EMBRYONIC EXPRESSION OF *CARP-OVX1*

Henri W. J. STROBAND, Carine STEVENS, Geertruy TE KRONNIE,
Johannis SAMALLO and Henk SCHIPPER
Department of Experimental Animal Morphology and Cell Biology, Agricultural University, Wageningen Institute of Animal Science, Marijkeweg 40, 6709 PG Wageningen, The Netherlands.

Homeobox genes of animal species contain a well conserved sequence, the homeobox, which codes for a DNA-binding part of the gene products. These genes have shown to be key elements during ontogeny by specification of positions in the embryo. The best known examples are the Hox genes, clustered in 4 complexes in vertebrates, which show specific differential expression along the anterior-posterior axis.

In an attempt to clone *Antennapedia*-related homeobox genes and to study their expression patterns in the carp, we produced a probe by PCR using primers derived from conserved sequences of the *Antennapedia* homeobox, using carp genomic DNA as a template. An early segmentation stage cDNA library was screened, and positive clones were used for *in situ* hybridization and dideoxy chain termination sequence analysis as described earlier (Stroband et al., 1995).

Among the positive clones, several represented genes belonging to the Hox-complexes, as expected. One of the other positive clones was a homologue of *Ovx1* (Bürglin, 1993). Other vertebrate *Ovx1* homologues are: chicken *CHox7* (Fainsod and Gruenbaum, 1989), human *GBX1* (Matsui et al., 1993, Lin et al., 1996), mouse *Gbx-2* (Boullet et al., 1995) and goldfish *G9-2* (Levine and Schechter, 1993). Furthermore, an *Ovx1* homologue (*Cts-Ovx1*) was found in annelids (Dick and Buss, 1994).

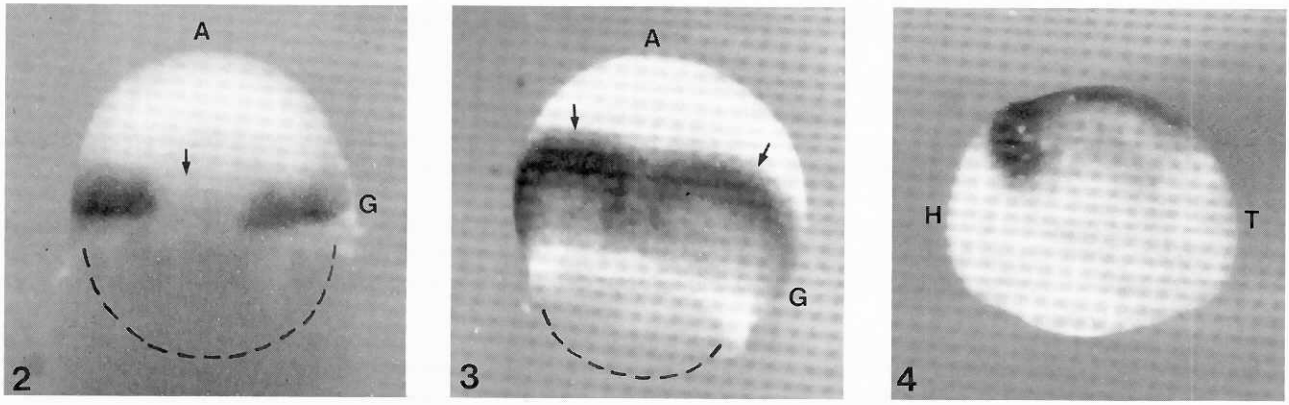
```
gacgaactcaagggctctgaactcctcaatttcacggaaacttttcaggcggttgcaggc gaaaccaaactctatagttcagatgatgagaaactggacctgaaatcagctgaggccgcg  120
D  E  L  K  G  S  E  L  L  N  F  T  E  T  F  Q  A  V  A  G   E  T  K  L  Y  S  S  D  D  E  K  L  D  L  K  S  A  E  A  A
tgcagtgacagggaggacagttcagccgacagcgagaacgaaagcttctccgacgggaac acctgcgcttctgcgtcccagaaaagcaaactcaaaggcggctcacaggacgcgttaccg  240
C  S  D  R  E  D  S  S  A  D  S  E  N  E  S  F  S  D  G  N   T  C  A  S  A  S  Q  K  S  K  L  K  G  G  S  Q  D  A  L  P
ccgggcagctcggcaggaaagagccggaggaggcggactgctttcactagcgaacagctg ctggaactcgagaaggagtttcactgtaagaagtatctgtccctcaccgagcgctctcag  360
P  G  S  S  A  G  K  S  R  R  R  R  T  A  F  T  S  E  Q  L   L  E  L  E  K  E  F  H  C  K  K  Y  L  S  L  T  E  R  S  Q
atcgcgcacgcgctcaaactcagcgaggtccaggtcaaaatctggttccagaaccgcagg gccaagtggaaacggatcaaagccggaaacgtcaacaacagatccggtgagccccgtcaga  480
I  A  H  A  L  K  L  S  E  V  Q  V  K  I  W  F  Q  N  R  R   A  K  W  K  R  I  K  A  G  N  V  N  N  R  S  G  E  P  V  R
aaccccaaaatcgtggtgcccattcccgtgcatgtcaacaggttcacggtgcggagtcaa catcaacagatcgaaccgggcagcaggccatgaactcctcaatgaaaatgaccctgaact  600
N  P  K  I  V  V  P  I  P  V  H  V  N  R  F  T  V  R  S  Q   H  Q  Q  I  E  P  G  S  R  P  *
cctcaaacgaacacttacatgacccacgttcccacaggtcaagggtcacacactacagat gcatgaagcacaccttaaaggagaccattccagtatctagaaggatattcagtgacttttt  720
aggtaacacgttgtcgactttcatcaattaaaacacgagtcctattcaatgcttaacggg tcatcggtttacatagtctacattcagttctgaatagcctatatgaatcaaagtttatga  840
tatttgaacgtaggctaacacatgacctgttcagcatttaatatttttgaaagttattat aaggtgttaccgacttttatatctgttcaatatcgacattacgttcaggtttgttctttt  960
ccttctctcctctaaaatgtctttatttttaatttattatttttgtacatttgtaaatatt aaatgtgtccatgcgacacaaatgtgcactatacgagactgtaaagagacatttttttttt 1080
taaataagtatttataagttgctgtccagtgagtttttgcatgttgccatttgttgtcta attttcccgtatgattttaacgggccgttctcgttgtttcgaccatttttgcatgtgctc 1200
ttaaagcatttctgtcccttagaggcggaatatcccacaagttctttgtgaaaaaaaaaa cggtgatggaaataaataagctcaagaacacaatgacagagtgatatattcccatcgacg 1320
tcacaataaatgtgcaatatggttgcataaaactgttaaaaaaaaaaaaaaaaaaaaaaa aaaa                                                          1384
```

