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The MHC class I of Grass Carp (*Ctenopharyngodon idellus*) With Different Way to Present Peptide

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Abstract: Major Histocompatibility Complex class I (MHC class I) proteins are critical for immune defenses against viruses. Polygeny and polymorphism of alleles contribute to the breadth of the immune response. In previous study, researchers showed that a *MHC class I* gene termed Ctid-AB190929 was found from a grass carp. It was a expressed gene from cDNA library. Now, characterization of a genomic clone from the same grass carp constructed fosmid genome library allows us to further analysis the MHC class I region. This clone carried whole *MHC class I* and *tapasin* gene. This MHC class I termed Ctid-UBA0201 was the classical MHC class I by genomic analysis. But the amino acid sequences of Ctid-UBA0201 were differed from that of Ctid-AB190929. As lower vertebrates, appear to use a single expressed MHC class I locus to present a wide variety of peptide to antigen. The Ctid-UBA0201 could be the allelic gene of Ctid-AB190929. As the *tapasin* gene may be a unexpressed gene by genomic analysis, the Ctid-UBA0201 may present a wide variety of peptide without the *tapasin* gene to control the assembly. It may preserve genetic diversity which can be exchanged when the species face a new pathogen.

Key words: MHC I, tapasin, grass carp, peptide, assembly, China

INTRODUCTION

Major Histocompatibility Complex class I (MHC class I) proteins play a fundamental role in the presentation of endogenously derived peptides to CTLs. *MHC class I* genes are critical for immune defenses against viruses (Moon *et al*., 2005). Their genomic regions are the most dynamic part of the genome. Polygeny and polymorphism of their alleles contribute to the breadth of the immune response. The number of MHC class I loci can differ greatly in each species. Thus, far the genomic sequences of humans, mammals, reptiles, amphibians, birds and fish indicate that there are one or more class I loci in jawed vertebrates (Shiina *et al*., 1999; Flajnik and Kasahara, 2001; Kulski *et al*., 2002; Matsuo *et al*., 2002).

Teleost (bony) fish are the largest group of vertebrates, comprising about half of the total living vertebrates. As lower vertebrates, appear to use a single MHC class I locus to present a wide variety of peptide to antigen. Since, then the *MHC class I* genes have been reported in at least nine species, at least in the trout and Atlantic salmon, it was shown definitively that there is only a single expressed *MHC class I* gene (Hansen *et al*., 1996; Shum *et al*., 2001).

Grass carp (*Ctenopharyngodon idellus*) as one of the most important freshwater fish species in China can service as a model fish representing not only the tradtional fish cultured in pond but also the fish cleaning the excessive grass in the river. In previous study, one expressed *MHC class I* gene named Ctid-AB190929 have been found in grass carp (Hao *et al*., 2006).

To further understand the *MHC class I* gene in grass carp, a genomic clone containing the *MHC class I* and *tapasin* gene was found out from a grass carp fosmid library. The MHC class I was termed Ctid-UBA0201.

MATERIALS AND METHODS

Screening of a fosmid genomic library for *tapasin* and *MHC class I* gene: The fosmid genomic library CIGF from a adult grass carp (fish-C) was previously constructed (Jia *et al*., 2010). The CIGF was screened using the primer pairs derived from a conserved region of MHC class I exon 4 common to cDNA sequences previously identified in the same individual fish. Screening of the library and identifing one clone containing *tapasin* and *MHC class I* gene clone GIGF-0405E6.

Sequencing strategy: Because preliminary sequencing suggested that *tapasin* and *MHC class I* gene could be in clone GIGF-0405E6. The clone was further sequenced by the Shotgun Method. First, fosmid DNA sample was extracted and broken up by ultrasonication. Subsequently, subcloned libraries of T-vectors were constructed from the clone GIGF-0405E6 as described

previously by Taghian and Nickoloff (1996). The sequences of 1000 positive clones having an insert size of 2-3 kb from library were determined with the ABI PRISM Big Dye Primer Cycle Sequencing Ready Reaction kit (Applied Biosystems) and the ABI 377 DNA sequencer using T7 and SP6 sequence primers (The Beijing Genomics Institute).

Finally, determining the order of the subcloning fragment in the final 33 kb contig was accomplished with Phrap (www.phrap.org/consed/consed.html#howToGe). The sequence of the entire fosmid clone is deposited in GenBank under the accession number EF584535.

