

REVIEW

Evolution of the vertebrate jaw: comparative embryology and molecular developmental biology reveal the factors behind evolutionary novelty

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Abstract

It is generally believed that the jaw arose through the simple transformation of an ancestral rostral gill arch. The gnathostome jaw differentiates from *Hox*-free crest cells in the mandibular arch, and this is also apparent in the lamprey. The basic *Hox* code, including the *Hox*-free default state in the mandibular arch, may have been present in the common ancestor, and jaw patterning appears to have been secondarily constructed in the gnathostomes. The distribution of the cephalic neural crest cells is similar in the early pharyngula of gnathostomes and lampreys, but different cell subsets form the oral apparatus in each group through epithelial–mesenchymal interactions: and this heterotopy is likely to have been an important evolutionary change that permitted jaw differentiation. This theory implies that the premandibular crest cells differentiate into the upper lip, or the dorsal subdivision of the oral apparatus in the lamprey, whereas the equivalent cell population forms the trabecula of the skull base in gnathostomes. Because the gnathostome oral apparatus is derived exclusively from the mandibular arch, the concepts ‘oral’ and ‘mandibular’ must be dissociated. The ‘lamprey trabecula’ develops from mandibular mesoderm, and is not homologous with the gnathostome trabecula, which develops from premandibular crest cells. Thus the jaw evolved as an evolutionary novelty through tissue rearrangements and topographical changes in tissue interactions.

Key words evolutionary novelty; *Hox* genes; jaw; lamprey; neural crest; pharynx.

Introduction: the mandibular arch and the jaw

The head of the vertebrate embryo is characterized by the possession of neural crest-derived ectomesenchyme and the pharyngeal arches (PAs), which are primarily equivalent to the gill arches. The skeletal elements in the PAs are derived exclusively from the ectomesenchyme, not from the mesoderm (Le Lièvre, 1974, 1978; reviewed by Le Douarin, 1982; Gans & Northcutt, 1983; Hall & Hörstadius, 1988; Noden, 1988; Hall, 1999; Le Douarin & Kalcheim, 1999; Kimmel et al. 2001; Morriss-Kay, 2001; Trainor et al. 2003). The jaw in gnathostomes (jawed vertebrates) is one of the earliest

innovations in the evolution of vertebrates and is derived from the mandibular arch (MA). Evolution of the jaw therefore can be viewed as the establishment of a developmental programme for the ectomesenchyme of the MA to form a dorsoventrally articulated pattern, consisting of upper and lower jaws. However, the evolutionary scenario of the jaw, or the history of changes in the developmental programmes to create the jaws, remains largely unknown. The lamprey, a jawless vertebrate, is thought to represent the outgroup to the jawed vertebrates and may suggest the ancestral developmental programmes shared by the common ancestor, as well as the changes introduced to form the jaw in gnathostome lineages.

According to the classic morphological concept, the jaw in gnathostomes is assumed to have arisen by transforming one of the rostral gill arches of the ancestral vertebrate (reviewed by Sewertzoff, 1911, 1928; Goodrich, 1930; Gregory, 1933; de Beer, 1937; Romer,

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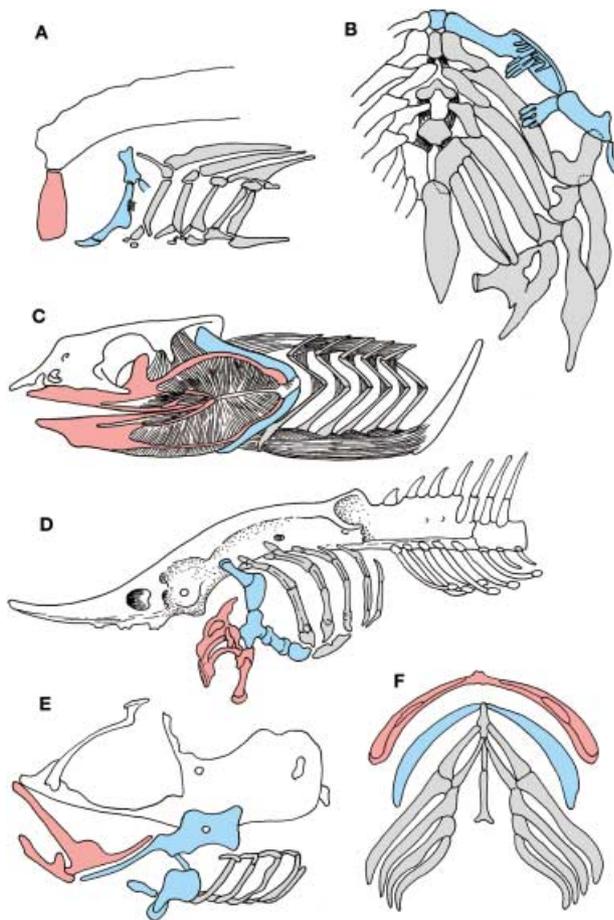


Fig. 1 Visceral skeletal systems in various gnathostomes. Visceral skeletons of *Chimaera monstrosa* (A: Holocephali), *Callorhynchus* (B: Holocephali), *Chlamidoselachus* (C: Elasmobranchii), *Acipenser sturio* (D: Chondrostei), *Gasterosteus aculeatus* (E: Teleostei) and *Triron cristatus* (F: Urodela) are shown. Mandibular arches are coloured pink, the hyoid arch light blue, and the more posterior respiratory arches (real branchial arches) grey. Note that, in each gnathostome species shown here, mandibular and hyoid arches are morphologically differentiated to take distinct shapes, whereas the branchial arches look similar to each other. Redrawn from Edgeworth (1935) (A, B, E, F) and Gregory (1933) (C and D).

1966; Moy-Thomas & Miles, 1971; Romer & Parsons, 1977; Jarvik, 1980; Mallatt, 1984, 1996; Carroll, 1988; Janvier, 1996; Kuratani et al. 2001). However, the fossil record has not revealed any ancestral animals with an undifferentiated series of gill arches in their pharynx. Moreover, in all the gnathostome embryos observed so far, PA1 and PA2 can be recognized as modified from the rest of the arches (branchial arches), and specifically called the mandibular (MA) and hyoid arches (HA), respectively (Fig. 1; and see Gregory, 1933; Edgeworth, 1935; de Beer, 1937; Romer, 1966; Jarvik, 1980).

