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Spatio-temporal expression patterns of anterior *Hox* genes during Nile tilapia (*Oreochromis niloticus*) embryonic development

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ABSTRACT

Hox genes encode transcription factors that function to pattern regional tissue identities along the anterior-posterior axis during animal embryonic development. Divergent nested *Hox* gene expression patterns within the posterior pharyngeal arches may play an important role in patterning morphological variation in the pharyngeal jaw apparatus (PJA) between evolutionarily divergent teleost fishes. Recent gene expression studies have shown the expression patterns from all *Hox* paralog group (PG) 2–6 genes in the posterior pharyngeal arches (PAs) for the Japanese medaka (*Oryzias latipes*) and from most genes of these PGs for the Nile tilapia (*Oreochromis niloticus*). While several orthologous *Hox* genes exhibit divergent spatial and temporal expression patterns between these two teleost species in the posterior PAs, several tilapia *Hox* gene expression patterns from PG3-6 must be documented for a full comparative study. Here we present the spatio-temporal expression patterns of *hoxb3b*, *c3a*, *b4a*, *a5a*, *b5a*, *b5b*, *b6a* and *b6b* in the neural tube and posterior PAs of the Nile tilapia. We show that several of these tilapia *Hox* genes exhibit divergent expression patterns in the posterior PAs from their medaka orthologs. We also compare these gene expression patterns to orthologs in other gnathostome vertebrates, including the dogfish shark.

1. Results and discussion

The nested expression of *Hox* genes along the antero-posterior axis of vertebrate and arthropod embryos has been proposed to provide a combinatorial code resulting in the specification of regional identity (Kessel and Gruss, 1991). Accordingly, variations in this *Hox* code may have resulted in the diversification of animal morphologies. We previously described a clear but incomplete combinatorial code of *Hox* gene expression in the precursors of the cichlid pharyngeal jaw apparatus (PJA) – pharyngeal arches (PA) 3 to 7 (Le Pabic et al., 2009). The PJA constitutes a set of internal jaws distinct from the oral jaws and which display great morphological variation across all ray-finned fish lineages (Nelson, 2006). A highly efficient PJA was hypothesized to have facilitated the explosive adaptive radiation of cichlids in the rift lakes of East Africa (Liem, 1973), which from the perspective of molecular determination of structure, may have resulted from changes in the PJA-associated *Hox* code.

Here, we present the pharyngeal arch and neural tube expression patterns of *hoxb3b*, *c3a*, *b4a*, *a5a*, *b5a*, *b5b*, *b6a* and *b6b* of the cichlid Nile tilapia (*Oreochromis niloticus*, order Perciformes) and compare them to their strict orthologs of medaka (*Oryzias latipes*, order Beloniformes) and, when appropriate, other gnathostome vertebrates. Expression patterns are reported at post-migratory cranial neural crest (CNC) developmental stages. At 48 and 54 hours post-fertilization (hpf) the two posterior-most arches are still unsegmented, and referred to as PA6/7 thereafter (Le Pabic et al., 2009). At 66 and 72 hpf, pharyngeal segmentation is complete, resulting in seven pharyngeal arches separated by endodermal pouches (Le Pabic et al., 2009). At the developmental stages mentioned above, the rhombomeres of the hindbrain are easily distinguished morphologically without the aid of rhombomere (r)-specific molecular markers. Further, we used other morphological landmarks to aid in rhombomere assignment, including the midbrain/hindbrain boundary and otic vesicle (OV). We found that several of the nested tilapia *Hox* expression patterns in the posterior arches observed in this study were divergent from those exhibited by medaka. We also compared the tilapia *Hox* PG3-6 expression patterns to those of other gnathostome vertebrates in the neural tube, namely the hindbrain.

Abbreviations: CNC, cranial neural crest; E, eye; hpf, hours post-fertilization; OV, otic vesicle; PA, pharyngeal arch; PG, paralog group; PJA, pharyngeal jaw apparatus; r, rhombomere.

