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Embryology of the lamprey and evolution of the vertebrate jaw: insights from molecular and developmental perspectives

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Evolution of the vertebrate jaw has been reviewed and discussed based on the developmental pattern of the Japanese marine lamprey, *Lampetra japonica*. Though it never forms a jointed jaw apparatus, the *L. japonica* embryo exhibits the typical embryonic structure as well as the conserved regulatory gene expression patterns of vertebrates. The lamprey therefore shares the phylotype of vertebrates, the conserved embryonic pattern that appears at pharyngula stage, rather than representing an intermediate evolutionary state. Both gnathostomes and lampreys exhibit a tripartite configuration of the rostral-most crest-derived ectomesenchyme, each part occupying an anatomically equivalent site. Differentiated oral structure becomes apparent in post-pharyngula development. Due to the solid nasohypophyseal plate, the post-optic ectomesenchyme of the lamprey fails to grow rostromedially to form the medial nasal septum as in gnathostomes, but forms the upper lip instead. The gnathostome jaw may thus have arisen through a process of ontogenetic repatterning, in which a heterotopic shift of mesenchyme–epithelial relationships would have been involved. Further identification of shifts in tissue interaction and expression of regulatory genes are necessary to describe the evolution of the jaw fully from the standpoint of evolutionary developmental biology.

Keywords: lamprey; embryo; pharynx; mandibular arch; premandibular region; evolution

1. INTRODUCTION

The evolutionary origin of the vertebrate jaw is a long-standing question of comparative zoology. The jaw is generally regarded as the rostral-most pharyngeal arch that has gone through an enormous modification; this arch was enlarged and articulated dorsoventrally to acquire the function of biting. The jaw is, therefore, a serial homologue of the branchial arches, a part of the visceral skeleton (for reviews, see Mallatt 1996; Hall 1998). The pharyngeal arch from which both the upper and lower jaws develop is called the mandibular arch, specifically innervated by the posterior component of the trigeminal nerve. Irrespective of its clear embryological origin, the sequence of steps in the evolutionary origin of the jaw is still enigmatic, partly due to the absence of data on the ancestral, intermediate condition. The only living jawless vertebrates are lampreys and hagfishes, whose phylogenetic relationships are also still enigmatic (for reviews, see Forey 1984; Yalden 1985; Mallatt & Sullivan 1998; Janvier 1996; Kuraku *et al*. 1999). These animals both possess a characteristic apparatus called velum in the oral region, adapted for filter feeding or parasitism.

Comparative embryology searches for developmental features conserved among different animals in order to postulate the ancestral conditions. Characters shared between two given species are assumed to have been possessed by their common ancestor, unless other evidence indicates convergent origins. A difference in developmental process, on the other hand, does not necessarily identify the key event that caused the diversification of these animals. Such difficulties associated with the comparative method are even more crucial for the origin of the jaw, since the lamprey embryology is almost the only comparison that we can now use as the sister group of gnathostomes. In this paper, evolutionary and embryological morphogenesis of the vertebrate mandibular arch will be reviewed, based mainly on new developmental data on a Japanese marine lamprey, *Lampetra japonica*, to interpret the evolutionary change that may have happened in the developmental programme of the ancestral agnathan vertebrates, allowing the evolution of this innovative and revolutionary feature of gnathostomes, the jaw.

2. PHYLOTYPE AND HEAD MESOMERES

One major idiosyncrasy that biased the understanding of lamprey development in classical embryology was the mesomerism of the head, since this animal appears to have pre-otic myotomes reminiscent of the amphioxus condition (figure 1*a*). The regionalized cephalic mesoderm of the lamprey was misleadingly compared with the segmentation of trunk somites (Koltzoff 1901; Neal 1918;

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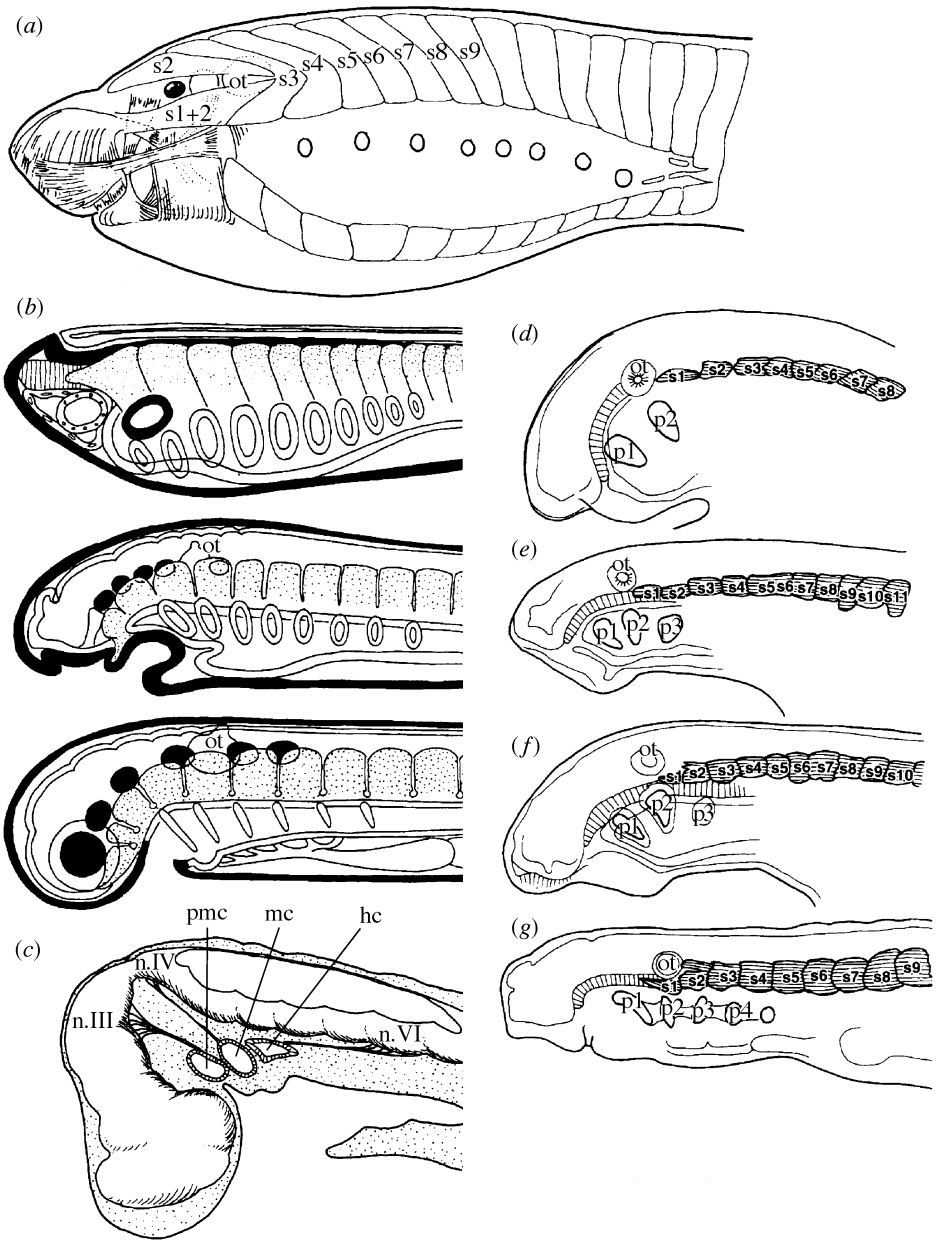

Figure 1. Embryology and evolution of the mesoderm. (*a*) Larval muscle morphology of the ammocoete larva. Rostral myotomes are located rostral to the otic vesicle (ot). (*b*) Evolution of mesomerism—a classical hypothesis. Top, larva of amphioxus; middle, ammocoete larva of the lamprey; and bottom, shark embryo. Comparative embryology equated rostral myotomes of amphioxus with vertebrate mesodermal blocks. Modified from Neal & Rand (1936). (*c*) Head cavities of a shark embryo. Each cavity is associated with a cranial nerve that innervates the extrinsic eye muscles derived from the cavity. Also modified from Neal & Rand (1936). (*d–g*) Developmental sequence of myotomes in the lamprey. Note that post-otically originating myotomes move rostrally during embryogenesis. ot, otocyst; p1–4, pharyngeal pouches; s1–11, myotomes with numbers of somites.

Damas 1944; Neal & Rand 1936; for a review, see Kuratani *et al*. 1999), and this interpretation had a pervasive influence in textbooks of comparative embryology (Neal & Rand 1936; Jarvik 1980; Jefferies 1986).

The basic idea of head segmentation stems in part from the head cavities, the epithelial mesodermal primordia of extrinsic eye muscles, which were first discovered in chondrichthyan embryos (figure 1*b*,*c*). Three pairs are usually recognized in cartilaginous fish embryos (from anterior to posterior: premandibular, mandibular and hyoid cavities), each associated with a pair of cranial nerves (III, IV and VI, respectively; figure 1*c*) that innervate the eye muscles. More posterior cavities tend to be lost in more derived groups (reviewed by Brachet 1935; Kuratani *et al*. 2000). A characteristic feature of the head cavities is that they exist as overt epithelial cysts embedded in a loose mesenchyme. Although the lamprey head mesoderm appears to arise as enterocoelic epithelium, it does not exhibit this common feature of head cavities, and hence head cavities are probably a derived feature of cartilaginous fishes (Kuratani *et al*. 1999; Kuratani & Horigome 2000). The cephalic mesoderm of the lamprey appears to be unsegmented initially, and only secondarily regionalized by surrounding embryonic structures such as the otocyst and pharyngeal pouches, a feature that is shared by most vertebrate embryos (figure 2*a*,*c*). Furthermore, the rostral myotomes originate post-otically from the true somites and migrate rostrally in later development

