

Mapping the transferrin gene in tilapia

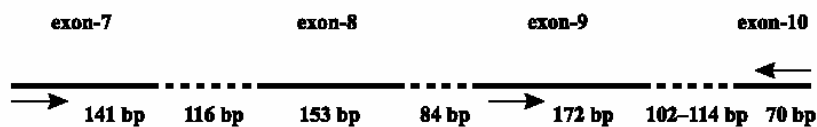
A. Cnaani*, **M. Ron***, **B.-Y. Lee[†]**, **G. Hulata***, **T. D. Kocher[†]** and **E. Seroussi***

*Institute of Animal Science, Agricultural Research Organization, PO Box 6, Bet-Dagan, 50250, Israel. [†]Hubbard Center for Genome Studies, University of New Hampshire, 35 Colovos Road, Durham, NH 03824, USA

Accepted 15 September 2001

Description: Transferrin is an iron binding glycoprotein that plays a central role in the transport of iron between sites of

Figure 1 Part of the transferrin gene in tilapia (solid line marks the exons and dashed line marks the introns). The arrows mark the primers used in this study.



absorption, storage and utilization in all vertebrate organisms. In animals, transferrin exhibits a high degree of polymorphism and is used for evolutionary and population studies. In fish, transferrin has an important role in the innate immune system. Differential disease resistance was correlated to allozyme genotypes and the level of transferrin in blood is used as a health parameter¹.

Tilapias (*Oreochromis* spp., family: Cichlidae, order: Perciformes) are common food fish bred in many parts of the world. The sequence of the transferrin gene is not known in these species or in any other perciform fish. Hence mapping of this gene may be important for genetic improvement for disease resistance in tilapia.

We searched the GenBank database for known sequences for the transferrin gene in fish from three different orders: bastard halibut (*Paralichthys olivaceus*) of the order Pleuronectiformes, Pacific salmon (*Oncorhynchus kisutch*) of the order Salmoniformes and Japanese medaka (*Oryzias latipes*) of the order Belontiiformes (GenBank accession numbers D88801, D89084 and D64033, respectively). The sequences were compared using the GAP4 software² and conserved regions were identified. Polymerase chain reaction (PCR) primers (ex7trF and ex10trR) were designed in the conserved regions of exon 7 and 10.

PCR conditions: Amplification reactions were performed in a 10- μ l reaction volume containing PCR buffer with 2 mM MgCl₂, 1 U *Taq* DNA polymerase (Quantum Biotechnologies, Inc.), 187.5 μ M each dNTP, 1 mM tetramethylammonium (TMAC), 5 μ M each primer and 60 ng of genomic DNA. The amplification conditions were as follows: 92 °C for 40 s, 60 °C for 40 s, 72 °C for 1 min, for 30 cycles.

Sequencing and identification of length polymorphism: Deoxyribonucleic acid (DNA) from *Oreochromis mossambicus* and *O. aureus* was PCR amplified. Products were purified from agarose gel (1%) using High Pure PCR Product Purification Kit (Roche, Mannheim, Germany). The sequence reactions were conducted using the Big-DyeTM Terminator Cycle Sequencing kit (Applied Biosystems) and a reverse or forward PCR primer. The sequences were recorded with an automated DNA sequencer (ABI 377, Applied Biosystems). The sequences were placed in the EMBL database under the accession numbers AJ312311, AJ318861 and AJ318862. Blast analysis using sequence from exon 7 to exon 10 as a query, revealed homology to the Japanese medaka transferrin. Highest identity (82%) was in exon 8 (168 bp). Sequence similarity enabled determination of exon-intron borders for these four exons in tilapia (Fig. 1). BlastP analysis using the amino acid sequence encoded by these four exons revealed 72% identity and 85% similarity to the Japanese medaka transferrin. Length polymorphism between

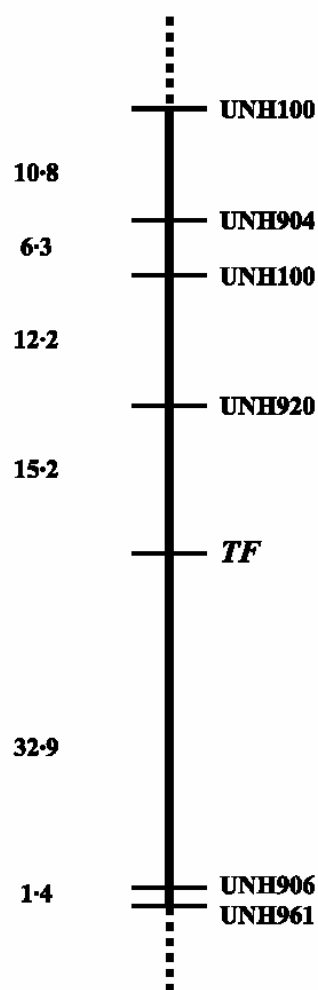


Figure 2 Map location of the transferrin gene (*TF*) in linkage group 21, with the intermap distances (in Kosambi cM, log₁₀_like = -190.82) between the surrounding microsatellites.

the two species was found in intron 9. To facilitate analysis of this polymorphic site, an additional primer was designed (ex9trF).

