Evolutionary biology

Lamprey Hox genes and the evolution of jaws

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n vertebrates with jaws (gnathostomes), the jaws are formed from the first pharyngeal arch (PA1), which does not express homeobox (*Hox*) genes. Cohn¹ describes expression of the *HoxL6* gene in the PA1 of the lamprey *Lampetra fluviatilis*,

a jawless (agnathan) vertebrate, and postulates that a retreat of *Hox* expression from PA1 might have favoured the evolution of jaws in the gnathostome lineage after the split from agnathans¹. Here we examine the distribution of *Hox* genes in another lamprey species, *Lethenteron japonicum*, and find that none are expressed in the PA1. We conclude that Cohn's finding is not a general feature within the lamprey group and is therefore unlikely to be related to jawlessness.

In gnathostome embryos, Hox genes display rostral boundaries of expression that are collinear with their relative chromosomal positions^{2,3}. Nested collinear Hox expression patterns display sharp anterior boundaries that map to distinct hindbrain segments (rhombomeres) and pharyngeal arches that give rise to the neuralcrest-derived skeletal structures in the head and neck region⁴. Notable exceptions are the first rhombomere (r1) and the jawforming first pharyngeal arch (PA1), which do not express Hox genes. Analysis of Hox expression patterns in the jawless lamprey may therefore provide insight into how the gnathostome Hox code has been established and its relevance to the evolution of craniofacial structures in vertebrates.

We isolated 11 *Hox* complementary DNAs from embryos of the Japanese lamprey *L. japonicum* (*Lj*). Alignment of the deduced amino-acid sequences of isolated cDNAs, as well as phylogenetic comparisons, revealed that they belong to paralogue groups (PG) 2 to 9, designated *LjHox2*, -3d, -4x, -4w, -5i, -5w, -6w, -6/7m, -8p, -Q8 and -9r, respectively (GenBank accession numbers: AY497314, AB125269–AB125278; Fig. 1a). On the basis of comparison of the nucleotide sequences, *LjHox4w*, *LjHox5w*, *LjHox5w*, *LjHox4w*, *LjHox5w*,

orthologues of *Hox4x*, *Hox4w*, *Hox5w*, *Hox6w* and *HoxQ8*, respectively, in *Petromyzon marinus*⁵⁻⁷, a lamprey species belonging to a different genus. *P. marinus* has three or four *Hox* clusters⁵⁻⁷. Our results from *L. japonicum* are in keeping with this, as we never isolated

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amphioxus Hox2	IADF. V.LK (24) - VNSSR.L. VF.NT.LY.K.VCKP.K
LJHOX2 hom shark Hox42	PPEREK (52) - NGGS. LNT. L
human HoyA2	PER 131 - GORT I NT I. K C B V AL B V V U BOTOTI
human HoxB2	APEF EX (38) - GGLS L. NT L. K. C. P. V. AL. D. V. V. H. BOTON
PG3	
amphioxus Hox3	TKI
LjHox3d	LSAS.ASA.LVC.P.V.M.NL.N
horn shark HoxA3	KQIFE <mark>S</mark> (25) PAS ASA.LVC.PV.M.NL.N
human HoxA3	KQIFES(24)QASASA.LVC.P.V.M.NL.NYDQKG
human HoxB3	KQIFES (48) - SAASASA.LVC.PV.M.NL.N.SYDQKAN
human HoxD3	KQIFE8 (22) PASVSA.LVC.PV.M.NL.NYDQKAN
PG4	
P marinus HoxyA	
P.marinus Hoxw4	PVV
LiHox4x	MVV
LjHox4w	PVVKI(10)GEL.SQ.V
horn shark HoxA4	PIVKI(10)GEPSQ.V
human HoxA4	PVVKI(10)GEPSQ.V
human HoxB4	PVVRKV(10)GEP.SQ.VYYS
human HoxC4	PIVKI (10)GEP.SO.VYSSDHRL
DOF	AVV
amphioxus Hox5	TGDN., T.,
P.marinus Hoxw5	POIKL(8)SEGSY
LjHox5w	PQIKL(8) PEGSY
LjHox5i	PQI
hom shark HoxA5	PQI,RKL(8) PEG A
horn shark HoxD5	NQIKM(8)LEG.TY
human HoxA5	PQIRKL(8) PEG A Y
humanHoxB5	PQIFRKL(7) PDG. AYDNKL
DCG/DC	7 7
amphiorus Hove	PDVP PYG(6) - FFY OF T Y X Y L G FNYT
P marinus Hoxw6	(VPT ORM(A) - TDP OT S Y EINTI
L.fluviatilis HoxL6	(4) TDRQT.S.YEHNII
LjHox6w	VPIQRM(8) TDRQT.S.Y
horn shark HoxA6	TPI, QRM (0) PHGQT.T.F
human HoxA6	SPVQRM (8) ~.SHGQT.T.Y
human HoxB6	TPVQRM (8) = .PSGQT.T.YYY
human HoxC6	IQIQRM (8) - ADRQT.S.YESNL/
Linoxor/m	
hom shark HoxA7	FRI PNA POR OT V FTKAC
human HoxA7	FRI RSS PDR. OT. Y. EHKDI
human HoxB7	FRI RSS TDR
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P.marinus Hox8	PERRQT.S.Y
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-3d, -4x, -4w, -5i, -5w, -6w, -6/7m, -8p, -Q8 and *-9r*, respectively (GenBank accession numbers: AY497314, AB125269–AB125278; Fig. 1a). On the basis of comparison of the nucleotide sequences, *LjHox4x, LjHox4w, LjHox5w, LjHox6w* and *LjHox4w, LjHox5w, LjHox6w* and *LjHoxQ8* are the

more than three *Hox* genes from any single cognate group.