Sequence analysis: The edited GIGF-0405E6 final sequence were analyzed for coding regions using GENSCAN (http://genes.mit.edu/GENSCAN.html) and through homology searches using BLAST. *MHC class I* gene within the clones were compared with cDNA previously published. Polymorphisms and the phylogenetic tree were analyzed using DDBJ.

Promoter analysis: The promoter was analyzed using Genomatix MatInspector Software. Grass carp sequences were also aligned to known MHC class I Promoter zebrafish using ClusalW and conserved sites within the promoter regions were identified manually.

RESULTS AND DISCUSSION

The clone GIGF-0405E6 was further sequenced by the Shotgun Method. As an insert size of 2-3 kb was needed to construct subcloned libraries of T-vectors. The DNA sample of GIGF-0405E6 was extracted and broken up by ultrasonication. As Fig. 1 shows the size of DNA sample has been 2-3 kb by ultrasonication three times and 1-2 kb by ultrasonication five times.

Selecting the insert size from T-vectors for sequencing: Because subcloned libraries of T-vectors were constructed from the clone GIGF-0405E6 and the insert size was 2-3 kb by ultrasonication. Researchers selected insert size of 2-3 kb to sequence. As Fig. 2 shows the number of 1, 3, 4, 7, 8, 9 and 10 could be select to sequence.

The homology analysis with zebrafish: The edited GIGF-0405E6 final sequence were analyzed for coding regions through homology searches using BLAST (Fig. 3). The GIGF-0405E6 carried whole *MHC class I* and *tapasin* gene.

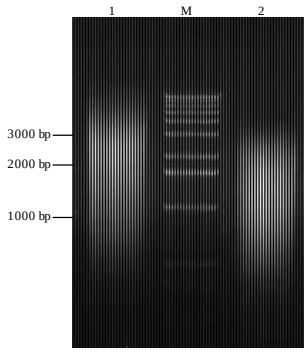

Fig. 1: Ultrasonication of the clone GIGF-0405E6; M: 1 kb marker; 1: Three times by ultrasonication and 2: Five times by ultrasonication

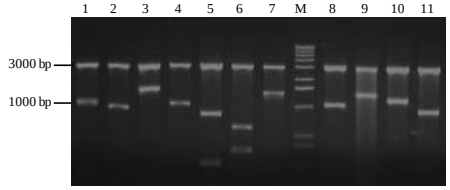

Fig. 2: Enzymatic digestion of the recombination T-vectors by restricted enzymic EcoRI; M: 1 kb marker and 1-11: Recombination T-vectors of digested by EcoRI

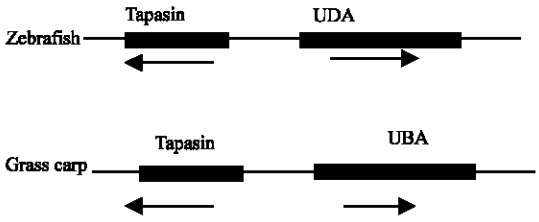

Fig. 3: Predicted genes of GCFL-0405E6 with zebrafish. Arrows indicate the transcriptional orientation of the gene

Organization of the exon-intron of *Ctid-UBA* gene: A complete contig of 33 kb contained part of the tapasin and *Ctid-UBA0201* genes in EF584535 (www.ncbi.nlm.nih.gov/nuccore/EF584535.1) (Fig. 4). The tapasin gene started at 7160 bp and ended at 1382 bp, consisting of 5778 bp. Exon 6 contained 93 bp. Exon 1 was 61 bp and encoded a signal peptide. Exon 2 was 196 bp. Intron 1 was 3644 bp. Exon 3 contained 249 bp. Intron 2 was 88 bp. Exon 4 was 360 bp.Intron 3 was 165 bp. Exon 4 was 360 bp. Intron 4 was 153 bp. Exon 5 was 93 bp. Intron 5 was 93 bp. Exon 6 was 4056 bp. Intron 6 was 249 bp.