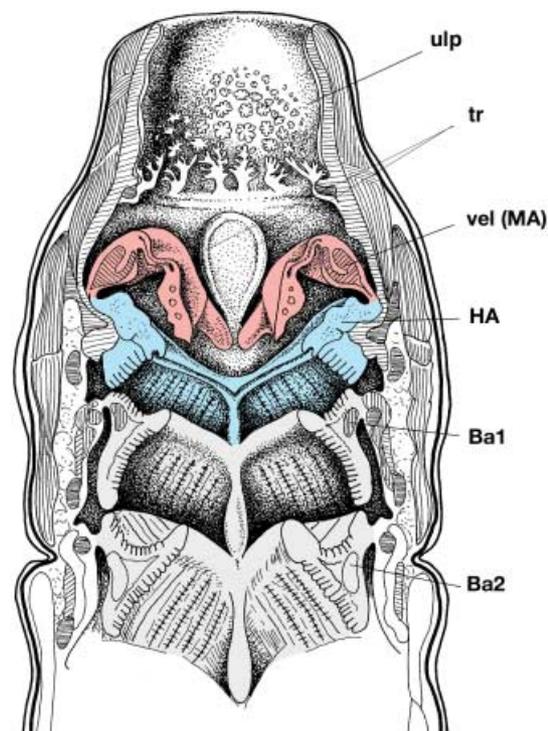


Fig. 2 Pharyngeal anatomy of the ammocoete larva of the lamprey. The pharynx of the lamprey larva has been cut horizontally and its dorsal half is illustrated from the ventral view. Mandibular, hyoid (HA), and two branchial arches (Ba1–2) are shown. The arches are coloured as in Fig. 1. Abbreviations: *tr*, trabecula of the lamprey; *ulp*, upper lip; *vel*, velum. Redrawn from Gaskell (1908).

This is also true for the lamprey, a modern agnathan (jawless) vertebrate (Kuratani et al. 2001). In this animal, the MA differentiates into the velum, the pumping apparatus that lets water into the pharynx, as well as the lower lip, which resembles the gnathostome lower jaw (Fig. 2; Mallatt, 1996; Kuratani et al. 2001; Shigetani et al. 2002; see below). An ancestral animal with simple gill arches with no mandibular or hyoid identities is purely hypothetical. Recent embryological and molecular developmental analyses of lampreys, the living agnathans, have suggested instead a more complicated scenario for the evolution of the gnathostome jaw.

Mandibular arch and the *Hox*-free default state

The idea that the jaw is a transformed PA fits the developmental sequence of the gnathostome embryo better than the actual fossil record. A specific class of homeobox-containing genes, called *Hox* genes, are expressed

sequentially along the anteroposterior axis of the embryonic pharynx, thereby constituting a nested pattern of gene expression, or the 'Hox code' in the ectomesenchyme (Fig. 3A; Hunt et al. 1991a,b). *Hox* genes in amniotes are arranged tandemly in four clusters, each of which is found on a different chromosome (reviewed by McGinnis & Krumlauf, 1992). There is a tendency called 'spatial collinearity' in that the genes located in the 3' direction of a cluster are more likely to be up-regulated in the anterior part of the embryo, whereas the more 5' genes are transcribed towards the posterior part of the embryo. Thus, each one of the PAs carries a different and specific subset of *Hox* transcripts that determines its specific developmental pathway (Fig. 3; Hunt et al. 1991a,b). *Hox* genes, encoding transcription factors, play developmental roles as the 'homeotic selector' genes by providing positional cues to the ectomesenchyme filling each PA. It is important to note that there are no *Hox* genes expressed in the MA (Fig. 3A).

Part of the jaw-patterning programme in gnathostomes appears to be regulated by the 'Hox-free default' state of the MA, as has been shown by a number of experiments. First, gain- and loss-of-function experiments on *Hoxa-2*, a *Hox* gene expressed in the HA and posterior to it, result in the transformation of the MA into the HA, and that of the HA into the MA, respectively (Gendron-Maguire et al. 1993; Rijli et al. 1993; Grammatopoulos et al. 2000; Pasqualetti et al. 2000). This is consistent with the 'law of posterior prevalence' (Lufkin et al. 1992), in which loss of function of a *Hox* gene leads to anteriorization, whereas gain-of-function leads to the posteriorization of morphological identities. Second, transplantation of the *Hox*-free neural crest into the rhombomere 4 level of the hindbrain, which is destined to become the HA, leads to the duplication of MA skeletal elements in the HA domain (Noden, 1983; Couly et al. 1998). The growth factor FGF8 (fibroblast growth factor 8), released from the embryonic mid-hindbrain boundary, possibly inhibits expression of *Hox* genes in the rostral hindbrain crest (Trainor et al. 2002). Thus, in the gnathostome developmental process, the differentiation of the jaw from the rostral crest cells is permitted by the absence of the *Hox* transcripts from the crest cells in the MA. The question then arises as to whether the *Hox*-free state of the MA was established at the outset of gnathostome evolution, or if it was already present in agnathans, or even in cephalochordates (e.g. amphioxus).

