs in the ancestral vertebrate. This is consistent with the recent finding of Dupret et al. (2014) that basal placoderms possessed a neurocranium that more closely resembled that of cyclostomes, lampreys in particular. It thus appears that the prechordal/chordal distinction of the neurocranium is a newly acquired feature of the neurocranium specific to crown gnathostomes.

From jawless to jawed states of evolution

Numerous studies have attempted to explain the origin of gnathostome jaws, which consists of upper and lower jaws under several different scenarios. Classical theories assumed that the jaw was simply obtained by the dorsoventral division of one of the rostral visceral arches that we recognize now as the mandibular arch. This theory, however, is not supported by fossil evidence (undivided mandibular arch forming the oral apparatus). Importantly, the jawless vertebrates, including ostracoderms and cyclostomes possess an oral apparatus that is well differentiated dorsoventrally, and incorporates premandibular ectomesenchymal components in its dorsal part (Cerny et al., 2004). In that sense, both the cyclostomes and gnathostomes have similar oral apparatuses, although not homologous to each other. The neo-classical theory of Mallatt (2008) also takes the developmental role of premandibular component into consideration in jaw formation, but only heterotopy is capable of explaining the simultaneous acquisition of prechordal cranium (trabecular cartilage) and jaw (Shigetani et al., 2002, 2005; reviewed by Kuratani, 2012).

From the molecular developmental perspective, evolution of the visceral arch skeleton depends of the regulation of Dlx genes. Namely, the nested expression of the Dlx genes, or the Dlx code, specify the pharyngeal arch ectomesenchyme along the dorsoventral axis in jawed vertebrate embryos (Depew et al., 2002). Based on this dorsoventral specification, a part of the upper jaw and the entire lower jaws can arise from the mandibular arch. The Dlx code appears to be an ancestral trait for jawed vertebrate development (Gillis et al., 2013), but cyclostome embryos do not show the same nested pattern (Myojin et al., 2001; Neidert et al., 2001; Cerny et al., 2010; Kuraku et al., 2010; Fujimoto et al., 2013; Oisi et al., 2013b).

In the fossil record, the dorsoventrally differentiated type of the visceral arch skeleton, typically observed in osteichthyans, appears to represent the ancestral type that was obtained before the divergence of chondrichthyans and osteichthyans, and was likely present in placoderms (Fig. 4; Pradel et al., 2014). With respect to the mandibular arch, it appears that the Dlx code is responsible for the dorsoventral specification of the arch (Depew et al., 2002; Sato et al., 2008; Kitazawa et al., 2015), but it is unknown whether the code is a prerequisite for segmentation and articulation of a visceral arch skeleton (Fig. 4). It may be that the nested Dlx code was established after the segmentation of the visceral arch skeleton, which initially would have been patterned in a dorsoventrally symmetrical manner, as suggested by Sewertzoff (1928, 1931) (Fig. 4). Importantly, the branchial
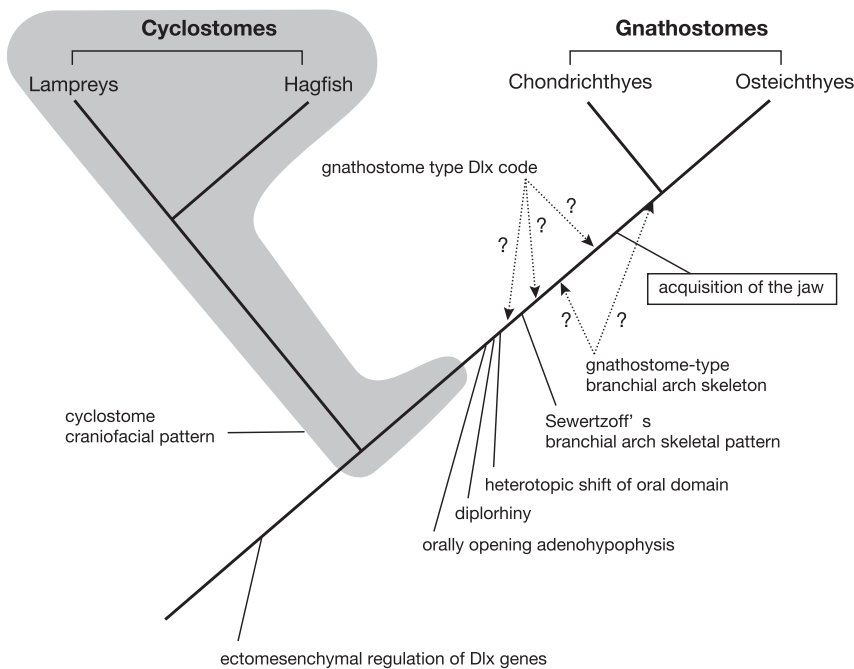
Fig. 4. Hypothetical evolutionary sequence of the craniofacial developmental pattern of vertebrates. The gray region represents distribution of the cyclostome craniofacial pattern on the phylogenetic tree. This developmental pattern extends into stem gnathostomes with single nostrils and adenohypophysis opening into the nasal cavity. The craniofacial morphotype for crown gnathostomes is thought to have been established in a stepwise manner, involving the acquisition of diplorhiny, shift of adenohypophyseal opening into the oral cavity, etc., before the acquisition of the jaw.