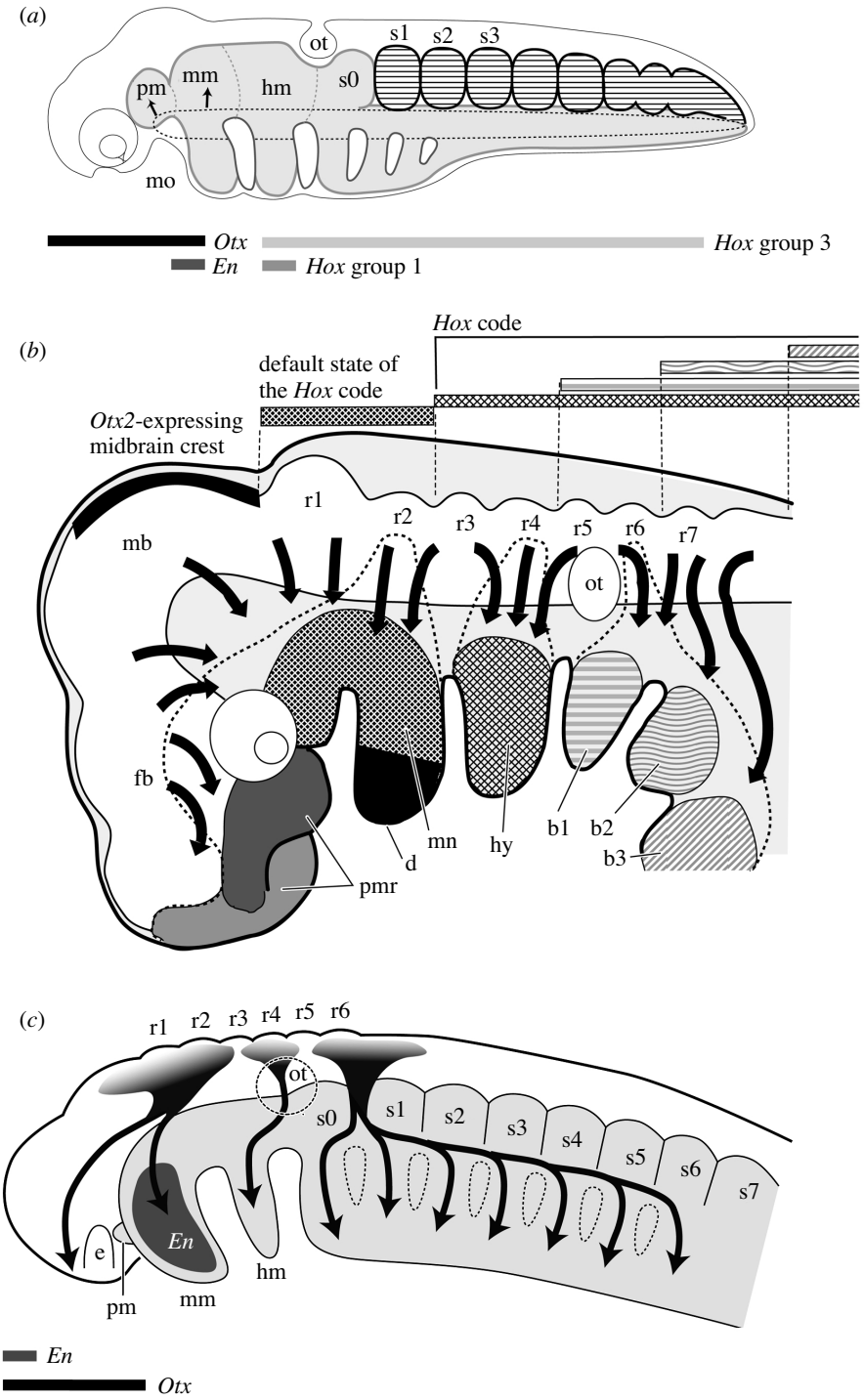

Figure 2. Vertebrate phylotype and lamprey development. (*a*) Mesodermal morphology and gene expression patterns of vertebrates. In all the vertebrate species, mesoderm is segmented only in the post-otic region, and the unsegmented head mesoderm is secondarily regionalized by surrounding structures, including pharyngeal pouches and the otocyst. (*b*) The gene expression pattern along the neuraxis and neural crest cell distribution in gnathostome embryos. The morphogenetic pattern along the neuraxis is carried by migrating crest cells that populate the ventral part of the head. Broken lines indicate three crest cell populations of the cephalic crest cells. Posterior pharyngeal arch ectomesenchyme appears to be specified by the *Hox* code, or the nested pattern of *Hox* gene expression, and the proximal part of the mandibular arch mesenchyme by the default state of this code. The distal portion of the mandibular arch receives crest cells from the posterior midbrain neural crest that expresses *Otx2*, which functions in patterning of the distal skeleton of the arch. There is also a crest-cell-filled region rostral to the mandibular arch, which can be called the premandibular region. Modified from Kuratani *et al*. (1997*b*). (*c*) Mesodermal and crest cell distribution pattern is similar in the lamprey pharyngula embryo. Localized expression patterns of several regulatory genes, including *LjOtxA* (neural tube rostral to the midbrain–hindbrain boundary) and *En* (mandibular arch mesoderm) are conserved at this stage. Abbreviations: b1–3, branchial arches; d, dentary-forming region of the mandibular arch; e, eye; fb, forebrain; hm, hyoid mesoderm; hy, hyoid arch; mb, midbrain; mm, mandibular (arch) mesoderm; mn, mandibular arch; ot, otocyst; pm, premandibular mesoderm; pmr, premandibular region; r1–r7, rhombomeres; s0–7, somites or myotomes.

(figure 1*a*,*d*–*g*). Thus, the lamprey head at no stage represents an intermediate evolutionary state, but rather it resembles gnathostome embryos, showing the shared embryonic pattern of head mesoderm (figure 2*a*).

Whether or not the vertebrate head mesoderm possesses intrinsic segmental patterns is another question. This problem has gained even more significance since the mechanism of segmentation is now being dealt with in terms of molecular biology (e.g. Müller *et al*. 1996). Importantly, there is a clear difference between the head mesoderm and trunk mesoderm in all the vertebrate species whose development is known, including the lamprey.

3. NEURAL CREST AND VISCERAL SKELETON

The vertebrate visceral skeleton is characteristically formed by neural-crest-derived ectomesenchyme (for a review, see Le Douarin 1982; Noden 1988; Kuratani *et al*. 1997*b*). Although non-vertebrate chordates exhibit similar body plans to that of vertebrates, the former lack the neural crest and crest-derived skeletal structures. The neural crest cells are specific to vertebrates and are often regarded as the fourth germ layer that permitted the elaboration of the vertebrate head (for a review, see Hall 1998) by contributing to mesenchymal components and the peripheral nervous system (Gans & Northcutt 1983). In the lamprey also, neural crest origin of the pharyngeal arch skeleton is plausible (Newth 1956; Langille & Hall 1988; Smith & Hall 1990; for a review, see Janvier 1993; but also see Von Kupffer 1895; Schalk 1913).

Advances in molecular embryology have accumulated data showing the molecular and genetic mechanisms that govern neural-crest-cell differentiation and patterning in gnathostomes (for a review, see Hunt *et al*. 1991; McGinnis & Krumlauf 1992). Typical examples are the *Hox* cluster and *Otx* genes whose expression patterns direct local specification of the branchial arch skeleton (figure 2*b*); each segment of pharyngeal ectomesenchyme carries its specific combination of homeobox gene expression patterns, thereby determining the developmental fate of the corresponding pharyngeal arch cartilage (figure 2*b*; Rijli *et al*. 1993). In jaw development, the expression pattern of another homeobox gene, *Otx* (vertebrate cognate of *Drosophila orthodenticle*), is most curious; murine *Otx2* is expressed rostral to the midbrain–hindbrain boundary (MHB), and the lower jaw was specifically diminished by the genetic disruption of murine *Otx2* (figure 2*b*; Matsuo *et al*. 1995; for a review, see Kuratani *et al*. 1997*b*). It has also been shown in amniote embryos that midbrain-derived crest cells populate the ventral portion of the mandibular arch, precursors of the lower jaw (Osumi-Yamashita *et al*. 1994; Köntges & Lumsden 1996). Cognates of various regulatory genes, including the homeobox genes, are now being isolated in various deuterostome species, allowing us to begin to interpret the evolution of the jaw in terms of molecular developmental mechanisms.

Profoundly related to the specification of crest cells and expression of homeobox genes is the neuromeric organization of the neural tube. Among neuromeres, those in the hindbrain or the rhombomeres (Orr 1887; Lumsden & Keynes 1989) are best known in terms of their molecular developmental significance (Hunt & Krumlauf 1991; Hunt *et al*. 1991). In the lamprey, as in gnathostomes, six to seven rhombomeres can be counted in the late pharyngula (figure 2*c*; Kuratani *et al*. 1997*a*, 1998; Horigome *et al*. 1999). As observed in various vertebrate embryos, the cranial nerve roots develop on the even-numbered rhombomeres. Odd-numbered rhombomeres—rhombomere (r) 3 and r5—do not possess cranial nerve roots or, in younger stages, are not accompanied by cephalic crest cells on their lateral aspects (figure 2*c*). Unlike the spinal nerves that develop in the trunk, the morphological patterning of the gnathostome cranial (branchiomeric) nerves appears to depend on the specific association of neural crest cells with even-numbered rhombomeres (Kuratani & Eichele 1993). This even–odd rule of lamprey cranial nerve root positioning suggests a shared patterning mechanism in this process (figures 1*c* and 3; Horigome *et al*. 1999).

In earlier developmental stages, the lamprey cephalic crest cells apparently have ubiquitous origins along the neuraxis as in gnathostomes (Sechrist *et al*. 1993; Shigetani *et al*. 1995; Horigome *et al*. 1999; but also see Lumsden *et al*. 1991). As shown by focal injections of a lipophilic dye, DiI, the premigratory neural crest is well organized anteroposteriorly along the neuraxis in the lamprey, as has been found in various vertebrates (figure 3).

Similar to gnathostomes, midbrain crest cells populate the lamprey mandibular arch despite its lack of a lower jaw (Horigome *et al*. 1999). Thus, *Otx* might have a conserved function in the specification of the mandibular arch crest cells of vertebrates. Two laboratories have isolated the lamprey homologue(s) of *Otx* genes, *LjOtxA* and *LjOtxB* in *L. japonica* (Ueki *et al*. 1998), and *PmOtx* in *Petromyzon marinus* (Tomsa & Langeland 1999). Of these, *LjOtxA* and *PmOtx* are expressed rostral to the MHB and also in crest cells that populate the mandibular arch, showing the same pattern found in gnathostome embryos (figure 2*c*; Horigome *et al*. 1999; Tomsa & Langelland 1999); *LjOtxB* is not expressed in the rostral brain except for the eye and epiphysis (Ueki *et al*. 1998). Localized expression of the *Otx* gene, therefore, specifies the mandibular arch as a part of the vertebrate phylotype and shows no new patterns in gnathostomes that could help explain the origin of the jaw.

Similar conservation is observed in the *En*-like expression pattern in the mandibular arch mesoderm (figure 2*c*; Holland *et al*. 1993), *Dlx1/6* in the forebrain domains (Myojin *et al*. 2001), and also in that of *Pax9* cognate in the pharyngeal pouch (see figure 5*d*; Ogasawara *et al*. 2000). Also included in this category would be the *Hox* code of the lamprey embryo, which is still unknown (Pendleton *et al*. 1993). Transgenic analyses, however, have indicated conserved regulation of one of the lamprey *Hox* genes (Carr *et al*. 1998).

