

cDNA cloning, tissues, embryos and larvae expression analysis of Sox10 in half-smooth tongue-sole, *Cynoglossus semilaevis*

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ABSTRACT

A half-smooth tongue-sole, *Cynoglossus semilaevis* Sox10 (Accession no.: EU070763) was isolated from brain of tongue sole by using homologous cloning and RACE method. The complete cDNA of the tongue sole Sox10 contains a 35 bp 5'UTR, a 1338 bp open reading frame (ORF) encoding 445 amino acids and a 1155 bp 3'UTR. A condensed phylogenetic tree was constructed based on the amino acid sequences of tongue sole Sox10 and other well-defined vertebrate Sox. The overall topology of the tree showed the tongue sole Sox10 clusters with all Sox10. Alignment of amino acid residues of the tongue sole Sox10 gene with those from other vertebrate indicated high level conservation of amino acid sequence. The RT-PCR analysis demonstrated that the tongue sole Sox10 was highly expressed in brain, gills, skin and eyes, intermediately in spleen, heart, head–kidney and muscles, weakly expressed in kidneys and intestine and no expression in liver and gonad. The Sox10 was also expressed weakly in germ cell and zygote. We cannot detect the expression of the Sox10 in 8-cells stage. However it resumed expression weakly from blastula stage to middle of gastrula. And it expressed highly from neurula stage to 25 dah (day after hatching). It suggested that the Sox10 was involved in the development of embryos and larvae in tongue sole.

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1. Introduction

The Sox gene family encodes a large family of transcription factors, and there are more than 30 Sox genes in the vertebrates (<http://www.ncbi.nlm.nih.gov>). Their precise roles are not well understood, although many are presumed to function in cell fate specification (Pevny and Lovell-Badge, 1997). For example, the founding family member, Sry, is likely to be responsible for Sertoli cell specification, and thus male sex determination in mammals (Capel, 2000; Gubbay et al., 1990). SOX10 is a transcription factor defective in the Dom (Dominant megacolon) mouse and in the human Shah-Waardenburg syndrome (Southard-Smith et al., 1998; Herbarth et al., 1998; Pingault et al., 1998), and Sox10 is clearly an important transcriptional regulator in neural crest cell (NCC) development (Kelsh, 2006). It has been suggested that peripheral nervous system and pigmentation defects result from loss of NCCs (Southard-Smith et al., 1998; Kapur, 1999).

Now, the complete Sox10 cDNA of *Takifugu rubripes*, *Danio rerio* and partial cDNA of *Astatotilapia burtoni*, *Oncorhynchus keta*, *Oreochromis niloticus*, and *Paramisgurnus dabryanus* were submitted to Genbank in the teleosts (<http://www.ncbi.nlm.nih.gov>). And the reports about Sox10 were only seen in the zebrafish, in which Sox10 is expressed in developing pre-migratory neural crest and plays an important role in specifying nonectomesenchymal (neurons, glia and pigment) neural crest (Dutton et al., 2001) in the teleosts. However, no data is available on the tissue, embryos and larvae expression in the teleosts. In order to clarify the mechanism of the Sox10, we isolated the cDNA and characterized the expression of Sox10 in the tissues, embryos and larvae of the tongue sole.

2. Materials and methods

2.1. Materials

For the cloning and measurement of Sox10 mRNA in the tongue sole, the gonads, liver, spleen, kidneys, brain, heart, muscle, head–kidneys, gills, skin, intestine, and eyes were collected from the two year old fish. They were snap frozen in liquid nitrogen, and stored at –80 °C until use. In addition, the germ cell, zygote, 8-cells stage, blastula stage, early of gastrula, middle of gastrula, neurula stage, tail-

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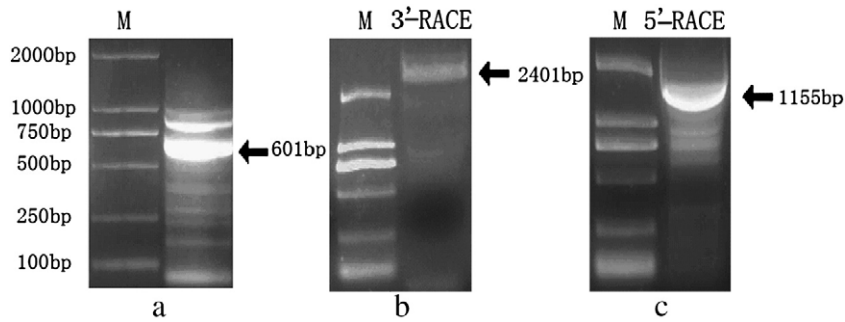