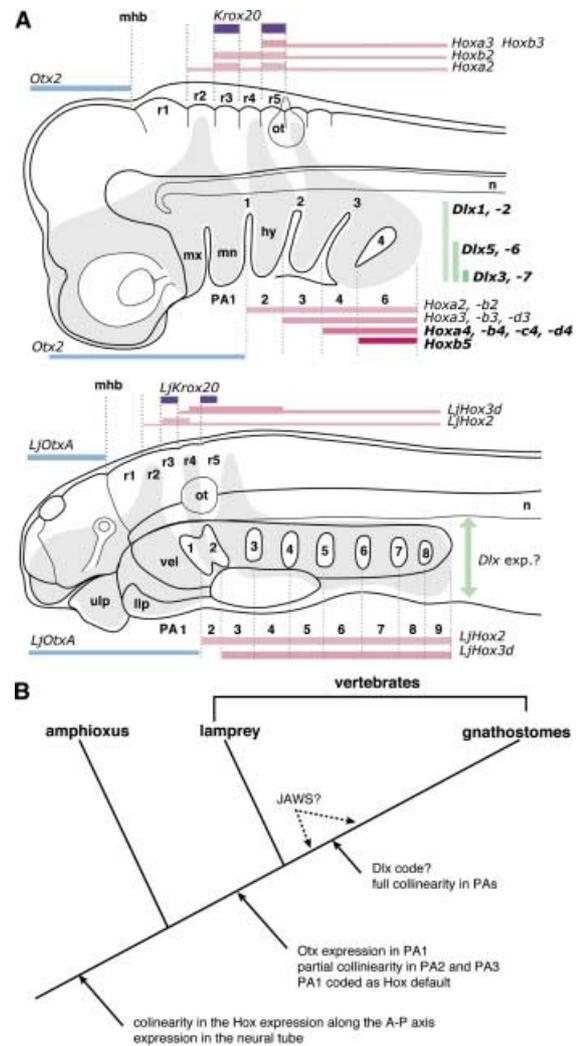


Fig. 3 Evolution of the gene expression patterns and the origin of the jaw. (A) Simplified *Hox* codes in gnathostomes (top) and the lamprey larva (bottom) are summarized. Gnathostome-specific regulatory gene expression domains are shown in bold, and crest cells in grey. In gnathostomes (in this figure, amniotes), distinct sets of *Hox* transcripts are distributed in a nested pattern in the pharyngeal arches (PAs) with the mandibular arch (PA1) defined by the *Hox*-free default state and by the expression of *Otx* cognates. Along the dorsoventral axis of the arches, a *Dlx* code is established to differentiate the dorsoventral pattern of each PA. In the lamprey, such nested expression of *Dlx* genes has not been detected. Note that the PA1 is commonly at the state of *Hox*-free default, and PG2 and PG3 *Hox* genes have the same rostral boundaries of expression along the PAs between lamprey and gnathostomes, implying the deep origin of the three morphological identities to differentiate PA1, PA2 and the rest of the arches. (B) Evolutionary changes in regulations of *Otx* expression, *Dlx*-, and *Hox* codes have been placed along the phylogenetic tree. Abbreviations: *hy*, hyoid arch; *llp*, lower lip; *mhb*, mid-hindbrain boundary; *mn*, mandibular process; *mx*, maxillary process; *n*, notochord; *ot*, otic vesicle; *r1-5*, rhombomeres; *ulp*, upper lip; *vel*, velum; 1-8, pharyngeal slits or pouches. Based on Murakami et al. (2004) and Takio et al. (2004).

In the above context, Cohn (2002) reported in a preliminary study on a lamprey species, *Lampetra fluviatilis*, that one of the *Hox* genes, *HoxL6*, was developmentally up-regulated throughout the PAs, implying that the presence of *Hox* transcripts in the agnathan MA inhibited the differentiation of the jaw in this animal group. There is no doubt that an MA can be identified morphologically in these animals, even if they lack jaws (de Beer, 1937; reviewed by Kuratani et al. 2001). By contrast, recent analyses by Takio et al. (2004) did not confirm this scenario: 11 *Hox* genes were isolated from a species, *Lethenteron japonicum*, including the orthologue of *HoxL6*, but none of the genes was expressed in the embryonic MA. This is consistent with the finding that *LjFgf8/17* (the lamprey cognate for *Fgf8*) is expressed in the mid-hindbrain boundary, as in gnathostome embryos (Fig. 3; Murakami et al. 2001; Shigetani et al. 2002), and with the generally accepted notion for *Hox* code evolution in chordates (Schilling & Knight, 2001, and references therein). Moreover, *Hox2* was clearly expressed in the crest cells of the HA and in the more posterior PAs, and similarly, *Hox3* was expressed in the crest cells of the PA3 and more posterior regions, reminiscent of the nested, collinear pattern of the gnathostome *Hox* code (Fig. 3). Although the difference of *Hox6* expression between *Lampetra fluviatilis* and *Lethenteron japonicum* maybe due to a species- or genus-specific difference in the regulatory mechanism for *Hox6*, at the very least it is conceivable that some agnathans and gnathostomes share the same basic *Hox* code (*Hox*-free default state of PA1; PG2 and PG3 genes expressed in PA1 and PA2, respectively). Importantly, almost all the vertebrate species possess more or less differentiated MAs (jaws in gnathostomes; velum and lower lip in the lamprey) and HAs, followed by respiratory branchial arches that are similar across species (Figs 1 and 2). It seems most likely that this type of 'primitive' *Hox* code was already established in the common ancestor of the lamprey and gnathostomes – with differentiated PA1 and PA2 – with distinctive identities as opposed to the morphologically identical, more posterior PAs (Fig. 3). In this connection, it is interesting to note that the Cambrian fossil animal *Haikouella* appeared to have possessed an oral apparatus that resembled that of the lamprey ammocoete larva (Mallatt & Chen, 2003; Mallatt et al. 2003; but also see Shu et al. 2003): well-differentiated oral apparatus, which would have been at least in part differentiated from the MA in this animal, is consistent with the deep origin of the *Hox*-free default MA.

This scenario further implies that evolution of the developmental programme that forms the jaw involved changes in molecular mechanisms downstream of the shared *Hox* code of ancestral vertebrates, again consistent with the idea that the MA is morphologically equivalent as a developmental unit between the lamprey and gnathostomes by having the same topographical relationships with other embryonic structures, as well as with the same positional value defined by the absence of *Hox* transcripts (Figs 3 and 5; see also Kuratani et al. 2001, for morphological value of the vertebrate MA).

