

## Cloning of CYP4F7, a Kidney-Specific P450 in the Sea Bass *Dicentrarchus labrax*

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**A cDNA sequence coding for a cytochrome P450 of the CYP4F subfamily was isolated from total RNA of sea bass kidney by rapid amplification of cDNA ends. The full length sequence coded for a protein of 526 amino acids. The amino acid sequence shared 39% to 56% residue identities with the mammalian CYP4F sequences, and thus was named CYP4F7 (accession number AF123541). RNA blot analysis using CYP4F7 cDNA as a probe indicated that the corresponding mRNA was only detected in kidney. Expression in the kidney was constitutive, and no induction of this mRNA was detected in this or other tissues, with any of the inducers tested, including peroxisome proliferators.** © 1999 Academic Press

**Key Words:** CYP4 family; cytochrome P450; *Dicentrarchus labrax*; kidney; molecular cloning.

Members of the cytochrome P450 family CYP4 are found in a wide variety of organisms and tissues. The role of mammalian CYP4 enzymes in  $\omega$ - and ( $\omega$ -1)-hydroxylation of fatty acids and prostaglandins is well established (1-2). Most of them are expressed preferentially in liver and kidney. Their expression is regulated both by endogenous substances, such as hormones (3, 4), and by some xenobiotics, the peroxisome proliferators in particular (5). The peroxisome proliferators include compounds with no obvious structural similarity: hypolipidaemic drugs (such as clofibrate), industrial plasticizers (such as di-2-ethylhexylphthalate), herbicides (such as chlorophenoxyacetic acid), insecticides (such as dimethrin), anti-fungal agents (such as bifonazole), wood preservatives (such as chlorophenolates), and several other industrial chemicals (6).

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Abbreviations used: 2,4-D, 2,4-dichlorophenoxy acetic acid; CYP, cytochrome P450; DEHP, di(2-ethylhexyl) phthalate; DIG, digoxigenin; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; RACE-PCR, rapid amplification of cDNA ends-polymerase chain reaction.

To our knowledge, members of the CYP4F subfamily have been identified only in rats and humans. Purification or expression of CYP4F2 (7) and CYP4F3 (8) in heterologous systems have demonstrated that LTB<sub>4</sub>  $\omega$ -hydroxylation was specifically catalysed by these enzymes. The metabolic role of the other CYP4F enzymes is still unknown. LTB<sub>4</sub>, which is derived from arachidonic acid, acts as a mediator of inflammation through the activation of polymorphonuclear leukocytes (9). The catabolism of LTB<sub>4</sub> and thus its inactivation leads to stop the inflammation process. The  $\omega$ -hydroxylation catalysed by CYP4F isoforms is the first step in the degradation of LTB<sub>4</sub>, which is further catabolized by  $\beta$ -oxidation in peroxisomes (10). As LTB<sub>4</sub> degradation occurs primarily in neutrophils or in liver, LTB<sub>4</sub>  $\omega$ -hydroxylases have been isolated from these tissues. Thus, CYP4F2 is highly expressed in human liver (11), and CYP4F3 in neutrophils (8).

Several P450 enzymes have been described in fish (12, 13). P450 from families CYP1, 2, 3, 11, 17, and 19 were isolated. Most of them have been purified or cloned from the rainbow trout, *Oncorhynchus mykiss* (13). As CYP1A enzyme activities or mRNA concentrations are used as a biomarker to estimate xenobiotic exposure in aquatic environments, CYP1A isoforms have been cloned in numerous freshwater and marine fishes (7 various species). Cytochromes P450 from the family 4 were isolated from rainbow trout and sea bass (14, 15). Their physiological role is still unknown. The relative tissue distribution and regulation of the CYP4T2 isoform cloned from the sea bass, *Dicentrarchus labrax*, present original features as compared to mammals. This gene is predominantly expressed in kidney and small intestine, and is induced by DEHP and 2,4-D but not by clofibrate (15).

We report here the molecular cloning of another CYP4 cDNA sequence from kidney of sea bass. Surprisingly the deduced amino acid sequence shared from 39% to 56% identity with the mammalian CYP4F sequences, and thus this gene was named CYP4F7. We



have investigated the tissue localization of this gene and its induction features by various xenobiotics, including peroxisome proliferators.

## MATERIAL AND METHODS

**Chemicals.** Clofibrate, DEHP, 2,4-D, benzo[a]pyrene, prochloraz, and all other chemicals were purchased from Sigma-Aldrich Chimie (France). Restriction enzymes were from Roche Diagnostics (Germany).

**Animals.** Sexually immature sea bass (*Dicentrarchus labrax*, 100-200 g) were obtained from the fish farm "Cannes Aquaculture" (France) and placed in aquaria containing 120 l of artificial sea water (28‰ salinity) at 18°C. Fish were acclimated for at least 2 weeks to reduce possible stress-related responses. Each group of 5 fishes was treated daily for 3 days by intraperitoneal injection of clofibrate (100 mg/kg body weight/day), DEHP (1.5 g/kg body weight/day), 2,4-D (20 mg/kg body weight/day), prochloraz (20 mg/kg body weight/day), benzo[a]pyrene (10 mg/kg body weight/day), or NaCl 9‰ (control).

