## A EMOPAMIL BINDING PROTEIN (*Cs-ebp*) INVOLVED IN THE GROWTH PERFORMANCE OF CHINESE TONGUE SOLE POSSIBLY THROUGH THE REGULATION ON CHOLESTEROL BIOSYNTHESIS



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Sexual size dimorphism (SSD) with faster-growing females challenges sustainable aquaculture of Chinese tongue sole (*Cynoglossus semilaevis*), an economically important flatfish in northeastern Asia. Females grow faster than males, and eventually reached over twice in weight and length. We explored the steroid pathway's role in SSD using comparative transcriptomics. Notably, genes involved in this pathway, such as emopamil-binding protein (*ebp*) and its downstream genes, lathosterol 5-desaturase (*sc5d*), 7-dehydrocholesterol- $\Delta$ 7 reductase (*dhcr7*) and 24-dehydrocholesterol Reductase (*dhcr24*), were highly expressed in female individuals of *C. semilaevis*. EBP is the key catalyzing enzymes in cholesterol biosynthesis. Its mutation caused abnormal bone development and growth retardation in humans. In the study, we characterized the *C.semilaevis* EBP gene (*Cs-ebp*) and revealed its possible involvement in the regulation of growth performance in teleost.

1. EBP domain was conserved in mammals and teleost. 2. Cs-ebp exhibited female-dominant expression in

C.semilaevis EBP was grouped together with those of two flatfish species.



gonads and livers. Its abundance in ovaries were in accordance with the ovarian development and oocyte growth.



Fig.2 Tissue distribution of *Cs-ebp* gene and its temporal and spatial expression in gonad.

3. *Cs-ebp* transcription was regulated by transcriptional factors JUNB and POU1F1. *Cs-ebp* gene knockdown triggered the down-regulation of its downstream genes in cholesterol biosynthesis pathway (*sc5d*, *dhcr7*, and *dhcr24*), and genes related to estrogen biosynthesis (*cyp19a1a*) and body growth (*akt1* and *bmp2b*).

