BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **251,** 213–219 (1998)
ARTICLE NO. RC989429

Molecular Cloning of a Phthalate-Inducible CYP4 Gene (CYP4T2) in Kidney from the Sea Bass, *Dicentrarchus labrax*

C. Sabourault,*,† J.-B. Bergé,* M. Lafaurie,† J.-P. Girard,† and M. Amichot*
**INRA-IFR 38 "Réponse des Organismes aux Stress Physico-Chimiques et Biologiques," BP 2078, 123 Bd Francis Meilland, 06606 Antibes Cedex, France; and †Laboratoire de Physiologie et Toxicologie Environnementales, Faculté des Sciences, Université de Nice-Sophia Antipolis, BP 71, 06108 Nice Cedex 02, France*

Received September 4, 1998

A full-length cDNA sequence was isolated from kidney total RNA of di(2-ethylhexyl) phthalate-treated sea bass by reverse-transcriptase polymerase chain reaction and then rapid amplification of cDNA ends. The deduced amino acid sequence, which has been named CYP4T2, shared 69 and 54.4% amino acid identity with rainbow trout CYP4T1 and rat CYP4B1, respectively. RNA blot analysis using the CYP4T2 cDNA as a probe indicated that the mRNA was rather abundant in kidney, and less so in liver, small intestine, and brain. Treatment of sea bass with peroxisome proliferators showed marked tissue-specific induction. CYP4 inducers clofibrate, di(2-ethylhexyl) phthalate (DEHP), and 2,4-dichlorophenoxy acetic acid (2,4-D) were administered by intraperitoneal injection. The strongest induction was found in kidney after a DEHP treatment (6.5-fold) or a 2,4-D treatment (9-fold), while no induction was observed in liver.

Key Words: **2,4-D; clofibrate; cytochrome P450; DEHP; *Dicentrarchus labrax;* induction; kidney; molecular cloning.**

Cytochromes P450 (CYP) enzymes are involved in both synthetic and catabolic reactions. CYP enzymes metabolize endogenous substrates including fatty acids, prostaglandins, leukotrienes, steroids, and vitamins, that can affect cellular functions (1, 2). They also play an important role in detoxifying xenobiotics, although some xenobiotics can be oxidized to reactive intermediates that may be potentially toxic, carcinogenic, or mutagenic. The CYP superfamily currently consists of over 800 genes (3, 4), divided into families and subfamilies. Many individual CYP isozymes have distinct but broad substrate specificities, and there is a great deal of overlap between different CYP enzymes. The CYP enzymes can be substantially induced in response to specific xenobiotics (5). These inductions are mediated mainly by transcriptional mechanisms (2, 5, 6), except for the CYP2E1 gene, which is also induced by protein stabilization (7).

CYP4 is one of the oldest P450 gene families. Several CYP4 genes have been isolated in both mammals and insects. In addition, a partial cDNA sequence named CYP4T1 was cloned from trout liver (8). The CYP4 gene family consists of 22 subfamilies, totaling 108 sequences (4). This family encodes isozymes that catalyse various activities such as ω or (ω-1)-hydroxylation of lauric acid, arachidonic acid, leukotrienes, or prostaglandins. These isozymes show both constitutive and inducible expression. Mammalian CYP4 genes may be constitutively expressed in the liver, the kidney, and sometimes in the lung (9). CYP4 expression is regulated also by xenobiotics, principally peroxisome proliferators, which have no obvious structural similarity (10). The best studied are hypolipidaemic drugs that belong to the fibrate class, such as clofibrate, phthalate ester plasticizers, such as di(2-ethylhexyl) phthalate (DEHP), and aryl phenoxy herbicides, such as 2,4-dichlorophenoxy acetic acid (2,4-D). Interestingly, all the identified CYP4 genes contain a 13-residue sequence around position 315 (I helix), which is extremely conserved and found exclusively in CYP4. This peptide sequence defines the CYP4 family (11, 12).

Our previous studies have demonstrated that lauric acid hydroxylase activity in sea bass kidney microsomes is induced by a DEHP treatment, and to a lesser extent by a clofibrate treatment of fish (13). These observations have led us to clone a CYP4 gene

Abbreviations used: 2,4-D, 2,4-dichlorophenoxy acetic acid; CYP, cytochrome P450; DEHP, di(2-ethylhexyl) phthalate; DIG, digoxigenin; LAH, lauric acid hydroxylase; RACE-PCR, rapid amplification of cDNA ends polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction.