**Poly (A)<sup>+</sup> mRNA isolation.** Total RNAs from DEHP-treated sea bass kidney were extracted by the guanidine thiocyanate method as described by Chomczynski and Sacchi (16). Poly (A)<sup>+</sup> mRNAs were isolated by affinity chromatography on an oligo(dT)-cellulose column as described in Sambrook *et al.* (17).

**Molecular cloning of CYP4F7.** Isolation of 5' and 3' ends of the gene was performed using the Marathon cDNA Amplification Kit (Clontech, USA). A library of adaptor-ligated double stranded cDNA was constructed from kidney poly (A)<sup>+</sup> mRNAs according to the manufacturer's instructions. The 3'-end fragment (857 bp) was obtained by RACE-PCR using a primer (3'-EGAD) designed from the "laurate box" of the CYP4T2 gene (15). The sequence of the primers used to clone the 3'-end were "3'-EGAD" (sense, 5'-GGGCCATGACACCACAGCCAGTGCG-3') and "AP1" (antisense, 5'-GCCCTATAGTGAGTCGTATTAGGATGG-3'). A CYP4F7 gene specific primer (5'-NWEDL) was designed from the 3' cDNA fragment previously cloned to generate the 5' fragment. The sequence of the primers used to clone the 5'-end were "AP1" (sense, 5'-CCATCCTAATACGACTCACTATAGGGC-3') and "5'-NWEDL" (antisense, 5'-GGGAAGGTTAGACAGATCCTCCAC-3'). 3' and 5' RACE-PCR reactions were performed using the Advantage klenTaq Polymerase Mix (Clontech, USA) on a ThermojeT thermal cycler (Eurogentec, Belgium) with the following program: 94°C for 1 min, 30 cycles of 94°C, 30 sec, and 68°C, 4 min, followed by a final 10 min extension at 68°C. Fragments of expected size were purified on a agarose gel then subcloned by inserting into the EcoRI site of the plasmid vector pCR 2.1 (Invitrogen, The Netherlands). Plasmid DNA from the clones of interest was purified using the QIAprep Spin Miniprep Kit (Qiagen Inc., USA) before sequencing.

**DNA sequencing.** cDNA sequencing was performed by Genome Express (Grenoble, France). cDNA sequencing was done by Dye Deoxy Terminator Cycle Sequencing (Applied Biosystems, Weiterstadt, Germany) according to the manufacturer's instructions. The reactions were performed on a GeneAmp 9600 thermal cycler (Perkin Elmer/Cetus), and were primed with universal primers (M13 reverse primer and M13 forward (-20) primer). Sequences were analysed on the Applied Biosystems DNA Sequencing System (model 373A).

**DNA sequence analysis.** The nucleotide sequence obtained was compared to the EMBL and GenBank databases using the BLAST 2.0 program (18). This cDNA sequence was then aligned with the human CYP4F2 (U02388) and CYP4F3 (Q08477), and the rat CYP4F1 (M94548), CYP4F4 (U39206), CYP4F5 (U39207), and CYP4F6 (U39208) sequences using the CLUSTAL X program (version 1.64b, 02/1998). The CYP4F7 sequence was also compared to all other full length CYP4 sequences available in GenBank. Sequences were retrieved using GenBank accession numbers from the D. Nel-

son web site (19). A phylogenetic tree was constructed with the distance-matrix method using the Clustal X program.

**Northern blot analysis.** Total RNAs from sea bass tissues were separated by electrophoresis on a 1.2% denaturing agarose gel, transferred to nylon membranes (Roche Diagnostics), and UV cross-linked. Digoxigenin (DIG)-labeled CYP4F7 probes (3', 5'-cDNA fragments, or full length sequence) was prepared by PCR using the specific required primers, and the DIG DNA Labeling Mix (Roche Diagnostics). A cDNA encoding for the sea bass ribosomal protein rpl 17A (Sabourault, unpublished results; GenBank AF 074720) was used as an internal standard for normalizing the quantity of mRNA on the gel (15). A DIG-labeled rpl 17 cDNA probe was prepared using the same procedure. Prehybridization and hybridization (14-16 h at 50°C), washes (at 65°C), and chemiluminescent detection were performed according to the manufacturer's instructions of the DIG system (20, 21).

The resulting films were scanned and analysed using the NIH Image 1.62 software (available by anonymous FTP from zippy.nimh.nih.gov). The integrated values were normalized with rpl 17 mRNA. Various exposure times were needed to avoid saturation of the signal due to rpl 17 mRNA, and therefore aberrant results in densitometry.