The *Ctid-UBA0201* gene started at 4505 bp and ended at 20,980 bp, consisting of 16,475 bp. Exon 1 contained 49 bp and encoded a signal peptide. Exon 2 was

261 bp and encoded the α 1 domain. Intron 1 was 1018 bp. Exon 3 contained 276 bp and encoded the α 2 domain. Intron 2 was 12,285 bp. Exon 4 was 288 bp and encoded the α 3 domain. Intron 3 was 1572 bp. Exon 4 was 99 bp and encoded the TM domain. Intron 4 was 335 bp. Exon 5 was 18 bp and encoded the CY1 domain. Intron 5 was 172 bp. Exon 6 was 53 bp and encoded the CY2 domain. Intron 6, located between exon 6 and 7 was 249 bp. The *Ctid-UBA 0201* gene encoded 342 aa (Fig. 5).

The promoter in Ctid-UBA 0201: Promoter organization was analyzed in the genomic sequences 800 bp before the start codons of the *Ctid-UBA0201* gene. GAAA motifs were found at positions -733, -654, -384 and -314. An IFN-Stimulated Regulatory Element (ISRE) sequence was found at position -242. The SXY box, composed of an S-box, X1 X2 and EnhB (Y) is a common promoter for expression of the *MHC class I*, *MHC class II* and *α2m* genes and was found at positions -203, -164 and -146 in Ctid-UBA 0201. The results indicate that a common promoter region exists in *Ctid-UBA0201* gene.

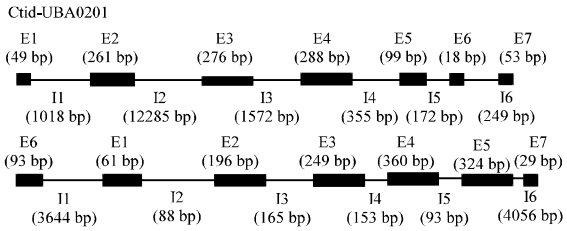

Fig. 4: The exon-intron organization of Ctid-UBA0201and tapasin. The exon sizes are noted above the sequences and the intron sizes are noted

High polymorphism of the MHC class I of grass carp: To investigate the allelic polymorphism of *Ctid-UBA 0201* genes, 26 grass carp were used to clone the *MHC class I* genes using allele-specific primers vertebrates. The α1 and α2 domains of the expressed *MHC class I* genes contained 85-89 and 91-93 aa, respectively. The amino acid identities of MHC class I ranged from 64.53-83.63%. The motif (Y,Y, R, T, K,W,Y and Y) which is crucial for peptide binding was showed with asterisks. Dashes indicate sequence identity (Fig. 6).

In previous study, researchers have constructed and characterized the genome fosmid library with excellent quality of grass carp and named this fosmid clone library as CIGF. From this library, clone GIGF-0405E6 had been screened. Though the sequence of the entire clone GIGF-0405E6 could be get by clone walking, it needs too much time (Bradeen *et al*., 2003). Subcloned library of T-vectors was constructed from the clone GIGF-0405E6. About 1000 positive clones having an insert size of 2-3 kb from this

-780 -770 -760 -750 -740 -730
TCACAGTGCA TTAACAAATA CAACTTTTGA TTTAATAATG TATCAGTAAA TGTT**GAAA**TT
-720 -710 -700 -690 -680 -670
AACATTAAGA TTAATAAATG CTGTTGAAGT ATTGTTCATT CTTAGTACAT GTAGTTAACA
-660 -650 -640 -630 -620 -610
CATGATAACA AAT**GAAA**GCT TATTGTAAAG TGTTACCAAT ATCACTATAT AACAAACAAG
-600 -590 -580 -570 -560 -550
GTAAATTAAT ACAATGCATT GTATCAACAC TATTGAAGTG AGCTGTAACT TGTAACGCTA
-540 -530 -520 -510 -500 -490
TTTAAAAAAG ATGGACATCG GTTTATAATT TGTACATGCA TTTATTTTGT TAATCAAATT
-480 -470 -460 -450 -440 -430
AATTGTTCAA AATAATTTTT ATAATAACTA CCAACTCCCA TAAACAAACA TGATATTTCT
-420 -410 -400 -390 -380 -370
ATTATGTGAA CATTTTAAGT AAATGTTATA TATTAAATTA AAA**GAAA**ATC TGTTGATAAA
-360 -350 -340 -330 -320 -310
GATGACACCA GCATGCTTTA ACCTGGTCAC AAATCAGATA ATAACCCTGA GGA**GAAA**TGT
-300 -290 -280 -270 -260 -250
TGACTTTTCT AACTTTATTT ATTTATTTAT TTATTTATTT ATTTACCTTT AATATGTC***CT***
ISRE -230 -220 -210 **S-box** -200 -190
TTATATTTGT GCACAAGACT GCTGCCGCCA CTATCCTGAT ***CAACCCAGTT*** AAGGAAGTAG
-180 -170 **X2-box** **X1-box** **(Y) EnhB** -140 -130
CTCTTAGTAA CTTGTGACGT ***TGAACGTACC*** ***TGGAATATCT*** ***ATTGG***CTGGA ATTTCAGAGA
-120 -110 -100 -90 -80 -70
CTATTTAGGC GTTCTCTCA***G*** ***AAA***CATTTCA TCAGTATATC GCCAGACATC TGAAACGTCT
-60 -50 -40 -30 **TATA** -20 -10
AGAACAAATA TTTCAATTAA TTTCTCTAGA ACGATTTTTA **CAAAAA**CTGT TTCTCATGCA
1
ACAGCAAGGA **TG**