specific cellular and molecular event. These muscles do not form in the body wall, but are positioned in close association with visceral structures and develop from *Lbx1*-expressing myoblasts that migrate over long distances (migrating muscle progenitor: see Alvares et al., 2003 and Dietrich et al., 1999; reviewed by Sambasivan et al., 2011). Lampreys also develop hypobranchial-like muscles, but they lack some of the properties of typical gnathostome hypobranchial muscles (for the hypobranchial muscle-homologue in the lamprey see; Kuratani et al., 2002; Kusakabe and Kuratani, 2007; Kuratani, 2008a; Kusakabe et al., 2011; for the hagfish hypobranchial-like muscle see Oisi et al., 2015). For example, the 'lamprey hypobranchial muscle' arises in superficial position of the pharyngeal wall (arches), not in the oropharyngeal floor, and its precursors migrate more dorsally than those of gnathostome embryos, in which the hypoglossal cord grows within the dorsal portion of the pericardial wall. The embryonic developmental pattern of the hypobranchial-like muscle of the hagfish is even more unlike that of gnathostomes (Oisi et al., 2015). It is unknown whether the lamprey hypobranchial muscle, which is comparatively more similar to gnathostomes, represents an ancestral pattern for cyclostomes. The occipital-hypobranchial system may be a gnathostome-specific novelty, involving a topographical shift of morphological patterns and change in gene regulation. Functionally, mandibular arch-derived lingual apparatus in cyclostomes would serve similar oropharyngeal apparatuses, which may not have led to the invention of the tongue, a derivative of the hypobranchial musculature.

Peculiarity of the hagfish?

In our report on the cyclostome pattern of hagfish and lamprey embryos, we proposed that hagfish would be more diverged than lamprey with respect to the anatomical pattern of adults (Oisi et al., 2013a, 2015; see also Kuratani, 2008a). This is supported by the fact that the lamprey has long been regarded as closer to gnathostomes in a number of morphological traits than is the hagfish (reviewed by Janvier, 1996). Indeed, hagfish were once classified outside the vertebrates due to the absence of vertebrae (but, see Ota et al., 2011, 2013, 2014). However, it should be noted that the apparent resemblances between lampreys and modern gnathostomes are often superficial and subjective. For example, the resemblance of upper and lower lips of ammocoete larvae of lampreys to the upper and lower jaws in gnathostomes does not represent a homology, but a systematic topographical shift of craniofacial ectomesenchyme as assumed in the transition from jawless to jawed states (Shigetani et al., 2002, 2005; reviewed by Kuratani et al., 2013). Thus, it is hard to determine whether the oral apparatus of hagfish or lamprey is more similar to that of the

arch skeleton of the lamprey, and possibly of the hagfish as well, develops in a dorsoventrally symmetrical pattern, consistent with the expression patterns of their Dlx genes (Cerny et al., 2010; but see Yao et al., 2011 for the dorsoventral polarity of the lamprey mandibular arch). Thus, before the heterotopic shift of the oral ectomesenchyme permitted by the acquisition of diplorhiny, the visceral arch skeleton may have been dorsoventrally specified. Unfortunately, however, anatomical pattern of the visceral arch endoskeleton is not well understood in stem gnathostomes (reviewed by Janvier, 1996).

