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Piscine (*Sparus aurata*) Transthyretin cDNA Cloning and Characterization^a

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Transthyretin (TTR) is one of the three plasma proteins that participate in the transport of thyroid hormones, thyroxine (T₄) and triiodothyronine (T₃); it is also involved in the carriage of retinol through the mediation of retinol-binding protein.¹ The liver and choroid plexus are the major sites of TTR synthesis in mammals, birds, and diprotodont marsupials; in reptiles, TTR is only synthesized in the choroid plexus.² cDNA coding for TTR has been cloned from several mammalian, reptilian, and avian species and is highly conserved especially in the domains involved in binding to thyroid hormones.³

TTR expression has never been reported in fish, in which albumin is considered to be the main carrier for T₃ and T₄.² We report the cloning of a TTR cDNA from sea bream (*Sparus aurata*) and demonstrate the presence of TTR mRNA in the liver of this fish.

CLONING AND CHARACTERIZATION OF A FULL-LENGTH SEA BREAM TTR

A full-length sea bream cDNA with high homology to previously isolated TTR cDNAs was cloned from a fish liver cDNA library constructed in Lambda-ZAP (Stratagene). The sequence obtained spans 630 bp and contains an open reading frame encoding a protein of 130 amino acids preceded by a presegment of 20 amino acids. The consensus AATAAA polyadenylation signal is located 120 bases downstream from the first in-frame stop codon. Comparison of the deduced amino acid sequence with some previously isolated TTR cDNAs demonstrated it is 47%, 47%, 54%, and 49% homologous to human,⁴ rat,⁵ chicken,⁶ and lizard,⁷ respectively. Although the overall homology is not very high, all the amino acids involved in the formation of the central channel of transthyretin, to which T₃ and T₄ bind, Lys 15, Leu 17, Glu 54, His 56, Thr 106, Ala 108, Leu 110, Ser 115, Thr 119, and Val 121⁸ are conserved except for Ser 117 which has had a conservative substitution by threonine.

Northern blot analysis (FIG. 1) revealed that this protein is encoded by a single transcript in the liver. No message could be detected in kidney and brain using the same amount of RNA.

To our knowledge, this is the first experimental evidence that mRNA encoding a protein that shares a high degree of similarity to mammalian, reptilian, and avian TTR is expressed in fish liver. The conservation of the binding sites for T₃ and T₄ suggests that it may be involved in the transportation of these hormones which are important for normal fish development. This is also an indication that TTR expres-

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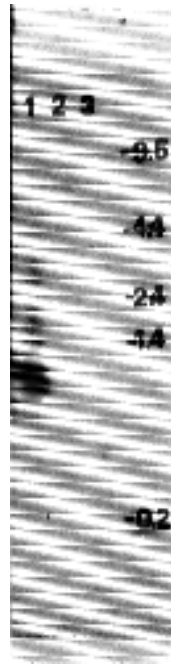


FIGURE 1. Thirty micrograms of total RNA from liver, kidney, and brain were separated on an agarose/MOPS/formaldehyde gel and blotted onto a nylon membrane. This filter was hybridized overnight at 55°C with a full-length sea bream transthyretin cDNA labeled with [³²P] dCTP, washed under high stringency conditions, and autoradiographed. *Lane 1: liver; lane 2: brain; lane 3: kidney.* Kb markers are indicated on the *right hand side*.

sion in vertebrate liver occurred long before that in the choroid plexus. Further studies to elucidate the function of this protein in fish are underway.

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