해양과학기술논문집 제14권 제1호(2005)
Bull. Mar. Sci. & Tech., 14(1) : 57-62(2005)

BULLETIN OF
RIMST MARINE SCIENCE AND TECHHNOLOGY

Cloning of cytochrome P450 1A (*CYP1A*) genes from the hermaphrodite fish *Rivulus marmoratus* and the Japanese medaka *Oryzias latipes*

Il-Chan Kim [a], Young Ja Kim [b], Yong-Dal Yoon [c], Shoji Kawamura [d], Yong-Sung Lee [a], Jae-Seong Lee [b,*]

[a] *Department of Biochemistry, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea*
[b] *Department of Environmental Science, Graduate School, Hanyang University, Seoul 133-791, Republic of Korea*
[c] *Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, Republic of Korea*
[d] *Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Chiba 277-8562, Japan*

Abstract

To use two small fish *Rivulus marmoratus* (Cyprinodontiformes, Rivulidae) and the Japanese medaka *Oryzias latipes* (Belloniformes) as testing models in molecular ecotoxicology, we have cloned the cytochrome P450 1A (*CYP1A*) gene after screening of both genomic DNA libraries, and sequenced 11,863 and 7,243 bp including all the exons and introns with promoter regions, respectively. The *Rivulus* and the medaka *CYP1A* gene consisted of seven exons (including non-coding exons) with high homology to mammals. In the promoter region, *Rivulus CYP1A* gene has seven xenobiotic response elements (XREs) and two metal response elements (MREs), while the Japanese medaka *CYP1A* gene has six XREs and four MREs. Interestingly, medaka *CYP1A* gene has a number of MREs at the promoter, which may affect its response on metal exposure. We describe here the gene structure of both fish *CYP1A* genes.

Keywords: *Rivulus marmoratus*; Medaka; *Oryzias latipes*; CYP1A

* Corresponding author. Tel.: +82-2-2290-0769; fax: +82-2-2294-6270.
E-mail address: jslee2@hanyang.ac.kr (J.-S. Lee).

0141-1136/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2004.03.006

The fish cytochrome P450 1A (*CYP1A*) gene has been cloned and sequenced from many organisms for use in assessing contamination in the aquatic environment (Fent, 2003; Meyer, Nacci, & Di Guilio, 2002 Moore et al., 2003; Williams, Lech, & Buhler, 1998). Recently, its utility in identifying the action of suspected endocrine-disrupting chemicals has been emphasized (Schlezinger & Stegeman, 2001). However, the sequence similarity between *CYP1A* genes is not very high, resulting in moderately low interspecies cross-reactivity between *CYP1A* antibodies (Cousinou, Nilsen, Lopez-Barea, & Dorado, 2000), although certain antibodies will bind to *CYP1A* proteins between different genera or orders. It may therefore be necessary to obtain the sequence of *CYP1A* to use a new testing organism for reverse transcriptase-polymerase chain reaction (RT-PCR) and/or Western blots for laboratory or cage-experimental *CYP1A* induction (Cousinou et al., 2000). In this connection, we cloned the *CYP1A* gene from the self-fertilizing fish. *Rivulus* and the Japanese medaka *Oryzias latipes* both of which are well-known species for environmental toxicology studies (Lee, Park, Choe, & Chipman, 2000, 2002; Rotchell, Lee, Chipman, & Ostrander, 2001). In this paper, we show the genomic structure of both fish *CYP1A* genes and suggest that the Japanese medaka would be useful for testing heavy metals as well as endocrine-disrupting chemicals based on the response elements in its promoter region.

To clone the *CYP1A* gene of both fish, we screened *Rivulus* λGEM-11 genomic DNA library (from Jae-Seong Lee's lab) and medaka (HNI strain) λEMBL3 genomic DNA library (from Shoji Kawamura's lab). About 3×10^5 genomic clones were screened by plaque hybridization using a radiolabelled *Rivulus CYP1A* partial probe according to the plaque hybridization method. Autoradiography was carried out with Kodak X-ray film and an intensifying screen at −70 °C for 48 h. *CYP1A* positive clones were repurified. Ten *Rivulus* and eight medaka *CYP1A* clones were isolated from the primary, secondary and tertiary screening of these genomic DNA libraries. Using the consensus primers (Fish-*CYP1A*-F, 5′-CAT CCG TGA CAT CAC TGA CTC-3′ and Fish-*CYP1A*-R, 5′-GCC GTA TTC TGG GGT CAT GTC-3′) for *CYP1A*, we amplified both *CYP1A* genes and sequenced them. After obtaining the amplified internal sequences of both *CYP1A* genes by PCR, we were able to isolate the amplified long-PCR products of both *CYP1A* clones using additional primers (internal primers from the previous PCR products amplified using consensus primers and the Sp6/T7 primer from λphage multicloning site). This avoided time-consuming work in purifying *Rivulus* and medaka λ*CYP1A* clones by a conventional method. We subcloned them to pCR2.1 vector (Invitrogen) and sequenced with an automated DNA sequencer. To analyze the gene structure of both *CYP1A* genes, we used the BLAST search program of NCBI and compared them to the existing *CYP1A* sequences of other species.