## RESULTS AND DISCUSSION

Several clones were obtained during isolation of the CYP4T2 cDNA by 3' RACE-PCR reactions (15). Among these clones was the CYP4T2 3'-fragment, and a new CYP4 sequence, which has been named CYP4F7 (accession number AF123541) by Dr. D. Nelson (P450 Gene Superfamily Nomenclature Committee). The sequence of this new cDNA revealed that the 3'-primer (positions 1062-1086) covered a very homologous region between the 2 genes (15). The other part of the CYP4F7 sequence was obtained by RACE-PCR using a 5'-gene specific primer (positions 1191-1215). The full length sequence (1917 bp) of this new CYP4 gene contained an open reading frame (positions 82-1659) followed by a TGA termination codon (positions 1660-1663) and a 3'-untranslated sequence of 255 bp (Fig. 1). This open reading frame encoded a protein of 526 amino acids with an deduced molecular weight of 60,326.

This sequence was compared to other CYP4F sequences. The sea bass CYP4F7 predicted protein sequence shared 54% amino acid identity with the human CYP4F2. The alignment of all CYP4F amino acid sequences showed several domains that are conserved among human, rat, and sea bass sequences (Fig. 2). The 5 regions of highest conservation are found at positions 150-162, 199-208, 319-334, 368-389, and 456-473. The region [456-473] contains the residues surrounding the heme-binding cysteine (position 467) conserved in all P450s. The region [319-339] may correspond to the "laurate box" initially described by Bradfield *et al.* (22), which is found exclusively in CYP4 sequences. There are however 3 differences in this 13-amino acid peptide. Residue 325 is Ala in all CYP4F sequences, but is Val in all other CYP4. Residue 326 is Asn in sea bass CYP4F7 but is Asp in all other CYP4,