Another change in the cranial pattern that gnathostomes have experienced was the incorporation of the occipital somites. As has previously been discussed, the cartilaginous vertebral column was an ancestral trait for all the vertebrates, and hagfish appear to have lost it except for the tail region (Neumayer, 1938; Ota et al., 2011). If this is the case, the vertebral column was possessed by the common ancestor of vertebrates, and the acquisition of the occipital was one of the first evolutionary changes introduced into the gnathostome vertebral column. Because the occipital and hypobranchial muscles innervated by the spinal nerve arising at the same segmental level as the occipital appeared simultaneously in evolution, these phenomena may have been developmentally coupled to each other. Although these morphological inventions are seen as position-specific modifications of somitic derivatives and spinal nerves, they may not be Hox code-dependent transformations. For example, like the fin muscles, the hypobranchial muscles differ from other trunk muscles in that these are formed through a

common ancestor of cyclostomes.

Similarly, although the branchial arch cartilages appear to be quite differentiated in the hagfish, presence of external and internal skeletal elements may also be shared by the elasmobranchs (Mallatt, 1984); however, the internal branchial arch skeletons are entirely absent in the lamprey. Rostral shift of the esophagus during lamprey metamorphosis also represents a lamprey-specific trait. For the reasons stated above, it is safer to state that lampreys and hagfish are both highly specialized and to recognize that determining which of the two groups more closely represents the ancestral condition is difficult, given all the peculiar traits that have been recognized in the hagfish.

The difficulty to determine whether the hagfish or lamprey is morphologically more plesiomorphic is partly attributable to the paucity of information about the developmental patterns of ostracoderms, the most suitable outgroup to be compared. It is also true that the molecular background of the cyclostome pattern is very limited, as compared to the experimental model vertebrates, which are all crown gnathostomes. In addition, molecular evolutionary studies suggest that hagfish and lampreys share several evolutionary events that took place after the latest common ancestor of vertebrates (Kuraku et al., 1999; Ota and Kuratani, 2010; Pancer et al., 2005; Fujimoto et al., 2013). For example, the topology of collagen and Dlx genes phylogenetic trees indicate that these genes were duplicated in the lineage of extant cyclostomes species, suggesting the cyclostome genomes experienced totally different evolutionary events, which did not occur in the gnathostome genome (Ota and Kuratani, 2010; Fujimoto et al., 2013). This genomic evolutionary evidence may explain potential developmental constraints where cyclostomes may not be able to possess a dorsoventrally articulated and differentiated visceral arch skeleton. A similar cyclostome genomic event was also reported in the evolution of the immune system (Pancer et al., 2005). Thus, the plesiomorphic nature of the cyclostome morphotype should also be questioned.

In the traditional comparative morphological framework, cyclostomes and crown gnathostomes were explained to possess two different morphotypes that diverged from each other (Sewertzoff, 1931; Jollie, 1977). The latter scenario suggests that the two morphotypes (cyclostomes and crown gnathostomes) are defined by their own derived features. However, it is noteworthy that some monorhinous stem gnathostomes (especially osteostracans) developed crania that more closely resembled the lamprey (not necessarily cyclostomes) than the crown gnathostomes (Fig. 4). The realistic question, therefore, is whether the cyclostome pattern of craniogenesis depends on a cyclostome-specific developmental program, or can also be seen as an ancestral (plesiomorphic) program, as suggested above. To clarify this, further comparative genome analysis between hagfish and lampreys is needed (see Smith et al., 2013). After finding the molecular entity, the evolutionary process of the highly specialized cranial morphology of cyclostomes will be further clarified by detailed molecular developmental study, which will allow us to elucidate the evolutionary sequence of the vertebrate cranium.

