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Differential expression of *hoxa2a* and *hoxa2b* genes during striped bass embryonic development

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Abstract

Here, we report the cloning and expression analysis of two previously uncharacterized paralogs group 2 *Hox* genes, striped bass *hoxa2a* and *hoxa2b*, and the developmental regulatory gene *egr2*. We demonstrate that both *Hox* genes are expressed in the rhombomeres of the developing hindbrain and the pharyngeal arches albeit with different spatio-temporal distributions relative to one another. While both *hoxa2a* and *hoxa2b* share the r1/r2 anterior boundary of expression characteristic of the *hoxa2* paralog genes of other species, *hoxa2a* gene expression extends throughout the hindbrain, whereas *hoxa2b* gene expression is restricted to the r2–r5 region. *Egr2*, which is used in this study as an early developmental marker of rhombomeres 3 and 5, is expressed in two distinct bands with a location and spacing typical for these two rhombomeres in other species. Within the pharyngeal arches, *hoxa2a* is expressed at higher levels in the second pharyngeal arch, while *hoxa2b* is more strongly expressed in the posterior arches. Further, *hoxa2b* expression within the arches becomes undetectable at 60 hpf, while *hoxa2a* expression is maintained at least up until the beginning of chondrogenesis. Comparison of the striped bass HoxA cluster paralog group 2 (PG2) genes to their orthologs and trans-orthologs shows that the striped bass *hoxa2a* gene expression pattern is similar to the overall expression pattern described for the *hoxa2* genes in the lobe-finned fish lineage and for the *hoxa2b* gene from zebrafish. It is notable that the pharyngeal arch expression pattern of the striped bass *hoxa2a* gene is more divergent from its sister paralog, *hoxa2b*, than from the zebrafish *hoxa2b* gene. Overall, our results suggest that differences in the Hox PG2 gene complement of striped bass and zebrafish affects both their rhombomeric and pharyngeal arch expression patterns and may account for the similarities in pharyngeal arch expression between striped bass *hoxa2a* and zebrafish *hoxa2b*.

Keywords: Striped bass; *Morone saxatilis*; *Hox* gene expression; *hoxa2a*; *hoxa2b*; Embryonic development; Pharyngeal arches; Hindbrain

1. Results and discussion

Paralog group 2 (PG2) *Hox* genes function specifically in the embryonic hindbrain and pharyngeal arches of teleosts and tetrapods by conferring segmental identity of the rhombomeres and pharyngeal arches (Trainor and Krumlauf, 2000). Despite similarities in the expression patterns of tetrapod and teleost HoxA cluster PG2 genes, both temporal and spatial differences in expression have been observed. In the tetrapods *Hoxa2* hindbrain expression extends from r2 to r7, whereas in zebrafish the expression of *hoxa2b* is restricted to the region of r2–r5 (Prince and Lumsden, 1994; Vieille-Grosjean et al., 1997; Prince et al., 1998; Maconochie et al., 1999; Pasqualetti et al., 2000; Baltzinger et al., 2005). In zebrafish both *hoxa2b*, the only *hoxa2* paralog group member in this teleost, and *hoxb2a* are expressed and function redundantly to pattern the second pharyngeal arch tissues (hyoid arch) (Hunter and Prince, 2002). In contrast, in tetrapods only *Hoxa2* expression is maintained in the second pharyngeal arch (Sham et al., 1993; Prince and Lumsden, 1994; Vesque et al., 1996; Vieille-Grosjean et al., 1997; Prince et al., 1998; Yan et al., 1998; Maconochie et al., 1999; Pasqualetti et al., 2000; Baltzinger et al., 2005; Santagati et al., 2005). The unique zebrafish *Hox* PG2 gene expression pattern raises the question of whether teleost *Hox* PG2 gene expression is generally different from the tetrapods.

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Here, we report the cloning and expression analysis of two previously uncharacterized PG2 *Hox* genes, striped bass *hoxa2a* and *hoxa2b*. We also report the cloning and early expression of the striped bass *egr2* gene, which is used in this study as a marker to define the location of specific rhombomeres during early embryogenesis.

