EXPRESSION OF *HOXB-3* IN CARP *(CYPRINUS CARPIO)* EMBRYOS

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Homeobox genes give positional information by creating regional diversity along the embryonic anterior-posterior (a-p) axis of all the animal species studied to date. They contain a well conserved sequence, the homeobox, a 180 base pairs (bp) sequence, which codes for a DNA binding helix-turn-helix motif in the gene products. Hox genes belong to the homeobox gene family and in vertebrates they are organized in 4 clusters and are aligned along the chromosome in the same order as they are expressed along the a-p axis (Gehring *et al.*, 1994).

In an attempt to clone *Antennapedia*-related homeobox genes and to study their expression patterns in carp, an *Antennapedia*-derived homeobox-specific probe was used to screen a carp early segmentation stage cDNA library (Stroband *et al.*, 1995).

One of the isolated clones had a length of about 1000 bp, with an open reading frame. After sequence analysis it showed a 100% deduced homeodomain identity with mouse *Hox-2.7* (Sham *et al.*, 1992), Chick *Hoxb-3* (Rex and Scotting, 1994), *Xenopus Hoxb-3* (Godsave *et al.*, 1994), zebrafish (only a part of the *Hoxb-3* homeobox has been sequenced) (Misof *et al.*, 1996) and man *HOX2G* (Acampora *et al.*, 1989). Therefore we named the clone *Hoxb-3*. The clone has not yet been sequenced for both strands except for the homeobox and the upstream sequence. Therefore, the sequences downstream of the homeobox are not certain. The sequencing results to date are shown in figure 1. The carp hexapeptide is identical to that of mouse, *Xenopus*, chick, and man *Hoxb-3* genes. Comparison of this sequence with the complete nucleotide sequences of the above mentioned species revealed that about 300 to 400 bp of the 5' end are missing. The 3' end appears to be complete as we have found a possible polyadenylation signal and a polyA tail.

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tccaagtcgtcctctctggccccaaacccagctctcacaaagcaaatctttccttggatgaaggagtcgactcagaacaccaagcaaaaaaacagctccctagtgcaagctcacgtaacg 120
S  K  S  S  S  L  A  P  N  P  A  L  T  K  Q  I  F  P  W  M  K  E  S  T  Q  N  T  K  Q  K  N  S  S  L  V  Q  A  H  V  T
cggagagcagcggtggtgagaaaagctccgggttctgcagcttccaagagagcacgcactgcttacaccagcgctcagctggtggagctcgagaaggagtttcacttcaaccgatacctg 240
R  R  A  A  V  V  R  K  A  P  G  S  A  A  S  K  R  A  R  T  A  Y  T  S  A  Q  L  V  E  L  E  K  E  F  H  F  N  R  Y  L
tgccggccgaggcgcgtggagatggccaatctgctcaatcttagcgagcggcagatcaagatttggttccagaacaggaggatgaagtacaagaaggatcaaaagttgaaaggattcggc 360
C  R  P  R  R  V  E  M  A  N  L  L  N  L  S  E  R  Q  I  K  I  W  F  Q  N  R  R  M  K  Y  K  K  D  Q  K  L  K  G  F  G
tcgtctcaggggggccatcccacaggaagcccacctctcccaatgcagtcctcagctggtttcatgaactctatgcactcaatgggcagttatgatgctccctccccaccatcttttaac 480
S  S  Q  G  G  H  P  T  G  S  P  P  L  P  M  Q  S  S  A  G  F  M  N  S  M  H  S  M  G  S  Y  D  A  P  S  P  P  S  F  N
aagccccatcaaaatgcatacgccatgtcaacagcctatcagaacccaatgaaaggtgccaccccaacagaagtatggaaacactgcaccggattatgacccacacggctacagggtaac 600
K  P  H  Q  N  A  Y  A  M  S  T  A  Y  Q  N  P  M  K  G  A  T  P  T  E  V  W  K  H  C  T  G  L  End
agtggcacatatggacacaaacatgatagggtagcctgtgtttgtgggaggtagcctatgttgatgaccatgcctgccactgggccttctatgtatggcctcaatcacctaccacaccac 720
caatcggcaagcatggactacaacggtnccacacagatgtcggcgaaccagcaccatggaccgtgtgacccacatccgtcatacacggatctgtctgcgcaccatccttctcagggtaga 840
atccaagaagcacccaagttgactcatctgtagcaggtttggacaaaaaataaaaaaaaaaaaaaaaaaaaactggagactagctccgtngncnnnggnaattcggnnnnnaaaaaaaaaa 960
aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa                                                1015
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Figure 1: cDNA sequence and deduced amino acid sequence of carp *Hoxb-3*. The homeobox is underlined and bold, the conserved hexapeptide is underlined and the polyadenylation site is printed in bold.