All the *LjHox* genes are strongly expressed at the pharyngula stage. In the neural tube, *LjHox* PG2–8 genes display offset rostral expression borders, moving from anterior to posterior (Fig. 2a–i). The rostral boundary of *LjHox2* expression is in the rhombomer r2 (results not shown), which is similar to that occurring in gnathostomes, where *Hoxa2* is the only *Hox* gene expressed in r2 (refs 4, 8).

The anterior border of LjHox3d expression occurs in the middle of r4 (ref. 9). More posteriorly, LjHox4w, LjHox6w and LjHoxQ8, which may be linked in the same cluster^{6,7}, display clear collinear expression patterns (Fig. 2d, f, i). This suggests that collinear *Hox* expression in the embryonic central nervous system represents an ancestral character for vertebrates. It is notable that partial *Hox* collinearity is already present in the neural tube of protochordates¹⁰.

By contrast, in the pharyngeal arches only LjHox2 and LjHox3d display conserved collinear expression patterns in the neuralcrest-derived ectomesenchyme (Fig. 2j-m). As in gnathostomes, the rostral expression boundaries of LiHox2 and LiHox3d are present in PA2 and PA3, respectively. LjHox4x is instead expressed at low levels, with no clear anterior boundary (Fig. 2c,n), whereas LiHox4w and Hox PG5-8 are not expressed in the pharyngeal arch ectomesenchyme (Fig. 2d-i), despite their collinear patterns in the neural tube. In the pharyngeal endoderm, Hox PG2 and PG3 genes are not expressed, whereas *LjHox4w*, *-5i*, *-6w* and *-Q8* are all co-expressed in the most caudal pouch (Fig. 2d-f, i-k, o).

None of the *LjHox* genes was expressed in the PA1 at any of the stages that we analysed (Fig. 2, and data not shown), in contrast to the findings of Cohn in Lampetra *fluviatilis*¹. In gnathostomes, the ectopic expression of Hoxa2 in the Hox-free PA1 suppresses jaw development, whereas Hoxa2 inactivation results in the transformation of PA2 into jaw-related structures^{11–13}. The expression pattern of LjHox2 is strictly conserved, which is consistent with lamprey PA1 and PA2 being patterned by molecular mechanisms that are similar to those in gnathostomes¹⁴. In agreement with this, Fgf8, which is expressed

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Figure 2 Expression patterns of *Hox* genes in *Lethenteron japonicum (Lj)* as revealed by *in situ* hybridization and whole-mount immunostaining. **a–i**, Expression of *LjHox* genes in stage-26 larvae. Arrowheads indicate rostral expression boundaries. **j**, **k**, Expression of *LjHox2* (**j**) and *LjHox3d* (**k**) in horizontal sections of stage-25 embryos. **I**, High-magnification micrograph of pharyngeal arches (PAs). Positively staining cells correspond to the neural-crest-derived ectomesenchyme (em). **m**, *LjHox3d*-positive neural crest cells populate PA3. **n**, **o**, Expression of *LjHox4* (**n**) in the posterior pharyngeal ectomesenchyme (arrowheads) and *LjHox6w* (**o**) in the endodermal pharyngeal pouch 8. Key: mp, muscle plate; n, notochord; nt, neural tube; ph, pharynx; 1–8, pharyngeal pouches. Scale bars: a-i, 0.1 mm; I,k, 0.1 mm; I,m, 0.05 mm; n,o, 0.1 mm. Full methodological details are available from the authors.

at the mid–hindbrain boundary in gnathostomes and prevents *Hox* expression in PA1 (ref. 15), is also expressed at the lamprey mid–hindbrain boundary¹⁴. Moreover, the nucleotide sequences of *LjHox6w* and *HoxL6* (ref. 1) display a high degree of homology, indicating that these genes are orthologous (Fig. 1b).

The discrepancy between Cohn's analysis and ours remains unclear. One possibility is that it might be due to genus- or speciesspecific regulatory differences in lamprey Hox6 expression patterns. In any event, the Hox6 expression in PA1 observed in L. fluviatilis does not appear to be a general feature within the lamprey group and is therefore not functionally relevant to jawlessness. Rather, the lack of Hox expression in the lamprey PA1 may reflect the fact that in both lampreys and gnathostomes the rostral-most pharyngeal arch forms highly specialized structures that are morphologically distinct from those of more posterior arches.

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