0006-291X/98 $25.00

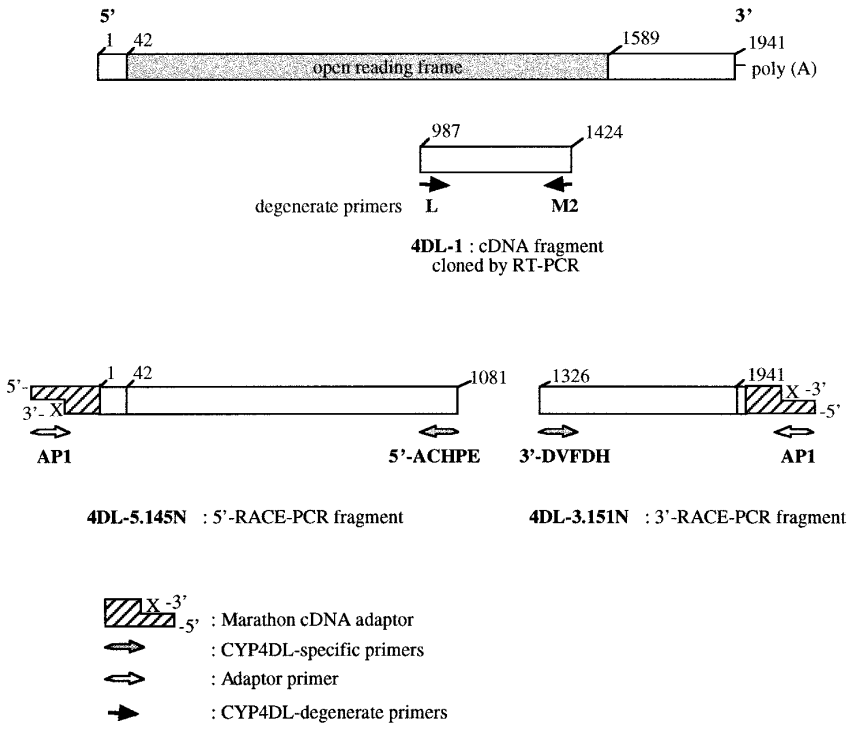


FIG. 1. Cloning procedure for sea bass CYP4T2. A cDNA fragment was amplified from kidney mRNA by RT-PCR experiments. The full-length sequence of CYP4T2 was obtained using the RACE-PCR technology. Primers used in RT-PCR and RACE-PCR are shown.

(CYP4T2) and to investigate the expression of this CYP4T2 gene in several organs from untreated or peroxisome proliferators-treated sea bass.

MATERIAL AND METHODS

Chemicals. Clofibrate, DEHP, 2,4-D and all other chemicals were purchased from Sigma-Aldrich Chimie (France). Restriction enzymes were from Boehringer Mannheim (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 (salinity 28‰) at 18°C. Fish were acclimated for at least two weeks to reduce possible stress-related responses. Fish in 10 groups were treated daily for three days by intraperitoneal injection of clofibrate (100 mg/kg body weight/day), DEHP (1500 mg/kg body weight/day), 2,4-D (20 mg/kg body weight/day), or NaCl 9‰ (control).

Poly(A)$^+$ mRNA isolation. Total RNA from DEHP-treated sea bass kidney was extracted by the guanidine thiocyanate method as described by Chomczynski and Sacchi (14). Poly(A)$^+$ mRNAs were isolated by chromatography on an oligo(dT)-cellulose column as described in Sambrook, *et al.* (15).