In all the cases described to date, the lamprey cognates of genes involved in mandibular development are expressed in comparable embryonic regions, suggesting homology of these regions in gnathostomes and lampreys at certain levels (figure 2; see Hall (1998) for embryonic criteria of homology and gene expression). As compared with gnathostomes, therefore, both morphology and gene expression at the phylotypic stage of the lamprey embryo

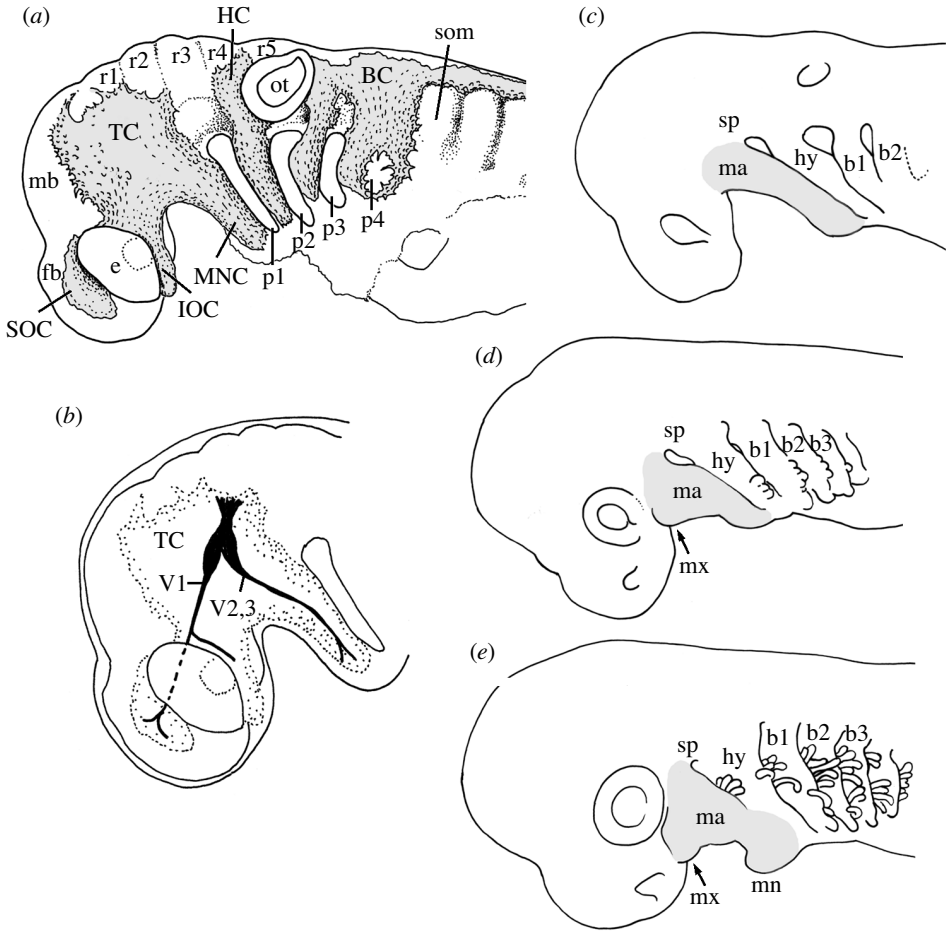

Figure 3. Development of the gnathostome mandibular arch. (*a*) The distribution pattern of cephalic crest cells in the *Scylliorhinus torazame* embryo at the same stage as (*c*). A scanning electron micrograph of this specimen appears in Kuratani & Horigome (2000). Note that the trigeminal crest cells (TC) consist of three subpopulations termed supra-optic (SOC), infra-optic (IOC) and mandibular arch (MNC) crest cells. The IOC cells are often neglected or misleadingly identified as maxillary process mesenchyme, which is of mandibular arch origin. (*b*) Schematized ditribution pattern of trigeminal nerve in the shark embryo. The trigeminal nerve branches grow parallel to the TC distribution. (*c–e*) Development of the mandibular arch in the shark. Three stages of shark pharyngula are shown. (*c*) Embryo (9.5 mm) of *Scylliorhinus torazame* representing a basic pharyngula morphology. (*d*) Pharyngula (17 mm) of the same species as (*a*). (*e*) Embryo (35 mm) of *Cephaloscyllium* sp., showing the differentiating upper and lower jaws. Note that at the early stage of the pharyngula, the mandibular arch (ma) appears as a simple bar-like structure just like more caudal pharyngeal arches. It is later in development that the maxillary portion (mx) arises from the dorsal portion of the mandibular arch (ma), as shown in (*d*) and (*e*). Based on Kuratani & Horigome (2000). Abbreviations: b1–b3, branchial arches; BC, branchial crest cells; fb, forebrain; HC, hyoid crest cells; hy, hyoid arch; ma, mandibular arch; IOC, infra-optic crest cells; mb, midbrain; MNC, mandibular arch crest cells; mx, maxillary process; p1–4, pharyngeal pouches; r1–5, rhombomeres; SOC, supra-optic crest cells; som, somite; sp, spiracle; TC, trigeminal crest cells.

do not exhibit any obvious difference that directly indicates the developmental change leading to the origin of the jaw in gnathostomes. The evolution of the jaw thus may be largely dependent on later epigenetic events such as local tissue interactions after the phylotypic stage. Typical examples are epithelial–mesenchymal interactions in craniofacial development that involve crest-derived ectomesenchyme.

4. MANDIBULAR CREST CELLS IN GNATHOSTOMES

Figure 3*b–d* shows a developmental sequence of shark pharyngula embryos in which the simple bar-like mandibular arch (figure 3*b*) differentiates into upper and lower jaws (see Kuratani & Horigome (2000) for shark development). It is from the dorsal portion of the mandibular crest cells that the maxillary process secondarily grows rostrally (figure 3*b–d*). It is not a simple task to define mandibular crest cells, partly because the cells are part of a more extensive crest cell population, the trigeminal crest cells, and partly because the regionalization mechanism of the mandibular arch-specific crest cells is still unclear. The name of the trigeminal crest cells stems from the fact that this entire region corresponds to the innervation area of the trigeminal nerve (figure 3*b*; see Kuratani 1997; Kuratani & Tanaka 1990).

The peripheral innervation pattern of the trigeminal nerve branches is in accordance with crest cell migration and growth (figures 2*c* and 3*a*; and see also figure 9*c*; Johnston 1966; Noden 1975; Kuratani 1997). For example, the ophthalmic nerve of the trigeminal nerve complex is distributed in the region rostral to the mandibular arch, and the innervation domain of the maxillomandibular nerve (trigeminal branches 2 and 3) corresponds with the

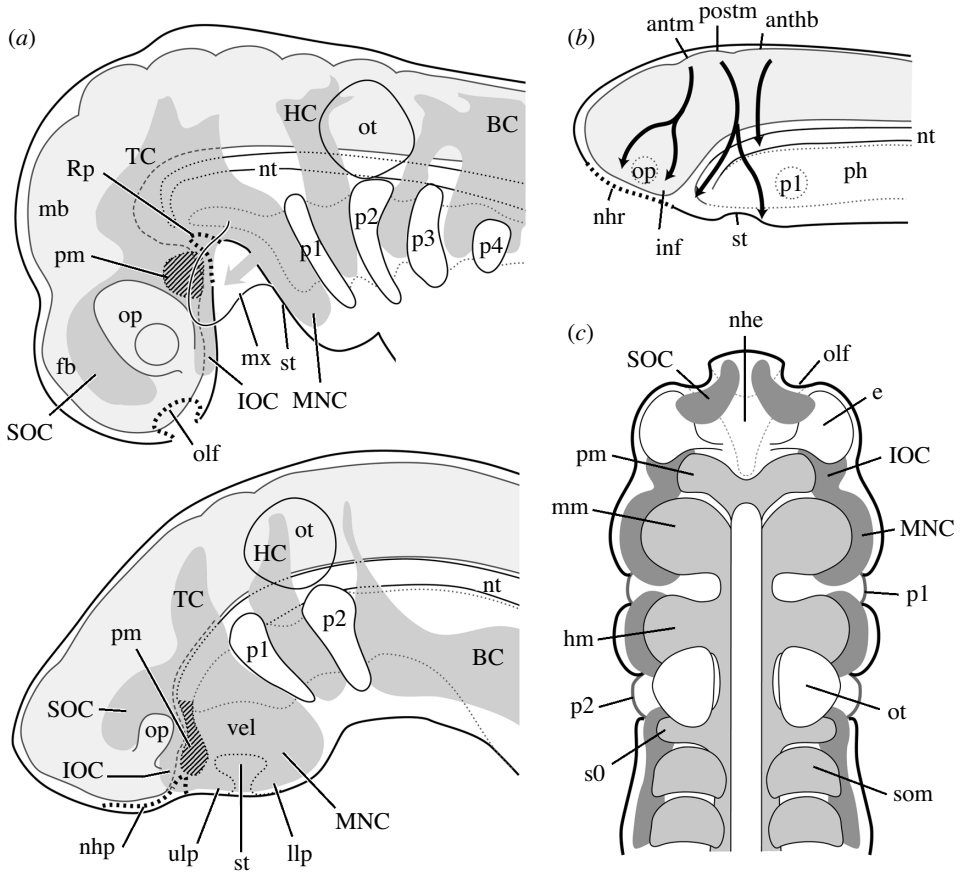

Figure 4. Comparative anatomy of the cephalic crest cells. (*a*) Distribution of the trigeminal crest cells is conserved at pharyngula stages between gnathostomes (top) and lamprey larva (bottom). (*b*) Hypothetical origins of trigeminal crest cells. In the premandibular region, there are two trigeminal crest cell subpopulations (SOC and IOC). Of these, IOC cells are located lateral to the premandibular mesoderm and closely related to the hypophysis. The mandibular arch receives crest cells from the anterior hindbrain and posterior midbrain. The premandibular crest cells that occur both rostral and caudal to the eye originate from the anterior midbrain and more rostral region of the neural crest. (*c*). Schematized anatomy of the vertebrate embryo with three cephalic crest cell populations and regionalized cephalic mesoderm. Note the topographical relationships between IOC cells, nasohypophyseal plate and the premandibular mesoderm. Abbreviations: anthb, anterior hindbrain; antm, anterior midbrain; BC, branchial crest cells; e, eye; HC, hyoid crest cells; hm, hyoid mesoderm; inf, infundibulum; IOC, infra-optic crest cells; llp, lower lip; mb, midbrain; mm, mandibular mesoderm; MNC, mandibular crest cells; mx, maxillary process; nhp, nasohypophyseal plate; nhr, nasohypophyseal region; nhe, nasohypophyseal ectoderm; nt, notochord; olf, olfactory epithelium; op, optic vesicle; ot, otocyst; p1–4, pharyngeal pouches; ph, pharynx; pm, premandibular mesoderm; postm, posterior midbrain; Rp, Rathke's pouch; s0, somite 0; SOC, supra-optic crest cells; som, somite; st, stomodeum; TC, trigeminal crest cells; ulp, upper lip; vel, velum.

mandibular arch derivatives (Goodrich 1930). A specific chemoattractant derived from specific parts of the craniofacial mesenchyme has been suggested to be behind the morphological correspondence between the sensory fibres and ectomesenchyme (for a review, see Lumsden 1988; O'Connor & Tessier-Lavigne 1999).

Observation of the early distribution pattern of the trigeminal crest cells in the early shark embryo allows us to divide this cell population into three subpopulations (figure 3*a*): the supra-optic crest (SOC) cells corresponding to the rostral branch of the ophthalmic nerve; infra-optic crest (IOC) cells corresponding with the ventral branch of the ophthalmic nerve; and the mandibular crest (MNC) cells in the mandibular arch proper (figures 3*a*,*b* and 4*a*). The SOC and IOC cells are also collectively called premandibular crest cells for their positions rostral to the mandibular region (see §§5 and 6). Every crest cell subpopulation occupies a characteristic site in the embryonic head, implying each is subject to a specific embryonic environment and tissue interactions (figure 4*a*). The tripartite morphology of the trigeminal crest cells in the shark is also found in other gnathostome embryos. In the chick, for example, the premandibular crest cells correspond with the cells that yield the prechordal neurocranial cartilage (Couly *et al*. 1993; Shigetani *et al*. 2000).

