J. Fish. Sci. Tech.. 6(2), 101-104, 2003

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Notes

Cloning and Characterization of the Putative Transferrin Receptor cDNA from the Olive Flounder (*Paralichthys olivaceus*)

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A cDNA clone for the olive flounder (*Paralichthys olivaceus*) transferrin receptor (fTfR) was isolated from a leukocytes cDNA library. The fTfR gene consisted of 2,319 bp encoding 773 amino acid residues. The amino acid sequence alignment of the fTfR showed that their size and hydrophobic profile are similar. In addition, the Tyr-Thr-Arg-Phe (YTRF) motif that is the recognition signal for high-efficiency endocytosis, is conserved very well. This motif is important for functional properties of TfR. The deduced amino acid sequence had 42.4-42.9% identities with the previously reported TfRs of vertebrates. The fTfR was expressed in the blood, kidney, spleen, and liver of healthy olive flounder by the Northern blot hybridization.

Key words: Transferrin receptor, cDNA, Olive flounder, *Paralichthys olivaceus*, Cloning

Transferrin receptor (TfR) is homodimer membrane protein that plays a major role in cellular iron uptake through binding and internalizing a carrier protein tranferrin. This protein has been characterized from a number of species including human and chicken (Gerhardt et al., 1991; McClelland et al., 1984). In fish, TfR was not reported although transferrin (Tf) gene was reported from some species (Hirono et al., 1995; Kim et al., 1997; Lee et al., 1998). The aim of the present study is to clone and sequence the transferrin receptor cDNA of olive flounder and to detect its expression within fish tissues.

The cDNA library was constructed from the olive flounder leukocytes by λ ZAP II phage vectors system (Stratagene, USA). This library was screened using a partial cDNA fragment of a TfR homologue previously identified by an EST analysis (Nam et al., 2000) as a probe. It was isolated 5 positive plaques containing full-length TfR cDNA, and sequenced using ThermoSequenase (Amersham Pharmacia, USA) with M13. Each determined sequence was compared with all sequences available in the DDBJ/EMBL/GenBank using the BLAST ver. 2.0. Total RNA of the peripheral blood, brain, kidney, spleen, liver, muscle, and intestine were isolated by Trizol (GibcoBRL, USA), and Northern blot hybridization was done with the cloned cDNA fragment that had been labeled with [α-32P] dCTP using a random primer labeling kit (Takara, Japan).

The olive flounder transferrin receptor (fTfR) cDNA consisted of 3,158 bp encoding 773 amino acid residues (Fig. 1). This gene was organized into a small N-terminal cytoplasmic domain, a single transmembrane region, and a large C-terminal extracellular domain. The fTfR indicated a conservation of the YTRF (Tyr-Thr-Arg-Phe) motif structured for high-efficiency endocytosis, which is one of the important characteristics of the TfR (Collawn et al., 1993). Gironè et al. (1991) examined the effect of point mutations within the TfR cytoplasmic tail internalization domain (YTRF) on the rate of endocytosis and showed that most of these point mutations caused an inhibition of receptor internalization. The transmembrane region was composed of 21 amino acids between 78 and 99 of amino acid residues, which contained 17 hydrophobic amino acids. No other part of the sequence appeared to cross the membrane, and it can could support that the receptor crossed the lipid bilayer only once. Mammalian TfR include the three N-linked glycosylation that is the most important modification for the correct folding and transport of the protein to the cell surface (Hoe and Hunt, 1992). Although fTfR do not have the glycosylation of the last N-linked site in hTfR (Asn