Striped bass *hoxa2a*, *hoxa2b*, and *egr2* partial cDNAs were amplified by RT-PCR from mRNA prepared from 24 h post fertilization (hpf) striped bass embryos. The primers for *Hox* gene amplification, A2exon1 and A2exon2, were designed to amplify a 537 bp region that includes the exon/intron splice sites. PCR products were cloned in the pGEM®-T-Easy Vector (Promega), according to manufacturer's instructions. Analysis of the putative *Hox* gene recombinant clones by restriction endonuclease digestion and DNA sequencing revealed the presence of two inserts of slightly different sizes and sequences. Clone A2-1 contained a 537 bp long insert, whereas clone A2-4 contained a 552 bp long insert. Sequence alignment of strictly overlapping sequence from A2-1 and A2-4 using ClustalX (Thompson et al., 1997) revealed an overall similarity of only 74%, indicative that the two sequences were not allelic variants but were products of different genes (Misof and Wagner, 1996). A

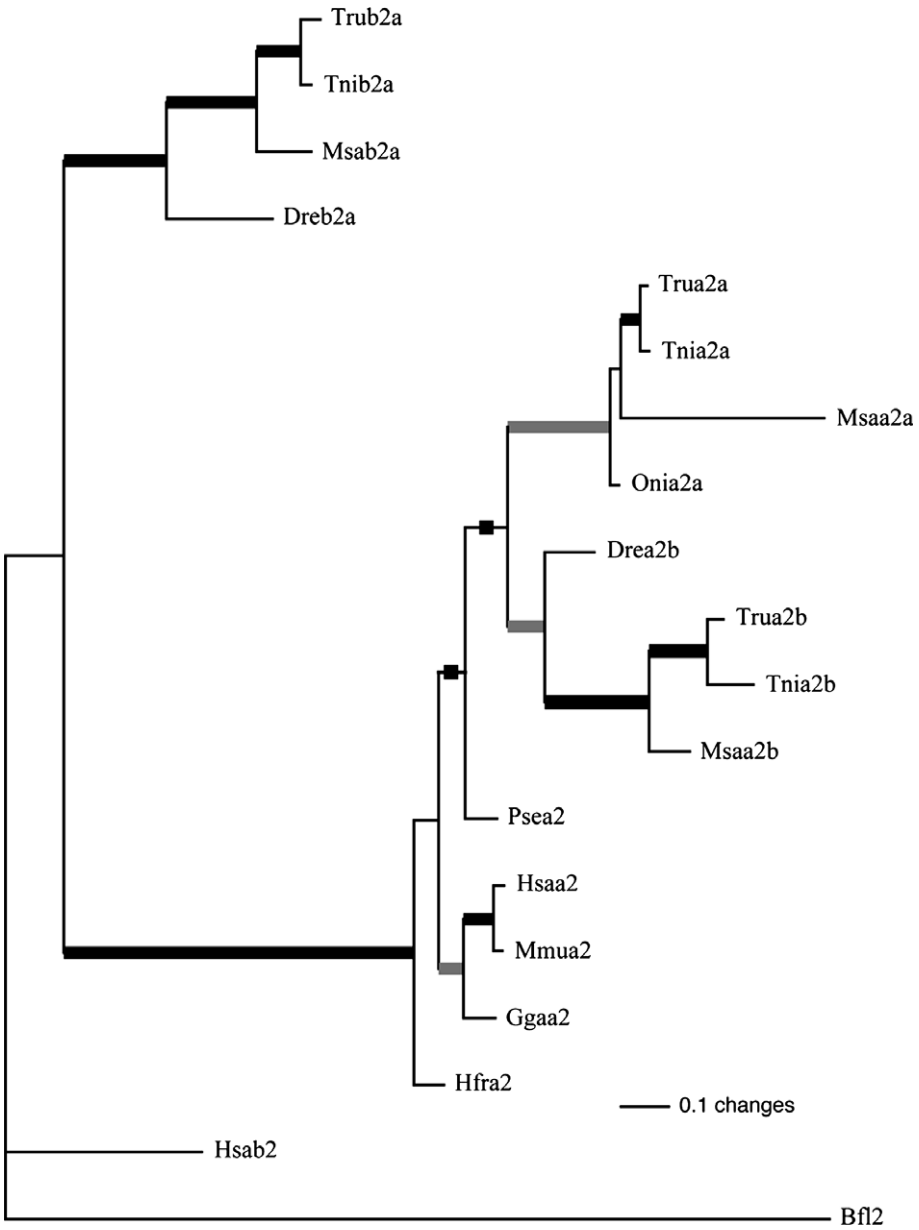

Fig. 1. Phylogenetic tree of vertebrate paralogous group 2 *Hox* genes obtained via Bayesian analysis of the single cluster of Amphioxus (Bfl), clusters A and/or B of human (Hsa), mouse (Mmu), chick (Gga), horn shark (Hfr), bichir (Pse), and clusters Aa, Ab, and Ba from Japanese (Tru) and fresh water (Tni) pufferfishes, tilapia (Oni), zebrafish (Dre), and striped bass (Msa). Thick black lines indicate nodes supported by a greater than 95% posterior clade probability and bootstrap value. Thick gray lines indicate node supported by a greater than 95% posterior clade probability. Black square boxes indicate node supported by an 80–85% posterior clade probability.