Fig. 5: The promoter in Ctid-UBA 0201. Putative transcription factorbinding sites are indicated. Enhancer elements including the ISRE are underlined and conserved motifs involved in the constitutive expression of MHC class I sequences are indicated above in bold

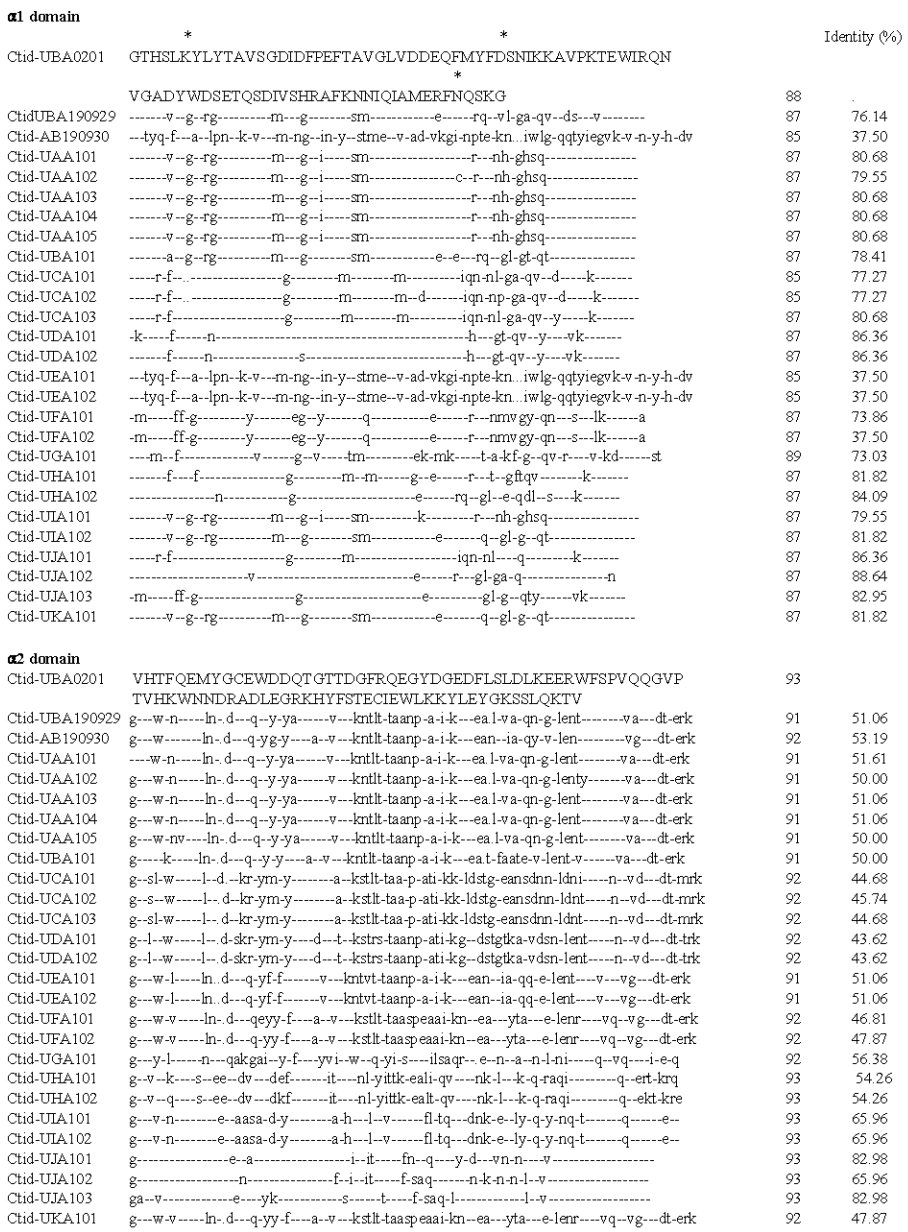

Fig. 6: Comparison of the PBD domains of Ctid-UBA0201 amino acid sequences to the MHC class I of other grass carp

library were selected to sequence. It ensured the each bases of clone GIGF-0405E6 was covered 8-10 times. So, the sequence of the entire fosmid clone had excellent quality.

Similar to chickens, ducks and mammals, the MHC class I regions in most bony fish are composed of seven exons and six introns (Hansen *et al*., 1999). In this study, the *Ctid-UBA0201* gene also has seven exons and six introns. Some reports have confirmed that in bony fish the *MHC class I* genes can also confer resistance to pathogens of the bacterial and viral (Chen *et al*., 2010). The polymorphic residues of the Peptide-Bindingdomain (PBD) of MHC class I are the key to resist the pathogens. The PBD is made up of the α1 and α2 domains. Comparison of the PBD domains of Ctid-UBA0201 amino acid sequences to the classical MHC class I of other grass carp reported in the previous study (Yang *et al*., 2006). The α 1 and α 2 domains of the *Ctid-UBA0201* genes contained 85-89 and 91-93 aa, respectively. The amino acididentities of Ctid-UBA0201 rangedfrom 64.53-83.63%. The motif (Y, Y, R, T, K, W, Y and Y) which is crucial for peptide binding was conserved in the *Ctid-UBA0201*

genes. These finding imply that *Ctid-UBA 0201* gene has the characteristics of the classical *MHC class I* gene.