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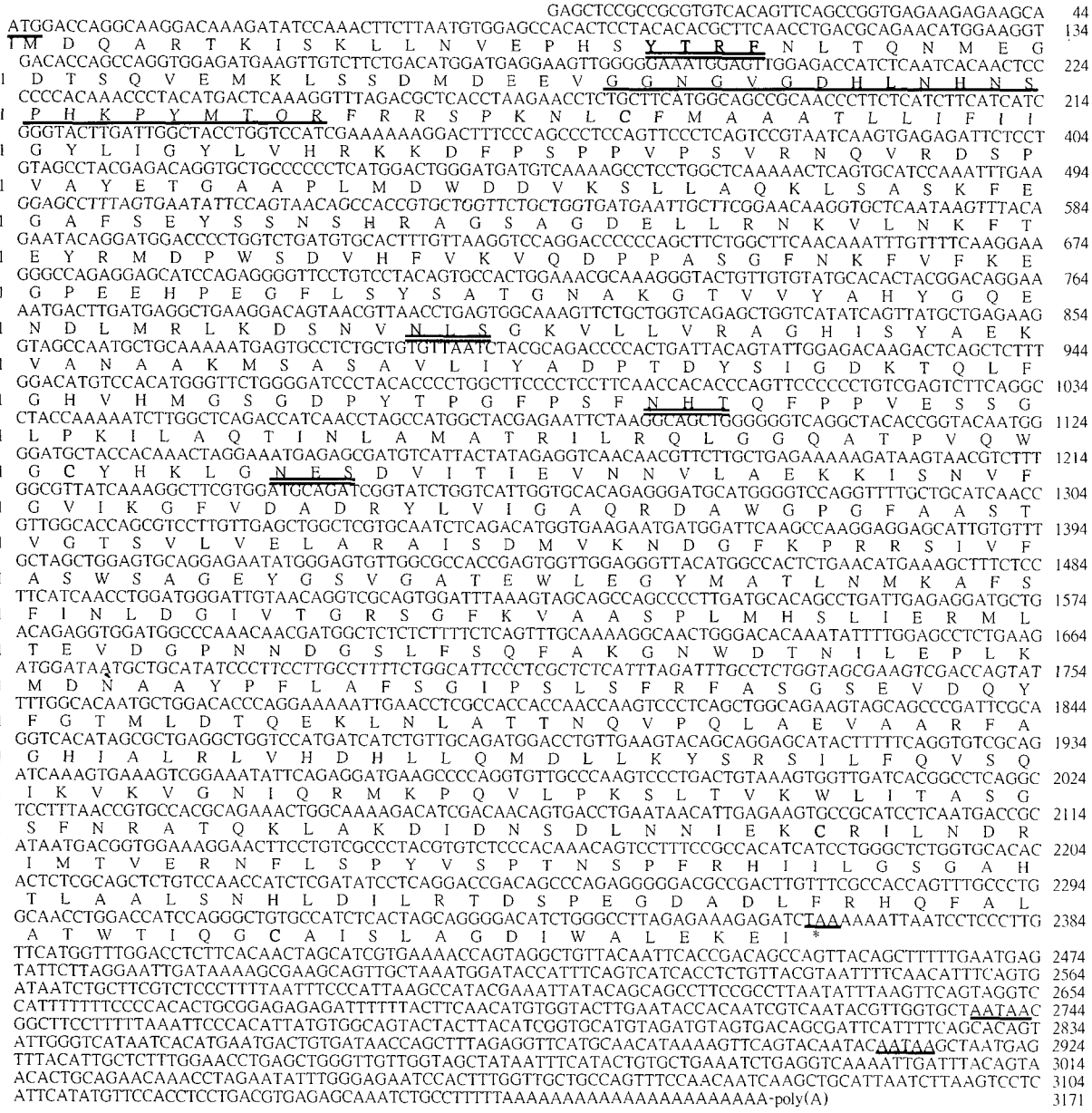

Fig. 1. Nucleotide sequences of the olive flounder, *Paralichthys olivaceus* transferrin receptor gene cDNA and its deduced amino acid residues. The putative transmembrane domain is singly overlined and potential N-linked glycosylation sites are underlined twice. Cys codons are marked with bold-face letter, and the internalization recognition signal is marked with a bold-face letters and underlined twice. The start codon ATG, stop codon TAA, and polyadenylation signal AATAA are a single underlined.

727) that is the most critical to the structure of the hTfR (Yang et al., 1993), the other two glycosylation sites are conserved in the fTfR. Four cysteine residues were distributed throughout the fTfR sequence. The fTfR did not conserve the Cys 89 and Cys 98 of the hTfR formed intermolecular disulfide bonds. However, mutant receptors in which these cysteine residues were altered to serine were transported normally to the cell surface and were functionally active (Jing and Trowbridge, 1987).

The fTfR shows 42.4-42.9% identities with other TfRs (Table 1). This percentage is very low but similar to the homology of cTfR with other mammalian TfR (50.6-52.3).