Figure 1: cDNA sequence and deduced aminoacid sequence of *carp-Ovx1*. The homeodomain and the polyadenylation site are underlined. The sequence is available from the EMBL database under Accession number X99910.

The sequence of this 1.4 kb clone, named *carp-Ovx1*, is given in fig.1. It contains part of the protein coding sequence and an untranslated region of 786 basepairs (bp). A polyadenylation signal is present at the 3' end, indicating that this UTR is complete. An open reading frame of 570 bp, coding for 190 aminoacids (aa) can be deduced from the reading frame of the homeobox, which was found between positions 262 and 441. In the table (right) the sequence of the homeobox of *carp-Ovx1* is compared with that of five other genes. % Identity are given. For *G9-2* and *Cts-Ovx1* only part of the homeobox sequence was published. Downstream from the homeobox 43 aa were deduced, which show 86.1% identity with *GBX1* (bp 79.9%) and 85.8% identity to *Gbx2* (bp 68.3%). Concerning *Chox7*, only the first 18 aa are largely identical, probably because of a mistake in the *Chox7* sequence published, as also suggested by Matsui et al. (1993): exclusion of one base from the *Chox7* sequence leads to 88.4% identity in this area (bp homology 78.1%). The stop codon at position 570 was found at the comparable location in *GBX1 and Gbx2*. From the 8 aa directly upstream to the homeobox only 1 differs between *Chox7* and *carp-Ovx1* (bp homology 80%). No homologies could be detected further upstream. We found 87 aa upstream to the homeobox, but the sequence is incomplete, since there are no in frame start codon and 5' UTR in the sequence. The complete cDNA sequence of GBX2 includes 2151 bp, and codes for 347 amino acids (Lin et al., 1996). About similar figures were described for its mouse homologue *Gbx2* (Bouillet et al, 1995).

Gene	aa	bp
G9-2	100	100
CHox7	100	82.8
GBX1	98.3	80.6
Gbx2	98.3	87.3
Cts-Ovx1	85.2	72.8

In situ hybridization (ISH) showed a localized expression pattern of *carp-Ovx1* during gastrulation. Around the 50% epiboly stage, when the formation of the hypoblast by involution of cells around the margin of the blastoderm was about to start, the mRNA was detected in the germ ring. Most label was found in its dorsal part, with exception of the, most dorsally located, embryonic shield (fig.2). Sections revealed the presence of label in all cells of the germ ring in the regions of gene expression. During subsequent



Figures 2-4: ISH of carp gastrulae with *carp-Ovx1* antisense RNA probe. Fig. 2 shows a dorsal view of a 50% epiboly stage embryo. The germ ring shows expression but in the embryonic shield area (arrow) almost no staining was found. Fig. 3: 70% epiboly, dorsal view. Strong expression in two bands (arrows) on a distance from the germring. Fig. 4: Bud stage, lateral view. Band of strong expression shifted further anteriorly. X 40. A = animal pole; G = germring; H = head; T = tail. From the embryos of figs. 2 and 3 the yolk was removed.

