

# Compartments in the lamprey embryonic brain as revealed by regulatory gene expression and the distribution of reticulospinal neurons

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**ABSTRACT:** The vertebrate neural tube consists of a series of neuromeres along its anteroposterior axis. Between amphioxus that possesses no neuromeres and gnathostomes, the lamprey occupies a critical position in the phylogeny for the origin of the segmented brain. To clarify the rhombomeric configuration of the Japanese lamprey, *Lampetra japonica*, we injected rhodamine- and fluorescein-labeled dextrans into the larval spinal cord, and retrogradely labeled the reticulospinal neurons. We also isolated prosomere marker genes from the embryonic cDNA library of *L. japonica*, and performed *in situ* hybridization on the embryonic brain. Of the genes examined, *LjOtxA*, *LjPax6*, *LjPax2/5/8*, *LjDlx1/6*, and *LjTTF-1* were expressed in clearly demarcated polygonal domains. In the telencephalon, *LjDlx1/6*, *LjPax6*, and a putative paralogue of *LjEmx* were expressed in different domains; the *LjEmx* paralogue was expressed in the dorsal region, and *LjDlx1/6* and *LjPax6* in a complementary fashion of the middle part. These expression patterns implied existence of a tripartite configuration of the lamprey telencephalon similar to that in gnathostomes. All these evidences strongly suggest that the segmental and compartmental architecture of the vertebrate brain was already established before the divergence of agnathans and gnathostomes. © 2002 Elsevier Science Inc.

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The gnathostome neural tube consists of a series of segmental bulges, known as neuromeres, along the anteroposterior axis. The segments in the hindbrain are called rhombomeres, and those in the forebrain, prosomeres [18]. The neuromeres are thought to represent basic components of the gnathostome brain, whereas no equivalent segments are apparent in amphioxus, the sister group of vertebrates. No doubt, the origin of this neuromeric pattern is the key to understanding vertebrate brain evolution. Development of the lamprey brain is thus an intriguing issue in the context of evolutionary developmental biology. Immunohistochemical analyses of the adult lamprey brain have revealed the presence of a segmental organization along the neuraxis similar to that of gnathostomes [14,15]. The development of neuromeres in the lamprey was also suggested by the classical comparative embryologists [1],

but was only partly confirmed in a recent immunohistochemical study [6]. To be able to describe the neuromeric development of the lamprey brain, we artificially fertilized eggs and obtained embryos of a Japanese lamprey, *Lampetra japonica*.

## RHOMBOMERES IN THE LAMPREY

In gnathostomes, rhombomeric metamerism is reflected in several morphological traits; cephalic crest cells and cranial nerve roots are attached to even-numbered rhombomeres, and the neuronal developmental pattern also follows the segments of the hindbrain [9]. In aquatic gnathostomes, reticulospinal neurons are arranged in a segmental pattern corresponding to the rhombomeres [8,10]. Of these, the Mauthner neuron, which is involved in escape behavior, always develops in rhombomere 4 (r4).

As detected by retrograde labeling with fluorescent dextran amines, reticular neurons including the inferior, middle, superior, and mesencephalic reticular nuclei, were arranged along the AP axis of the hindbrain in the stage 30 lamprey larva. Because rhombomeres appear only transiently in this animal [4,6], a metameric pattern of these neurons was not confirmed at this stage. In younger embryos, however, the earliest reticular neurons were detected at stage 24, as several distinct clusters along the neuraxis: a middle reticular group was found in r4, superior reticular cells were located in r2 and ithmic reticular cells were placed in r1. As in gnathostomes, Mauthner neurons of the lamprey emerged in r4 as in gnathostomes. Thus, the developmental program of the lamprey reticular neurons initially follows a metameric pattern, which becomes obscured at larval stages. Furthermore, we identified that in the lamprey the branchial motoneurons including trigeminal, facial, glossopharyngeal and vagus motor nuclei also developed segmentally, similarly to gnathostomes.