The expression of the *Hoxb-3* gene was studied by *in situ* hybridization (ISH) on carp embryos of different developmental stages (1 somite (1 S), 5/6 S, 10/11S, 15/16 S, 24h, and 36h). mRNA expression was first observed during early segmentation. At the 1 S stage, strongest expression was present in two bands perpendicular to and on either side of the non-expressing midline. Furthermore, two stripes ran posteriorly from those bands on either side and parallel to the median axis (fig 2A).Sections showed that the two anterior bands were positioned in the part of the neurectoderm predestined to be a part of the hindbrain. At the posterior end, the longitudinal stripes fork (fig 2A). This is probably due to the fact that convergence has not been completed yet. In this part of the stripes, three to five bands, the number probably depending on small differences in the developmental stage of individual embryos, were present. These bands ran in an angle of about 40 degrees to the a-p axis, and appeared to be located in the paraxial mesoderm. The pattern of expression in this area, as shown in whole mount (Fig 2A) and sections (Fig 2B), makes it likely that the bands each represent a part of the pre-somite mesoderm posterior to the first pair of somites. At this early stage of somite formation the first somites expressed *Hoxb-3*. However, in none of the later stages that were studied expression was found in the paraxial mesoderm. Therefore, *Hoxb-3* might have a function in specifying only the first pairs of somites.

From the 5/6 S stage onwards the expression in the neurectoderm showed its anterior boundary at the r4/5 junction. This could be judged from the position of the boundary with respect to that of the otic vesicle, and the analysis of the expression of *Hoxb-3* in other vertebrates agrees with these observations (Sham *et al.*, 1992; Rex and Scotting, 1994: Godsave *et al.*, 1994). To confirm the anterior expression boundary in carp, we performed a double ISH using the *Hoxb-3* and the carp *Hoxb-1* probe. *Hoxb-1* is expressed in r4 but not in r5, r6 or r7 (Stevens *et al.*, 1996). We found that indeed the area of *Hoxb-3* expression lies immediately posterior to the area in which *Hoxb-1* expression was present, as expected (see Fig 2C). From the 5/6 S stage the expression in the nervous system did not extend throughout the neural tube but was limited to r5, r6 and r7. Cross-sections through r5 and more posteriorly located rhombomeres of various segmentation stages revealed differential expression in the neural tube. Staining was