Dlx and *Otx* genes

The shared developmental features identified between the lamprey and gnathostomes are most likely to represent the ancestral programme possessed by the common ancestor (Fig. 3B; also see Trainor et al. 2003, for a similar method of speculation), whereas there can also be programmes that have arisen or lost only in one of the lineages. It is important to realize that molecular phylogenetic analyses of regulatory gene cognates often indicate the polarity of changes along the phylogenetic tree, making the evolutionary scenario a more plausible one (reviewed by Kuratani et al. 2002). For a possible example of the gnathostome-specific derived feature (gnathostome synapomorphy), it is worth noting that gnathostome MA is also dorsoventrally patterned through the nested expression of *Dlx* genes along the dorsoventral axis of the arch (Depew et al. 2002). Thus, simultaneous disruption of *Dlx5* and *Dlx6* expressed in the ventral half of the MA leads to the mirror-image duplication of the upper jaw segment in the domain of the lower jaw. Preliminary data on the lamprey *Dlx* genes and their expression patterns, and the dorsoventrally symmetrical morphology of the lamprey branchial cartilages, indicate that the *Dlx* code for dorsoventral polarity is not a pan-vertebrate developmental feature: expression patterns of *Dlx* cognates are not localized in the MA, as seen in mouse embryos (Myojin et al. 2001; Neidert et al. 2001; also see Kuratani et al. 2002; Fig. 3).

Like the *Hox* code in vertebrates, expression of *Otx* cognates is highly conserved between gnathostomes and the lamprey, both in the neural tube and in the MA (Ueki et al. 1998; Tomsa & Langeland, 1999; Murakami et al. 2001). In the mouse embryo, *Otx2*-expression and *Hox*-free default states appear to pattern the distal and

proximal portions of the MA in a complementary fashion (Rijli et al. 1993; Gendron-Maguire et al. 1993; Matsuo et al. 1995; also see Kuratani et al. 1997, and Kuratani et al. 2001, 2002, for reviews). Such a division appears to be partly due to the migration and distribution patterns of the crest cells in MA, the rostral part of which preferentially receives cells from the *Otx2*-positive midbrain and segregates from the *Hox*-free crest originated from the rostral hindbrain (Osumi-Yamashita et al. 1994; see also Köntges & Lumsden, 1996, for chick development). It will be intriguing to determine if a similar complementary pattern exists in the lamprey MA during development. In this context, based on vital-dye labelling studies, the origin and migration patterns of crest cells in the MA are not identical between the lamprey and amniotes, as discussed below (Shigetani et al. 2002; McCauley & Bronner-Fraser, 2003).

The gnathostome jaw now seems to have arisen as one of several variations in differentiation programmes at the radiation of the ancestral vertebrates, based on the shared ground plan of craniofacial patterning with the shared basic expression patterns of some homeobox genes. However, the variation in gnathostome patterning also seems to involve a change in global interactions between mesenchyme and epithelium, as seen in the various types of craniofacial designs found in the Palaeozoic fossils (Janvier, 1996).

Oral apparatus and the mandibular arch: heterotopy and loss of homology

According to Wagner & Müller (2002), an 'evolutionary novelty' can be defined as a new structure that arises by overriding ancestral developmental constraints, so that morphological homology is lost between the novel and ancestral structures. Thus, the gnathostome jaw could be counted as an evolutionary novelty, as discussed below. In the following discussion, we have to bear in mind that the term 'mandibular arch' universally refers to an identical developmental unit among vertebrates (morphologically homologous throughout vertebrates), whereas the 'oral apparatus' or 'oral region' may differentiate from different regions of the embryonic head in each animal group.

Although the classical transformation theory of the jaw predicts the initially identical, undifferentiated pharyngeal arches, the cephalic crest cells (ectomesenchyme) never simply form single divided cell streams each filling a single PA. Instead, in all the vertebrate

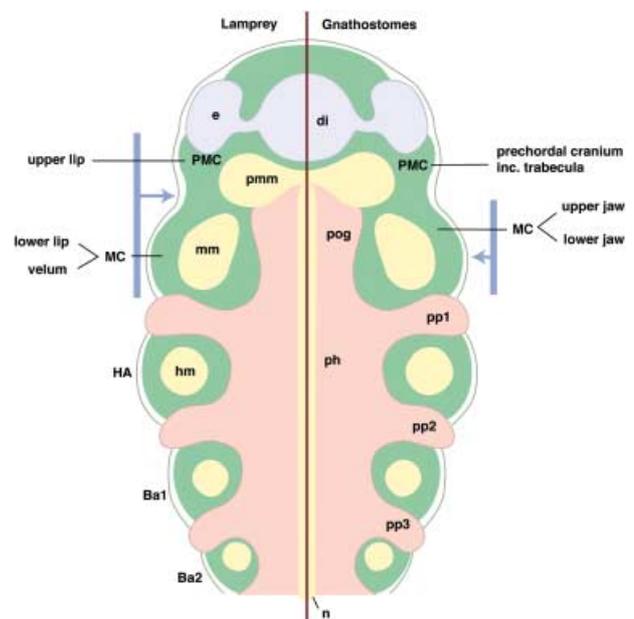


Fig. 4 Comparison of mesenchymal developmental patterning. In the lamprey and gnathostome embryonic heads, shared patterns of mesodermal (yellow) and ectomesenchymal (green) distribution can be detected. The endodermal derivatives are coloured pink. The crest cells rostral to the first pharyngeal pouch (pp1) are collectively called the trigeminal crest cells that can be divided into those in the mandibular arch (mandibular crest cells; MC) and the premandibular crest cells (PMc). The posterior region comprising the mandibular crest cells surrounds the premandibular mesoderm (pmm) that arises rostral to the tip of the notochord (n). Different subsets of the trigeminal crest cells are patterned on this shared pattern on mesenchymal distribution, as the oral apparatus in each animal groups arises through different distribution of growth factors such as FGF8 and BMP4 that define the mouth (shown by blue bars). In the lamprey, the upper lip arises from PMc and the lower lip from MC, whereas in gnathostomes both the upper and the lower jaw arise from MC, and the PMc differentiate into the prechordal cranial elements. Blue arrows indicate the position of mouth openings. Abbreviations: Ba1–2, branchial arches; di, diencephalon; e, eye; hm, hyoid mesoderm; ph, pharynx; pp1–3, pharyngeal pouches; pog, preoral gut.