REFERENCES

Adachi N, Kuratani S (2012) Development of head and trunk mesoderm in a dogfish, *Scyliorhinus torazame*. I. Embryology and morphology of the head cavities and related structures. Evol Dev 14: 234–256

Alvares LE, Schubert FR, Thorpe C, Mootoosamy RC, Cheng L, Parkyn G, et al. (2003) Intrinsic, Hox-dependent cues determine the fate of skeletal muscle precursors. Dev Cell 5: 379–390

Balfour FM (1877) The development of the elasmobranch fishes. J Anat Physiol 11: 406

Cerny R, Lwigale P, Ericsson R, Meulemans D, Epperlein HH, Bronner-Fraser M (2004) Developmental origins and evolution of jaws: new interpretation of "maxillary" and "mandibular." Dev Biol 276: 225–236

Cerny R, Cattell M, Sauka-Spengler T, Bronner-Fraser M, Yu F, Medeiros DM (2010) Evidence for the prepattern/cooption model of vertebrate jaw evolution. Proc Nat Acad Sci USA 107: 17262–17267

Couly GF, Coltey PM, Le Douarin NM (1993) The triple origin of skull in higher vertebrates: A study in quail-chick chimeras. Development 117: 409–429

Damas H (1944) Recherches sur le développment de *Lampetra fluviatilis* L. - contribution à l'étude de la cephalogénèse des vertébrés. Arch Biol Paris 55: 1–289

Dean B (1899) On the embryology of *Bdellostoma stouti*. A genera account of myxinoid development from the egg and segmentation to hatching. Festschrift zum 70ten Geburststag Carl von Kupffer: 220–276 (Gustav Fischer)

de Beer GR (1937) The Development of the Vertebrate Skull. Oxford Univ. Press

Depew MJ, Lufkin T, Rubenstein JL (2002) Specification of jaw subdivisions by *Dlx* genes. Science 298: 371–373

Delarbre C, Gallut C, Barriel V, Janvier P, Gachelin G (2002) Complete Mitochondrial DNA of the hagfish, Eptatretus burgeri: The comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. Mol Phylogenet Evol 22: 184–192

Dietrich S, Abou-Rebyeh F, Brohmann H, Bladt F, Sonnenberg-Riethmacher E, Yamaai T, Lumsden A, Brand-Saberi B, Birchmeier C (1999) The role of SF/HGF and c-Met in the development of skeletal muscle. Development 126: 1621–1629

Dupret V, Sanchez S, Goujet D, Tafforeau P, Ahlberg PE (2014) A primitive placoderm sheds light on the origin of the jawed vertebrate face. Nature 507: 500–503

Froriep A (1882) Über ein Ganglion des Hypoglossus und Wirbelanlagen in der Occipitalregion. Arch Anat Physiol 1882: 279–302

Froriep A (1883) Zur Entwickelungsgeschichte der Wirbelsäule, insbesondere des Atlas und Epistropheus und der Occipital Region. I. Beobachtung an Hühnerembryonen. Arch Anat Physiol 1883: 177–234

Froriep A (1886) Zur Entwickelungsgeschichte der Wirbelsäule insbesondere des Atlas und Epistropheus und der Occipitalregion. II. Beobachtung an Säugetierembryonen. Arch Anat Physiol Anat Abt 1886: 69–150

Froriep A (1905a) Die occipitalen Urwirbel der Amnioten im Vergleich mit denen der Selachier. Verh Anat Ges 1905: 111–120

Froriep A (1905b) Sur la genése de la partie occipitale du crâne. CR Ass des Anat 7: 156

Fujimoto S, Oisi Y, Kuraku S, Ota K, Kuratani S (2013) Non-parsimonious evolution of hagfish *Dlx* genes. BMC Evol Biol 13: 15