CAAACTGATC CTACGCTTCT TCTTTAACCA AATGGTCGAT GCTGACATTT ATGAGAATAG AGCAAAATTA TTCGGACAAA ACAGCCACAT GAAAACAGAC -2251 AAGTGAAAGT TCAGGTAATA TGACACTTAG AAATACCATA AAAATATAAT AACAGGGCCA GAAGGGAAAA CAAGTTAAAT TAGAATATGA CACAAATTA -2151 AATAGAATAT ATTTTCTGTG CGGTTGAATG CAGTCGAAGC CAAGCACCCA <u>GCACTGT</u>TTT CTTAACCAAA GCACAGTTTA GCATTTAAAA TTATACGAAA TGTATCTAAT AGTAGTAGTA AGCGCAAACAA ACGCCGCAGA GTCGTCCTTC TAAGCCCCATA TAGCCTGTI CCCAGTAGCT CGCCGTGATC GTATAGTGGT TAGTACTCTG CGTTGTGGCC GCAGCAACCC CGGTTCGAAT CCGGGTCACG TTACAGATGA CTGGATACAA TTCTGTGATG AGCAGTGATT CCTAAACAGT CAGAATCAGG AACATACATT TGTGAGCAAA ACTTGGACAG GTCCTGGAGA -1651 ATTTGTATGT GCAAGTAACA AGCTTACCTA CATTTCTGTC CAGGAGCCAG AGTGGACATT GTTGAAGCTT GCAGGTTTCT ACATTGAAAA CAAACTGGAC -1551 TGGGCTAAGT CCTGGTAGTA AATGCCAACA G<u>ATTCCACAA</u> <u>A</u>CGTATGCAT AAGACTCTGT AACACAAACC ATTTTCTTCT GGGTATCAAT AAAATATTTT -1451 TTATTTAAGA ACGGTATTTG TTTTGGATCC TTTTTGAAAA GGTGAAAAAA AAACATCTCC CTGCCGTGTC AGGGACTATT CATCCCTGCC GTGACCCGGA -1351 TTC ACCGG GGTTGCTGCG GCCACAACGC AGAGTACTAA CCACTATACG ATCACGGCGA GCTACTGGGA GCGTGACGTG -1251 AAAACCAAAT TGAATGACAT TTGGTAATAC GTTTTAATCA CCTGAGATTT GGCCGACAAA CGAAGGGGTA TATTTTACAG TCTAAAATAT -1151 TGTTCGTTTA TAATTTAGAT GACTTACGTG GCTGTCTCCT TCATGTTGTC TCACTACTTT TGTCCTTAAG GGATACCCCA GGAAAGTACT TTACATTTTA -1051 AAGTACTCAC TTTAAAGTTT AACGTAAGGA ATGCACGACG AACCCGGAA<u>a</u> <u>tggcg</u>aatta gaactaagcg gtggtggcac tggatcaaca actcctatat TTTTGGATTA AAATGTCGTA TTTTCTCTAC GTACTTTGGC GTGGAAGCTC GIGTTTGTAC AAGTGTCCTC TAAATAAATC TACCATACAG TCAGACGTAG AAATACAACA CTTGACTAAT CTTCTTCGTT TTGTCTTTGC TCTTGAATAA GGCAAAATGT CTCAA<u>CGAGT</u> <u>CA</u>GGAAACAC TGAAAGTGCT TTAAATAGTA -751 AACTTAAAAT TAGAAAGAAG GAAAAATAAA <u>tatatt</u>aaa gacaataaaa aaacagtaag gagggt<mark>c6gs</mark> aaattaaaaa aataaataag gtgacaa<u>ggg</u> TATA box gcgtggtttg ctgaggcgcc ccatatgcat tggtggccgc cgacttcatt gtcaatgatc cgaggtaaag ac<u>ccaatca</u>g aaatcctgaa gatcacacat GC box GCTGCACACT GCCCAGTCAT TGGTCGATGC AGCAGTTCGC CCTTCCCATT GC<u>TATGCTAA</u> AGTCCCTCAC TGACTGCTCG GTTAAGAAAA AAGAATTAGG pou1F1a TTGAGCTGAA AAGTCTGCAG CAGGACAGGT AAGAGCAGCC CATTCTGACC CTTTTATGAT AAATAATTAA TAAGGAAAAAA CGAAAATTTT AATGAAGTAA TTTTTTTTAG AAAGTTCTGT TTACGTTCTT TAGCTAAACA TTGCTAAACC ATTGTATGTT TATTTCAACA AAGAGTTTAT GAGCACAATT AAATTTTTCT GTGAAAATAC TGAAACTCAT TACTCTGTTT TTTTTTTTT GCCTGAAAAA AAATTTTTTA ATTA<u>ATTICC</u> AGACTATTGA GTCTAGTTTG TAGTAATGAT **Stat4** TGTGTAGACG TGTTAGGAGT TTTCTAGAAA ATTTGGATTG ACTGAATGAG AAGTAGTAAC TGAAGAGTTG GTGGTTGTAC CT<u>GCTGCTG</u>A ATAATGATAT GCTGATGTGA TTCCTCCCAC AGACCATGGC CTCCCCCGTC TCCTACAGAC CATG



Fig.3 The effect of transcription factor on Cs-ebp promoter and the inhibitory effects of Cs-ebp siRNA in C. semilaevis ovarian cells.

## **Conclusions:**

- 1. Emopamil binding protein (*Cs-ebp*) showed high expression in gonads in 6 months post hatching, and the expression was female biased.
- 2. Cs-ebp RNAi led to decreased transcription of genes involved in steroid and estrogen biosynthesis, and body growth.
- 3. By screening the transcription factors on *Cs-ebp* promoter, we found it is positively regulated by transcription factors JUNB and POU1F1.
- 4. Based on these findings, *Cs-ebp* is a potential regulator in sexual size dimorphism of Chinese tongue sole, possibly through PI3K/Akt and TGF-β/Bmp signaling pathway.
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