Mapping the transferrin gene in tilapia

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Accepted 15 September 2001

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

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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 allelic genotypes and the level of transferrin in blood is used as a health parameter.

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: baldsail halibut (Paralichthys olivaceus) of the order Pleuronectiformes, Pacific salmon (Oncorhynchus kisutch) of the order Salmoniformes and Japanese medaka (Oryzias latipes) of the order Beloniformes (GenBank accession numbers D88801, D89084 and D64033, respectively). The sequences were compared using the GAP4 software 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-μl reaction volume containing PCR buffer with 2 mM MgCl₂, 1 U Taq DNA polymerase (Quantum Biotechnologies, Inc.), 187.5 μM each dNTP, 1 mM tetramethylammonium (TMAC), 5 μM 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-Dye™ Terminator Cycle Sequencing kit (Applied Biosystems) and a reverse or forward PCR primer. 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 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'-CTGCTTGTGAGATACACAC-3'
- ex9trF: 5'-CCATATGGGTGTGCGTGG-3'
- ex10trR: 5'-GAACGAGCCACACTTCCAG-3'
Polymorphism and Mendelian inheritance: The PCR products of
primers \textit{ex9tr} and \textit{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 (ver-
sion 2.0) software (Applied Biosystems). A family, consisting
of a female homozygous to a 320-bp allele and a male homozy-
gous to a 308-bp allele, two heterozygous \textit{F}_1 and
their 129 \textit{F}_2 offspring was genotyped. This family serves as a
reference for the tilapia linkage mapping and was already
genotyped for 505 polymorphic microsatellite markers\textsuperscript{3}.

Linkage mapping: Linkage analysis was performed using Cimap
(version 2.4) hosted at the Sanger Center. Preliminary local-
ization 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.

\begin{verbatim}
cctctggcgaagcttaccaAGACCACTATTGACAACTATGAA
ACCTGCTCCCTGCGCCAGTGCGCCAGTCTACGCGTGTCTACTC
GCAAGGATCCACGCTGCGGCGTCTTATCTGGGAGACCTTCC
ACCGAGTTCAGCAGCAACGATAGGAAGTGGCAAGTAAAC
ATCTCATTTCTGCATATTGTATCTCTCTCTGTTTTCAGACGCT
\alpha AGACACACTATTTGACAACTATGAAACCTGCTCTGCGGCA
GATGGCCAGCCTACGCTGTTTCTACTCGCAAAGATCCACACGC
TGGCGGCTTTAGACACCTATTGAGAAACTATGAAACCTGCT
CCCTGAGCCAGTGCGGCGTCTACGCGTGTCTACTCGCAAGG
ATCCACAGCTGCGCCAGTTTATCTGGGAGACCTTCCAGGCA
TTCTAGACCTGACAGTATGGGATGCTAGTCAGAAATACATTC
TTTCAGCTATTTCTGATTTTCTGTTTACACAGAGTAAGAG
ACCTATTACGCTATTAGGTGCTGACCAGCATGCTAGTAC
ACCAAGGGCAAGTGTGACAGTGGACATACAGCAGTGCTCT
GGGGAGGGGCGTCACTGCATTTAGAAGTCTGCGGAGCCAG
AGTGGGAAGTGTGGGAGAAGTGGTAAACTTGG
AGGAATATCTAAAATTCTTTATTTATTGAAAAATTATTTGAGA
TTTTTTTTCTCTCTGGGCCATCAGTGTAGAAGCCAAGGCACTG
ACACGTTGGAGCACTCAGCAGTGGTCTGCGGACGGGATCGCA
CCTCCATTGAGATGGGAACTACAGTGGTTCTGCT
\end{verbatim}

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

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