embryos observed, there are three distinct crest cell populations, and the most rostral one not only populates the MA, but also expands rostrally to the entire pharynx (Noden, 1988; Osumi-Yamashita et al. 1994; Kuratani, 1997; Graham, 2001; Kuratani et al. 2001; Graham et al. 2004; reviewed by Horigome et al. 1999, and Kuratani et al. 2001; Figs 4 and 5 top). At the early pharyngula stage, when cephalic crest cells cease emigration, the distribution pattern of the crest cells is very similar between gnathostome and the lamprey – the cephalic crest cells form three major streams of migration,

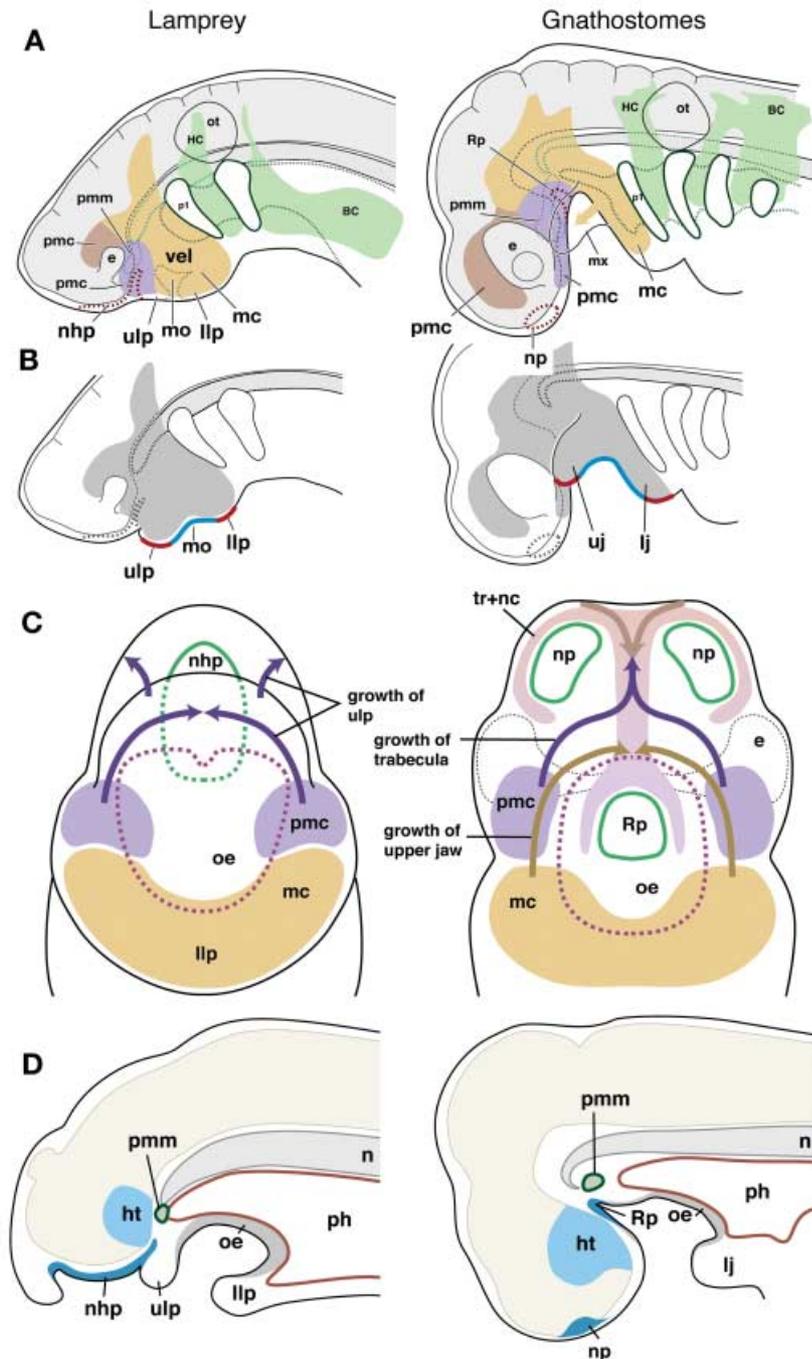


Fig. 5 Comparisons of oral patterning. Distribution patterns of cephalic crest (A), expression of *Fgf8/17* and *Bmp2/4* cognates in the ectoderm (B), ventral view of embryonic oral regions (C), and the medial sagittal sections (D) are schematically represented. (A) In the early pharyngula, cephalic crest cells form three distinct cell populations called trigeminal, hyoid (HC) and branchial crest cells (BC). The trigeminal crest cell population can be further subdivided into the mandibular crest cells (mc) and two domains of premandibular crest cells (pmc), based on the topographical relationships with the first pharyngeal pouch (p1), eye (e), premandibular mesoderm (pmm) and the mandibular mesoderm. Note that in the gnathostome embryo the maxillary process (mx) or the upper jaw primordium secondarily grows from the dorsal part of the mandibular arch (arrow; for descriptions see Kuratani & Horigome, 2000; Kuratani et al. 2001). (B) Expressions of *Fgf8/17* (blue lines) and *Bmp2/4* (red lines) are shown on embryos at similar stages to those in A. (C,D) In the lamprey, nasal placodes and hypophysial placodes develop as a single primordium, the nasohypophysial plate (nhp) that lies rostral to the oral ectoderm (oe). Because of the presence of this plate, the premandibular crest cells (pmc) form the upper lip (ulp) that grows beneath the plate as the floor of the nasohypophysial duct. In the gnathostomes, the hypophysis arises as a part of the oral ectoderm, or Rathke's pouch (Rp), which develops separate from the nasal placodes (np), leaving a median space, which permits the premandibular (pmc) and mandibular crest cells (mc) to grow to form the trabecula and upper jaw, respectively. For induction of the hypophysis, the posterior part of the nasohypophysial plate in the lamprey has to grow posteriorly to reach the hypothalamic anlage (ht). The same topographical relationship is established early in gnathostome development. Abbreviations: *lj*, lower jaw; *llp*, lower lip; *mo*, mouth; *n*, notochord; *ot*, otic vesicle; *ph*, pharynx; *vel*, velum.