Gai Z, Donoghue PC, Zhu M, Janvier P, Stampanoni M (2011) Fossil jawless fish from China foreshadows early jawed vertebrate anatomy. Nature 476: 324–327

Gee H (1996) Before the Backbone: Views on the Origin of the Vertebrates. Chapman and Hall, London

Gegenbaur C (1871) Ueber die Kopfnerven von *Hexanchus* und ihre Verhältniss zur "Wirbeltheorie" des Schädels. Jena Z Med Naturwiss 6: 497–599

Gegenbaur C (1872) Untersuchungen zur vergleichenden Anatomie der Wirbelthiere. 3. Heft: Das Kopfskelet der Selachier, als Grundlage zur Beurtheilung der Genese des Kopfskeletes der Wirbelthiere. Wilhelm Engelmann

Gillis JA, Modrell MS, Baker CV (2013) Developmental evidence for serial homology of the vertebrate jaw and gill arch skeleton. Nature Commun 4: 1436

Goodrich ES (1909) Vertebrata Craniata. First Fascicle, Cyclostomes and Fishes, Adam and Charles Black

Goodrich ES (1930) Studies on the Structure and Development of Vertebrates. McMillan, London

Haeckel E (1874) Anthropogenie oder Entwickelungsgeschichte des Menschen. Keimes- und Stammesgeschichte. Wilhelm Engelmann, Germany

Hanken J, Hall BK (1993) The Skull vols. 1–3. Univ. of Chicago Press, Chicago

Heintz A (1963) Phylogenetic aspect of myxinoids. In "The Biology of Myxine" Ed by A Brodal, R Fänge, Universitetsforlaget, Oslo, pp 9–21

Higashiyama H, Kuratani S (2014) On the maxillary nerve. J Morphol 275: 17–38

Hirasawa T, Kuratani S (2015) Evolution of the vertebrate skeleton - morphology, embryology and development. Zool Lett 1: 2

Holmgren N (1946) On two embryos of *Myxine glutinosa*. Acta Zool: 1–90

Holmgren N, Stensiö EA (1936) Kranium und visceral Skelett der Akranier, Cyclostomen und Fische. In "Handbuch der Vergleichenden Anatomie und Morphologie der Wirbeltiere" Ed by L Bolk, E Göppert, E Kallius, W Lubosch, Urban & Schwarzenberg, Berlin und Wien, Bd 4, pp 233–499

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

Huxley TH (1858) The Croonian Lecture: On the theory of the vertebrate skull. Proc Zool Soc London 9: 381–457

Janvier P (1996) Early Vertebrate. Clarendon Press, Oxford

Johnels AG (1948) On the development and morphology of the skeleton of the head of *Petromyzon*. Acta Zool 29: 140–279

Jollie MT (1977) Segmentation of the vertebrate head. Am Zool 17: 323–333

Kitazawa T, Takechi M, Hirasawa T, Hirai T, Narboux-Nême N, Kume H, et al. (2015) Developmental genetic bases behind the independent origin of the tympanic membrane in mammals and diapsids. Nat Commun 6: 6853

Koltzoff NK (1901) Entwicklungsgeschichte des Kopfes von *Petromyzon planeri*. Bull Soc Nat Moscou 15: 259–289

Kuraku S, Hoshiyama D, Katoh K, Suga H, Miyata T (1999) Monophyly of lampreys and hagfishes supported by nuclear DNA-coded genes. J Mol Evol 49: 729–735

Kuraku S, Meyer A, Kuratani S (2008) Timing of genome duplications: Did cyclostomes diverge before, or after? Mol Biol Evol 26: 47–59

Kuraku S, Ota GK, Kuratani S (2009) Jawless Fishes (Cyclostomata). In "Timetree of Life" Ed by SB Hedges, S Kumar, Oxford Univ Press, Oxford, pp 315–319

Kuraku S, Takio Y, Sugahara F, Takechi M, Kuratani S (2010) Evolution of oropharyngeal patterning mechanisms involving Dlx and endothelins in vertebrates. Dev Biol 341: 315–323