One good example to show the modular nature of each subpopulation is found in the *small eye* (*Sey*) mutant in mice. This mutation in the paired homeobox gene, *Pax6*, which is expressed in the neural tube and the eye, results in the specific loss of the SOC cells (Osumi-Yamashita *et al*. 1997). The loss has been shown to be caused by a defect in migratory environments, not in the crest cells themselves, implying an epigenetic process in craniofacial specifications, based on local tissue interactions. In a similar context, a secreted molecule, fibroblast growth factor 8 (FGF8) expressed in overlying ectoderm appears to be required in premandibular–mandibular (IOC–MNC)

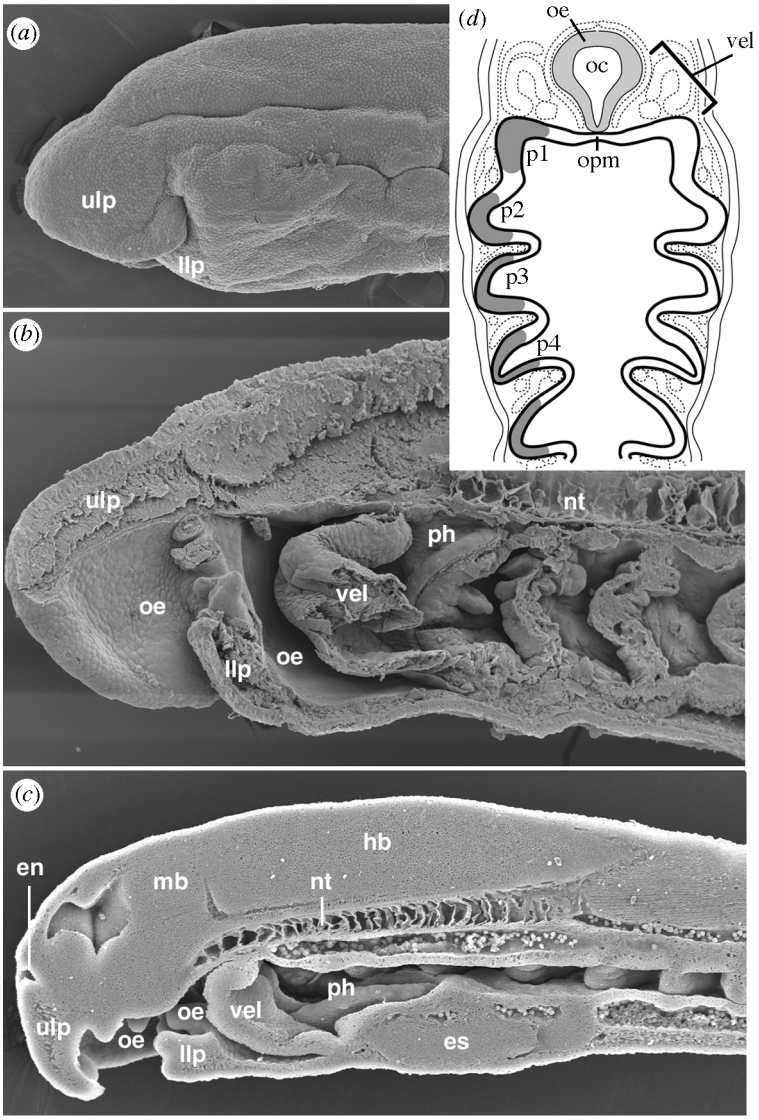

Figure 5. Basic structure of the mouth of ammocoete larva of *L. japonica*. Scanning electron micrographs of ammocoete larvae. (*a*) A lateral view of stage 29.5 *L. japonica*. (*b*) Medial view of a stage 30 larva that has been sagittally sectioned. (*c*) A stage 26 larva that has been sagittally sectioned. Note the position of the velum that has arisen between the pharynx and the oral cavity. The rostral end of the notochord is at the level of the oral ectoderm. (*d*) Expression of *LjPax9*. Expression of the lamprey cognate of *Pax9*, *LjPax9*, is shown in the horizontal section. Based on Ogasawara *et al*. (1999, 2000). Note that the rostral-most indentation of the pharyngeal endoderm expresses *LjPax9*, implying the morphological nature of this endodermal structure as a pharyngeal pouch. Abbreviations: en, external nostril; es, endostyle; hb, hindbrain; llp, lower lip; mb, midbrain; nt, notochord; oc, oral cavity; oe, oral ectoderm; opm, oropharyngeal membrane; p1–4, pharyngeal pouches; ph, pharynx; ulp, upper lip; vel, velum.

regionalization (Shigetani *et al*. 2000). In the chick embryo, this growth factor is initially present in a part of the head ectoderm that corresponds with the future mandibular region. A recent experiment in the mouse based on *Cre/LoxP* technique to produce a stage and tissue-specific disruption of FGF8 resulted in mandibular defects (Trumpp *et al*. 1999).

Observations of dissected embryos with scanning electron microscope and focal injections of DiI provide general ideas about neural crest development in the lamprey embryo (Horigome *et al*. 1999). The tripartite configuration of the trigeminal crest cells is also found in the lamprey larva based on topographical relationships between ectomesenchyme and surrounding structures: SOC, IOC and MNC cells are identified in a stage 25 larva (figure 4*a*; see also Horigome *et al*. 1999; developmental stages based on Tahara 1988). The initial distribution pattern of the craniofacial ectomesenchyme seems, therefore, to be conserved among vertebrates (figure 4*c*). The neuraxial origins of each ectomesenchymal portion remain unclear, although the premandibular components (SOC and IOC) appear to originate from the rostral midbrain and the more rostral neural crest in amniote embryos (figure 4*b*; Shigetani *et al*. 2000; Y. Nobusada & S. Kuratani, unpublished data). Which part of the oral apparatus originates from these ectomesenchymal portions is the next topic and is crucial for understanding how the velar apparatus may have evolved.

5. MOUTH FORMATION IN THE LAMPREY

The ammocoete mouth exhibits three major components rostral to the first pharyngeal pouch, namely the upper and lower lips and the velum (figure 5). This does

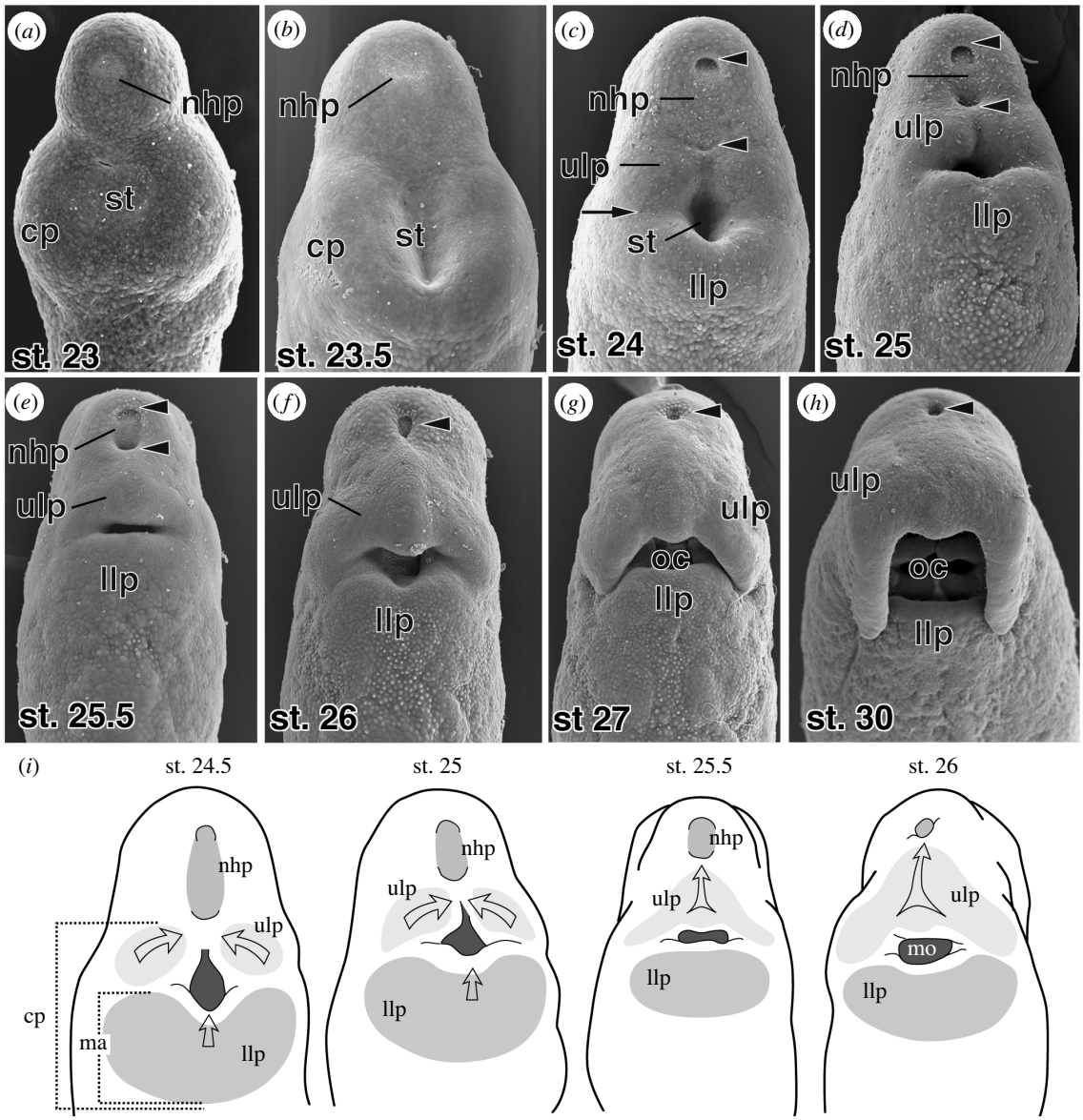

Figure 6. Developmental sequence of oral and pre-oral regions in staged *L. japonica*. (*a*,*b*) In stage 23 and 23.5 embryos, a pair of cheek processes has appeared on the head. Between the processes is a depression indicating the stomodaeum (st). Note that the stomodaeum lies behind the future nasohypophyseal plate (nhp). (*c*) By stage 24, the cheek process has been divided into rostral and caudal halves by a groove (arrow). (*d–h*) The upper lip primordia (ulp) gradually fuse together in the middle and grow rostrally. Simultaneously, the nasohypophyseal plate (nhp, arrowheads) is embedded to form the definitive external nares. Note that the lower lip retracts caudally from stage 25.5. (*e*) Expansion of the upper lip and translocation of the external nares (arrowheads) are shown. (*i*) Developmental sequence of the upper lip of *L. japonica*. Directions of the mesenchymal growth as suggested by successive surface morphology of the head oral region (open arrows). The upper lip appears to form from a pair of processes (or the rostral half of the cheek process) that fuse in the middle and grow further rostrally. The upper lip thus created forms a floor beneath the nasohypophyseal plate that grows from a caudal to a rostral direction. Abbreviations: cp, cheek process; llp, lower lip; ma, mandibular arch; mo, mouth; nhp, nasohypophyseal plate; oc, oral cavity; st, stomodaeum; ulp, upper lip.