Fig. 1. Electrophoresis of the *Cynoglossus semilaevis* Sox10 PCR products by degenerate primers (a), 3'-RACE (b) and 5'-RACE (c).

bud stage, heart beating stage, hatching, 1 day after hatching(dah), 12 dah, 19 dah, and 25 dah of tongue sole were also collected. The fish were obtained from the Haiyang 863 High-Tech Experimental Base, Haiyang city, China.

2.2. RNA extraction and cDNA cloning

Total RNA extraction and reverse transcription were carried out as described (Chen et al., 2001). A pair of degenerate primers (P1: 5'-

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1  acgcggggctgtctgaggtggagctcagccccggcATGTCGGACGACAGCCGCTCCATGTCTCCGGGTGAGTCTCT
      M S D D S R S M S P G Q S
76  CCTCCGGGGCGACGGGGCGGAGAGACTCGCCCTCCACGGGCATCAGCAGGTGCGGCTGGCGGCTTGGACGACG
   S S G A T G G G D S P L H G H Q Q V R L A A L D D
151  CTTCGGCGCAGTGCCTCCGCCGATCCGACGACGAGGACGAGCGTTTCCCGCGGGGATCCGGGAAGCAGTGA
   A S A Q C S S A R S D D E D E R F P A G I R E A V
226  GTCAGGTGCTCGACTGCTACGACTGGACCTGGTGCAGATGCCGTGCGTGTCAACAGCGGAGGGGAGTCCAAGC
   S Q V L D C Y D W T L V P M P V R V N S G G E S K
301  CGCACGCTGAAGAGACCCATGAACGCCTTCATGGTTTGGGCGCAGGCGCGCAGGAAAGTGGCGGATCAGCACC
   P H V K R P M N A F M V W A Q A A R R K L A D Q H
376  CGCACCTGCACACGCGAGCTGAGCAAGACCTGGGGAAGCTGTGAGACTTCTCAATGAAAGAGACAAGCGAC
   P H L H N A E L S K T L G K L W R L L N E R D K R
451  CCTTCATCGAGGAGCAGAGAGGCTGAGGAAGCAACAAGAAGGACTACCAGACTACAAGTACCAGCCACGCC
   P F I E E A E R L R K Q H K K D Y P D Y K Y Q P R
526  GGCCTAAGAACGGGAAGATTGGTTCGGGTCAGGAAGTGAGGCCGACGCAATCCGAGGGTACAGTCTCCCAA
   R R K N G K I G S G S E A D G N S E H S P P
601  CACCTCCGACGACTCCTAAGACGGAGCCTCAGTCTGGAAAAGCAGGGGATGGAAAAGAGACGGGGAGGAAACG
   T P P T T P K T E P Q S G K A G D G K R D C G G G N
676  TGGCTCCCGGGTAAACGCTGGACTGGAGGGAAGCTCAAGTCCCGCAGGAGCTGGGAAACACACATTGACTTTG
   V G S R G N A G L E G S S S A A G A G K P H I D F
751  GTAATGTGGACATTGGTGAATCAGCCACGAGGTGATGGCCAAATGGAGTCAATTTGACGTCAATGATTTGACC
   G N V D I G E I S H E V M A N M E S F D V N E F D
826  AGTACTCCCCCAACGGCCACCCAGGGGTCCGACAGAGCACTGCAAGTGGTGGCGCCGACCATCACCAGCAT
   Q Y L P P N G H P G V G Q S T A G G A A A P S P A
901  CGCCCTACTCCTACGGCATCTCCTCAGTCTGGCAGCAGCCAGTGACACTCAGCAGCCTGGTTGTCCAAGCAGC
   S P Y S Y G I S S A L A A A S G H S A A W L S K Q
976  ATCAGCAGTCCCCCAACAACACACAGCTCCCTCTGGGCTCAGACCCCTCAAGGCCACACATCAAGATGAGA
   H Q Q L P P Q H H S S P L G S D P S K A H I K S E
1051  GCGGAAGCACAGGGGTCACCTTTCAGAGAAGCGGTCTCAGCAGGTTCCACGTCACCTTACGCCCTCTGACCCCTC
   S G S T G G H F A E A V S A G S H V T Y A P T L
1126  CTCACTACAGCTTGTCTTCCCTCTCTGGCCTCCAGGGCACAGTTTGTGAATACGTCGACCACAGGGTCTGTG