RT-PCR cloning of a cDNA fragment. RNA was reverse-transcribed using a 18-mer oligo(dt) primer and the First Strand cDNA Synthesis Kit (Clontech, MA, USA). cDNA was amplified by PCR in a 50 μl reaction mix containing 75 mM tris-HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 1.5 mM $MgCl_2$, 125 μM dNTPs, 40 ng of cDNA, 0.5 μM of each of the sense and antisense primers, and 0.5 U of Goldstar DNA Polymerase (Eurogentec, Belgium). Oligonucleotides for PCR were based on two conserved amino acid domains designed from the alignment of several CYP4 sequences: CYP4A1 (*Rattus norvegicus*, M14972), CYP4B1 (*Oryctolagus cuniculus*, M29852), CYP4C1 (*Blaberus discoidalis*, M63798), CYP4D1 (*Drosophila melanogaster*, X67645) and CYP4F1 (*Rattus norvegicus*, M94548). The first domain (sense primer, named L) is specific to the CYP4 family members (11). The second (antisense primer, named M2) corresponds to the heme-binding region (cysteine) and several amino acids of this motif are conserved in all CYP proteins (12). Partially degenerate primers were designed based on these two conserved sequences, and on estimates of codon usage in *Dicentrarchus labrax*. The sequence of the sense primer (L) was 5′-GAY-ACN-TTY-ATG-TTY-GAR-GG-3′ and the sequence of the antisense primer (M2) was 5′-CCD-ATG-CAR-TTD-CGD-GVD-CC-3′ (according to the IUB codes). PCR reactions were performed on a ThermojeT thermal cycler (Eurogentec, Belgium) with the following program: 94°C for 3 min, 35 cycles of 94°C, 1 min, 54°C, 1 min, and 70°C, 1 min, followed by a final 10 min extension at 70°C. cDNA was purified from a 1.5% agarose gel using the Geneclean III Kit (Bio 101, Inc., CA, USA). Gel-purified cDNA (named 4DL-1) was subcloned into the pGEM-T vector (Promega, WI, USA) according to the manufacturer's instructions. Plasmid DNA from positive individual clones was purified by banding in CsCl (17) then sequenced.

Cloning of the 5′ and 3′ ends by RACE-PCR. Isolation of 5′ and 3′ ends of the gene was performed using the Marathon cDNA Amplification Kit (Clontech, MA, USA). A library of adaptor-ligated double strand cDNA was constructed from kidney poly $(A)^+$ mRNAs according to the manufacturer's instructions. Gene specific primers were designed from the cDNA fragment first cloned (4DL-1, 438 bp) to generate 5′ and 3′ fragments, as shown in Fig. 1. The primers used to clone the 5′ end were "AP1" (sense, 5′-CCATCCTAATACGACTCACTATAGGGC-3′) and "5′-ACHPE" (antisense, 5′-CAAATCTTCTGGTGTTCTGGGTGGCAGGCC-3′) and those used to clone the 3′ end were "3′-DVFDH" (sense, 5′-GATGTCTTTGACCACTGGCGTTTC-3′) and "AP1" (antisense,