and the most rostral cell population (trigeminal crest cells) is distributed in the MA as well as in the premandibular (PM) region (Fig. 5A; Horigome et al. 1999; Shigetani et al. 2000). Here the term 'premandibular' does not imply the presence of a 'premandibular arch' once assumed by comparative morphologists, but simply indicates the position rostral to the MA. [In this review,

the premandibular crest cells are defined as those crest cells that surround the premandibular mesoderm, as opposed to the MA crest cells that surround the mandibular mesoderm. Owing to the absence of the pharyngeal pouch that normally limits the MA rostrally, only the mesodermal component can be used as a landmark. This terminology inevitably relates to the

| | Division of the ectomesenchyme | | Developmental fates | |
|--|---|--|--|---|
| | | | Ammocoete larva of the lamprey | Gnathostomes |
| Trigeminal crest cells | Premandibular crest cells | Preoptic crest cells (located rostral to the optic vesicle) | Part of nasal cartilage? | A part of trabecula? Lateral wall of the nasal capsule? |
| | | Postoptic crest cells (surrounding the premandibular mesoderm; posterior to the optic vesicle) | Upper lip skeleton (through the function of <i>Dlx1</i> cognate) | Major part of the trabecula? (<i>Dlx1</i> cognate not expressed) |
| | Mandibular crest cells (surrounding the mandibular mesoderm; posteriorly limited by the first pharyngeal pouch) | Skeletal elements in the velum and the lower lip (through the function of <i>Dlx1</i> cognate) | Skeletal elements of both the upper and lower jaws (through the function of <i>Dlx1</i> cognate) | |
| Hyoid crest cells (in the hyoid arch; surrounding the hyoid mesoderm) | | | Hyoid arch skeletal elements | Hyoid arch skeletal elements |
| Branchial crest cells = postotic crest cells = circumpharyngeal crest cells (Kuratani, 1997) (populating all the branchial arches or the postotic pharyngeal arches) | | | Branchial arch skeletal elements | Branchial arch skeletal elements |

Fig. 6 Comparisons of equivalent ectomesenchymal regions between the lamprey and gnathostomes. Terminology for the crest cell populations and subpopulations is based on the topographical distribution of the crest cells with respect to the other embryonic structures such as mesoderm and pharyngeal pouches, not in terms of their developmental fates. This division defines the morphological homology of ectomesenchymal portions, which can be applied throughout vertebrates, laid down at the early pharyngula stages (see Figs 4 and 5). Note, however, that different portions of ectomesenchyme are utilized to differentiate into the 'oral apparatus' in the lamprey and gnathostomes (shaded). Because of this heterotopic shift in tissue interactions, the ectomesenchymal part with the same name in the lamprey and gnathostomes do not always differentiate into the same skeletal elements (see Kuratani et al. 2001; Shigetani et al. 2002). For the homology of the cartilages called 'trabeculae' between the two animal groups, see Kuratani et al. (2004).

classical concept of head segmentation in vertebrates, which used to be based primarily on the segments in the head mesoderm (see de Beer, 1937; Jarvik, 1980; Jefferies, 1986). However, as has been discussed previously (Kuratani et al. 1999), neither lamprey nor gnathostome embryos show any sign of segmentation in the cephalic mesoderm, except for the late-forming premandibular mesoderm that is more or less separated from the rest of the cell population, and the lateral part of the head mesoderm, which is partially and secondarily divided by the growth of pharyngeal pouches. Thus, primary mesodermal segmentation is not assumed in the vertebrate head in this review. For more discussion on head segmentation and metamerism, see Kuratani (2003). The term 'trigeminal crest cells' stems from the fact that the distribution of these cells corresponds to the peripheral distribution patterns of the trigeminal nerve in the later embryo (see Kuratani, 1997; Kuratani et al. 2001; Fig. 6). The crest cells in the PM region are further subdivided into preoptic and postoptic cell populations (Figs 4, 5A and 6).

Importantly, at an early stage of pharyngular development, there is no clear boundary between the PM and MA regions (Kuratani et al. 1999; Shigetani et al.

2000, 2002), and the oral apparatus is formed in different ways between gnathostomes and the lamprey (it is composed of upper and lower jaws in the gnathostomes; of upper and lower lips in the ammocoete larva of the lamprey). Each domain of the trigeminal ectomesenchyme can be identified by its topographical relationships with the mesodermal components (reviewed by Kuratani et al. 2001; Figs 4–6). The MA crest cells surround the mandibular mesoderm and the postoptic part of the PM crest cells surrounds the premandibular mesoderm (primordia of the extrinsic eye muscles in gnathostomes; Koltzoff, 1901; see Boorman & Shimeld, 2002, for a case of specific gene expression in the premandibular mesoderm of lamprey embryos; and see Kuratani et al. 1999, for comparative embryology of this mesoderm). Interestingly, both the upper and the lower jaws develop from the MA crest cells, whereas in the lamprey the MA differentiates only into the velum and the lower lip, and the upper lip is derived from PM crest cells (Kuratani et al. 2001; Shigetani et al. 2002). Based on the basic architecture of the embryo therefore the term 'mandibular arch' should be reserved for the specific PA found in the same relative position in the embryo, as a developmental unit

that is specifically and topographically associated with the mandibular mesoderm, irrespective of its fate in later developmental stages, which can differ in each animal lineage. Thus, in gnathostomes, the oral apparatus called the 'jaw' develops from the MA, whereas the lamprey oral region also requires the PM region as an embryonic material (Figs 4–6). The term 'oral apparatus', on the other hand, implies only a functional resemblance of structures, which can be derived from varied sets of embryonic tissues. It is important to note that a gene expression pattern is not always associated with a homologous set of cell populations, as is discussed below.