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1.1. Tilapia hoxb3b gene expression pattern

Tilapia *hoxb3b* expression was observed in r4 of the hindbrain (Fig. 1A and B). Tilapia *hoxb3b* expression was first detected within the pharyngeal arches at 54 hpf in PA5 and 6/7 (Fig. 1B) but was not observed to be expressed in the pharyngeal arches after this timepoint. By comparison, similar hindbrain expression patterns were observed solely in r4 for medaka *hoxb3b* and Atlantic salmon *hoxb3bα*, but not for mouse, which shows a *Hoxb3* expression pattern with an anterior limit at the r4/r5 boundary (Mungpakdee et al., 2008; Tümpel et al., 2009; Davis and Stellwag, 2010). In the pharyngeal arches, medaka *hoxb3b* expression showed a more restricted expression pattern in the posterior pharyngeal arches as it was expressed in PA6/7 early in development and later in just PA7, but never in PA5 (Davis and Stellwag, 2010). Interestingly, in dogfish shark *Hoxb3* is expressed throughout the hindbrain with an anterior limit at the r3/r4 boundary and PA2/PA3 boundary, respectively, which matches the expression patterns of *hoxb3a* of tilapia and medaka (Le Pabic et al., 2009; Davis and Stellwag, 2010; Oulion et al., 2011).

1.2. Tilapia hoxc3a gene expression pattern

We did not observe *hoxc3a* expression in tilapia embryos at any developmental stage analyzed, even though we were able to amplify cDNAs from total RNAs of tilapia embryos (data not shown). This lack of expression was distinct from medaka *hoxc3a* (Davis and Stellwag, 2010), which showed faint expression in the neural tube posterior to the hindbrain, and from *hoxc3a* α and β of Atlantic salmon, which are both expressed in r7 and posteriorly (Mungpakdee et al., 2008).

1.3. Tilapia hoxb4a gene expression pattern

Tilapia *hoxb4a* transcripts were detected at low levels in the neural tube with an anterior limit at the r6/r7 boundary at 48 hpf (Fig. 2A) but this expression intensified by 54 hpf (Fig. 2B). No hindbrain expression was observed by 66 hpf (Fig. 2C). In the pharyngeal arches, tilapia *hoxb4a* expression was observed in PA5 and PA6/7 at 48 and 54 hpf (Fig. 2A and B) but was restricted to PA6 and PA7 by 66 hpf (Fig. 2C). By comparison, similar hindbrain expression patterns with anterior limits at the r6/r7 boundary were observed for *hoxb4a* of medaka and zebrafish and *Hoxb4* for mouse and the dogfish shark (Prince et al., 1998; Tümpel et al., 2009; Davis and Stellwag, 2010; Oulion et al., 2011). The expression pattern of tilapia *hoxb4a* in the pharyngeal arches was similar to medaka *hoxb4a* during early post-migratory CNC stages, as both genes were expressed in PA5 and PA6/7, but diverged from medaka *hoxb4a* expression later in development, as medaka *hoxb4a* was expressed in PA5, 6 and 7 (Davis and Stellwag, 2010). Dogfish shark *Hoxb4* was shown to be expressed with an anterior limit at the PA5/PA6 boundary, but only one developmental stage was recorded for this species (Oulion et al., 2011). Interestingly, zebrafish *hoxb4a* expression in the pharyngeal arches was shown to be divergent from *hoxb4a* of tilapia and medaka and *Hoxb4* of dogfish, as it was expressed in PA4-7 (Miller et al., 2004).