The segmental developmental plan of the lamprey hindbrain is, in every respect, comparable to that of zebrafish and *Xenopus*; rhombomeric organization is not restricted to some gnathostomes, but its origin seems to date back to the common ancestor of vertebrates. The rhombomeres may have served as the primitive developmental units to generate the segmental neuronal components in the vertebrate hindbrain. Because these characters apparently do not exist in amphioxus [22], the segmental components

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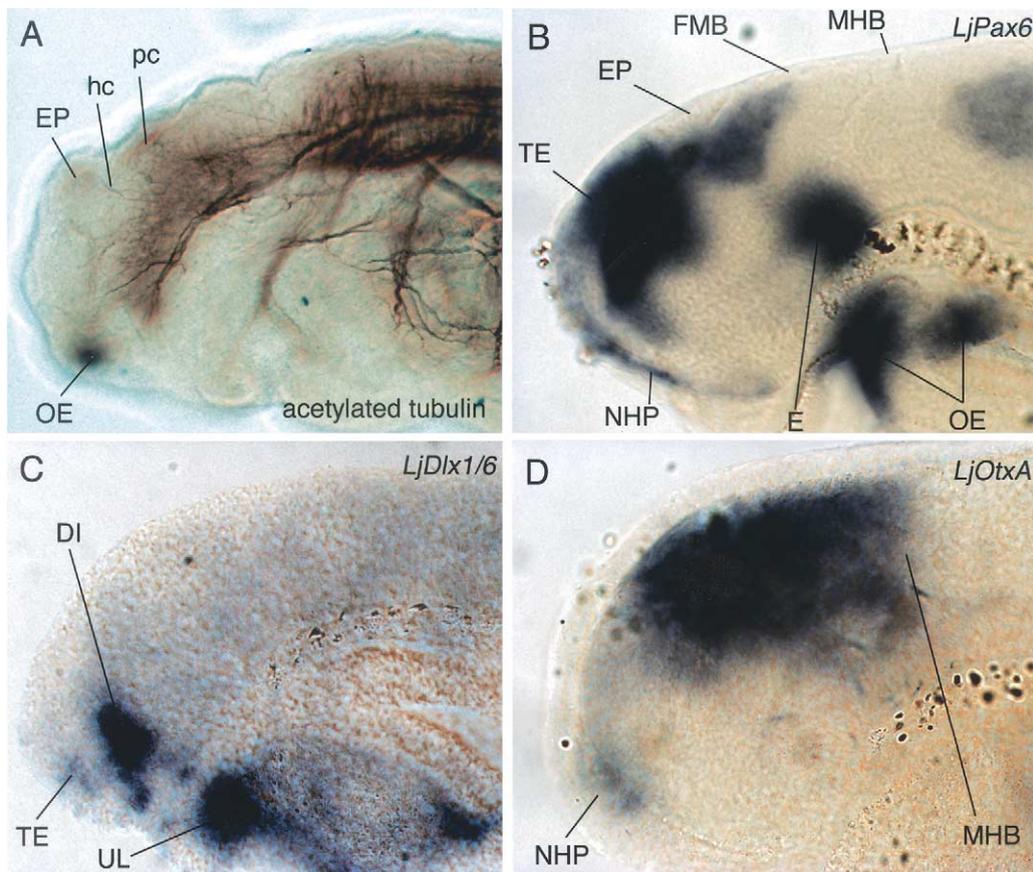


FIG. 1. Comparison of regulatory gene expression in the stage 26 lamprey brain. (A) Immunohistochemical staining of nerve tracts. The habenular commissure (hc), the posterior commissure (pc), and the epiphysis (EP) are stained. (B–D) Whole-mount *in situ* hybridization in lamprey embryos. (B) Expression of *LjPax6*. Transcripts are detected in the rostral to the forebrain-midbrain boundary (MHB), the telencephalon (TE), the eye (E), the nasohypophysial plate (NHP) and oral ectoderm (OE). (C) *LjDlx1/6* transcripts are detected in the telencephalon (TE), dorsal part of the rostral diencephalon (DI), and upper lip (UL). (D) *LjOtxA* expression is detected in the rostral to the midbrain-hindbrain boundary (MHB), and the nasohypophysial plate (NHP).

including reticular neurons, branchial motoneurons as well as the rhombomeres would have been acquired together in the early evolution.

#### NEUROMERIC COMPARTMENTS IN THE LAMPREY FOREBRAIN

A certain number of prosomeres has been described in the forebrain of various gnathostome species [5,18]. Some of them could be identified by the position of specific neuronal tracts, which are also commonly identified in various vertebrate species [3,7]. The posterior commissure, for example, is located in the pretectum or prosomere 1 (P1), and the habenular commissure in the dorsal thalamus (P2). To identify the neuronal components in the lamprey forebrain, we analyzed the position of morphologically detectable axonal tracts. Immunohistochemical analysis using the anti-acetylated tubulin antibody showed the existence of posterior and habenular commissures in the lamprey, suggesting that P1 and P2 do exist in the lamprey (Fig. 1A). However, the other more rostral prosomeres (P3 to P6) could not be shown by this method.