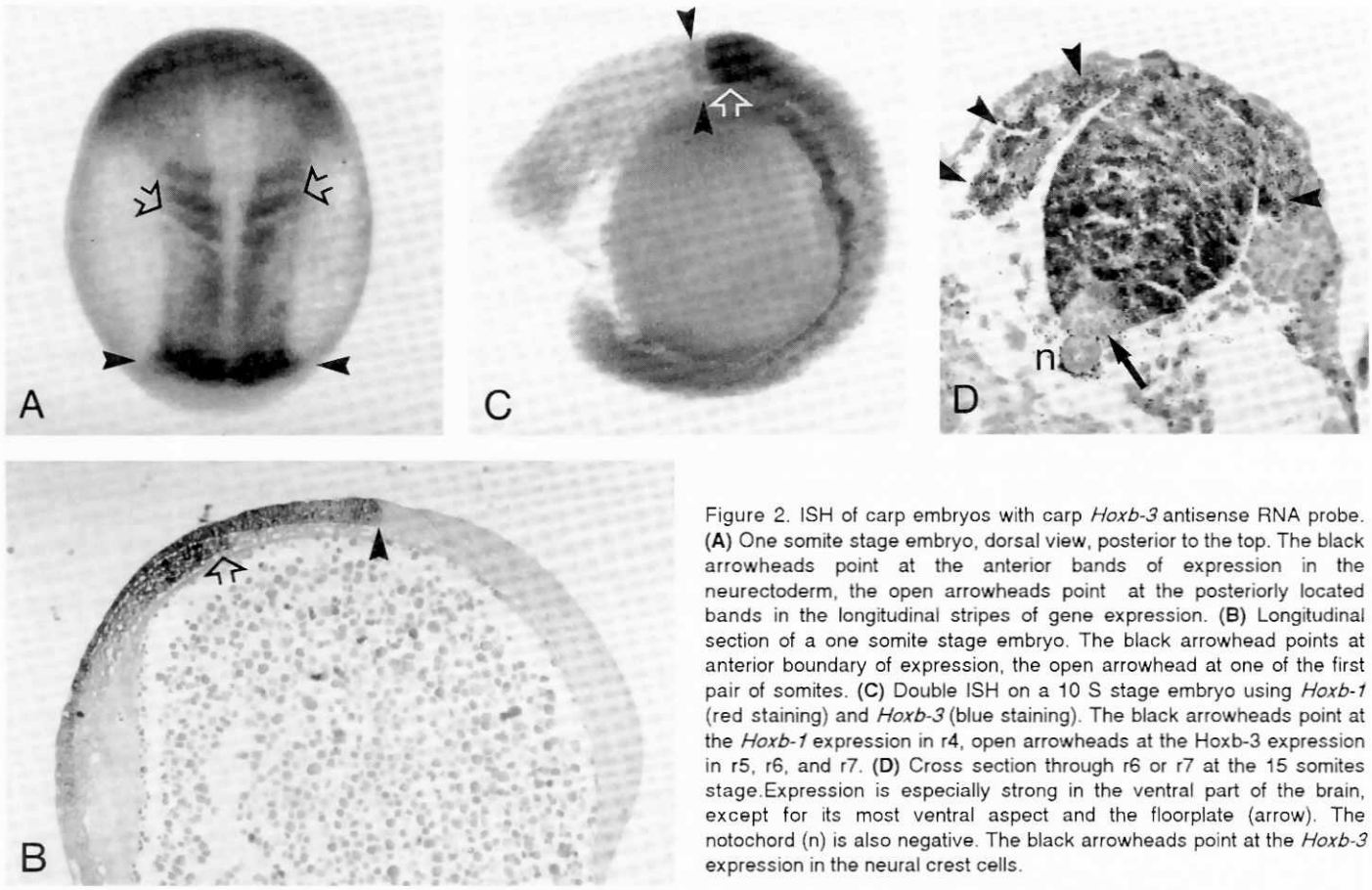

Figure 2. ISH of carp embryos with carp *Hoxb-3* antisense RNA probe. **(A)** One somite stage embryo, dorsal view, posterior to the top. The black arrowheads point at the anterior bands of expression in the neurectoderm, the open arrowheads point at the posteriorly located bands in the longitudinal stripes of gene expression. **(B)** Longitudinal section of a one somite stage embryo. The black arrowhead points at anterior boundary of expression, the open arrowhead at one of the first pair of somites. **(C)** Double ISH on a 10 S stage embryo using *Hoxb-1* (red staining) and *Hoxb-3* (blue staining). The black arrowheads point at the *Hoxb-1* expression in r4, open arrowheads at the Hoxb-3 expression in r5, r6, and r7. **(D)** Cross section through r6 or r7 at the 15 somites stage.Expression is especially strong in the ventral part of the brain, except for its most ventral aspect and the floorplate (arrow). The notochord (n) is also negative. The black arrowheads point at the *Hoxb-3* expression in the neural crest cells.

much stronger ventrally than dorsally, as can be seen in figure 2D. Also noticable are the negative notochord, floorplate, and the layer of cells above the floorplate. Lateral from the neurale tube a paired group of cells was found that also expressed *Hoxb-3*. These cells most likely represent the neural crest. Sham *et al.* (1992) also found *Hoxb-3* expression in mouse neural crest cells. At 24h and 36h expression of *Hoxb-3* was also found in gill arches. Migrating neural crest cells may be involved.

To test the possibility to use the carp *Hoxb-3* probe in zebrafish, a cyprinid species closely related to carp (Stroband *et al.*, 1995), a whole mount ISH was carried out using 14 S stage zebrafish embryos. The expression patterns in carp and zebrafish were identical, but futher studies of sections are needed.

In conclusion, the present study shows that homeobox sequences of Hoxb-3 genes are very well conserved between fish and other vertebrates and even lower animals, and that its pattern of expression also seems to be conserved. However, concerning the latter, futher study has to be done.

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