The sequences of both *CYP1A* genes were deposited in GenBank under the accession numbers of AY279213 (*Rivulus CYP1A* gene) and AY233000 (Japanese medaka *CYP1A* gene) (Fig. 1(a)). Both the *Rivulus* and the medaka *CYP1A* genes consisted of seven exons (including non-coding exons), and they spanned about 3 kb. The accepting and donor sequences of exon/intron boundary were according to the

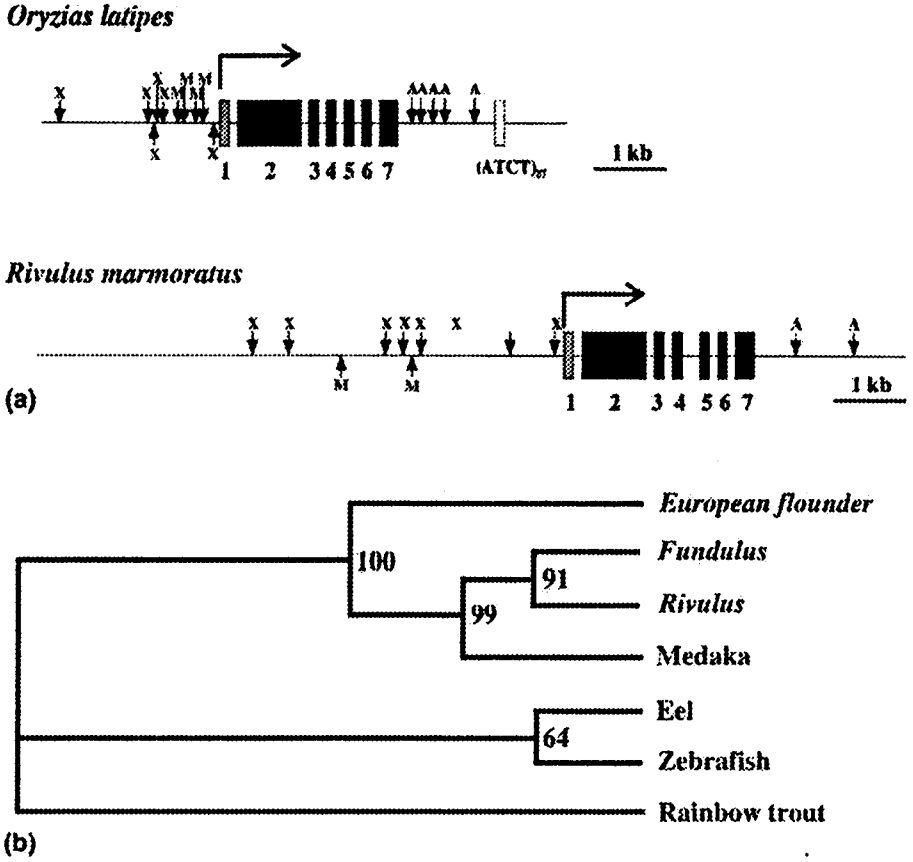

Fig. 1. (a) Schematic representation of the *Rivulus marmoratus* and the Japanese medaka *Oryzias latipes CYP1A* genes. The shadow and black boxes indicate a non-coding and the coding exons, respectively. X, Xenobiotic Response Element (XRE); M, Metal Response Element (MRE); A, Poly(A) signal sequence. (b) Dendrogram inferred from *CYP1A* in fishes. Numbers on clade indicate the bootstrap value.

GT/AG rule and were identical to those of other *CYP1A* genes. Therefore, *Rivulus* and medaka *CYP1A* genes are highly structurally conserved with those of other species. Poly(A) signal sequences of the *CYP1A* genes were located at 0.7 and 1.5 kb downstream for *Rivulus*, and there were five sites in 1 kb for medaka *CYP1A* gene. The 3′-untranslated region (UTR) of medaka *CYP1A* contained $(ATCT)_{27}$ repetitive sequences.