The expression patterns of specific regulatory genes are known to serve as prosomere markers [18,19]. In lampreys, embryonic

expression patterns have been reported for several regulatory genes, including *PmOtx*, *LjOtxA*, *LjDlx1/6*, *LjEmx*, *LjTTF1*, and *LjPax2/5/8* [11–13,20,21]. These genes were expressed in clearly demarcated polygonal domains in the lamprey brain. Of those, the *LjPax2/5/8* expression domain corresponded to the mid-hindbrain boundary (Fig. 3; [4,6,21]), from which point the *LjOtxA* domain extended rostrally into the entire midbrain and part of the prosencephalon (Figs. 1D, 3). These genes still did not allow us to identify the midbrain/P1 boundary. The gene *Pax6* would be useful for this purpose, because it is expressed in gnathostomes rostral to the midbrain/P1 boundary in a domain that extends rostrally to the P2/P3 boundary [2].

We screened the lamprey cDNA library and identified a cognate of *Pax6* (*LjPax6*), and performed *in situ* hybridization on embryonic and larval brains. As expected, *LjPax6* expression was developmentally regulated and expressed in restricted domains in the neural tube, and at stage 26, high level of expression was restricted to the forebrain. The caudal part of the *Pax6* expression domain overlapped the rostral and dorsal part of the *LjOtxA* domain (Figs. 1B, 3). The positions of the epiphysis, the posterior commissure and the habenular commissure overlapped the *LjOtxA*–*LjPax6* co-expressing domain, and should corre-