				gcc	cgg	gca	ggt	cag	cag	gct	aac	cgt	cga	gca	acc	tgg	acc	tct	gga	48
ttc	cca	tta	atc	tca	ctc	ttt	ttc	tgt	ctc	agg	<u>atg</u>	cct	ctc	ctc	cag	ggt	gtc		102	
											M	P	L	L	Q	G	V		7	
ctc	tct	cac	atc	ctc	agc	tgg	aca	ggc	ctc	tgt	cag	gtc	ctg	ttt	gtg	gtc	tgg		156	
L	S	H	I	L	S	W	T	G	L	C	Q	V	L	F	V	V	W		25	
aca	tta	ctg	gga	gct	gtg	gtt	gta	gtg	tgg	acg	gcg	aag	ctg	ctg	gtg	cga	cac		210	
T	L	L	G	A	V	V	V	V	W	T	A	K	L	L	V	R	H		43	
gtc	tgg	tac	aca	cac	agg	ctg	tcc	tgc	ttc	aac	aaa	cca	cat	gca	aac	tcc	tgg		264	
V	W	Y	T	H	R	L	S	C	F	N	K	P	H	A	N	S	W		61	
ctt	ttt	ggc	cac	ctg	ggc	cag	atg	cag	agc	aca	gaa	gaa	ggc	ctc	cag	cat	gtg		318	
L	F	G	H	L	G	Q	M	Q	S	T	E	E	G	L	Q	H	V		79	
gat	gag	ttg	gtg	cag	acg	tac	aag	cac	tct	tgc	agc	tgg	ttc	ctc	ggc	cct	ttc		372	
D	E	L	V	Q	T	Y	K	H	S	C	S	W	F	L	G	P	F		97	
tac	cac	ctg	gtc	aga	ctc	ttc	cac	cgc	gac	tac	gtc	aaa	cct	ctg	cta	atg	gca		426	
Y	H	L	V	R	L	F	H	P	D	Y	V	K	P	L	L	M	A		115	
cct	gcc	agc	att	aca	gta	aaa	gat	gag	ctc	atc	tat	ggc	cat	cta	cgt	cca	tgg		480	
P	A	S	I	T	V	K	D	E	L	I	Y	G	H	L	R	P	W		133	
ctt	gga	cag	agt	ctg	ttg	cta	agc	aac	ggg	gag	gag	tgg	tcc	cgc	aag	aga	cgg		534	
L	G	Q	S	L	L	L	S	N	G	E	E	W	S	R	K	R	R		151	
ctg	ctg	act	cca	gct	ttt	cat	ttt	gat	att	ctg	agg	aac	tac	gtt	gcc	ata	ttt		588	
L	L	T	P	A	F	H	F	D	I	L	R	N	Y	V	A	I	F		169	
aac	acc	tca	act	aac	acc	atg	cat	gac	aag	tgg	cgc	cac	cat	ttg	gca	gta	ggc		642	
N	T	S	T	N	T	M	H	D	K	W	R	H	H	L	A	V	G		187	
acc	act	aat	cta	gag	atg	ttt	gac	cac	atc	act	ctg	atg	aca	ctg	gac	agt	tta		696	
T	T	N	L	E	M	F	D	H	I	T	L	M	T	L	D	S	L		205	
ctg	aaa	tgt	gcc	ttt	agc	tac	aac	agc	aac	tgc	cag	cag	tcc	acc	agt	gag	tat		750	
L	K	C	A	F	S	Y	N	S	N	C	Q	Q	S	T	S	E	Y		223	
gtg	tca	gcc	ata	gtg	gag	ctg	agt	gac	ctg	ata	ata	gat	cgc	cgc	caa	aag	att		804	
V	S	A	I	V	E	L	S	D	L	I	I	D	R	R	Q	K	I		241	
tta	cac	cac	tgg	gac	tgg	att	tac	tgg	aaa	act	aag	cag	ggg	aaa	cga	ttc	aaa		858	
L	H	H	W	D	W	I	Y	W	K	T	K	Q	G	K	R	F	K		259	
aag	gcc	tta	agc	att	gtg	cac	agg	ttt	acc	aga	gag	gtg	gtt	cag	aag	cgc	cgt		912	
K	A	L	S	I	V	H	R	F	T	R	E	V	V	Q	K	R	R		277	
gcc	ctg	atc	agc	caa	cag	agg	gag	aca	gaa	acc	aca	gca	cca	cag	aaa	aag	aaa		966	
A	L	I	S	Q	Q	R	E	T	E	T	T	A	P	Q	K	K	K		295	
gac	ttt	gtg	gac	att	ata	ctg	ctt	tca	agg	gat	gag	gat	gga	caa	ggc	cta	aca		1020	
D	F	V	D	I	I	L	L	S	R	D	E	D	G	Q	G	L	T		313	
gat	gag	gag	ata	cag	gct	<u>gag</u>	<u>gcc</u>	<u>aac</u>	<u>acc</u>	<u>ttc</u>	<u>atg</u>	<u>ttc</u>	<u>gca</u>	<u>ggt</u>	<u>cat</u>	<u>gac</u>	<u>aca</u>		1074	
D	E	E	I	Q	A	<u>E</u>	<u>A</u>	<u>N</u>	<u>T</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>A</u>	<u>G</u>	<u>H</u>	<u>D</u>	<u>T</u>		331	
aca	gcc	agt	cgc	atc	tgc	tgg	acg	ctg	tat	aat	tta	gca	cgc	cac	gac	caa	tat		1128	
<b>T</b>	A	S	A	I	C	W	T	L	Y	N	L	A	R	H	D	Q	Y		349	
cag	gag	aaa	tgc	agg	cag	gaa	gtg	atg	gag	ctg	atg	gaa	gga	cga	gac	aga	cat		1182	
Q	E	K	C	R	Q	E	V	M	E	L	M	E	G	R	D	R	H		367	
gaa	ata	gag	tgg	gag	gat	ctg	tct	aac	ctt	ccc	ttc	acc	acc	atg	tgc	atc	aga		1236	
E	I	E	W	E	D	L	S	N	L	P	F	T	T	M	C	I	R		385	
gag	tct	ctc	agg	ctg	cac	tct	cct	gtg	cag	gct	gta	aca	agg	aag	tac	acc	cag		1290	
E	S	L	R	L	H	S	P	V	Q	A	V	T	R	K	Y	T	Q		403	
gac	atg	aca	ctg	cca	ggg	gat	tgt	aca	gtg	cca	aag	ggt	gcc	atc	tgt	ttg	gtc		1344	
D	M	T	L	P	G	D	C	T	V	P	K	G	A	I	C	L	V		421	
agt	att	tat	gga	aca	cac	cac	aac	cgc	act	gtt	tgg	aca	aac	cca	cat	gag	ttt		1398	
S	I	Y	G	T	H	H	N	P	T	V	W	T	N	P	H	E	F		439	
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H	P	L	R	F	D	P	T	N	K	E	G	L	A	A	H	A	F		457	
atc	ccc	ttt	tcc	tca	ggc	ccc	agg	aac	tgc	att	ggt	cag	aag	ttc	gcc	ctg	gca		1506	
I	P	F	S	S	G	P	R	N	<b>S</b>	I	G	Q	K	F	A	L	A		475	
gag	act	tgc	agt	cgt	ggc	ttt	gac	tct	gct	cag	gtt	tgc	tct	gac	cct	ggg		1560		
E	T	S	S	R	G	G	F	D	S	A	Q	V	S	S	D	P	G		493	
ggt	gaa	ccc	cga	agt	tgg	gcc	cag	ctc	tgg	gga	agt	tgc	cgc	tct	gcc	cca	gct		1614	
G	E	P	R	S	W	A	Q	L	W	G	S	S	P	S	A	P	A		511	
tgt	cct	cgc	tgc	aga	ggg	gag	gca	tgt	ggc	tgc	agg	tgg	agc	ctc	<u>tga</u>	ccc	cgg		1668	
C	P	A	C	R	G	E	A	C	G	C	R	W	S	L					526	
aca	agg	atg	aat	gat	cca	cac	aaa	ggc	aaa	aaa	atg	att	tat	gcg	tat	tgc	att		1722	
gtg	ttt	aaa	tgc	att	tgc	ttt	ctg	cag	agc	aac	ctg	tat	aac	gat	agc	ttt	att		1776	
att	acc	ctg	aaa	tta	ctg	acc	tta	cag	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	agc		1830	
ggc	cgt	taa	att	ctg	ggc	aaa	cgt	ggc	agc	taa	gcg	cgt	cgc	gcg	cgt	gcg	gcg		1884	
gcg	cgt	gcg	ctg	taa	taa	gtg	gtg	ctg	ggc	tgg									1917	