In the amniote MA, BMP4 in the distal ectoderm induces the expression of the target gene, *Msx1*, in the underlying ectomesenchyme, and the proximally distributed FGF8 induces *Dlx1* expression in the proximal ectomesenchyme (Barlow & Francis-West, 1997; Neubüser et al. 1997; Tucker et al. 1998; Shigetani et al. 2000). Shigetani et al. (2002) have also found that molecules involved in the proximodistal patterning of the gnathostome jaw are apparently used in similar patterning of the upper and lower lips of the lamprey larva, as if lips and jaws were homologous to each other (Fig. 5B). Namely, *LjFgf8/17* (the lamprey cognate of *Fgf8*) and *LjDlx1/6* (the lamprey cognate of *Dlx1*) are expressed widely in the oral ectoderm and mesenchyme, respectively. Moreover, *LjBmp2/4a* (the lamprey cognate of *Bmp4*) and *LjMsxA* (the lamprey cognate of *Msx1*) are expressed in the tips of lips, respectively (Fig. 5B; also see Shigetani et al. 2002). As the functions of ectomesenchymally expressed homeobox genes are most prominent in the MA-derivatives in gnathostomes (Satokata & Maas, 1994; Martin et al. 1995; Qiu et al. 1995, 1997; Yamada et al. 1995; reviewed by Hall, 1998), and apparently masked by the *Hox* gene expression in HA and posterior PAs (Mallo & Gridley, 1996), the *Hox*-free default state of the lamprey MA is again consistent with the apparently similar functions of these gene cognates in the lamprey (proximodistal specification in oral patterning). In the above comparison, homologous sets of genes are not expressed in homologous embryonic materials, but rather are associated with functionally similar structures, namely the oral apparatus (see below for a similar discussion on adeno-hypophysial differentiation). To resume the agnathan mode of *Dlx1* gene expression, which denotes the oral and pharyngeal region in the ectomesenchyme, the domain of the upstream factor, FGF8, has to be expanded in the gnathostome embryo.

Actually, implantation of an FGF8-soaked bead into the PM region mimics the lamprey-type *Dlx1* expression in the chick embryo (Shigetani et al. 2000, 2002).

These experimental results imply that epithelial–mesenchymal interactions have been topographically shifted in the transition from the lamprey-like agnathan to the gnathostome states, based on a shared pattern of embryonic tissues. As a result, morphological homology was apparently lost between the lamprey and gnathostome oral apparatus; expression patterns of orthologous genes are not associated with morphologically equivalent cell populations. This evolutionary implication is curious: even if these genes always functioned in defining the oral apparatus, their regulation does not seem to be restricted to the same (homologous) embryonic component during this transition (see Manzanares & Nieto, 2003, for a similar discussion on gene usage). Such a situation simultaneously implies that the invention of the jaw deserves to be understood in the context of evolutionary novelty as defined by Wagner & Müller (2002): because the newly acquired pattern is not homologous with the ancestral pattern, the former was brought about by overriding ancestral developmental constraints, not simply modifying it for adaptation.

Alternatively, it is also possible that lips and jaws are homologous, derived from homologous cell populations with homologous gene expressions. In this case, however, morphological identities of crest cell populations cannot rely on the mesodermal components that are shared in vertebrates (Kuratani et al. 1999). Furthermore, the developmental nature of the lamprey trabecula, the premandibular cartilage, does present a conundrum and cannot be explained using this consideration, as will be discussed below.

Trabecula cranii and hypophysis

The above heterotopic scenario of jaw evolution leads us to question the homology of the so-called 'trabecular cartilage' reported in various vertebrate embryos and larvae. In many, the trabecular cartilage has been illustrated to arise as a pair of rod-like primordia, rostral to the MA domain, and for this mode of development, this cartilage has often been equated with the pharyngeal arch skeleton as a remnant of the premandibular arch (reviewed by de Beer, 1931, 1937; Kuratani et al. 1997, 2001). However, there is no clear embryological or palaeontological evidence to support

the presence of the premandibular arch as a basic component of the vertebrate head. Regardless, in gnathostome development, this cartilage appears most likely to develop from the PM ectomesenchyme (Le Lièvre & Le Douarin, 1975; Couly et al. 1993), and is then incorporated into the rostral part of the cranial base. As opposed to the more caudal part of the neurocranium, which is derived from the mesoderm and requires the presence of the notochord to chondrify, the trabecula derivatives and some associated cartilages similarly derived from the crest are called the 'prechordal cranium' (Couly et al. 1993). If this cartilage is derived from the PM crest cells in gnathostomes, can it also develop in the lamprey, which uses the PM crest cells to differentiate into the upper lip? A pair of rod-like cartilages actually develops beneath the brain in the chondrocranial base of the lamprey, and they are termed 'trabeculae' (Johnels, 1948; see McBurney & Wright, 1996, for its histogenesis).

By injecting vital dyes into the mandibular mesoderm in the young lamprey embryo, it has been shown that both the trigeminal nerve-innervated muscles and the trabecular cartilage primordium were labelled (Kuratani et al. 2004). The latter was seen as a strand of mesenchymal condensation lateral to the notochord. As noted above this is a site that is more suitable for the mesodermally derived neurocranium, which does require the notochord (Couly et al. 1993). Moreover, at the initial stage of its development, the trabecula has been found at the level of the MA (dorsal to the first aortic arch) by Johnels (1948). Therefore, it seems that the so-called trabecula in the lamprey might represent a rostrally elongated parachordal cartilage, not a crest-derived prechordal skeletal element (see Fig. 6), although it cannot be ruled out that the rostral part of the lamprey trabecula may receive contributions from the neural crest, or there would be a cryptic boundary in the rostral part of the lamprey trabecula, delineating the mesodermally derived and crest-derived parts as seen in the gnathostome neurocranium. Moreover, several studies have so far alluded to the contribution of the neural crest to the lamprey trabecula (Newth, 1956; Langille & Hall, 1988; but also see Newth, 1951). Importantly, although the detailed mapping of the lamprey cranium is still incomplete, the mesodermal contribution to the lamprey trabecula is consistent with the heterotopy theory of jaw evolution.