Northern blot hybridization analysis showed that the olive flounder TfR was expressed in the blood,

Table 1. Comparison of amino acid sequences from different species (%)*

	Olive flounder	Chicken	Chinese hamster	Mouse
Chicken	42.9			
Chinese hamster	42.5	52.3		
Mouse	42.4	50.6	83.3	
Human	42.5	51.7	78.5	76.8

*Percentage of identical amino acids was calculated using GENETYX-MAC program. References for the predicted amino acid sequences are as follows: cTfR (Gerhardt et al., 1991), chTfR (Collawn et al., 1993) mTfR (Grego et al., 1985), and hTfR (McClelland et al., 1984).

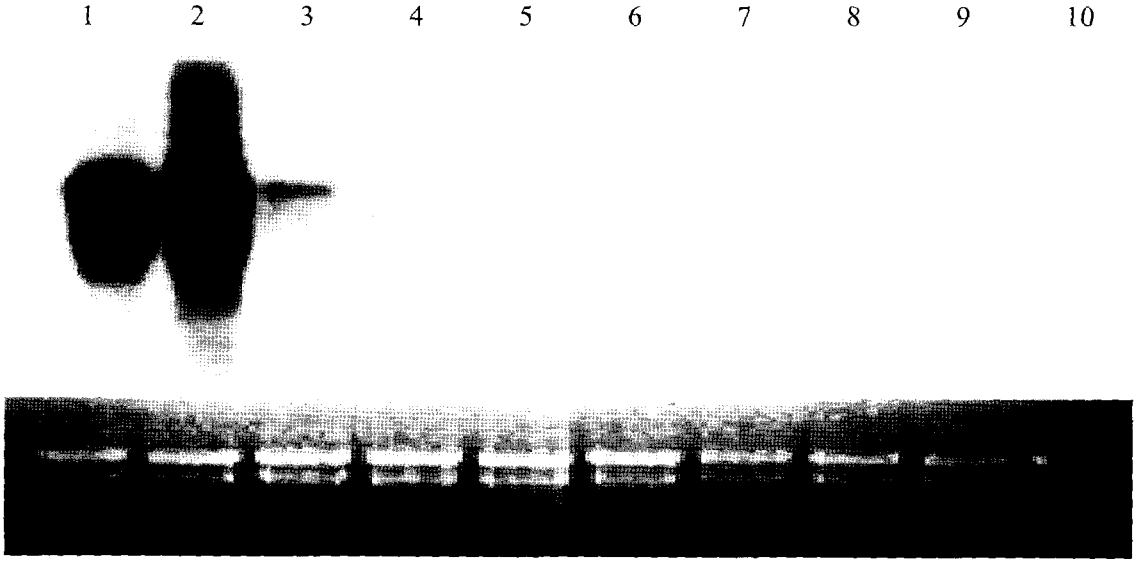

Fig. 2. Northern blot hybridization of the olive flounder, *Paralichthys olivaceus* transferrin receptor. Total RNA (20 μg) extracted from the blood (lane 1), kidney (lane 2), spleen (lane 3), intestine (lane 4), liver (lane 5), peritoneal cavity fluid (lane 6), skin mucus (lane 7), muscle (lane 8), brain (lane 9), and testis (lane 10) was electrophoresed in a 1% agarose gel, and transferred to a nylon membrane and hybridized with [^{32}P] labeled olive flounder transferrin receptor cDNA. Ribosomal RNA (28S and 18S) as a control for equivalent loading and RNA integrity are shown at the bottom.

kidney, spleen, and liver, but not in the testis, brain, muscle, mucus, peritoneal cavity fluid, and intestine (Fig. 2). Although all cell types have these transmembrane glycoproteins, most of them are revealed that the cells could express iron-uptake regulation during rapid proliferating such as stimulated T-cell and embryonic tissues (Hirata et al., 1986). Also, the TfR was highly expressed in maturing erythroid cells, trophoblasts, hepatocytes, Kupffer cell, and tissue macrophages (Iacopetta and Morgan, 1983; Testa and Morgan, 1993; Young and Hisen, 1981).

Acknowledgements

The authors thank to Professor T. Aoki of the Laboratory of Genetics and Biochemistry, Department of Aquatic Biosciences, Tokyo University of Fisheries, Japan for providing the first author (K.M. Won) with an opportunity to work on this theme.

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(Received March 2003, Accepted June 2003)