Primer sequences:

ex7trF: 5'- CTGCTGTGCAAGGATAACACC -3'

ex9trF: 5'- CCATTAGGTGGTGTGCTGTG -3'

ex10trR: 5'- GAACCAGACCACACTTCCAG -3'

Polymorphism and Mendelian inheritance: The PCR products of primers ex9trF and ex10trR were separated in acrylamide gel (4%). Genotypes were determined by automated sizing of PCR fragments using an ABI 377 automated sequencer (Applied Biosystems) and Genescan (version 3.1) and Genotyper (version 2.0) software (Applied Biosystems). A family, consisting *O. aureus* female homozygous to a 320-bp allele and *O. niloticus* male homozygous to a 308-bp allele, two heterozygous F_1 and their 129 F_2 offspring was genotyped. This family serves as a reference for the tilapia linkage mapping and was already genotyped for 505 polymorphic microsatellite markers³.

Linkage mapping: Linkage analysis was performed using Crimap (version 2.4) hosted at the Sanger Center. Preliminary localization was performed with the two-point command with a LOD of 3.0. Final ordering of the linkage group was performed using Build and confirmed by Flips. Transferrin was found linked to marker UNH920 in linkage group 21 (Fig. 2).

The transferrin gene sequence with the primer used in this study. The sequence in the database does not include the external primers.

ctgctgtgcaaggataaacaccAGAGCACCTATTGACAACCTATGAA
 ACCTGCTCCCTGGCCAGAGTGCCAGCTCACGCTGTTGTTACTC
 GCAAGGATCCACAGCTGGCCGACTTTATCTGGGAGACCCTTC
 ACCGAGTTCAGACTGACCACGTAGGAAGTCCACTGAATAAC
 ATCTCATTTTCAGCGTATTCTGATTCCTTGTGTTTCAGCAGCCT
 æAGAGCACCTATTGACAACCTATGAAACCTGCTCCCTGGCCA
 GAGTGCCAGCTCACGCTGTTGTTACTCGCAAGGATCCACAGC
 TGGCCGACTTTAGAGCACCTATTGACAACCTATGAAACCTGCT
 CCCTGGCCAGAGTGCCAGCTCACGCTGTTGTTACTCGCAAGG
 ATCCACAGCTGGCCGACTTTATCTGGGAGACCCTTCACCGAG
 TTCAGACTGACCACGTAGGAAGTCCACTGAATAACATCTCA
 TTTCAGCGTATTCTGATTCCTTGTGTTTCAGCAGCCTæAGAG
 CACCTATTGACCATTAGGTGGTGTGCTGTGGCCATGCTGAG
 ACCAAGGGCAAGTGTGACACGTGGAGCATCAGCAGTGTGTCT
 GGGGACGGGGTCAACACCTCCATTGAATGCCAGAGCGCCTCT
 ACAGTTGAAGAGTGCCTGAAGAAGATTATGGTAAACTCTGG
 AGGAAATATCAAAATCTTTATTTTATTTTATTTTATTTATTTA
 TTTTTTGCTCCACT\$GGCCATGCTGAGACCAAGGGCAAGTGTG
 ACACGTGGAGCATCAGCAGTGTGTCTGGGGACGGGGTCAACA
 CCTCCATTGAATctggaaagtgtggtctggttc

Acknowledgements This study was supported by the Israeli Science Foundation (Grant no. 418/99-1).

References

- 1 Yano T. (1996) In: *The Fish Immune System*. (Eds by G. Iwama & T. Nakanishi), Academic Press, San Diego, CA, USA.
- 2 Staden R. *et al.* (2000). *Methods Mol Biol* **132**, 115.
- 3 Lee B.-Y. *et al.* (2001) *Abstracts of the Plant & Animal Genome IX Conference*. W616, p. 215. San Diego, CA, USA.

Correspondence: A. Cnaani (e-mail: cnaani@agri.huji.ac.il)