The lamprey-specific use of PM crest cells is made possible by a unique patterning of the nasohypophysial

placode. In the gnathostomes, nasal placodes are patterned as a pair of structures beneath the forebrain, rostral to the Rathke's pouch anlage (which differentiates as a part of the oral ectoderm). Such placodal morphology allows premandibular crest cells of gnathostomes to invade rostrally in the cranial base to form the prechordal cranium (Fig. 5C, right). In the lamprey, by contrast, nasal and hypophysial placodes initially form a single ectodermal plate rostral to the oral ectoderm (Fig. 5C; for embryology see Gorbman & Tamarin, 1985; Kuratani et al. 2001; Uchida et al. 2003). Thus the premandibular crest cells in the lamprey cannot grow rostrally to form a median septum in the cranial base as seen in the gnathostomes; instead the upper lip primordia arise behind this hypophysial plate and grow beneath the plate to form the floor of the nostril, or the nasohypophysial duct (Fig. 5A,C; see Kuratani et al. 2001). The hagfish, which follows a similar developmental pattern of placodes, has an equivalent duct that opens into the pharynx, unlike the lamprey (Gorbman, 1983; Gorbman & Tamarin, 1986; Janvier, 1996). It is important to realize that the hypophysis arises through interaction between the ectoderm and the ventral diencephalon, or the hypothalamic anlage. Again, the topographical relationships of tissues form a central problem. With respect to the mouth openings (oral ectoderm) and nasal placodes, the hypophysial primordia arise in non-equivalent topographies between the lamprey and gnathostomes – again the morphological homology is lost in a strict sense. Still, the primordia have to come into contact with the same inducer, or the hypothalamic anlage, to differentiate as the hypophysis in both animal groups. A close relationship between the oral ectoderm and hypothalamic anlage is established early in amniote development (Couly & Le Douarin, 1985; reviewed by Uchida et al. 2003). This relationship does not greatly change through later development. In the lamprey, by contrast, the hypophysial placode has to grow towards the hypothalamus secondarily in the late embryonic period. No similar pattern of tissue growth appears in gnathostome development.

Uchida et al. (2003) examined regulatory gene expression patterns and speculated on the developmental patterning of the hypophysis in the lamprey. For example, *Pitx* genes are known to specify the rostral ectoderm during early gnathostome embryogenesis, and play essential roles in development of the hypophysis (Szeto et al. 1999). In the lamprey, the *Pitx*

and *Pax6* cognates are also expressed in rostral ectoderm, and the expression domain becomes divided anteroposteriorly into two parts, the nasohypophysial plate and the oral ectoderm, by the secondary growth of the upper lip primordia. Similarly, *TTF-1*, a marker gene for the gnathostome hypothalamus, is also expressed in an equivalent portion of the brain anlage in the lamprey (Fig. 5D; Murakami et al. 2001; Ogasawara et al. 2001; Uchida et al. 2003). Of the genes that have been examined thus far, the expression of transcription factors, which act in a cell-autonomous manner, is associated with the equivalent cell type or specific structure in both lamprey and gnathostomes. In contrast, the expression patterns of genes encoding non cell-autonomously functioning signalling molecules, such as growth factors, are not comparable between the lamprey and gnathostomes (Uchida et al. 2003). Unlike the mouse, neither the *Fgf8/17* or the *Bmp2/4* cognates are expressed in the lamprey hypothalamus, whereas the *Fgf8/17* cognate is expressed in the hypophysial placode of the lamprey.

As already seen in the relatively expanded expression domain of *Fgf8* (*LjFgf8/17*) in the lamprey, changes in the distribution patterns of signalling molecules in the two animal groups may explain the different topography and behaviour of embryonic tissues: it is conceivable that these growth factor-encoding genes have to be regulated differently in those embryos with non-comparable topography to realize identical tissue interactions. Morphological homology may be lost during evolutionary changes of oral patterning, but the cell-autonomous developmental roles of regulatory genes tend to be conserved and are thus always attached to specific cell types or structures, such as *Msx* cognates always expressed in the tips of oral fringes (lips and jaws). It may rather be the changes in regulation of signalling molecule-encoding genes that form the base for heterotopy.

In conclusion, comparative embryology and molecular developmental biology of the lamprey embryo have allowed us to distinguish between the common features in development shared by lampreys and gnathostomes, and unique developmental programmes possessed by each of these animal lineages. As seen in the basic *Hox* code in PAs and the *Hox*-free default state of the MA, these shared developmental programmes are most likely to have been established in their common ancestor in the Cambrian sea, whereas the unique features in gnathostome embryos are likely

to indicate the evolutionary changes in developmental programmes behind the acquisition of the jaw, unless the features were secondarily lost in the lineage of the lamprey. As has been discussed elsewhere (Kuratani et al. 2001), the craniofacial pattern with a pair of nostrils (diplorhiny) in gnathostomes appears to be apomorphic with respect to that with single nostril (monorhiny) in many of agnathans (also see Janvier, 1996). In this transition of developmental programmes, there is a tendency that cell-autonomously functioning genes, mostly transcription factor-encoding genes, are always associated with the functionally equivalent structures or cell types, whereas the non-cell-autonomously functioning genes, such as growth factor-encoding genes tend to shift their regulation topographically, possibly as the molecular basis for heterotopy. In this way, a specific function is not always associated with the same cell lineage or homologous embryonic cell population, and morphological homology is often lost during this type of evolution. The gnathostome jaw therefore is apparently an 'evolutionary innovation' by the definition of Wagner & Müller (2002), being made possible by a heterotopic shift of gene regulation. For the same reason, the morphological concepts 'oral' and 'mandibular' must be dissociated in the discussion of vertebrate history.

As far as the 'jaw' is defined as a derivative from the 'mandibular arch', the jaw homologue cannot be found in the lamprey, no matter how well the larval lips resemble jaws. As suggested from some fossil records (Janvier, 1996), agnathans would have already enjoyed a dorsoventrally movable oral apparatus patterned through identical molecular cascades (FGF8–BMP4 signalling cascades onto *Hox*-negative ectomesenchyme) that now pattern the vertebrate jaw. In other words, shape and function were already there, but the place to create them was not fixed. This is reminiscent of the hypothesis by Janvier (1996) that the morphological pattern of the gnathostome head, including the patterning of the mouth, nose and hypophysis, would have been merely one of the various possible evolutionary experiments invented in the Palaeozoic era. With the advance of genomic sciences, it may become possible in the near future to compare the regulation of genes in various animals at the genomic level in an evolutionary developmental context, and we will be able to relate such changes directly to the heterotopic changes in embryonic patterning programmes at the morphological level.

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