**FIG. 1.** Nucleotide and deduced amino acid sequences of the CYP4F7 transcript. Putative translation start codon (ATG), and stop codon (TGA) are underlined. The 13-amino acid peptide, which is characteristic of the CYP4 family, is boxed. The putative heme-binding cysteine residue is shaded.

including the CYP4F sequences. Residue 331, which is Glu in most CYP4 sequences (except for the *Drosophila* CYP4D1) is variable in the CYP4F sequences (Glu,

Gly, or Ala for CYP4F7). This 13-amino acid peptide was described to be closely related to the substrate recognition domain (22), despite the diversity of sub-



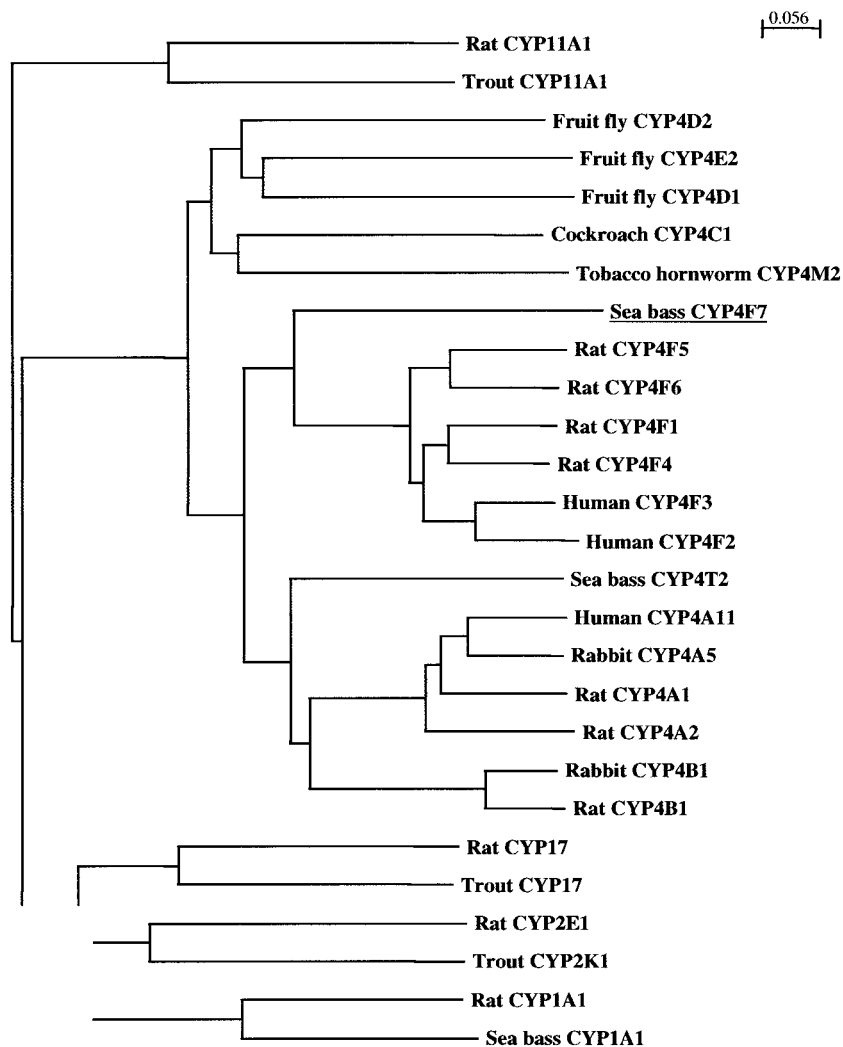
CYP4F2 Human	..MSQLSLWLGGLCDVAASFWLLLLLLVGASWLLAHVLAWTYAFYDNCRRRC	PQPPRRNF	FWGHQGMVN	68
CYP4F3 Human	..MPQLSLSLGLWPMAASFWLLLLLLVGASWLLARILAWTYTFYDNCRRRC	PQPPKRN	FLGHLGLIH	68
CYP4F4 Rat	..MPQLDLWGLRLLETSLPWLLELLIGASWLLVRVLTQTYIFYRTYQHLC	PQPPKWN	FLGHLGMIT	68
CYP4F1 Rat	..MSQLSLWLGGLGPEVAFPPWQTLTLLFGASWILAQILTQIYAAYNFRRLRG	PQPPKRN	LMGHVGMVT	68
CYP4F6 Rat	..MLQLSLRLGMSLTASFWHLLLLGASWILARILAWIYTFYDNCRRRC	PQPPKPS	FWGHLTLMK	68
CYP4F5 Rat	..MPWLTVSGLDLGSVVTSTWHLLLLGAASWILARILAWTYSFCNCSSRLRC	PQSPKRN	FLGHLGTIQ	68
CYP4F7 Sea bass	MPLLQGVLSHILSWTGLCQVLFVWVWTLGAVVVVWTAKLVRHVWYTHRLSC	NKPHANS	FLFGLGQM	70
CYP4F2 Human	PTTEMRVLTLQVATYPQGGFKVWMMGISPLLSLCHPDIIRSVINASAIAPK	DKFFYS	FLFPWLDG	138
CYP4F3 Human	SSSELLYTQSLACTFGDMCCWVWGFHWAIVRIFHPTIYIKPVLFAPAI	VPKDKVYF	SLFKPWLDG	138
CYP4F4 Rat	PTTQLKQVTKLVATYPQGFMTWLPFILPIITLCHPDVIRSVLSAS	SVALKE	VIFYSP	138
CYP4F1 Rat	PTTQLKELTRLVGTYPQGFMLWIGFMVVPITLCHSDIVRSILNAS	AAVALK	DVIFYT	138
CYP4F6 Rat	NNTEQMFIHLGRNFRDIHLSWVGFVYPILRLVHPNVIAPLLQASAA	VAPKEM	TLYGFL	138
CYP4F5 Rat	SNTEMRVLVTEMGQTFRDIHLCWLPVIVPVLRLVDPAFVAPLLQAP	ALVAPK	DTTFLR	138
CYP4F7 Sea bass	STTEMLQHVDELVQTYKHSCSWFLGFYHVLVRLFHPDYVKPLLMA	PASITV	KDEL	140
CYP4F2 Human	AADKSRHSMMLTPAFHNLKPKMKLYNESVNIHAKWQLLASEGSAC	DNFEHIS	MLTDSLQ	208
CYP4F3 Human	AAEKSRHSMMLTPAFHNLKPKMKLYNESVNIHAKWQLLASEGSARL	DNFEHIS	MLTDSLQ	208
CYP4F4 Rat	DDDKSCHHMLTPAFHNLKPKVKLFNDSTNIHAKWQLDLAGSARL	DNFKNI	SLMLTDSLQ	208
CYP4F1 Rat	AADKSRHSMMLTPAFHNLKPKVKLFNDSTNIHAKWQLLASEGS	SSRDN	FEHVS	208
CYP4F6 Rat	AAEKSNHSMMLTPAFHNLKPKSVNTNHAKWQLRLTAKSARL	DNFEHIS	MLTDSLQ	208
CYP4F5 Rat	SDDKSRHSMMLTPAFHNLKPKVKLFNQSVNIHAKWHLCLGCSARL	EMFENIS	MLTDSLQ	208
CYP4F7 Sea bass	NNEESRKMLLTSAPFDILRNVAFLMLTSTNDNRHHLAVGTTN	EVVDHIT	MLTDSLQ	210
CYP4F2 Human	SFDHHEKPSVIAALSLALVSKHHEILLHIDFLYLLPDQQRERR	CRLVND	TDVIRE	278