   P H Y S S A F P S L A S R A Q F A E Y V D H Q G S
1201  GGTCTGACTACGCCCCTCCAGCCAGGCTTCGGGGCTGATTTCAGCCTTCTCCTACATGGGCCCTTCCAGAGGC
   G S Y Y A H S S Q A S G L Y S A F S Y M G P S Q R
1276  CCCTGTACAGCCCACTCTGACCCCGTAGTGCACCGCAGTCCGACGCCCCACACAGTGGGATCAGCCCGTCT
   P L Y T P I S D P A S A P Q S H S P T Q W D Q P V
1351  ACACAACACTGTGCGGCCATGACagataaacaagaccggagggactggtgacaggttcagccccatgtcagacc
   Y T T L S R P *
1426  agaggagcagcagcgtgacaacaacaagatgtcagatcaaaagctcaaaagtggtctgagctgtggggagtggtg
   ccggcaccggtggaggaggtggtggggagcaggaggacagaactcctccgctcagttcccagaggtacaaatgctg
1576  ccacggcccaagtaggagcttgagcgtggagccccagcgtggtgcggtatcagggggcgtcaggtggaa
   atcaaaagtgtaactttttcgtgaagtcagaataatctctcaaacatgaagcatttcaggtttcaggaacctt
1726  cgacaacatcattacgagtgatagaaactgaaagaccagctgttcgaggtcacattccaatcaaaggtgggtg
   cttcctgtggtgcttcccttgaagatcccaagcagagatgatcaatgtcctattagaagaaggtcagaggtca
1876  atcatcacaattgatttttagaagaacagacactgatctgtggccatctccagcacttatgagcagaatctctg
   ttcctttttaccagaacaagactgcagcgaagtcagtggtgtaaccaagagtagtctaccaaacagactgttacaacatc
2026  ataggggtcattttggggtataccccgacaacatcgcagaaacctcattgttgccacatttctccaatgata
   tatggggcgtatagggtcacattcattattgtggaccagatgaaagtcaaaagcagcgtcatgtcctctctgtct
2176  gtgtactatcaggtcgggtgccaatcctctgctgtgagccatgtctacggcgacagccaacaataactcaaa
   ttattctgttaattgttgatttagtgcgctgtctgaacacctggagaacatgtaatttttggcttttttttaatt
2251  gtatataggtcagaataatcaagtaattagcgtaccagttattcaaaatacatgtaaaatactgtaaattga
   ccaaaggtttgagaccaaaacttacgtagaagaacagatcaggtttcctgtagacgtttctcccaacgatata
2476  ctccaattacctgtgtgacagtagttactgtatgaagttttatccagcttgagttttataaactctgactga
   2551  tgtcacttcacgtcctggtgtgtattcaggtgaccatcacatcattggtaaaaaaacatcaccatggttacaacatc
2626  gagattcatgtgcttttaattattgtatcaagtgcttccagccatatttatttccactgcacagctgctcactg
2701  caatttcctaccgcacagaaacccacatcctttattattaactcctactcaataataaacatgtattttta
2776  cattttgtcaatgtgagcgtgccaatattgagcatcagttaccatccggtactgttcaccaaacagatttagtgac
2851  cgaaattgtgcttgaatcgatgcaaaacatttccagaaaagttggtgagctttaaaaaaaaaaaaaaaaaaaaa
2926  aaaaaaaaaaa
    
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Fig. 2. cDNA nucleotide (GenBank Accession no.: EU070763) and predicted amino-acid sequences of the *Cynoglossus semilaevis* Sox10 Nucleotides are indicated above and numbered to the left of each lane (upper row). The deduced amino acid sequence is shown below the nucleotide sequence. Amino acids are numbered to the left of each lane (lower row). The start codon ATG is underlined; the stop codon is indicated by an asterisk; the polyA signal is shaded in gray; the lowercase indicated 5' and 3'UTR.

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