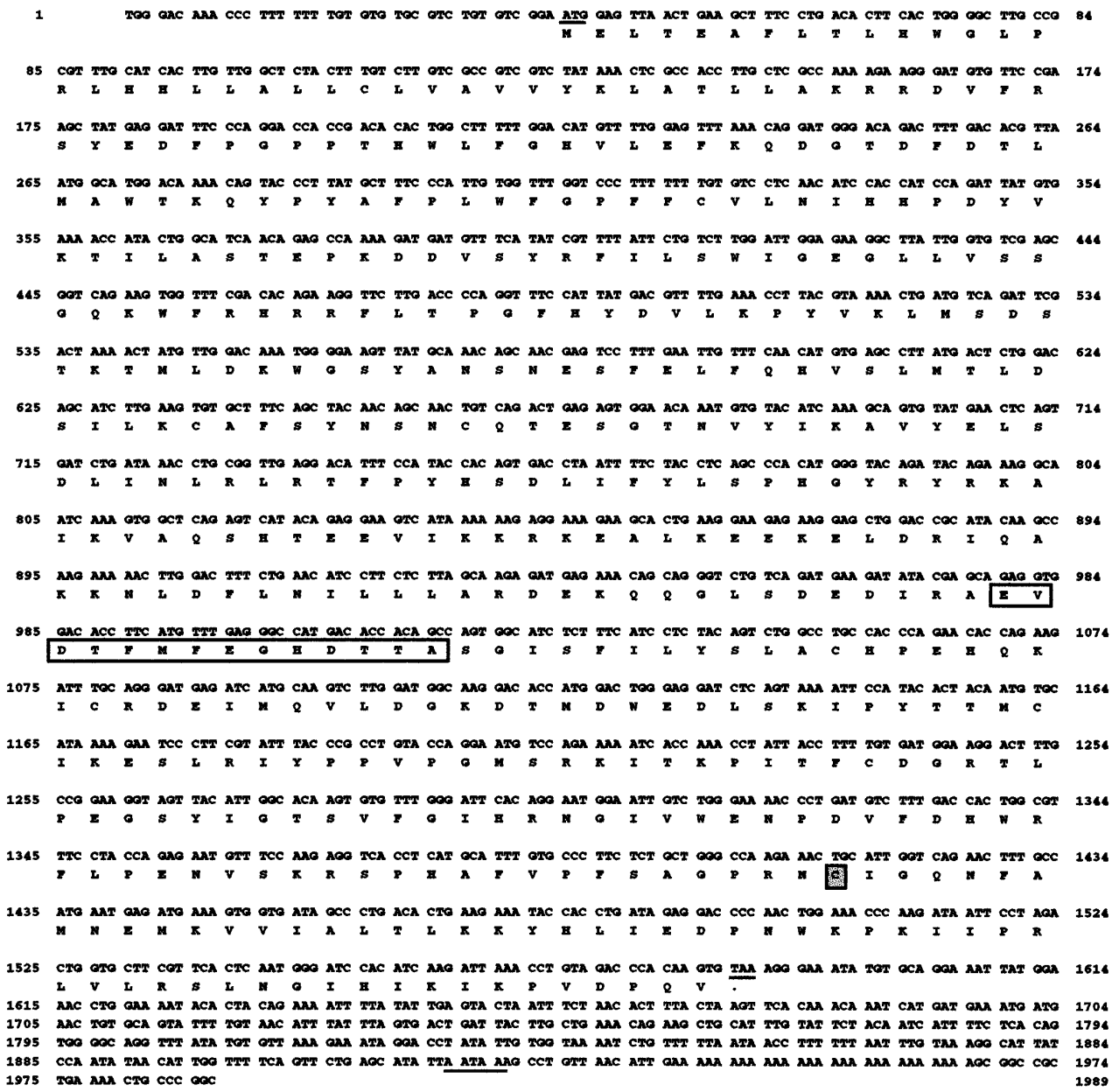


FIG. 2. Nucleotide and deduced amino acid sequences of the CYP4T2 transcript. Putative translation start codon (ATG), stop codon (TAA) and consensus polyadenylation site (AATAAA) are underlined. The amino acid sequence, which is characteristic of the CYP4 family, is enclosed in a box. The putative heme-binding cysteine residue is shaded.

5′-GCCCTATAGTGAGTCGTATTAGGATGG-3′). 3′ and 5′ RACE-PCR reactions were performed using Advantage klenTaq Polymerase Mix (Clontech, MA, 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. Gel-purified fragments of expected size were subcloned by inserting into the EcoRI site of the plasmid vector pCR 2.1 using the TA Cloning Kit (Invitrogen, NV Leek, Netherlands). Plasmid DNA from the clones of interest was purified using the QIAprep Spin Miniprep Kit (Qiagen Inc., CA) then sequenced.

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 by BLAST against the EMBL and GenBank databases (BLAST 2.0 program; 16). This cDNA sequence was compared to the rainbow trout CYP4T1 (U82983), the rat CYP4B1 (M29853), and the rabbit CYP4B1 (M29852) using the CLUSTAL X program (version 1.64b, 02/1998). A phylogenetic tree was constructed with

the distance-matrix method from a multiple alignment of 23 complete amino acid sequences using the Clustal X program.

Northern blot analysis. Total RNA from sea bass tissues was separated by electrophoresis on a 1.2% denaturating agarose gel then transferred to nylon membranes (Boehringer Mannheim) and UV cross-linked. A digoxigenin (DIG)-labeled CYP4T2 probe (the 438 bp cDNA fragment cloned into pGEM-T) was prepared by PCR using the primers L and M2, and the DIG DNA Labeling Mix (Boehringer Mannheim). A sea bass cDNA encoding for the ribosomal protein rpl 17A (rpl 17A-homologue) was isolated in the lab (Sabourault, unpublished results; GenBank AF 074720) and used as an internal standard for normalizing the quantity of mRNA on the gel. Rpl 17 is a ribosomal protein isolated in human, Drosophila, and yeast, which is constitutively expressed whatever the organ, the sex, or the treatment of animals. A DIG-labeled rpl 17 cDNA probe was prepared using the same procedure. Prehybridization and hybridization (overnight at 50°C), washes (at 65°C), and chemiluminescent detection were performed according to the DIG system (17, 18).