Kuratani S (2003) Evolutionary developmental biology and vertebrate head segmentation: a perspective from developmental constraint. Theory Biosci 122: 230–251

Kuratani S (2005) Developmental studies of the lamprey and hierarchical evolution towards the jaw. J Anat 207: 489–499

Kuratani S (2008a) Evolutionary developmental studies of cyclostomes and origin of the vertebrate neck. Dev Growth Differ 50: Suppl 1: 189–194

Kuratani S (2008b) Is the vertebrate head segmented? - Evolutionary and developmental considerations. Integ Comp Biol 48: 647–657

Kuratani S (2009) Modularity, comparative embryology and evodevo: Developmental dissection of evolving body plans. Dev Biol 332: 61–69

Kuratani S (2012) Evolution of the vertebrate jaw from developmental perspectives. Evol Dev 14: 76–92

Kuratani S, Adachi N (2016) What are head cavities? — A history of studies on the vertebrate head segmentation. Zool Sci 33: 213–228

Kuratani S, Ota K (2008) My favorite animal: hagfish: identifying ancestral developmental traits for vertebrates. BioEssays 30: 167–172

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: 1615–1632

Kuratani S, Kuraku S, Murakami Y (2002) Lamprey as an Evo-Devo model: lessons from comparative embryology and molecular phylogenetics. Genesis 34: 175–195

Kuratani S, Murakami Y, Nobusada Y, Kusakabe R, Hirano S (2004) Developmental fate of the mandibular mesoderm in the lamprey, *Lethenteron japonicum*: comparative morphology and development of the gnathostome jaw with special reference to the nature of trabecula cranii. J Exp Zool (Mol Dev Evol) 302B: 458–468

Kuratani S, Adachi N, Wada N, Oisi Y, Sugahara F (2013) Developmental and evolutionary significance of the mandibular arch and prechordal/premandibular cranium in vertebrates: revising the heterotopy scenario of gnathostome jaw evolution. J Anat 222: 41–55

Kusakabe R, Kuratani S (2007) Evolutionary perspectives from development of the mesodermal components in the lamprey. Dev Dyn 236: 2410–2420

Kusakabe R, Kuraku S, Kuratani S (2011) Expression and interaction of muscle-related genes in the lamprey imply the evolutionary scenario for vertebrate skeletal muscle, in association with the acquisition of the neck and fins. Dev Biol 350: 217–227

Langille RM, Hall BK (1988) Role of the neural crest in development of the trabeculae and branchial arches in embryonic sea lamprey, *Petromyzon marinus* (L). Development 102: 301–310

Lindstrom T (1949) On the cranial nerves of the cyclostomes with special reference to N. trigeminus. Act Zool Stockh 30: 315–

458

Mallatt J (1984) Early vertebrate evolution: Pharyngeal structure and the origin of gnathostomes. J Zool 204: 169–183

Mallatt J (2008) Origin of the vertebrate jaw: neoclassical ideas versus newer, development-based ideas. Zool Sci 25: 990–998

Mallatt J, Sullivan J (1998) 28S and 18S rDNA sequences support the monophyly of lampreys and hagfishes. Mol Biol Evol 15: 1706–1718

Mallatt J, Winchell CJ (2007) Ribosomal RNA genes and deuterostome phylogeny revisited: More cyclostomes, elasmobranchs, reptiles, and a brittle star. Mol Phylogenet Evol 43: 1005–1022

Marinelli W, Strenger A (1954) Vergleichende Anatomie und Morphologie der Wirbeltiere. 1. *Lampetra fluviatilis.* Franz Deuticke, Wien

Marinelli W, Strenger A (1956) Vergleichende Anatomie und Morphologie der Wirbeltiere. 2. *Myxine glutinosa*. Franz Deuticke, Wien

McBratney-Owen B, Iseki S, Bamforth SD, Olsen BR, Morriss-Kay GM (2008) Development and tissue origins of the mammalian cranial base. Dev Biol 322: 121–132

McCauley DW, Bronner-Fraser M (2002) Conservation of Pax gene expression in ectodermal placodes of the lamprey. Gene 287: 129–139