not necessarily mean, of course, that all three of these elements are of mandibular arch origin. The velum, or the pumping apparatus, is located between the endodermal pharynx and oral ectoderm, reminiscent of the gnathostome oropharyngeal membrane that completely ruptures during embryogenesis (Johnels 1948; figure 5*b*,*c*). It is located just rostral to an endodermal lateral pocket (figure 5*d*). The morphological identity of the latter structure as the first pharyngeal pouch is supported by comparative embryology (Goette 1901; Schalk 1913; Mallatt 1996) and by expression of *Pax9* (figure 5*d*; *LjPax9*; Ogasawara *et al*. 2000), a marker of the whole series of pharyngeal slits in deuterostomes (see Ogasawara *et al*. 1999). Moreover, the gene is also expressed in the mesenchyme of the velum, as murine *Pax9* is expressed in the mandibular arch mesenchyme. Classically established homology of the velum as a mandibular arch derivative thus seems to be likely (Ogasawara *et al*. 2000; for a review, see Carroll 1988; Mallat 1996; Janvier 1996). Through observation of developing lamprey embryos by scanning electron microscopy, the lower lip was seen to develop from the ventrocaudal portion of the cheek

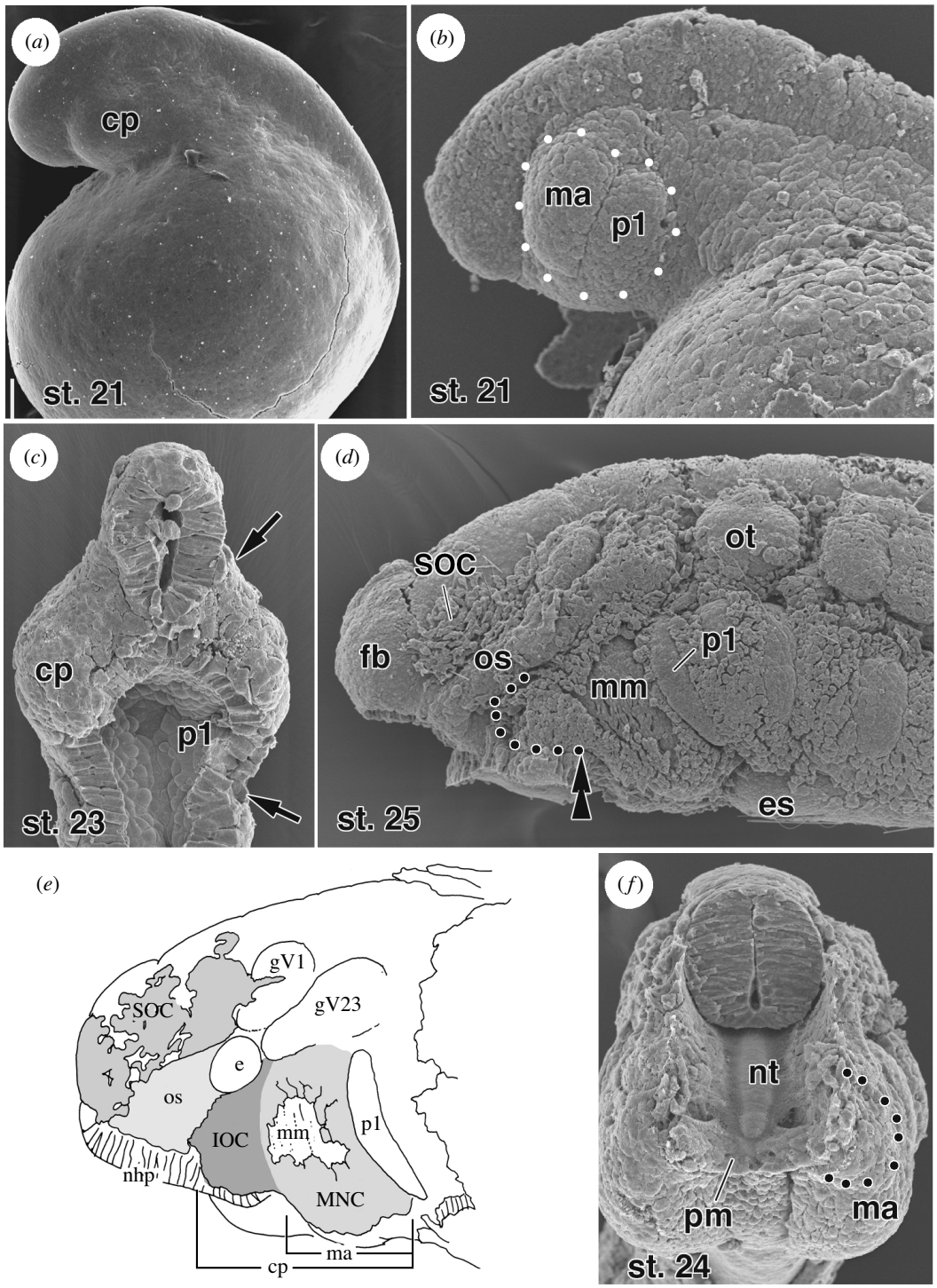

Figure 7. Identity of the upper-lip-forming mesenchyme. (*a*) Lateral view of a stage 21 lamprey embryo observed by scanning electron microscopy. The cheek process (cp) develops as a lateral protrusion in the head. (*b*) Ectoderm has been removed from the same stage embryo as (*a*). Note that the cheek process consists of mandibular arch mesoderm and the first pharyngeal pouch. (*c*) Ventral view of a dissected embryo. Arrows indicate the extent of the cheek process. (*d*) In a stage 25 embryo, the rostral portion of the cheek process has developed a small process rostral to the mandibular arch. (*e*) Schematic to show the regionalization of the cheek process mesenchyme. The upper-lip-forming mesenchyme corresponds with IOC cells, since, as seen in (*f*), the cells surround the newly forming mesodermal component, the premandibular mesoderm. For better resolution of crest-derived and mesodermal cells, see also fig. 5F of Kuratani *et al.* (1999). Abbreviations: cp, cheek process; es, endostyle; fb, forebrain; gV1, ophthalmic ganglion; gV23, maxillomandibular ganglion; IOC, infra-optic crest cells; ma, mandibular arch; mm, mandibular mesoderm; MNC, mandibular crest cells; nhp, nasohypophyseal plate; nt, notochord; os, optic stalk; ot, otocyst; pm, premandibular mesoderm; p1, first pharyngeal pouch; SOC, supra-optic crest cells; st, stomodaeum.

process developing on the lateral aspect of the head, due to the growth of both the mandibular arch and the first pharyngeal pouch (figures 6 and 7). Thus, the mandibular arch origin of the lower lip is again plausible.

Problematic is the origin of the upper lip. By its functional and morphological similarities, the upper lip of ammocoetes has often been compared with the structure of the same name in gnathostomes, which is a maxillary process derivative (for a review, see Mallatt 1996; see also Johnston 1905). However, although this structure arises as a part of the cheek process, this falls where the premandibular mesoderm appears by stage 24 (figure 7*a–c*; Kuratani *et al*. 1999). Given the universal homology of the premandibular mesoderm among vertebrates (topographical relationships, as well as its prechordal plate origin; Kuratani *et al*. 1999), the crest cells that form the ectomesenchyme of the lamprey upper lip appear most likely to correspond with the IOC cells (figure 7; also see fig. 5F of Kuratani *et al*. 1999). In addition, the cheek process can be equated with the mandibular arch of gnathostomes only up to stage 21 (figure 7*a–c*), and it becomes a composite structure including both the premandibular and mandibular elements after stage 24 (figure 7*d–f*).

The sensory innervation of the trigeminal branches seems to support the premandibular origin of the upper lip (see figure 9*c*).[1] As noted above, the craniofacial elements receive sensory fibres from specific trigeminal branches, as the maxillomandibular branches always innervate the mandibular arch derivatives. The ammocoete upper lip receives sensory fibres from a branch of the ophthalmic profundus nerve, as does the gnathostome frontonasal region (Johnston 1905; Kuratani *et al*. 1998; see figure 9*c*). In gnathostomes, there is no muscle except for the extrinsic eye muscles that are derived from the premandibular mesoderm. One of maxillomandibular nerve branches in the upper lip (see figure 9*c*) may partly be explained as innervation of the upper lip muscle that originally developed from the mandibular mesoderm. Evolution of the head mesoderm differentiation is thus profoundly connected to the question of the origin of the jaw.

6. PREMANDIBULAR ECTOMESENCHYME

Up to the stage when trigeminal crest cells are subdivided into three subpopulations, lamprey and gnathostome embryos do not show overt differences as far as basic topographical relationships of embryonic structures—such as the eye, pharyngeal pouches, mesodermal elements, notochord, etc.—are concerned. Here we see the phylotype of the vertebrate head in which equivalent cell populations are exposed to comparable embryonic environments. It is also at this stage that master control genes such as homeobox genes are expressed in a conserved fashion (Duboule 1994). After this period, however, an enormous change occurs in the growth and distribution patterns of the ectomesenchyme. The developmental process of the IOC cells is most conspicuously different between the two animal groups (figure 7*a*).

The classical concept of the premandibular region stems from the hypothesis that the paired trabecular cartilages of the ventral brain-case resemble a pharyngeal cartilage. By this notion these cartilages might represent another pharyngeal arch anterior to the mandibular arch that has been displaced dorsally (for a review, see Goodrich 1930; De Beer 1937; Kuratani *et al*. 1998). This idea gained popularity since the comparative anatomists and embryologists expected that each mesomere (head cavities in elasmobranchs) would correspond with one of the pharyngeal arches; the rostral-most head cavity did not seem to possess its pharyngeal arch counterpart. The term 'premandibular region' in the present review, however, does not imply branchiomerism of the head.

The trabecular cartilages in gnathostomes are now regarded as neural-crest-derived components of the neurocranium. An excellent experimental treatment of this question of cartilage would be that by Couly *et al*. (1993), who demonstrated that the prechordal neurocranium (partly trabecular cartilage derivative) of avian embryos is of crest origin (also see Le Lièvre 1978; Noden 1988). This part of the neurocranium, rostral to the hypophyseal foramen, is not accompanied by the notochord medially and mesoderm does not chondrify in the absence of the sonic hedgehog, a signalling molecule emanating from the notochord. These prechordal cranium-forming crest cells (SOC and IOC cells) are, however, embryologically more closely associated with the MNC cells than with the cephalic mesoderm at notochordal levels (figure 4*a*,*c*), and these crest cell populations are only secondarily dissociated from each other. Together with the more caudal ectomesenchyme, the premandibular crest cells form the ventral part of the cranium, the viscerocranium in a wide sense (reviewed by Kuratani *et al*. 1997*b*).

The IOC cells form the caudal component of the premandibular crest cells. Their position lateral to the hypophysis suggests that they differentiate into the trabecular cartilage of gnathostomes (see § 7). This is also consistent with the skeletal phenotype of the *Sey* mutant rat, in which SOC cells and the lateral nasal wall are missing, but which retains the nasal septum (= the trabecula derivative) (Osumi-Yamashita *et al*. 1997). The IOC cells should not be misinterpreted as the maxillary mesenchyme, which is a secondary growth of MNC cells (figure 3*c–e*).