The SXY is generally believed to be the shared promoter for MHC class I, MHC class II and α2m (Gobin *et al*., 1998). Analysis of the promoter region of Ctid-UBA 0201 showed that the promoter had this original feature in bony fish and then evolved in ducks, mammals and humans. The transcriptional regulatory regions were composed of five conserved domains: S-box, X1 3 2, EnhB (Y), GAAA and ISRE motifs. This finding implies that *Ctid-UBA 0201* gene can express. Because the Ctid-AB190929 and Ctid-UBA0201 was came from same grass carp and the Ctid-AB190929 was a expressed *MHC class I* gene from cDNA library. The amino acid sequences of Ctid-UBA0201 were differed from that of Ctid-AB190929. As lower vertebrates, appear to use a single MHC class I locus to present a wide variety of peptide to antigen. The *Ctid-UBA0201* could be the allelic gene of Ctid-AB190929.

In all species studied to date, the MHC is a large chromosomal region, containing many genes encoding proteins of immunological importance. Several cofactors are essential for proper MHC class I molecules assembly, such as proteasome, calnexin, calreticulin, ERP57, tapasin and TAP (Grandea and Van Kaer, 2001). Tapasin is supposed to play an important role in the quality control of MHC class I molecules assembly in endoplasmic reticulum. It is still unclear how tapasin controls the assembly of MHC class I molecules in ER. But pech argues that tapasin-mediated bridging of TAP-class I complexes is not necessarily sufficient for peptide loading (Peh *et al*., 2000).

In this study, researchers agree with the view of pech. The Ctid-UBA 0201 has the characteristics of the classical *MHC class I* gene but the tapasin gene may be a unexpressed gene by genomic analysis. So, the Ctid-UBA0201 may present a wide variety of peptide without the tapasin gene to control the assembly. It may preserve genetic diversity which can be exchanged when the species face a new pathogen.

CONCLUSION

The *Ctid-UBA 0201* gene is the classical *MHC class I* gene and the allelic gene of *Ctid-AB190929*. It may present a wide variety of peptide without the *tapasin* gene to control the assembly.

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