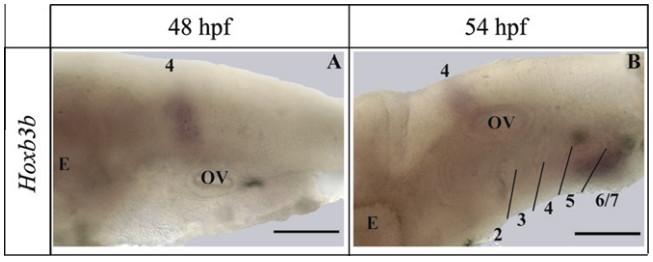

Fig. 1. Whole-mount *in situ* hybridization analysis of tilapia *hoxb3b* at 48 hpf (A) and 54 hpf (B). All embryos were mounted with their anterior sides to the left and their lateral sides toward the reader. Rhombomere numbers are indicated by black numbers above the dorsal sides of the embryos. Pharyngeal arches are delineated by vertical slanted lines on the ventral sides of the embryos and are indicated by black numbers below or on the ventral sides of the embryos. E, eye; OV, otic vesicle. Scale bars equal 0.1 mm.

1.4. Tilapia hoxa5a gene expression pattern

Tilapia *hoxa5a* transcripts were first detected in PA6 and 7 at 66 hpf (Fig. 3A) and persisted in PA6 and 7 at 72 hpf (Fig. 3B). At 72 hpf, tilapia *hoxa5a* was also detected within the neural tube posterior to the hindbrain (Fig. 3B). A similar neural tube and pharyngeal arch expression pattern was observed for *hoxa5a* of medaka and *Hoxa5* of dogfish shark (Davis and Stellwag, 2010; Oulion et al., 2011).

1.5. Tilapia hoxb5a gene expression pattern

Tilapia *hoxb5a* transcripts were first detected at 66 hpf in PA6 and 7 and in the neural tube posterior to the hindbrain (Fig. 3C). By 72 hpf, tilapia *hoxb5a* expression was still observed in PA6 and 7 but at lower levels when compared to expression at 66 hpf (Fig. 3D). By comparison, medaka *hoxb5a* was observed to be robustly expressed in PA5, 6 and 7 late in development (Davis and Stellwag, 2010). Medaka *hoxb5a* was also expressed in the neural tube posterior to the hindbrain (Davis and Stellwag, 2010). Dogfish shark *Hoxb5* was observed to be expressed in a similar manner in the pharyngeal arches and neural tube to that of tilapia *hoxb5a* (Oulion et al., 2011).

1.6. Tilapia hoxb5b gene expression pattern

Tilapia *hoxb5b* transcripts were first detected at 66 hpf in PA7 and in the neural tube posterior to the hindbrain (Fig. 3E). This expression pattern was maintained at 72 hpf (Fig. 3F). A similar expression pattern was observed in the pharyngeal arches and the neural tube for medaka *hoxb5b* (Davis and Stellwag, 2010).

1.7. Tilapia hoxb6a gene expression pattern

Tilapia *hoxb6a* transcripts were first detected exclusively in PA7 at 66 hpf (Fig. 4A). By 72 hpf, tilapia *hoxb6a* was maintained in PA7 but expanded to include the neural tube posterior to the hindbrain (Fig. 4B). A similar spatial expression pattern in the pharyngeal arches and neural tube was observed for medaka *hoxb6a* (Davis and Stellwag, 2010). Interestingly, no dogfish shark *Hoxb6* expression was observed in the pharyngeal arches (Oulion et al., 2011).

1.8. Tilapia hoxb6b gene expression pattern

Tilapia *hoxb6b* transcripts were first detected at 72 hpf in PA7 and in the neural tube posterior to the hindbrain (Fig. 4C and D). This expression pattern was also observed for medaka *hoxb6b* during late development (Davis and Stellwag, 2010).

1.9. Comparative Hox expression patterns in the rhombomeres and pharyngeal arches between tilapia and medaka

Our current expression pattern analysis of several *Hox* genes within the pharyngeal arches of tilapia in conjunction with data from previous *Hox* gene expression studies in tilapia (Le Pabic et al., 2007, 2009) and medaka (Davis et al., 2008; Davis and