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Short communication

Cloning and expression pattern of peroxisome proliferator-activated receptor α in the thicklip grey mullet *Chelon labrosus*

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Abstract

Aquatic organisms living in coastal and estuarine areas are exposed to diverse contaminants which can cause peroxisome proliferation. Peroxisome proliferators are agonists of peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily. We have recently demonstrated expression of the three PPAR isoforms in liver of mullet *Chelon labrosus* and other fish species by immunohistochemistry. The goal of the present study was first to clone PPARα and second to investigate its expression pattern in various tissues of mullet. PCR-based screening of mullet cDNA with PPARα specific degenerate primers resulted in amplification, subcloning and sequencing of a 1090 bp cDNA fragment (AY618315) that encodes mullet PPARα and exhibits highest amino acid identity to fish *Sparus aurata* PPARα (90%). Semi-quantitative RT-PCR was used to characterize the expression of PPARα in brain, muscle, liver, spleen, gill, heart and female gonad of juvenile and adult male and female mullet. For this, mullet 18S-rRNA (AY825252), β-actin (AY836368) and elongation factor α (AY836369) were cloned and used as internal reference for RT-PCR. Expression of PPARα was detected in all tissues, was highest in liver and lowest in adult male and female muscle.

Keywords: PPARα; Gene expression; Peroxisome proliferation; *Chelon labrosus*

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Genes belonging to the nuclear hormone receptor (NR) superfamily regulate on–off switches for transcription of genes implicated in fundamental biological processes in metazoans (Owen and Zelent, 2000). Of special interest are the NRs involved in regulation of peroxisome proliferation as both laboratory and field studies have shown that diverse organic contaminants provoke peroxisome proliferation in aquatic organisms (Cajaraville et al., 2003). In vertebrates, peroxisome-proliferator activated receptor (PPAR)α is the key transcription factor involved in peroxisome proliferation. PPARs are activated by fatty acids and their eicosanoid metabolites and upon heterodimerization with the retinoid X receptor (RXR), they bind to specific peroxisome proliferator response elements (PPRE) of numerous target genes. These genes, like the ones of the peroxisomal β-oxidation pathway, are mainly involved in lipid homeostasis (Forman et al., 1997).

To date, PPARs have been identified and cloned in a wide range of vertebrates including fish species. Recently, we reported expression of the three PPAR isotypes in the liver of thicklip grey mullet *Chelon labrosus* by immunohistochemistry (Ibabe et al., 2004). Mullets are widely distributed in European coastal areas, can survive highly polluted environments and thus could be used as sentinel of environmental pollution.

In order to gain knowledge about the molecular regulation of peroxisome proliferation in aquatic animals, we report the cloning of a fragment of PPARα in *C. labrosus* and its expression in different tissues of juvenile and adult male and female fish.

Mullets, *C. labrosus*, were collected in the leisure harbour of Arriluze, Bay of Biscay (43°20′N, 003°01′W) in December 2004. Total RNA was isolated (GibcoBRL TRIzol Reagent) and cDNA was obtained (SuperScript™ First-Strand Synthesis System, Invitrogen) from 1 μg of RNA from brain, muscle, liver, spleen, gill, heart and female gonad dissected from juveniles and mature male and female mullets.

cDNAs were PCR amplified (PCR procedure for targets up to 4 kb with Taq DNA Polymerase, Invitrogen) using degenerate primers designed to amplify conserved regions of PPARα from teleostei and other phyla. A short 283 bp fragment was first cloned using 5′-GATGGAGCCCAAGTTRCAG and 5′-CTTGATTTCCTGCACSAGC; then the 5′-end was extended using 5′-GCTTCAGGCTTCCACTACGG and 5′-TGCTCCGTGACCAGCTCTCG and the 3′-end using 5′-TTGCCACGCGCTTCAACTCT and 5′-TCAGTACATGTCYCTGTA. Non-degenerate primers were used to clone the housekeeping genes β-actin (5′-CTGGCATCACACCTTCTACAAC and 5′-AGCTCGTAGCTCTTCTCCAGG), 18S-rRNA (5′-GATGGTACTTTCTGTGCCTAC and 5′-GAGCTATCAATCTGTCAATCCT) and elongation factor 1-α (5′-GAGCGTGAGCGTGGTATCAC and 5′-GTGGAGTCCATCTTGTTGAC). Amplified fragments were visualized by 1.5% agarose gel electrophoresis, ligated to TOPO vector (Invitrogen), and automatically sequenced using vector primers.

Semi-quantitative RT-PCRs were run for 1 μg total RNA of each tissue, by monoplex-PCR using the Taq DNA polymerase (Invitrogen). All PCR primers were chosen within the sequences previously obtained to generate specific products around 200 bp; 5′-CTGGACGACAGCGACCTT and 5′-GCTCTCGGAGGTCAGCCAGT were used to amplify the PPARα gene, 5′-CAGATCATGTTCGAGACCTT and 5′-CATGAGGTAGTCTGTGAGGT for β-actin, 5′-CACATCCAAGGAAGGCAGCA and 5′-AAGATACGCTATTGGAGCTG for 18S-rRNA, and 5′-CAGGGATTTCATCAAGAACA and 5′-GTCCATCTTGTTGACACCA for EF1-α. Temperature cycling was performed for all housekeeping genes as follows: 1 cycle at 94 °C for 2 min, 30 cycles at 94, 60 and 68 °C for 30 s each time, followed by 68 °C for 8 min. For PPARα, 1 cycle at