

Linkage mapping of major histocompatibility complex class I loci in tilapia (*Oreochromis* spp.)

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Introduction: The genes of the major histocompatibility complex (MHC) code for highly polymorphic cell surface glycoprotein structures, involved in the specific immune response, by displaying both self and non-self peptides to T-lymphocytes. The MHC multi-gene family includes two major subfamilies, class I and class II. The polymorphic class I molecules are expressed ubiquitously on all nucleated cell surfaces and present peptides, derived from endogenously synthesized proteins to cytotoxic T-cells.^{1,2}

The MHC is located on chromosome 6 in human and chromosome 17 in the mouse as a single cluster of tightly linked genes,² while its position is unknown in most fish species. Results of studies in several fish species, from different orders, suggest that in all Euteleostei (bony fish) the class I and class II loci are not linked.³ Studies in east African cichlid fishes, including *Oreochromis niloticus*, provided evidence for at least 17 class I loci.⁴ Several haplotypes were observed with variable number of loci per haplotype. All loci were closely linked, indicating a single genetic cluster of MHC class I in tilapia.⁵ Mapping the MHC loci in tilapia is important for genetic studies of disease resistance and of immune responses in this commonly cultured species.

Experimental design: To map the MHC class I, we used polymerase chain reaction (PCR) primers flanking a polymorphic region within the MHC class I loci.³ Using this polymorphism, we genotyped a segregating family⁶ and analysed the linkage between MHC class I loci and previously mapped genetic markers.

PCR primers: Two PCR degenerate primers, E3T4F and E4T1R, designed by Sato *et al.* (2000) from exons 3 and 4 of *O. niloticus* MHC class I³ were used for amplification. The PCR fragments were purified from agarose gel (1%) using the Ultrafree[®]-DA kit (Millipore Corporation, Bedford, MA, USA), cloned using the pGEM[®]-T Easy Vector System (Promega Corporation, Madison, WI, USA) and sequenced using the BigDye[™] Terminator Cycle Sequencing kit (Applied Biosystems, Warrington, UK). The sequences were placed in the EMBL database under the accession numbers AJ577831, AJ577832, AJ577833, AJ577834, AJ577835 and AJ577836. Blast analysis against the GenBank database confirmed that the products are indeed part of the MHC class I. Following sequencing, the E3T4F primer was modified to include a T-nucleotide in the sixth base position and a 5' fluorescent dye label.

Primer sequences: E3T4F: 5'-TGTCYAGTGGGTGAAGAAG TAT-3' (sense)

E4T1R: 5'-GGRAGWCTTCTGRAGGAGAGACA-3' (antisense)

PCR conditions: Amplification reactions were performed in a 10 µl reaction volume containing PCR buffer with 2 mM MgCl₂, 1 U *Taq* DNA polymerase (JMR, London, UK), 187.5 µM each dNTP, 1 mM tetramethylammonium (TMAC), 5 µM each primer and 60 ng of genomic DNA. The amplification conditions were as follow: 92 °C for 40 s, 60 °C for 40 s, 72 °C for 1 min, for 30 cycles.

Length polymorphism and Mendelian inheritance: The primers amplified several loci. Four major bands were detected, showing Mendelian inheritance. With 440 and 466 bp bands considered as alleles of one loci and 480 and 506 bp bands considered as alleles of another loci. One µl of PCR products was added to 1.5 µl formamide-loading buffer and 0.5 µl GeneScan™-1000 ROX™ size standard (Applied Biosystems). After denaturation at 92 °C for 2 min, 1 µl of the solution was loaded onto acrylamide gel (4%) in an ABI 377 automated sequencer (Applied Biosystems) and DNA fragments sized by comparison with the internal standard using Genescan software (Version 3.1). Genotypes of individual samples were determined by Genotyper software (Version 2.0). *Oreochromis aureus* female homozygous to the 466 and 480 bp alleles and *O. niloticus* male homozygous for the 440 and 506 bp alleles, two heterozygous F₁ and their 109 F₂ offspring were genotyped. This family serves as a reference for the tilapia linkage mapping and was already genotyped for 505 polymorphic microsatellite markers.⁶ The two loci were completely linked, without a single recombination event in the genotyped family.

Linkage mapping: Linkage analysis was performed using CRIMAP (Version 2.4) (<http://linkage.rockefeller.edu/soft/crimap/>). Preliminary localization was performed with the TWOPOINT command with a LOD of 3.0. Ordering of markers in the linkage group was performed using ALL, FLIPS and BUILD. The markers were reanalysed with a LOD of 7.0 and final distance between markers was given using JoinMap 3.0. One marker with inconsistent behaviour (GM197) was eliminated to produce the map shown in Fig. 1. In both analyses, the MHC class I was found closely linked to the microsatellite markers UNH185 and GM531 (Fig. 1). These markers were located on linkage group 18 in the last published map.⁶ The latest updates to this map are available on the website (<http://hogs.unh.edu/comp/>).

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References

- Hughes A. L. & Yeager M. (1998) *Annu Rev Genet* **32**, 415–35.
- Manning M. J. & Nakanishi T. (1996) In: *The Fish Immune System* (Ed. by G. Iwama & T. Nakanishi), Academic Press, San Diego, CA, USA.
- Sato A. *et al.* (2000) *Immunogenetics* **51**, 108–16.
- Sato A. *et al.* (1997) *Immunogenetics* **46**, 63–72.
- Murray B. W. *et al.* (2000) *Mar Biotechnol* **2**, 437–48.
- Lee B.-Y. *et al.* (2001) Abstracts of the Plant & Animal Genome IX Conference. W616, pp. 215. Scherago Intl. Inc., San Diego, CA, USA, available at <http://www.intl-pag.org/9/>.

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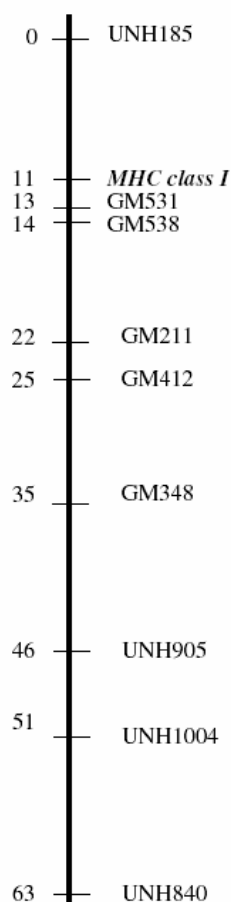


Figure 1 Map location of the MHC class I gene and surrounding microsatellites, with inter map distances in Kosambi cM.