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 the saturation of the signal due to rp l17 mRNA, and therefore aberrant results in densitometry.

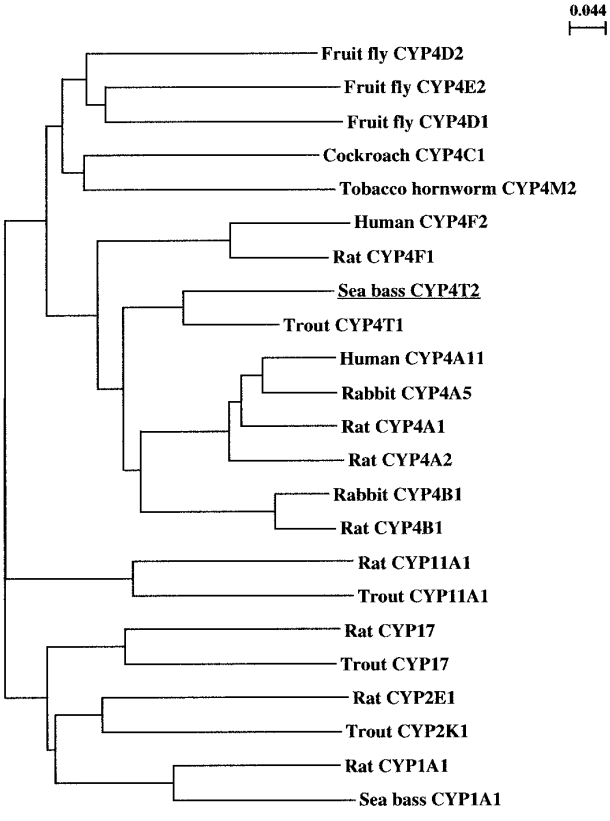


FIG. 3. Phylogenic tree of the full length CYP4 sequences. The tree inferred by the distance-matrix method using the Clustal X program is shown. Distances between genes are represented by the sum of their horizontal separation. The scale bar on the top indicates the distance corresponding to 10 differences in 100 positions. Sequences were retrieved using GenBank accession numbers from the Nelson Web Site (4).

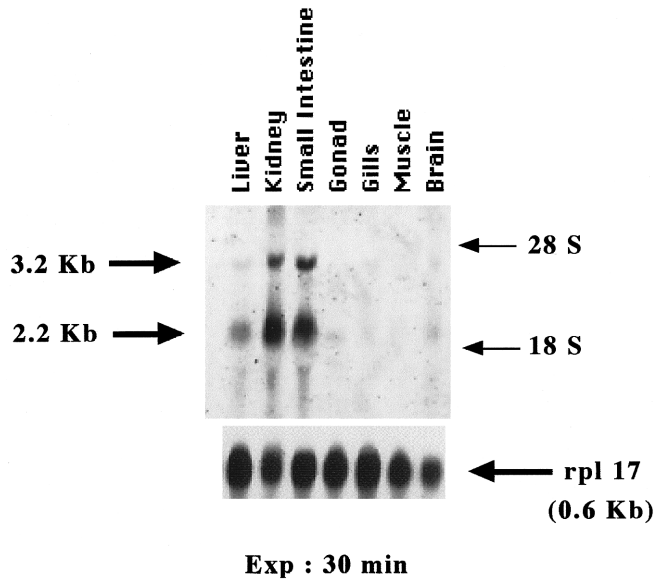
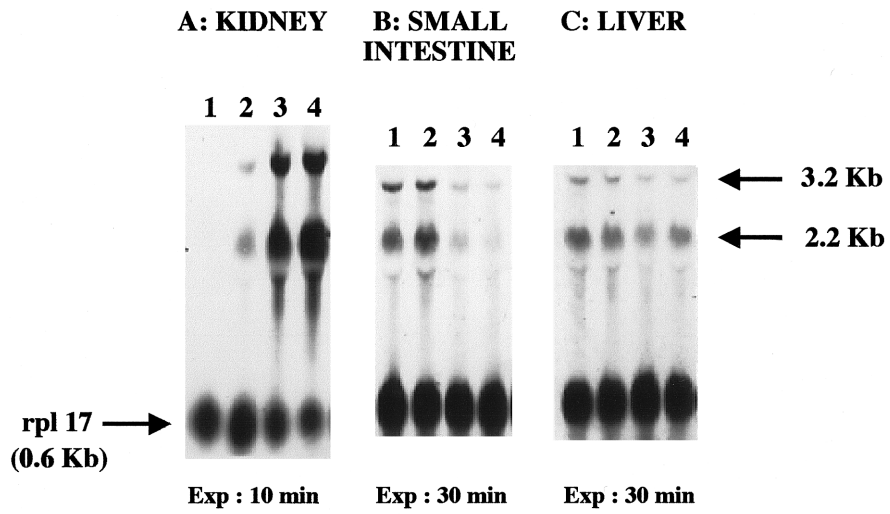