Comparison of IOC cells in lampreys and gnathostomes leads to one possible scenario to explain the evolution of the gnathostome jaw: in the gnathostomes, the premandibular crest cells extend rostrally into the space between the nasal placodes and Rathke's pouch (figures 8 and 9*a*). In the lamprey embryo, in contrast, the latter space does not exist since the olfactory epithelium and the hypophysis develop as a single median placode, the nasohypophyseal plate (NHP). Thus, the lamprey IOC cells can merely grow beneath the NHP to form the nasohypophyseal duct, which does not exist in gnathostomes (figures 8, 9*a* and 10). In other words, the configuration of the oral ectoderm differs between the two animal groups in its relation to the hypophysis and the nasal placode (figure 8*c–g*). The maxillary processes should meet in the middle in front of the hypophysis—the space that is not present in the lamprey embryo (figures 6–8). The differences found between gnathostome and lamprey embryos first become clear in late

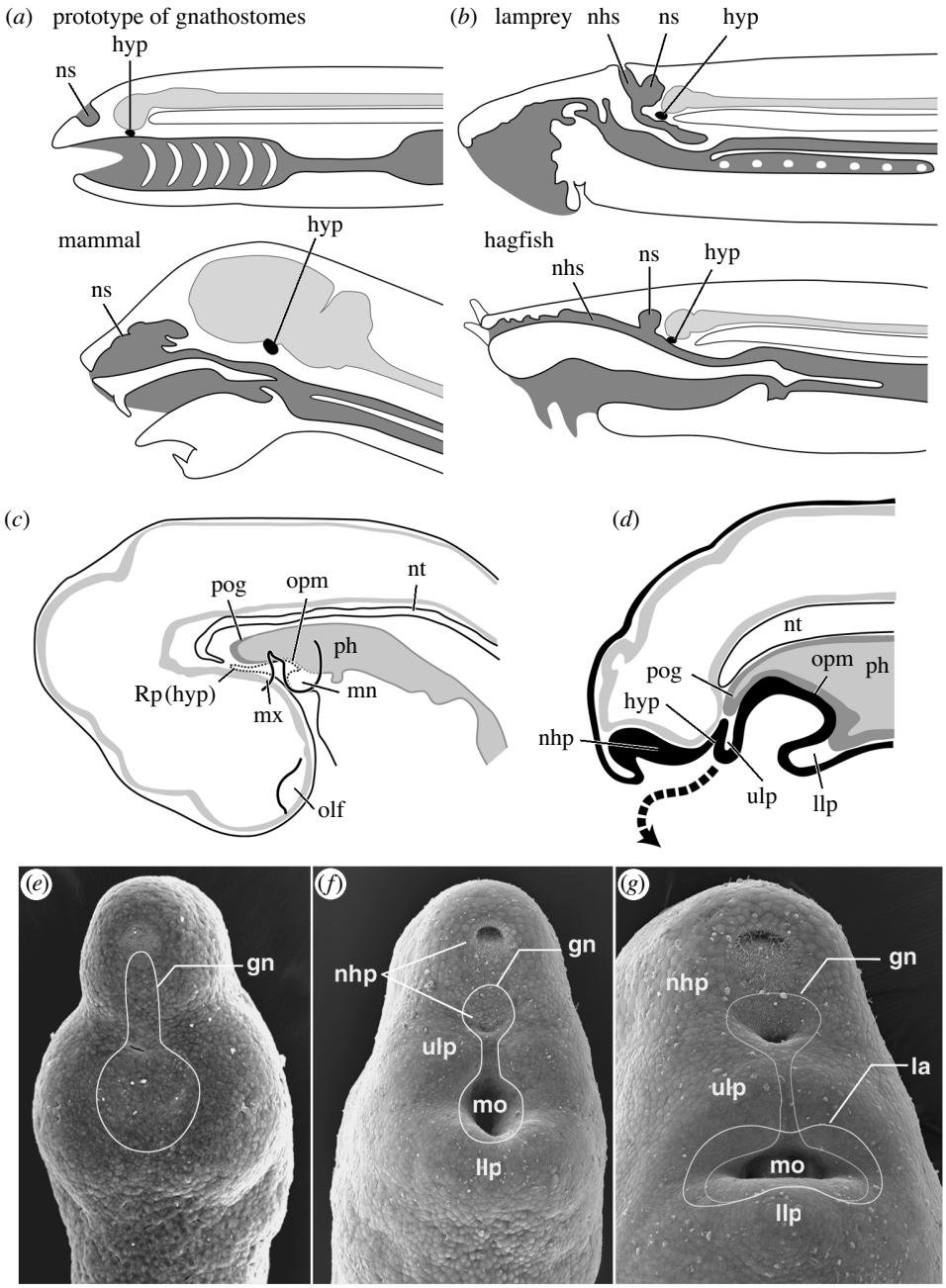

Figure 8. Morphological difference of the oral ectoderm between gnathostomes and ammocoete larva. (*a*) Basic anatomy of gnathostomes. Top, generalized scheme. Nasal cavity is primarily a blind sac, separated from the hypophysis. In mammals (bottom), as a secondary condition, the nasal cavity leads to the pharynx caudally, rostrally separated from the oral cavity by the secondary palate. (*b*) Anatomy of lampreys (top) and hagfishes (bottom). Nasal sacs are closely related to the hypophysis. Nasohypophyseal complexes are separated from the oral cavity and the pharynx by a primary structure that is homologous with the lamprey upper lip. (*c*) Embryonic development of the oral region in the chick embryo. The maxillary process (mx) is a secondary protrusion of the mandibular arch, located lateral to the hypophysis (Rp(hyp)) and caudal to the olfactory placode (olf). (*d*) In lamprey development, the upper lip arises caudal to the nasohypophyseal complex. (*e*) Ventral view of a stage 23 embryo. (*f*) Stage 24 embryo. The region corresponding to the gnathostome oral ectoderm is encircled by a line (gn). (*g*) Stage 25 *L. japonica* embryo. The most profound difference between the lamprey and gnathostomes is the expansion of the oral ectoderm. Due to the early separation of the nasohypophyseal complex in gnathostomes into the nasal placode and Rathke's pouch, the oral ectoderm can incorporate Rathke's pouch. In the ammocoete larva, in which the nasohypophyseal plate stays together, no space is left for the maxillary process to grow into. The oral ectoderm of gnathostomes (gn) would appear in a much wider region than the lamprey oral ectoderm (la) when the former is superimposed on the lamprey embryo. Abbreviations: gn, oral ectoderm in gnathostomes; hyp, hypophysis; la, oral ectoderm in the lamprey; llp, lower lip; mn, mandibular process of the gnathostome embryo; mo, mouth; mx, maxillary process; nhp, nasohypophyseal plate; nhs, hasohypophyseal sinus; ns, nasal sac; nt, notochord; olf, olfactory placode; opm, oropharyngeal membrane; ph, pharynx; pog, preoral gut; Rp, Rathke's pouch; ulp, upper lip.

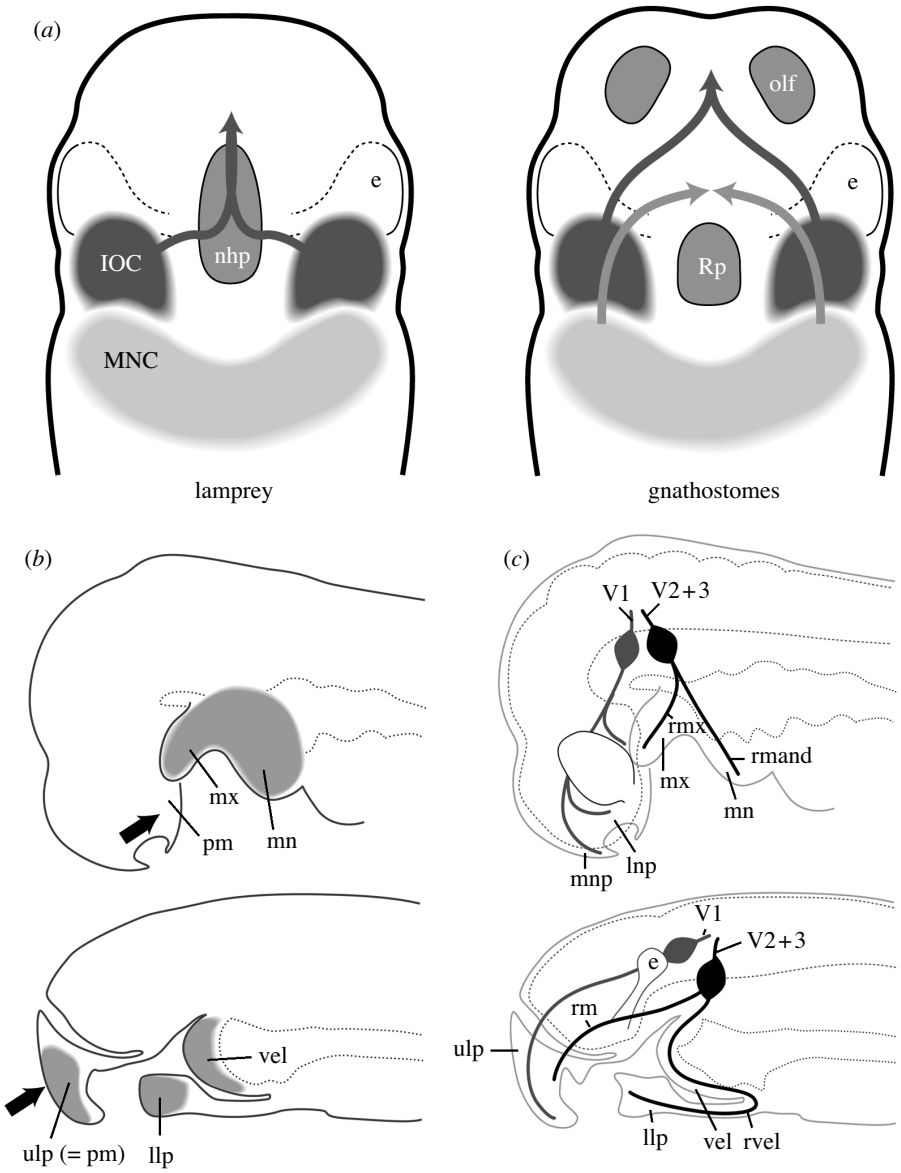

Figure 9. Comparison between lamprey and gnathostome embryos. (*a*) In the lamprey, the upper lip develops from the IOC cells that grow rostrally beneath the nasohypophyseal plate (nhp). In gnathostomes, IOC cells grow rostrally between Rathke's pouch (Rp) and the olfactory placode (olf), to form a part of the trabecular cartilage. The maxillary process develops from some of the mandibular crest cells, growing rostrally to form the upper jaw that fuses in the middle in front of the hypophysis. Note the difference of topographical relationships between oral ectoderm, nasohypophyseal complex and ectomesenchymal subpopulations. No direct homologies can be established in the oral parts between gnathostomes and lampreys. (*b*) Expression pattern of *Dlx1/6* genes. In the oral region of gnathostomes (top), *Dlx1* expression is restricted to the ectomesenchyme of the mandibular arch. In the lamprey embryo (below), *LjDlx1/6* is expressed also in the upper lip, which is a premandibular structure. (*c*) Comparison of peripheral branches of the trigeminal nerve. In gnathostomes (top), the ophthalmic nerve (V1) is primarily distributed in the premandibular region. In the lamprey embryo (below), the upper lip receives branches from both the ophthalmic (V1) and maxillomandibular (V2+3) nerves. Abbreviations: e, eye; IOC, infra-optic crest cells; llp, lower lip; lnp, lateral nasal prominence; mn, mandibular process; MNC, mandibular crest cells; mnp, medial nasal prominence; mx, maxillary process; nhp, nasohypophyseal plate; pm, premandibular region; rmand, mandibular branch; rmx, maxillary branch; rvel, velar branch; ulp, upper lip; vel, velum; V1, ophthalmic nerve; V2+3, maxillomandibular nerve.

embryogenesis, but one possible ultimate cause of the differences, or the timing of NHP separation, can be traced back to the stage before the establishment of the phylotype.