CYP4F3 Human	SFDHHEKPSVIAALSLALVTKHQQILLYIDFLYLLPDQQRERR	CRLVND	TDVIRE	278
CYP4F4 Rat	SFDNHEKPSVIAALSLAAVAKYQQLLHTLSLYQLHNR	AKCKLV	NDVIRE	278
CYP4F1 Rat	SFDNHEKPSVIAALSLAAVAKYQQLLHMULLYLPDMMYH	KCNLH	NDVIRE	278
CYP4F6 Rat	SFDNHEKPSVIAALSLIVKQRQPFYLYDFLYLLADCR	RRKCDV	NDVIRE	278
CYP4F5 Rat	GFDNHEKPSVIAALSLIIVKQRQPFYLYDFLYLLADCR	RRKCDV	NDVIRE	278
CYP4F7 Sea bass	SYNENQSTSTVIAALSLIIVKQRQPFYLYDFLYLLADCR	RRKCDV	NDVIRE	278
CYP4F2 Human	PSGVDDFLQAKAKS..KTLDFIVLAKDHEKESDEDIRAD	DFHGGHDTTAS	VSPGSC	346
CYP4F3 Human	PSGVDDFLQAKAKS..KTLDFIVLAKDHEKESDEDIRAD	DFHGGHDTTAS	FLKSWVLYH	346
CYP4F4 Rat	PSHEDDILKAKAKS..KTLDFIVLAKDHEKESDEDIRAD	DFHGGHDTTAS	GLSWILYN	346
CYP4F1 Rat	PDGGLDEFLKSKAKS..KTLDFIVLAKDHEKESDEDIRAD	DFHGGHDTTAS	GLSWILYN	346
CYP4F6 Rat	NTGVDLEFLKAKAKT..KTLDFIVLAKDHEKESDEDIRAD	DFHGGHDTTAS	GLSWILYN	346
CYP4F5 Rat	SSGOTDEFLESKTKSKSKTLDFIVLAKDHEKESDEDIRAD	DFHGGHDTTAS	ALSWILYN	348
CYP4F7 Sea bass	SQRETETTAPOKKK.....DEVIIISRDQGTREEQEANT	DFHGGHDTTAS	AICWTLYN	348
CYP4F2 Human	TQNTRSVCRQEVQELKDEPKKEIEMDDLAHFLPLTMCIKES	SLHLHP	VTVIS	416
CYP4F3 Human	HPEYQERCRQEVQELKDEPKKEIEMDDLAHFLPLTMCIKES	SLHLHP	VTVIS	416
CYP4F4 Rat	HPEYQERCRQEVRLLRDEPEEIEEMDDLAHFLPLTMCIKES	SLHLHP	VTVIS	416
CYP4F1 Rat	DPEYQERCRQEVQELLRDEPEEIEEMDDLAHFLPLTMCIKES	SLHLHP	VTVIS	416
CYP4F6 Rat	HPEYQERCRQEVRLLRDEPEEIEEMDDLAHFLPLTMCIKES	SLHLHP	VTVIS	416
CYP4F5 Rat	HPEYQERCRQEVRLLRDEPEEIEEMDDLAHFLPLTMCIKES	SLHLHP	VTVIS	418
CYP4F7 Sea bass	HDQYQEKCRQEVRLMEGDRHEIEEMDDLSNPLTMCIRESL	HLHSEV	QAVTKYT	415
CYP4F2 Human	GICCLISVFGTHNPAYVDPDEVYDFRFDPENIKERSPLA	IFPSAGPRNC	ICQTFAM	486
CYP4F3 Human	GICCLISVFGTHNPAYVDPDEVYDFRFDPENIKERSPLA	IFPSAGPRNC	ICQTFAM	486
CYP4F4 Rat	GVICIINIFATHNPTVDPDEVYDFRFDPENIKERSPLA	IFPSAGPRNC	ICQTFAM	486
CYP4F1 Rat	GICCLISVFGTHNPAYVDPDEVYDFRFDPENIKERSPLA	IFPSAGPRNC	ICQTFAM	486
CYP4F6 Rat	GNCVISIFGTHNPAYVDPDEVYDFRFDPENQKRSPLA	IFPSAGPRNC	ICQTFAM	486
CYP4F5 Rat	GNCVISIFGTHNPAYVDPDEVYDFRFDPENQKRSPLA	IFPSAGPRNC	ICQTFAM	488
CYP4F7 Sea bass	GAICLVSIYGTTHNPAYVDPDEVYDFRFDPENQKRSPLA	IFPSAGPRNC	ICQTFAM	485
CYP4F2 Human	LAFRVL DHTPEPRRSRSWSCAQRADFGCGWSP			518
CYP4F3 Human	LAFRVL DHTPEPRR			501
CYP4F4 Rat	LRFRVL DDKEPRRKPEILRAEGGLWLRVEPLSTQ			522
CYP4F1 Rat	LRFRLL DDKEPRRKPEILRAEGGLWLRVEPLTAGAQ			524
CYP4F6 Rat	LRFCVL DDKEPRRKPEILRAEGGLWLRVEPLSTVTSQLPWDLLAHPPTS			537
CYP4F5 Rat	LRFRVL DDKEPRRKPEILRAEGGLWLRMEPLSTDTQ			526
CYP4F7 Sea bass	AQVSSD GGEPRSWAQLWGSSPSAPACPACRGEACGRWSL			526