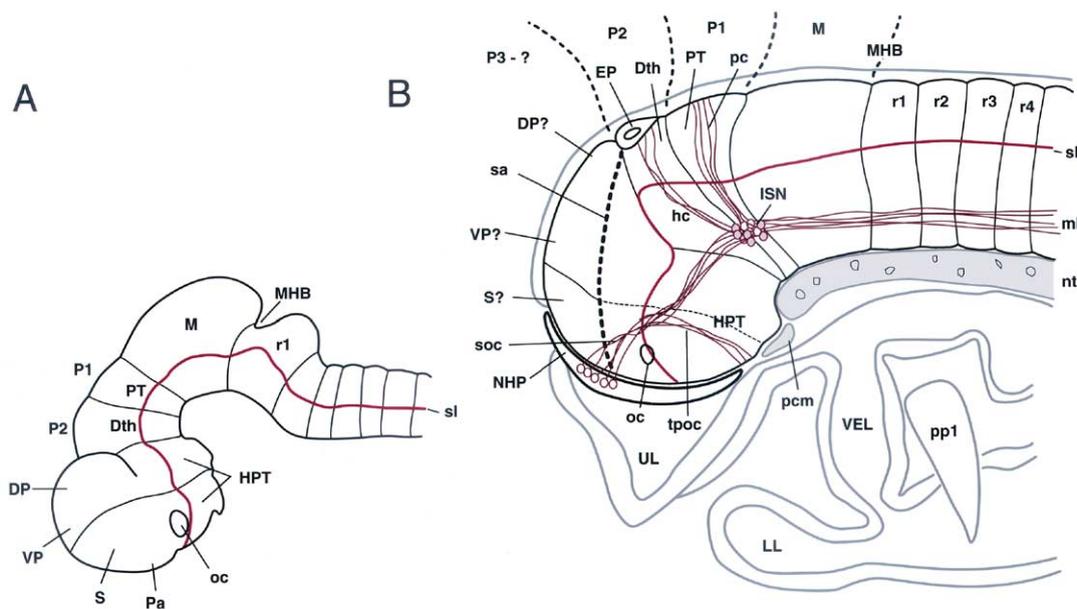


FIG. 2. Schematic representation of the neuromeres in the brain of the mouse and *Lampetra japonica*. Schematic illustration showing the developmental plan of the lamprey brain. (A–B) Comparison of the developmental plans between amniote and lamprey brains. On the basis of the gene expression patterns and nerve tracts described in this study, expected segments such as P1 and P2, as well as the sulcus limitans, are drawn on the stage 26 lamprey brain as solid lines (B). Note that the ventral limit of *Pax6* expression corresponds to the sulcus limitans both in the lamprey and the mouse (A). Dotted lines in (B) represent the hypothetical boundaries proposed in other studies but not confirmed in this study. Homologies between the domains are based on a comparison with the model proposed by Puelles and Rubenstein ([17]; A). No data were obtained in the present study to show more subdivisions rostral to the P2/P3 boundary. The region rostral to the sulcus intraencephalicus anterior (sa) has here been tentatively termed the telencephalon (T). Note that three gene expression domains are detected in this telencephalon, possibly corresponding to the dorsal and ventral pallium (DP and VP), and the striatum (S). Abbreviations: DP, dorsal pallium; Dth, dorsal thalamus; EP, epiphysis; hc, habenular commissure; HPT, hypothalamus; ISN, interstitial nucleus; LL, lower lip; M, midbrain; MHB, mid-hindbrain boundary; mlf, medial longitudinal fasciculus; NHP, nasohypophysial plate; nt, notochord; oc, optic chiasm; P1–P3, prosomeres; Pa, pallidum; pc, posterior commissure; pcm, prechordal mesoderm; pp1, pharyngeal pouch 1; r1–4, rhombomeres; S, striatum; sa, anterior intraencephalic sulcus; sl, sulcus limitans; soc, supraoptic commissure; tpo, postoptic tract; UL, upper lip; VEL, velum; VP, ventral pallium.

spond to the dorsal thalamus (P2) plus pretectum (P1; caudal part of dorsal diencephalon). This also indicates that the posterior limit of *LjPax6* domain corresponds to the forebrain-midbrain boundary (M/P1), whereas the rostral limit of the *LjOtxA-LjPax6* co-expressing domain corresponds to the P2/P3 boundary.

Rostral to the P2/P3 boundary, *LjPax6* and *LjDlx1/6* were co-expressed in the dorsal part of the rostral diencephalon (Figs. 1B,C, 3), whereas no expression was seen in the ventral diencephalon nor in the hypothalamus. The expression of *LjTTF-1* was restricted to the basal/paramedian hypothalamus, which lies ventral to the expression domains of *LjPax6* and *LjDlx1/6* (Fig. 3). Thus, the boundary between the *LjPax6-LjDlx1/6* and *LjTTF-1* domains corresponds to the alar-basal plate boundary assumed by Rubenstein et al. [18] in gnathostomes (Fig. 2).

It is generally accepted that the telencephalon in gnathostomes can be divided dorsoventrally into three major components, the pallium, an intermediate zone (the ventral pallium), and the sub-pallium (the striatum; [16]). In gnathostomes, *Emx* and *Pax6* genes are mainly expressed in the dorsal and lateral pallium, *Pax6* in the ventral pallium, and *Dlx* in the striatum (Fig. 2; [16]). In the lamprey telencephalon, the *Emx*-like gene expression domain was restricted to the dorsal telencephalon, possibly corresponding to the dorsal and lateral pallium of gnathostomes (Fig. 3). In the intermediate part of the telencephalon, a *LjPax6* but not an *Emx*-like gene expression was observed (Fig. 3). This region could

represent the ventral pallium of lamprey. The *LjDlx1/6* was expressed in the ventral telencephalon (Fig. 3). As in gnathostomes, this region contains the striatum of the adult lamprey [14]. These results support the tripartite configuration of the lamprey telencephalon similar to that in gnathostomes. However, there is a region in the gnathostome telencephalon that includes the pallidum, in which *Dlx* and *TTF1* are both expressed (Figs. 2, 3; [16]). In the lamprey telencephalon, *LjPax6* expression was not detected in the corresponding area (Fig. 3). The loss of *LjTTF1* expression in the ventral telencephalic region may be related to the apparent absence of a pallidum in this animal.