In the gnathostome also, the nasal placode and hypophysis can be mapped very closely to each other at early stages of development (Couly & Le Douarin 1988; Osumi-Yamashita *et al*. 1994). In subsequent developmental stages, however, they are soon separated from each other, possibly in part due to the rostral growth of the brain that vertically induces Rathke's pouch (Gleiberman *et al*. 1999).

Conserved patterns of the phylotypic stage are mainly correlated with segments or compartments that are typically seen in the rhombomeres or somites that show *Hox*-gene expression patterns. The most fundamental

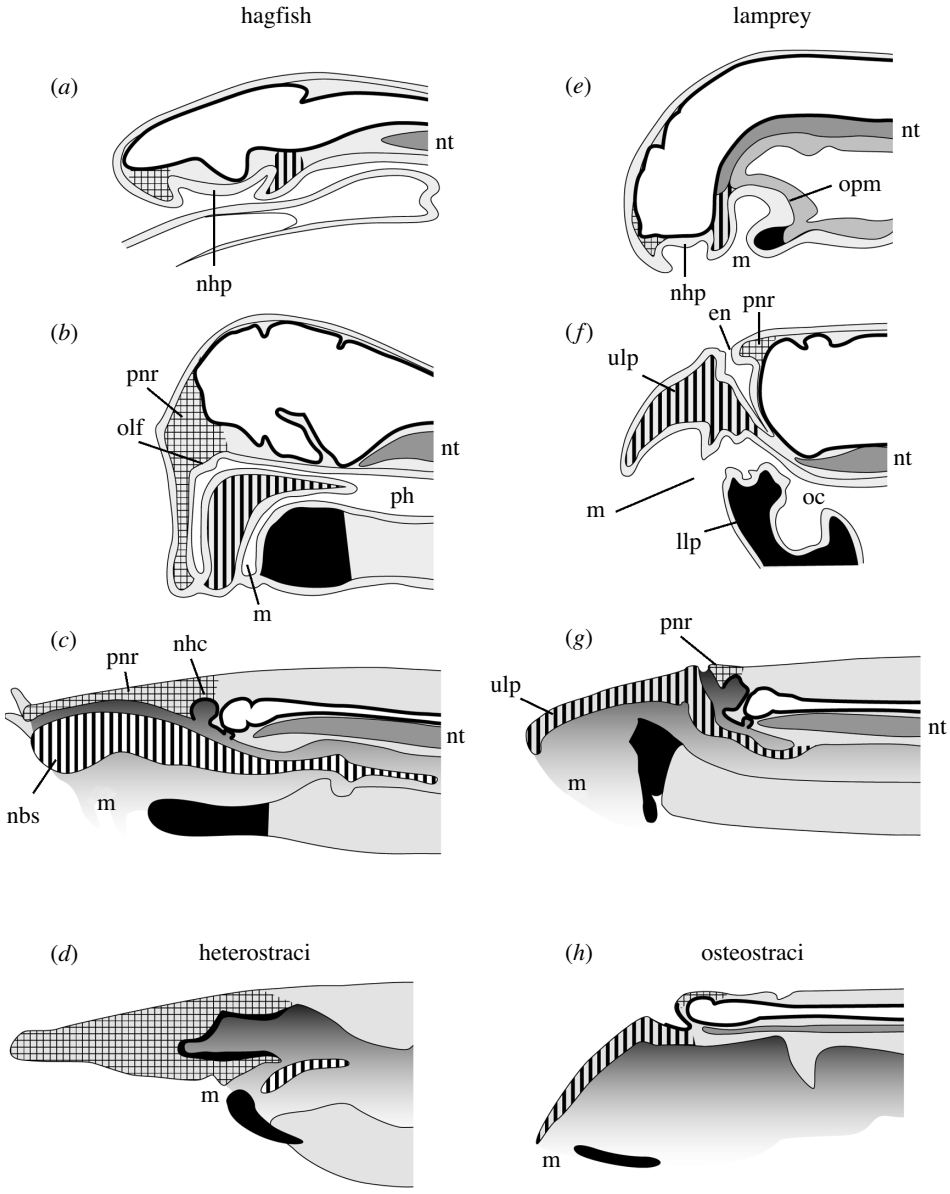

Figure 10. Mesenchymal architecture of oral region in agnathans. (*a–c*) Development of the oronasal regions in the hagfish. Although a question remains as to the origin of the nasohypophyseal complex, the topographical relationships between the nasohypophyseal ectoderm (nhp) and craniofacial ectomesenchyme show a striking similarity to those of the lamprey embryo (*e*). Comparable ectomesenchymal parts are marked by the same pattern. These embryonic stages may represent a shared pattern of ectomesenchyme found only in agnathans. Based on Gorbman (1983) and Heintz (1963). The fossil anatomy of heterostracans (*d*) is comparable with the adult anatomy of the hagfish (*c*) based on the hypothetical comparison of ectomesenchymal growth. By the same comparison, the ammocoete upper lip (ulp in (*f*)), or the dorsorostral portion of the sucker of the adult lamprey (*g*), can be homologized with the nasobuccal shelf (nbs) of the hagfish and heterostracans, and also with the rostral oral hood of osteostracans (*h*). Abbreviations: en, external nares; llp, lower lip; m, mouth; nbs, nasobuccal shelf; nhp, nasohypophyseal plate; nt, notochord; oc, oral cavity; olf, olfactory epithelium; opm, oropharyngeal membrane; pnr, prenasal region; ulp, upper lip.

divergences, on the other hand, seem to be brought about by epigenetic events of embryogenesis, mainly local tissue interactions that are not necessarily determined at preceding stages. A shift of timing (heterochrony) often causes the shift of cell and tissue relationships (heterotopy; for a review, see Hall 1998), as seen in the crest cell distribution in vertebrate embryos. If the above evolutionary scenario was actually involved in the origin of the gnathostome jaws, it may have been an example of the ontogenetic repatterning postulated by Wake & Roth (1989). Shift of tissue predicts an altered epithelium–mesenchymal interaction, a prerequisite for the fundamental ectomesenchymal patterning (FGFs and BMPs vertically inducing *Msx*, *Dlx*, etc.; see Shigetani *et al*. (2000) and references therein). As one example of the shifted interactions that would have resulted in the non-homologous pattern of expression, the lamprey *Dlx* gene (*LjDlx1/6*; Myojin *et al*. 2001) is expressed in all three oral elements of the ammocoete (figure 9*b*). If *Dlx* expression was conserved in all the vertebrates, the upper lip mesenchyme would not have expressed the gene, since the mesenchymal expression of *Dlx* genes is restricted to the mandibular and more posterior pharyngeal arches (Qiu *et al*. 1997);

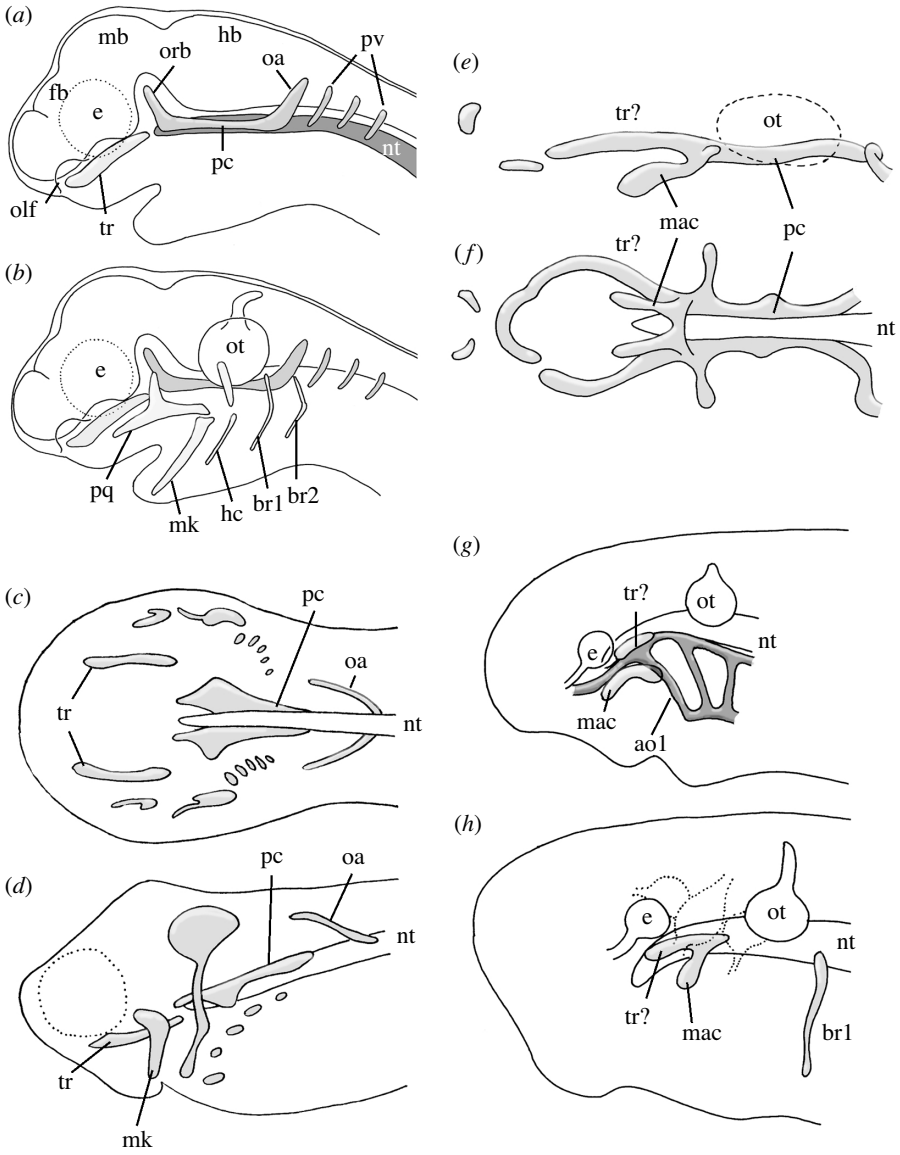

Figure 11. Comparison of trabecular cartilage between lamprey and gnathostome embryos. (*a*,*b*) Generalized chondrocranium of gnathostomes. (*c*,*d*) Early chondrocrania of *Polypterus*. Redrawn from De Beer (1937). (*e–h*) Chondrocrania of *Petromyzon*, described by Johnels (1948). Lateral (*e*) and ventral (*f*) views and graphic reconstructions (*g*,*h*). The trabecular cartilage—tr in (*a*), (*c*) and (*d*)—was initially described in gnathostomes as the pair of cartilage bars that are located rostral to the parachordal and orbital cartilages, or the mesodermal (=chordal) neurocranial elements (*a*). The trabecular cartilages are shown to be derived from the neural crest (Couly *et al*. 1993). In lamprey development, however, the cartilages called trabeculae—tr? in (*e*,*f*)—develop lateral to the notochord at the level of the first pharyngeal (mandibular) arch (*g*,*h*). Early development of this cartilage does not seem to support the premandibular nature of (at least a part of) the lamprey trabecula. Abbreviations: ao1, aortic arch 1; br1–2, cartilages in branchial arches 1–2; e, eye; fb, forebrain; hb, hindbrain; hc, hyoid arch cartilage; mac, mandibular arch cartilage; mb, midbrain; mk, Meckel's cartilage; nt, notochord; oa, occipital arch; olf, olfactory placode; ot, otocyst; pc, parachordal cartilage; pv, prevertebrae; tr, trabecular cartilages in gnathostomes; tr?, trabecular cartilages in the lamprey.

a parallel problem is the differential expansion of oral ectoderm. The expression patterns of *Dlx*-upstream genes (*Fgfs*) in the ectoderm will be extremely curious in the lamprey.