FIG. 2. Alignment of the deduced amino acid sequences of sea bass CYP4F7 with human CYP4F2 and CYP4F3, and rat CYP4F1, CYP4F4, CYP4F5, and CYP4F6. Residues conserved in all sequences are shaded.

strates (fatty acids, prostaglandins, leukotrienes) and the catalytic variability among CYP4s. The CYP4F7 sequence was compared to all other full CYP4 sequences available in GenBank to infer the phylogenetic relationships between the mammalian, insect, and fish CYP4 genes. These CYP4 sequences were also compared to representative mammalian and fish members of gene families CYP1, 2, 11, and 17. A phylogenetic

tree was generated using the distance-matrix method (Fig. 3). All vertebrate CYP4 sequences clustered together but the CYP4Fs formed a group quite distinct from the others (CYP4As, CYP4Bs, and CYP4T2). These data may be correlated with the fact that CYP4Fs have specific metabolic functions, quite different from those of the other CYP4s. Within the CYP4Fs subgroup, the fish sequence formed a separate branch.





**FIG. 3.** Phylogenetic tree of the full length CYP4 sequences inferred by the distance-matrix method using the Clustal X program. Distances between genes are represented by the sum of their horizontal separation. The scale bar indicates the distance corresponding to 10 differences in 100 positions.

Most of CYP4s are suspected to be involved primarily in endogenous metabolic pathways. Mammalian CYP4F catalyse  $\omega$ -hydroxylation, and thus inactivation of the potent proinflammatory agent LTB<sub>4</sub> (7, 8). They play an important role in regulating the biological activity of LTB<sub>4</sub> and their concentration in the organism (10). Thus, these enzymes might serve as a negative feedback mechanism involved in the modulation of some inflammatory responses. To date, CYP4F enzymes were isolated only in human and rat (8, 11, 23, 24). These enzymes are predominantly expressed in liver (7, 11, 23, 24), and polymorphonuclear leukocytes (8, 11), but also in kidney (11, 24), and brain (24).