## CONCLUSIONS

The present study has suggested that lampreys have both rhombomeres and prosomeres that are directly comparable to those in gnathostomes (Figs. 2, 3). This indicates that the morphogenetic plan of the anterior neural tube was established before the divergence of the agnathan and the gnathostome ancestors. A possible evolutionary scenario drawn from this new finding would be that the segmented vertebrate brain arose together with the segmental neuronal developmental pattern based on the already established AP organization of the neural tube of an amphioxus-like ancestor. Because many of the regulatory genes are expressed at similar neuraxial levels in the amphioxus and vertebrates, major changes

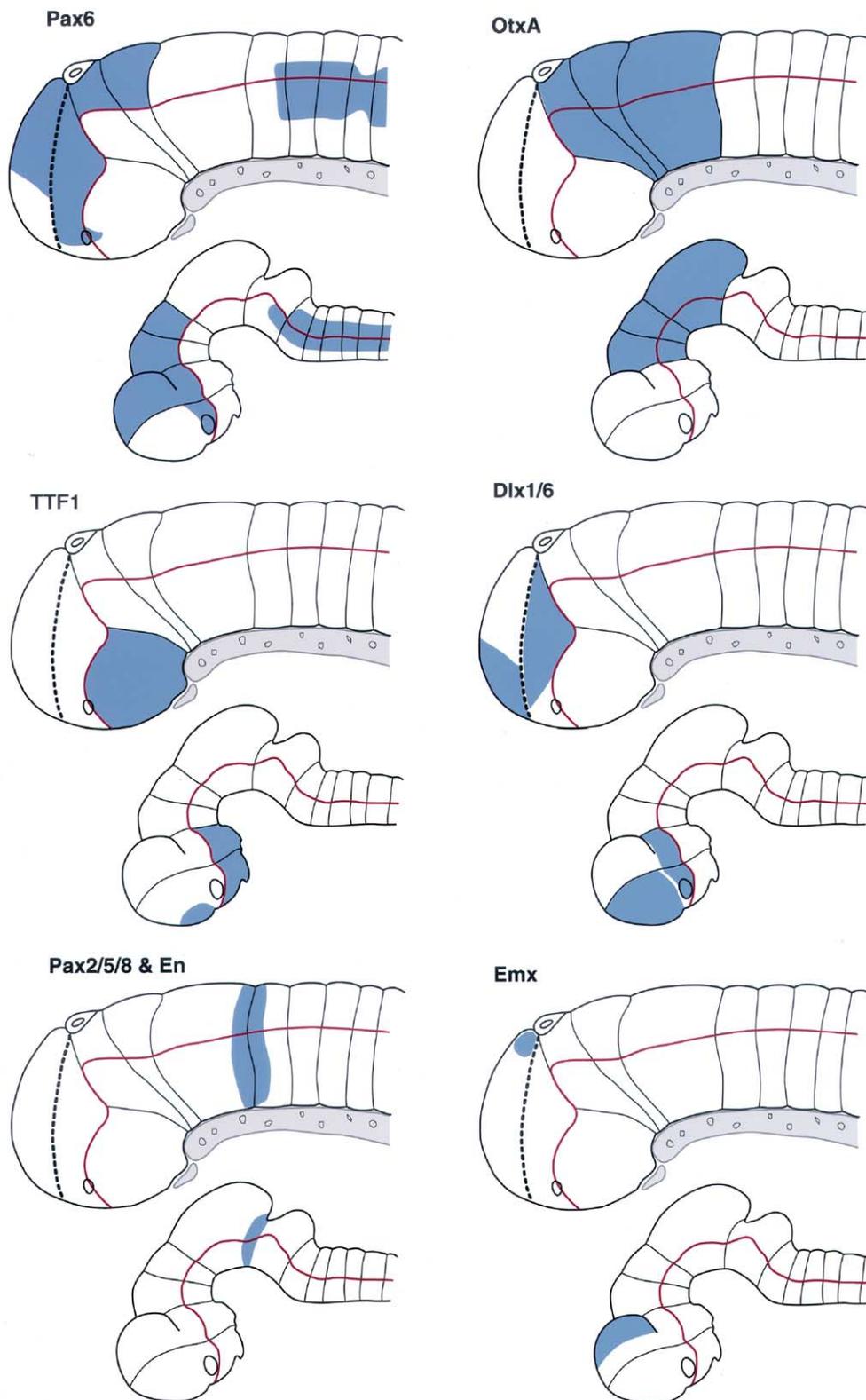


FIG. 3. Schematic comparison of the expression of neuromere marker genes in the lamprey (upper) and the mouse (lower).

in the ancestral vertebrates may possibly have involved the restriction of local cell lineages into compartments (neuromeres) that also would have affected the developmental pattern of early developing neurons.

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