7. AN UNSOLVED PROBLEM: THE TRABECULAR CARTILAGE

In the gnathostome embryo, a pair of cartilaginous rods develops on the ventral aspect of the forebrain extending rostrally from the rostral tip of the notochord. The nature of these cartilages, called the trabecular cartilages, has a long history of debate, especially in the context of the segmentation of the vertebrate head. A similar pair of cartilages also develops in the lamprey (Damas 1944; Johnels 1948). Although a few experimental studies have implied an origin from the neural crest (Newth 1956; Langille & Hall 1988), there still remains an ambiguity (see Newth 1951). If the lamprey upper lip is closely related to the gnathostome trabecula, what then is the nature of this pair of cartilages in the lamprey neurocranium?

Although the cartilage called the trabecula in the lamprey embryo apparently resembles the gnathostome trabecula and is assumed to be derived from the neural crest, there are several problems with regard to the

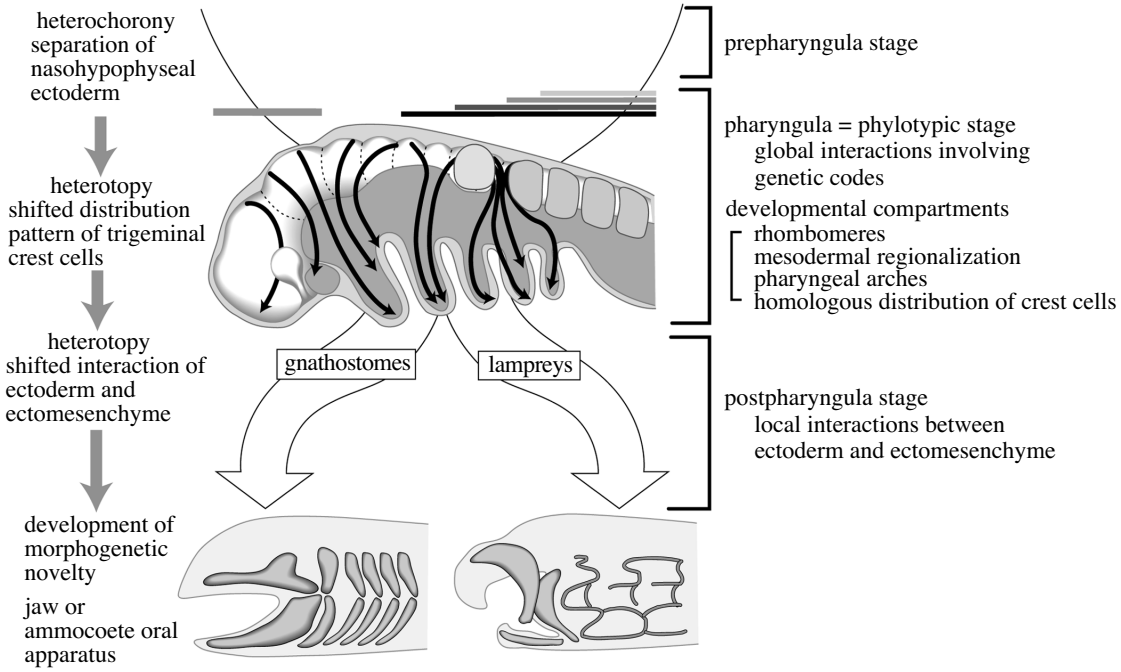

Figure 12. Developmental scheme of jaw evolution—a hypothesis. The basic body plan is shared at the pharyngula stage between the gnathostomes and lampreys, representing the vertebrate phylotype. This consists of neuromerical segmentation of the neural tube, pharyngeal arches, postotic somites, unsegmented cephalic mesoderm and three cephalic crest cell streams, as well as the tripartite trigeminal crest cell distribution. Also shared are the local expression of regulatory genes constituting a conservative gene expression pattern along the anteroposterior axis. From this common developmental design, extremely differentiated oral structures of gnathostomes and lampreys can arise. Development of the jaw would possibly be permitted by the accelerated separation of the nasohypophyseal ectoderm in gnathostomes, which takes place very early in ontogeny, before the establishment of the phylotype. Ontogenetic repatterning to create the jaw, therefore, does not seem to obliterate the vertebrate phylotype.

morphological identity of the former (figure 11). First, the lamprey trabecula initially develops lateral to the rostral part of the notochord (figure 11*g*,*h*; Johnels 1948), the position that is usually occupied by the cephalic mesoderm of gnathostomes (Goodrich 1930; De Beer 1937). This cartilage only secondarily extends rostrally beyond the rostral tip of the notochord (Johnels 1948). Therefore, the possibility cannot be ruled out that it represents rostrally extended parachordals (see Newth 1956). To support this, the lamprey trabecula in early stages is located dorsal to the cartilage primordium developing in the velum (mandibular arch derivative), namely, at the level of the mandibular arch (figure 11*g*,*h*). Thus, the homology of the lamprey trabecula with the cartilage of the same name in gnathostomes is unlikely. The lamprey cartilage is more similar to the rostrally extended parachordal cartilage or the mandibular arch element. As will be noted in § 8, the upper lip mesenchyme rather resembles the gnathostome trabecular cartilage in terms of embryonic topography. The transverse section presented by Johnels (1948) seems to suggest that the cells of this lamprey cartilage are mesodermal in origin: the definitive nature of this element will require a more detailed cell labelling study.

8. CONCLUSIONS AND POSSIBLE SCENARIO OF JAW EVOLUTION

From the above discussion, we conclude that the gnathostome jaw cannot be readily derived evolutionarily from any of the structures found in the ammocoete larva. When we compare their development, no simple morphological sequence appears to exist between the oral structures of these two groups of vertebrates. Rather we suggest that the evolution of the jaw involved systematic rearrangement of craniofacial mesenchyme that took place after the establishment of the vertebrate phylotype (figure 12). The different patterns of neural crest cell distribution in the two animal embryos, however, appear to be based on a conserved ectodermal prepattern (nasohypophyseal complex), which develops even before the phylotypic stage (figure 12). Such mesenchymal rearrangements obliterate classically proposed one-to-one structural homologies in the adult state. Probably, the origin of the gnathostome jaw will not be found in any particular structures in lampreys or in hagfishes. The velum would be homologous with the jaw only as a derivative of the mandibular arch, but neither of them would represent an ancestral condition of the other. It is even more questionable whether or not the ancestral form of gnathostomes had a differentiated velum (see Mallatt 1996; Janvier 1996). If it did, the position of the velum, similar to that of the gnathostome oropharyngeal membrane, invalidates the simple derivation of the upper and lower jaws from this structure. The above assumed rearrangements of the ectomesenchyme must have occurred in the basal lineage of gnathostomes after the splitting of the lamprey lineage.

As Mallatt (1996) had assumed that the biting jaw first evolved as an adaptation to osmoregulation, the jaw may

have been invented as the result of exaptation, the derivation of a new function by elaborating a pre-existing structure for another purpose. Whatever the common ancestor of the lamprey and gnathostomes may have looked like, it most likely possessed a neural-crest-derived premandibular ectomesenchyme closely associated with the NHP. Invention of the jaw subsequently required a space for the nasal septum and maxillary process to develop (figure 9), which might have been provided by subdivision of the NHP into the nasal placode and the hypophysis (diplorhiny, the state of bilaterally separated nasal openings, would also have been a prerequisite for this). Janvier (1996) has demonstrated various types of topographical relationships between nasal epithelium, hypophysis and the mouth among fossil and extant vertebrates. In this context it is interesting that in the chick mutant *talpid*[3], in which hypophyseal development is affected (lens differentiates in place of the adenohypophysis), the maxillary process also fails to develop (Ede & Kelly 1964),[2] suggesting that the space is required.

Dissociation of the olfactory system from the NHP suggests either the facilitation of olfaction (P. Janvier, personal communication), or endocrine facilitation. As shown embryologically as well as physiologically, the nasal epithelium and adenohypophysis are closely related to each other. Some releasing hormones are secreted from the nasal placode itself, or nasal-placode-derived cells. For example, luteinizing hormone releasing hormone (LHRH) neurons originate from the nasal placode and migrate secondarily into the hypothalamus in amniote embryos (Schwanzel-Fukuda & Pfaff 1989). It is plausible to assume that the NHP represents an ancestral condition of this nasohypophyseal complex (Couly *et al.* 1992) and the transition of the regulation of adenohypophysis from the nasal epithelium to the hypothalamus might have laid the groundwork for embryonic environments in which jaw primordia could have the chance to appear. In any case, it seems likely that the evolution of the jaw seemed only possible in the embryonic body plan in which diplorhiny and separation of the nasal epithelium and adenohypophyseal anlage took place very early in development; other agnathan groups possessed monorhiny as well as prenasal sinus, as in the lamprey and hagfish (figure 10; Janvier 1996). The gnathostomes would have represented one of such plans in the early vertebrate lineages.

The evolution of the gnathostome jaw thus appears to have been based on a shift of epigenetic regulation of genes (for a review, see Hall 1998). The clue to solve this problem, therefore, will not be obtained by comparative anatomy of adult structures, but rather by discrimination of conserved and newly acquired patterns of gene regulation, as well as by identification of the shifted tissue interactions that brought about the non-homologous expression patterns of regulatory genes. Experimental embryology indicates that the ectodermal distribution of signalling molecules (FGFs and BMPs), as well as tissue interactions (regulation of various mesenchymally expressed homeobox genes), will provide us with new evidence. Molecular developmental biology has taken only the initial steps into this old question of comparative zoology, but it has already suggested new directions in which a solution may lie.

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ENDNOTES

[1]Unlike the sensory innervation, the trigeminal motor neurons present a serious problem in morphological homologies. Song & Boord (1993), for example, have postulated homologies of oral musculature in gnathostomes, ammocoete larva, adult lamprey and hagfish, based on the nuclear organization of the trigeminal motor neurons. These comparisons, however, are consistent neither with the *Engrailed* expression pattern in the trigeminal muscle subsets (see Holland *et al.* (1993) and references therein) nor with the above-noted sensory innervation patterns. Nuclear organization of the trigeminal nervous system does not seem to serve as the basis of structural homologies.

[2]There still remains an ambiguity as to the germ-layer origin of the hagfish adenohypophysis (Gorbman 1983). At certain early stages of development, however, lamprey and hagfish embryos exhibit similar epithelial and mesenchymal morphology (figure 10*a*,*e*). Homologies of mesenchymal populations based on this shared pattern lead to the homology between the lamprey upper lip and the nasobuccal shelf of the hagfish, as supported by comparative anatomy (figure 10*c*,*g*). This may further support the basic perioral configurations recognized in fossil agnathans (figure 10*d*,*h*).

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