FIG. 4. Blot analysis of total RNA from various sea bass tissues. Total RNA (20 μg per lane) from various tissues were separated on a 1.2% denaturing agarose gel, transferred to a nylon membrane, and hybridized with a digoxigenin-labeled CYP4T2-specific probe. As a RNA loading control, the membrane was cohybridized with a specific cDNA-probe of rpl 17, which is constitutively expressed.

RESULTS AND DISCUSSION

To isolate an inducible CYP4 gene, we have used mRNA extracted from kidney of DEHP-treated sea bass in RT-PCR experiments. Partially degenerate primers were designed based on two highly conserved amino acid sequences: the 13-residue sequence characteristic of the CYP4 proteins and the residues surrounding the heme-binding cysteine, which are conserved in all P450 proteins. A 440 bp fragment was obtained from PCR amplification. The full length sequence of this mRNA transcript was obtained by the method of 3′- and 5′-rapid amplification of cDNA ends (RACE-PCR) (Fig. 1). We obtained therefore a combined cDNA sequence of 1941 bp. The open reading frame (1547 bp) encoded a 516 amino acid-protein (accession no. AF 045468) with a calculated M_r of 59,739 Da. The deduced amino acid sequence (Fig. 2) was classified as CYP4T2 by Dr. D. Nelson (University of Tennessee) from the P450 Gene Superfamily Nomenclature Committee. Sequence comparisons between trout CYP4T1 (8) and sea bass CYP4T2 showed 69% identity over the length of the partial CYP4T1 cDNA sequence. The closest mammalian CYP4 genes are the rat CYP4B1 and the rabbit CYP4B1, that showed 54.4 and 54.2% identity, respectively (Fig. 3). The CYP4T2 sequence was compared to all other full CYP4 sequences available in GenBank (except CYP4T1, which is a partial



FOLD INDUCTION				
Lane	1	2	3	4
Kidney :		1	6.5	9
Intestine :	1	1.5	0.2	0.17
Liver :	1	0.85	0.4	0.56

FIG. 5. Blot analysis of total RNA from liver, kidney, and small intestine of sea bass treated with peroxisome proliferators. Total RNA (20 μg per lane) was separated on a 1.2% denaturing agarose gel, transferred to a nylon membrane, and hybridized with a digoxigenin-labeled CYP4T2-specific probe. As a RNA loading control, the membrane was cohybridized with a specific cDNA-probe of rpl 17, which is constitutively expressed. (A) RNA from kidney; (B) RNA from small intestine; (C) RNA from liver. Lane 1, Nacl 9_; lane 2, Clofibrate-treated; lane 3, DEHP-treated; lane 4, 2,4-D-treated. The films were scanned and analysed by the NIH-Image program. The values normalized with rpl 17 mRNA are given.

cDNA sequence) 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). This tree showed that the sea bass CYP4T2 and the trout CYP4T1 sequences were assembled in a major branch that was quite distinct from the branches of mammalian CYP4. All the vertebrate CYP4 sequences clustered together in a branch distinct to that of the insect CYP4 sequences.