McCauley DW, Bronner-Fraser M (2003) Neural crest contributions to the lamprey head. Development 130: 2317–2327

Modrell MS, Hockman D, Uy B, Buckley D, Sauka-Spengler T, Bronner ME, Baker CV (2014) A fate-map for cranial sensory ganglia in the sea lamprey. Dev Biol 385: 405–416

Myojin M, Ueki T, Sugahara F, Murakami Y, Shigetani Y, Aizawa S, Hirano S, Kuratani S (2001) Isolation of *Dlx* and *Emx* gene cognates in an agnathan species, Lampetra japonica, and their expression patterns during embryonic and larval development: Conserved and diversified regulatory patterns of homeobox genes in vertebrate head evolution. J Exp Zool (Mol Dev Evol) 291: 68–84

Neidert AH, Virupannavar V, Hooker GW, Langeland JA (2001) Lamprey *Dlx* genes and early vertebrate evolution. Proc Natl Acad Sci USA 98: 1665–1670

Neumayer L (1938) Die Entwicklung des Kopfskelettes von *Bdellostoma*. St. L. Arch Ital Anat Embryol 40 Suppl: 1–222

Newth DR (1956) On the neural crest of the lamprey embryo. J Embryol Exp Morphol 4: 358–375

Noden DM (1988) Interactions and fates of avian craniofacial mesenchyme. Development 103: 121–140

Oisi Y, Ota KG, Kuraku S, Fujimoto S, Kuratani S (2013a) Craniofacial development of hagfishes and the evolution of vertebrates. Nature 493: 175–180

Oisi Y, Ota KG, Fujimoto S, Kuratani S (2013b) Development of the chondrocranium in hagfishes, with special reference to the early evolution of vertebrates. Zool Sci 30: 944–961

Oisi Y, Fujimoto S, Ota KG, Kuratani S (2015) On the peculiar morphology and development of the hypoglossal, glossopharyngeal and vagus nerves and hypobranchial muscles in the hagfish. Zool Lett 1: 6

Ota KG, Kuratani S (2006) History of scientific endeavours towards the hagfish embryology. Zool Sci 23: 403–418

Ota KG, Kuratani S (2007) Cyclostome embryology and early evolutionary history of vertebrates. Integ Comp Biol 47: 329–337

Ota KG, Kuratani S (2008) Developmental biology of hagfishes, with a report on newly obtained embryos of the Japanese inshore hagfish, *Eptatretus burgeri*. Zool Sci 25: 999–1011

Ota KG, Kuratani S (2010) Expression pattern of two collagen type 2 alpha1 genes in the Japanese inshore hagfish (*Eptatretus burgeri*) with special reference to the evolution of cartilaginous tissue. J Exp Zoolog B Mol Dev Evol 314: 157–165

Ota KG, Kuraku S, Kuratani S (2007) Hagfish embryology with reference to the evolution of the neural crest. Nature 446: 672–675

Ota G, Fujimoto S, Oisi Y, Kuratani S (2011) Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. Nat Commun 2: 373

Ota KG, Fujimoto S, Oisi Y, Kuratani S (2013) Late development of the hagfish vertebral elements. J Exp Zool (Mol Dev Evol) 320: 129–139

Ota KG, Oisi Y, Fujimoto S, Kuratani S (2014) The origin of developmental mechanisms underlying vertebral elements: implications from hagfish Evo Devo. Zoology 117: 77–80

Pancer Z, Saha NR, Kasamatsu J, Suzuki T, Amemiya CT, Kasahara M, Cooper MD (2005) Variable lymphocyte receptors in hagfish. Proc Natl Acad Sci USA 102: 9224–9229

Parker KW (1883a) On the Skeleton of the Marsipobranch Fishes. Part I. The Myxinoids (*Myxine*, and *Bdellostoma*). Phil Trans R Soc Lond 174: 373–409

Parker KW (1883b) On the Skeleton of the Marsipobranch Fishes. Part II. *Petromyzon*. Phil Trans R Soc Lond 174: 411–457