In order to investigate expression of this gene in sea bass tissues, Northern blot analysis were performed with full length, 5'-end, or 3'-end cDNAs of CYP4F7 as digoxigenin-labeled probes. The same results were ob-

tained with the various probes (not shown). As presented in Fig. 4, CYP4F7 transcripts are exclusively present in the kidney of untreated sea bass. The CYP4F7 cDNA hybridized to a unique band of 2.1 kb, which is the expected size for the CYP4F7 transcript. While the major site of expression for most CYPs in mammals is the liver, it appears that the kidney is a key organ for the expression of various P450s in fish (13, 15). CYP1A and CYP2K4 isoforms are strongly expressed in trunk kidney of male rainbow trout, and expression of CYP3A27 increases with age in this tissue (Sabourault, unpublished results, 13).

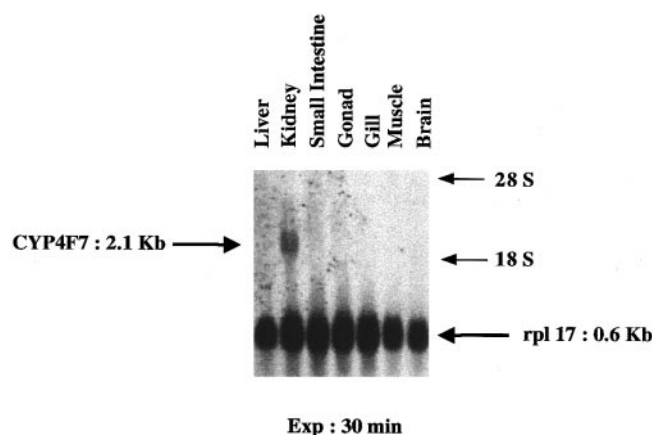
Sea bass were also treated with various xenobiotics, such as peroxisome proliferators, and some "classical inducers" of other CYP families (benzo[a]pyrene, prochloraz, ethanol). For each fish, RNAs were isolated from 7 organs and analysed. No CYP4F7 transcript



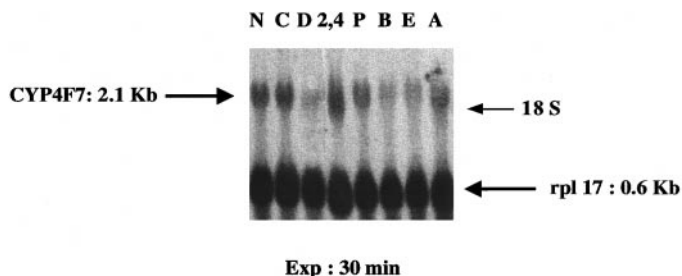
was detected in tissues other than kidney, even in treated sea bass (data not shown). No induction of CYP4F7 was seen in kidney of xenobiotic-treated animals. While the amounts of renal mRNAs in clofibrate- and 2,4-D-treated sea bass was slightly affected, a treatment of fish by prochloraz or acetone resulted in a 40% decrease of the transcripts (Fig. 5). The lowest amount of CYP4F7 mRNA was found after a treatment by DEHP, benzo[a]pyrene, or ethanol (40% of the control). It is noteworthy that ethanol inhibits the LTB<sub>4</sub> metabolism in rat hepatocytes (25), and that benzo[a]pyrene induce an inflammatory response in mouse skin (26). Thus it appeared that this isoform is not inducible by xenobiotics, unlike the sea bass CYP4T2, which was strongly induced by DEHP or 2,4D (15).

CYPs belonging to 7 families (CYP1, CYP2, CYP3, CYP4, CYP11, CYP17, and CYP19) have been isolated from fish, especially from rainbow trout (13). Most of them (except for the CYP1A) are involved in endogenous metabolism, such as fatty acids hydroxylation or steroid biosynthesis. In view of the biological importance of CYP4F genes in mammals, it is not so surprising to find a member of this CYP4 subfamily in fish. However, the tissue-specific expression of CYP4F7 in kidney is quite unexpected as compared to mammals. Little is known about LTB<sub>4</sub> generation and its biological activities in fish. Their potential pro-inflammatory ability has not been tested. Leukotrienes B<sub>4</sub> are produced by the macrophages and by the thrombocytes (platelet equivalent cells) in rainbow trout (27, 28). The presence of putative receptors for LTB<sub>4</sub> on trout macrophages was reported (29), suggesting a biological mechanism similar to that in mammals.

The tissue-specific and constitutive expression of the CYP4F7 gene in sea bass kidney, and the great homol-



**FIG. 4.** Blot analysis of total RNA from various sea bass tissues. Total RNA (20  $\mu$ g per lane) were separated on a 1.2% denaturing agarose gel, transferred to a nylon membrane, and hybridized with a digoxigenin-labeled CYP4F7-specific probe (5' end). As a loading control, the membrane was cohybridized with a rpl 17 probe, which is constitutively expressed.



**FIG. 5.** Blot analysis of total RNA from kidney of sea bass treated with various xenobiotics, including some peroxisome proliferators. N, control; C, Clofibrate; D, DEHP; 2,4, 2,4-D; P, Prochloraz; B, Benzo[a]pyrene; E, Ethanol; A, Acetone.

ogy with mammalian CYP4F isoforms suggest an important physiological role that remains to be established. Heterologous expression of the gene and the screening of potential substrates will be required to identify the physiological substrates and roles of this new CYP4.

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