Expression of CYP4T2 at the transcription level was investigated by Northern blot hybridization of total RNA from sea bass tissues using the 4DL-1 cDNA fragment (see Fig. 1) as a digoxigenin-labeled probe. Differences in sample loading were normalized by scans then by densitometry studies of the rpl 17-homologue mRNA (see materials and methods). At first, total RNA was prepared from various organs from untreated sea bass. CYP4T2 cDNA hybridized to two bands: a 2.2-kb transcript, which probably corresponds to CYP4T2 mRNA, and a thin band about 3.2 kb. The nature of this other mRNA is not known, but it is noteworthy that its expression followed the CYP4T2 expression exactly, according to the tissue type (Fig. 4) or the animal treatment (Fig. 5). There could be two different transcription start sites; identification of the CYP4T2 transcript size by RNase protection assay is currently in progress. However, despite the high stringency of the washes, the presence of a second highly homologous gene cannot be excluded. The CYP2K1 expression in mature rainbow trout liver and kidney (19) showed similar multiple mRNA hybridizations. While liver is the major site of expression of several CYP isozymes in mammals (5) and also in fish (20), the most abundant content of CYP4T2 mRNA was observed in kidney (Fig. 4). CYP4T2 mRNA was expressed to a lesser extent in liver, intestine, and brain (2-fold less than kidney for these organs), and it was detectable only in trace amounts in testis. No expression was observed in gill and muscle.

Treatment of sea bass with peroxisome proliferators resulted in marked tissue-specific induction. Rat and rabbit CYP4B1 are expressed in a tissue-specific manner and are differentially induced according to the species (21). While in most mammals CYP4 induction by peroxisome proliferators is strongest in liver, the strongest induction of sea bass CYP4T2 by these compounds was observed in kidney. In the kidney (Fig. 5A), clofibrate treatment caused a significant induction, while DEHP and 2,4-D increased the amount of the CYP4T2 mRNA (more than clofibrate, 6.5- and 9-fold respectively). On the other hand, the most potent inducer in the small intestine (Fig. 5B) was the clofibrate (1.5-fold induction), while a decrease was observed with DEHP (5-fold) and 2,4-D (6-fold). In contrast no induction was observed in liver (Fig. 5C) with any of the peroxisome proliferators tested. A small decrease in liver CYP4T2 mRNA was also observed with DEHP (2.3-fold) and 2,4-D (1.8-fold). Our previous studies on lauric acid hydroxylation in sea bass showed a strong induction of this activity in kidney of DEHP-treated fish. These results agreed with the results obtained from Northern blot experiments. Moreover, it appeared that the sea bass was a less responsive species to fibrate, such as the marmoset and the guinea pig (22, 23).

Induction of mammalian CYP4 mRNA by peroxisome proliferators is due to a transcriptional activation (24), which probably is mediated by the peroxisome proliferator-activated receptor (PPAR), as proposed by Isseman and Green (25). It is noteworthy that a PPARγ was recently isolated from Atlantic salmon liver (26), suggesting a similar regulation of CYP4 gene expression in fish and in mammals. Phthalate ester plasticizers represent widely-distributed environmental contaminants. Thus the induction of CYP4T2 mRNA in sea bass kidney by phthalates could be a first step in the development of efficient biomarkers for this class of marine pollutants (27).

The precise physiological role of the CYP4 proteins remains to be established. The liver is the major site for detoxication in mammals. Kidney CYP4 enzymes in rodents (28) and humans (29) are mostly involved in hydroxylations of endogenous substrates such as fatty acids, prostaglandins and arachidonic acid, rather than steroids and carcinogens. Fatty acids and eicosanoids play important roles in renal physiology (30). It appears that the kidney is the major site of CYP4T2 expression in fish, a result that agree with the high lauric acid (ω-1) hydroxylation rates in kidney microsomes from sea bass (13) and from rainbow trout (31). Our results indicate that CYP4 enzymes may function in renal physiology in fish. Determination of substrate specificities of CYP4T2 should help to define those functions.

ACKNOWLEDGMENTS

This research was supported in part by grants from the Commission of the European Union (BIOMAR EV5V-CT94-0550 et ENV4-CT96-0300), the Groupement de Recherche et d'Etude des Génomes (GREG), and the Conseil Scientifique des Iles de Lérins (CSIL). We are very grateful to Professor René Feyereisen (University of Arizona, Tucson, AZ) for critically reading the manuscript. We thank Dr. David R. Nelson (University of Tennessee, Memphis, TN) for his help in naming this gene, and Dr. John J. Stegeman (Woods Hole Oceanographic Institute, MA) for valuable discussion and revision of the manuscript. We gratefully acknowledge M. Charvoz (Cannes Aquaculture, Cannes, France) for the generous gift of sea bass. The authors thank Delphine Farconnet and the staff of Genome Express (Grenoble, France) for cDNA sequencing.