Parker WK, Bettany GT (1877) The Morphology of the Vertebrate Skull. MacMillan, London

Portmann A (1976) Einführung in die vergleichende Morphologie der Wirbeltiere. 5. Aufl. Schwabe & Co., Basel

Pradel A, Maisey JG, Tafforeau P, Mapes RH, Mallatt J (2014) A Palaeozoic shark with osteichthyan-like branchial arches. Nature 509: 608–611

Rathke MHR (1839) Entwickelungsgeschichte der Natter (*Coluber natrix*). Verlag der Gebrüder Bornträger, Koenigsberg

Romer AS, Parsons TS (1977) The Vertebrate Body. 5th ed, Saunders, Philadelphia

Sambasivan R, Kuratani S, Tajbakhsh S (2011) An eye on the head: the evolution and development of craniofacial muscles. Development 138: 2401–2415

Sato T, Kurihara Y, Asai R, Kawamura Y, Tonami K, Uchijima Y, et al. (2008) An endothelin-1 switch specifies maxillomandibular identity. Proc Nat Acad Sci USA 105: 18806–18811

Sewertzoff AN (1913) Das Visceralskelet der Cyclostomen. Anat Anz 82: 280–283

Sewertzoff AN (1928) Directions of evolution. Act Zool 9: 59–141

Sewertzoff AN (1931) Morphologische Gesetzmässigkeiten der Evolution. Gustav Fischer, Jena

Shigetani Y, Sugahara F, Kawakami Y, Murakami Y, Hirano S, Kuratani S (2002) Heterotopic shift of epithelial-mesenchymal interactions for vertebrate jaw evolution. Science 296: 1316–1319

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

Smith JJ, Kuraku S, Holt C, Sauka-Spengler T, Jiang N, Campbell MS, et al. (2013) Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. Nat Genet 45: 415–421

Stöhr P (1881) Zur Entwicklungsgeschichte des Annurenschädels. Z wiss Zool 36: 68–103

Strahan R (1960) Speculations on the evolution of the agnathan head. In "Proceedings of the Centenary and Bicentenary Congress of Biology" Ed by RD Purchon, Malaya Univ Press, London, pp 83–94

Suzuki DG, Fukumoto Y, Yoshimura M, Yamazaki Y, Kosaka J, Kuratani S, Wada H (2016, in press) Comparative morphology and development of extra-ocular muscles in the lamprey and gnathostomes reveal the ancestral state and developmental patterns of the vertebrate head. Zool Lett

Takezaki N, Figueroa F, Zaleska-Rutczynska Z, Klein J (2003) Molecular phylogeny of early vertebrates: monophyly of the agnathans as revealed by sequences of 35 genes. Mol Biol Evol 20: 287–292

Uchida K, Murakami Y, Kuraku S, Hirano S, Kuratani S (2003)

Development of the adenohypophysis in the lamprey: Evolution of epigenetic patterning programs in organogenesis. J Exp Zool (Mol Dev Evol) 300B: 32–47

Wada N, Nohno T, Kuratani S (2011) Dual origins of the prechordal cranium in the chicken embryo. Dev Biol 356: 529–540

Wedin B (1949) "The Anterior Mesoblast in Some Lower Vertebrates - A Comparative Study of the Ontogenetic Development of the Anterior Mesoblast in *Petromyzon*, *Etmopterus*, *Torpedo*, et al." Hakan Ohlsson Boktryckeri, Lund

van Wijhe JW (1882) Über die Mesodermsegmente und die Ent- wicklung der Nerven des Selachierkopfes. Ver. Akad. Wiss. Amsterdam, Groningen pp 1–50

Yalden DW (1985) Feeding mechanisms as evidence for cyclos- tome monophyly. Zool J Linn Soc 84: 291–300

Yao T, Ohtani K, Kuratani S, Wada H (2011) Development of lam- prey mucocartilage and its dorsal–ventral patterning by endothelin signaling, with insight into vertebrate jaw evolution. J Exp Zool (Mol Dev Evol) 316B: 339–346