

Q5RS-2000-30360 (fPPARs)

Cloning and functional analysis of fish peroxisome proliferators-activated receptors: The transcriptional control of lipid metabolism in farmed fish species.

The aim of the fPPARs project was to provide insight into the molecular mechanisms involved in the lipid and fatty acid metabolism in fish. This was to be achieved by the identification and study of the peroxisome proliferators-activated receptors (PPARs) in sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), plaice (*Pleuronectes platessa*), and Atlantic salmon (*Salmo salar*). PPARs are ligand-inducible transcription factors belonging to the nuclear hormone receptor superfamily. Three PPAR isotypes have been identified in mammals, birds and amphibians, termed PPAR α , PPAR γ and PPAR β or δ . Each isotype is a product of a separate gene and each one has a distinct tissue distribution. PPARs were originally identified and named as receptors that are activated by a diverse range of chemicals termed peroxisome proliferators. Subsequent work has led to the identification of various natural and synthetic PPAR ligands that include a number of unsaturated fatty acids, eicosanoids, hypolipidemic and antidiabetic drugs. The ligand binding properties of these transcription factors, along with their demonstrated function in the control of expression of key enzymes of fatty acid metabolism, resulted in their recognition as critical regulators of lipid homeostasis in mammals.

The possibility that PPARs fulfill similar functions in fish as they do in mammals implied that these receptors could serve as prime targets for nutritional/diet-based interventions to manipulate fatty acid and lipid metabolism in farmed fish species. Thus, important issues in finfish aquaculture nutrition, such as the fat accumulation in the tissues of farmed fish as well as the metabolism of n3-HUFAs in fish, could potentially be addressed through the study of PPARs in these species.

In addition to the identification and characterization of the PPAR genes and cDNAs from the fish species of interest, the fPPARs project also aimed to study the functional properties of the receptors, including tissue expression profile, DNA and ligand binding, and transcriptional activation. Furthermore, we aimed to develop appropriate *in vitro* assays, to clearly establish the role of PPARs in lipid metabolism in fish, as well as to examine *in vivo* the effect of induced PPAR expression and PPAR-dependent transcriptional activation on fat accumulation and lipid composition in fish tissues.

Our efforts resulted in the identification of genes and corresponding cDNAs encoding homologues of the three mammalian PPAR isotypes in all four species of fish that we examined. In fact, our study demonstrated a far more complex level of variety in PPAR isotype in teleosts than that observed in higher vertebrates. Thus, in sea bream, in addition to the PPAR α , PPAR β and PPAR γ homologues, we have identified a gene and its corresponding cDNA encoding a second form (isoform) of PPAR α . In Atlantic salmon we have identified a total of four genomic sequences potentially encoding distinct PPAR β isoforms. However, we have succeeded in identifying complete cDNA sequences corresponding to only two of these loci. Our analysis, in conjunction with the complete genome data available for *Fugu rubripes*, *Tetraodon nigroviridis*, and *Danio rerio*, allow us to conclude that all fish species harbor two PPAR α genes and a single PPAR γ gene in their genome. The percomorpha species appear to contain a single PPAR β gene, while in Atlantic salmon at least two functional PPAR β genes exist.

As far as the DNA binding properties of the receptors are concerned, we have demonstrated that, like their mammalian homologues, fish PPARs heterodimerize with the retinoid-X receptor (RXR) and bind to PPAR response elements (PPREs) of both mammalian and piscine origin. In the percomorpha fish species studied both the PPAR α and PPAR β isotypes exhibit tissue expression profile and transactivation properties similar to their mammalian counterparts. Of interest is the wide tissue distribution of PPAR γ in all four fish species, a profile that contrasts with the rather restricted tissue expression pattern of its mammalian homologue. Also notable is the fact that the piscine PPAR γ apparently fails to bind and consequently is not activated by highly specific mammalian PPAR γ ligands. We have proposed that this discrepancy results from the non-conservation in the fish receptor of amino acid residues implicated in interactions with the ligand and/or in interactions with co-activators, as these have been demonstrated by crystallographic studies of the mammalian PPAR γ . Of the two PPAR β isoforms identified in Atlantic salmon one, PPAR β 1, appears to share the transactivation properties of its percomorpha and mammalian homologue. On the other hand, the Atlantic salmon PPAR β 2 fails to be activated by known PPAR β ligands, while it suppresses the activity of PPAR β 1 when the two isoforms are co-transfected in cultured cell lines.

In sea bream we have studied the effects of fasting and feeding on the expression of the three PPAR isotypes in various tissues. Diet-based interventions (substitution of fish oils by vegetable oils in fish feed, supplementation of feed with conjugated linoleic acid) were tested on both sea bream and Atlantic salmon to examine potential effects on PPAR expression and fatty acid and lipid metabolism in various tissues of the fish. In parallel, the effect of these treatments on the expression of several presumed PPAR target genes [carnitine palmitoyltransferase I (CPT I), fatty acid elongase, Δ 6 fatty acid desaturase, glutathione-S transferase (GST)] was assessed. The results of the above analyses revealed that qualitative and quantitative aspects of the diet can have significant effects at the gene expression level of PPARs and consequently of PPAR target genes.

In addition to the PPAR genes and cDNAs, the project also resulted in the identification and molecular characterization of several other cDNAs and genes from the four species of interest. Thus, the CPT I cDNA was isolated from sea bream and Atlantic salmon, the GST cDNA was isolated from sea bream, the RXR α gene and cDNA was isolated from plaice, the α -amylase cDNA was isolated from sea bream and sea bass, and the acyl-CoA oxidase cDNA was isolated from sea bass.

In summary, the results of this project provide the molecular background for future in-depth studies of the transcriptional control of lipid metabolism in fish species.

References

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