REFERENCES

1. Nebert, D. W. (1990) *Nature* **347,** 709–710.
2. Denison, M. S., and Whitlock, J. P. (1995) *J. Biol. Chem.* **270,** 18175–18178.
3. Nelson, D. R., Koymanis, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W., Gunsalus, I. C., and Nebert, D. W. (1996) *Pharmacogenetics* **6,** 1–42.
4. Nelson Web Site: http://drnelson.utmem.edu/nelsonhomepage.html, June 24, 1998.
5. Gonzalez, F. J. (1989) *Pharmacol. Rev.* **40,** 243–288.
6. Okey, A. B. (1990) *Pharmacol. Ther.* **45,** 241–298.
7. Roberts, B. J., Song, B.-J., Soh, Y., Park, S. S., and Shoaf, S. E. (1995) *J. Biol. Chem.* **270,** 29632–29635.
8. Falckh, P. H., Wu, Q. K., and Ahokas, J. P. (1997) *Biochem. Biophys. Res. Commun.* **236,** 302–305.
9. Kusunose, M. (1993) *in* Cytochrome P450 (Omura, T., Ishimura, Y., and Fujii-Kuriyama, Y., Eds.), 2nd ed., pp. 127–141, Kodansha, VCH, Tokyo.
10. Reddy, J. K., and Rao, M. S. (1986) *Trends Pharmacol. Sci.* **7,** 438–443.
11. Bradfield, J. Y., Lee, Y.-H., and Keeley, L. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4558–4562.
12. Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C., Gotoh, O., Okuda, K., and Nebert, D. W. (1993) *DNA Cell Biol.* **12,** 1–51.
13. Sabourault, C., de Sousa, G., Amichot, M., Cuany, A., Rahmani, R., Salaün, J. P., Bergé, J.-B., Girard, J.-P., and Lafaurie, M. (1998) Submitted for publication.
14. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
15. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
16. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) *Nucleic Acids Res.* **25,** 3389–3402.
17. Boehringer Mannheim Gmbh (1993) The DIG System User's Guide for Filter Hybridization, Mannheim, Germany.
18. Engler-Blum, R., Meier, M., Frank, J., and Muller, G. A. (1993) *Anal. Biochem.* **210,** 235–244.
19. Buhler, D. R., Yang, Y. H., Dreher, T. W., Miranda, C. L., and Wang, J. L. (1994) *Arch. Biochem. Biophys.* **312,** 45–51.

20. Cok, I., Wang-Buhler, J.-L., Kedzierski, M. M., Miranda, C. L., Yang, Y.-H., and Buhler, D. R. (1998) *Biochem. Biophys. Res. Commun.* **244,** 790–795.
21. Gasser, R., and Philpot, R. M. (1989) *Mol. Pharmacol.* **35,** 617–625.
22. Makowska, J. M., Gibson, G. G., and Bonner, F. W. (1992) *J. Biochem. Toxicol.* **7,** 183–191.
23. Bell, D. R., Plant, N. J., Rider, C. G., Na, L., Brown, S., Ateitalla, I., Acharya, S. K., Davies, M. H., Elias, E., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., and Elcombe, C. R. (1993) *Biochem. J.* **294,** 173–180.
24. Hardwick, J. P., Song, B. J., Huberman, E., and Gonzalez, F. J. (1987) *J. Biol. Chem.* **262,** 801–810.
25. Issemann, I., and Green, S. (1990) *Nature* **347,** 645–650.
26. Ruyter, B., Andersen, O., Dehli, A., Ostlund Farrants, A. K., Gjoen, T., and Thomassen, M. S. (1997) *Biochim. Biophys. Acta* **1348,** 331–338.
27. Sabourault, C., Amichot, M. , Lafaurie, M. , Bergé, J.-B., and Girard, J.-P. (1998) 8th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Bordeaux, France, April 14–18.
28. Imaoka, S., Yamaguchi, Y., and Funae, Y. (1990) *Biochim. Biophys. Acta* **1036,** 18–23.
29. Imaoka, S., Ogawa, H., Kimura, S., and Gonzalez, F. J. (1993) *DNA Cell Biol.* **12,** 893–899.
30. Schwartzman, M., Ferreri, N. R., Carroll, M. A., Songu-Mize, E., and McGiff, J. C. (1985) *Nature* **314,** 620–622.
31. Williams, D. E., Okita, R. T., Buhler, D. R., and Masters, B. S. S. (1984) *Arch. Biochem. Biophys.* **231,** 503–510.