progression of epiboly, expression was progressively confined to dorsal areas, and after completion of epiboly it was restricted to the dorsal half of the embryo. At 70% epiboly, expression had shifted from the germ ring to a band at about 40% of the distance between the germ ring and the animal pole. Sections showed that in this area involuted cells as well as epiblast cells were stained, most intense staining being present in the epiblast. The front of the involuting cells had progressed further in animal direction than the position of the band with intense staining. From the band, expression faded away caudally, but left and right from the embryonic axis a narrow zone of stronger expression runned caudally to the germ ring (fig. 3). At the tailbud stage, the band of strong expression had shifted further in the direction of the animal pole (head) area and had narrowed further, so that only the dorsal third of this part of the embryo was labelled (fig.4). Again, sections showed expression especially in the epiblast. During subsequent somitogenesis expression was weak. Staining was visible near the midbrain/hindbrain border, in a yet not defined area lateral to the hindbrain and in the tailbud. Very weak labelling was found in the spinal cord, but this could not yet be confirmed in sections.

The expression in the germ ring, at the onset of involution, suggests a function of *carp-Ovx1* in gastrulation. However, involution starts at the dorsally localized embryonic shield. This area did not show any label. Expression was also weak in ventral areas. Therefore, it is not likely that *Ovx1* is needed for involution to occur. As other homeobox genes, *carp-Ovx1* shows differential expression along the anterior-posterior axis. The area of expression in the epiblasts shifts anteriorly during gastrulation. Since epiboly leads to movement of epiblast cells in the direction of the germ ring, i.e. caudally, it can be concluded that in subsequent stages of gastrulation different cell populations express *Ovx1*. According to the fate map of the closely related zebrafish (Kimmel et al., 1995), the cells expressing *Ovx1* at 50% epiboly might include presumptive endoderm and mesoderm cells. At later stages, strongest expression is found in cells with a neurectodermal fate. The gradual restriction of gene expression to more dorsal areas might well be the result of convergent movements of neurectodermal cells to the embryonic axis.

The pattern of expression of *carp-Ovx1* has much in common with that of the mouse gastrulation and brain specific gene *Gbx-2* (Bouillet et al., 1995), renamed *Ovx1* by Bürglin (1993). At the early headfold stage, expression was found in the ectoderm, but not in the headfold, and in mesoderm and endoderm it was restricted to the primitive streak area. Later, label was present at the midbrain/hindbrain boundary, in the foregut and first pharyngeal arch and in the tailbud. From day 9 expression was found in the brain, only caudal from the mesencephalon, and in the spinal cord. Together with the high measure of sequence identity in the homeobox and flanking area, these comparable expression patterns lead to the conclusion that *carp-Ovx1* and *Gbx-2* are very close homologues.

References.

Boullet, P., Chazaud, C., Oulad-Abdelghani, M., Dolle, P. and Chambon, P. (1995) Sequence and expression pattern of the *Stra7* (*Gbx2*) homeobox containing gene induced by retinoic acid in P19 embryonic carcinoma cells. Dev. Dyn. 204: 372-382.

Bürglin, T.R. (1993) A comprehensive classification of homeobox genes. In: Guidebook to the Homeobox Genes (D. Duboule, ed) Oxford University Press.

Dick, M.H. and Buss, L.W. (1994) A PCR-based survey of homeobox genes in ctenodrilus serratus (Annelida: Polychaeta). Mo. Phylogen. Evol. 3: 146-158.

Fainsod, A. and Gruenbaum, Y.(1989) A chicken homeobox gene with developmentally regulated expression. FEBS-letters 250: 381-385.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B. And Schilling, T.F. (1995) Stages of embryonic development of the zebrafish. Dev. Dyn. 203: 253-310.

Levine, E.M. and Schechter, N. (1993) Homeobox genes are expressed in the retina and brain of adult goldfish. Proc.Natl.Acad.Sci.USA 90: 2729-2733.

Lin, X., Swaroop, A., Vaccarino, F.M., Murtha, M.T., Haas, M., Ji, X., Ruddle, F.H. and Leckman, J.F. (1996) Characterization and sequence analysis of the human homeobox containing gene *GBX2*. Genomics 31: 335-342.

Matsui, T., Hirai, M., Wakita, M., Hirano, M. and Kurosawa, Y. (1993) Expression of a novel human homeobox-containing gene that maps to chromosome 7q36.1 in hematopoietic cells. FEBS-letters 322: 181-185..

Stroband, H.W.J., Stevens, C., Te Kronnie, G., Samallo, J., Schipper, H., Kramer, B. and Timmermans, L.P.M. (1995) Expression of *carp-cdx1*, a *caudal* homolog, in embryos of the carp, *Cyprinus carpio*. Roux's Arch. Dev. Biol. 204: 369-377.