The phylogenetic tree relating to fish *CYP1A* was constructed after we applied them to the ClustalW program (www.ebi.ac.uk/clustalw/index.html) with TreeView of PHYLIP, based on similarity of amino acid residues of both *CYP1A* genes to those of other fishes; *Fundulus* (Morrison, Weil, Karchner, Sogin, & Stegeman, 1998; AF026800), European flounder (Williams, Lee, Sheader, & Chipman, 2000; AJ132353), eel (Aoki, Itakura, Kato, & Sato, 1999; AB020414), rainbow trout (AF015660), and zebrafish (AB078927). Both of our fish *CYP1A* genes showed generally high similarity but some regions were poorly matched, which may result in non-specific binding to *CYP1A* antibodies derived from other species, even though the monoclonal antibody from John Stegeman's lab has had remarkable cross-reactivity with *CYP1A* from various species (personal communication). It may prove

necessary to clone each *CYP1A* gene and raise specific antibodies for each potential testing organism to obtain a good correlation of chemical-induced protein expression profiles by Western blotting for specific species.

In the *CYP1A* promoter region, *Rivulus CYP1A* gene has seven XREs consensus sequences in 7704 bp, which we expect to mediate the induction of this gene by polycyclic aromatic hydrocarbons, including some EDCs. We also found two MREs, giving rise to the possibility that there is some regulation of this gene by heavy metals (Fig. 1(a)). The European flounder *CYP1A* gene has two potential MREs (one is proximal and other is distal), this was the first report to date that a fish *CYP1A* gene promoter contained MRE sites (Williams et al., 2000).

However, the Japanese medaka *CYP1A* gene has four MREs and six XREs in a short sequence (2596 bp) of its promoter region (Figs. 1A and 2). It is interesting that the Japanese medaka *CYP1A* gene promoter included 4 MREs, which may potentially confer a response to metal exposure, as is found for the metallothionein gene. As shown in Fig. 1, we find that the XREs and MREs on the medaka *CYP1A* promoter are closely associated while other fishes' *CYP1A* promoters have their response elements spread out over a longer sequence. Also, in the medaka *CYP1A* gene promoter, there are additional elements, a PRL motif and an Sp1 binding site (Fig. 2). Therefore, we may speculate that the Japanese medaka could be an ideal model to determine the relationships between environmental contaminant-mediated gene induction and the effects on the whole organism. To make use of these characteristics of the medaka *CYP1A* gene promoter, we are constructing a transgenic medaka line using compact chemical response elements in a luciferase reporter gene construct.

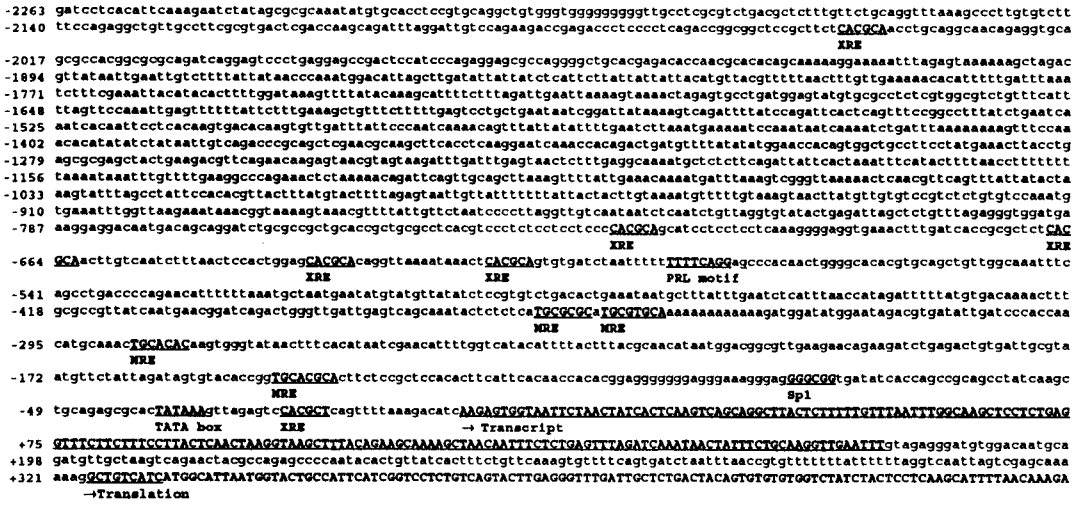

Fig. 2. Nucleotide sequence of the Japanese medaka *CYP1A* 5′-flanking region, exon 1 and intron 1. Nucleotides are numbered to the putative transcriptional start site, with negative numbers representing the 5′-flanking region. Nucleotides in the exons are represented in bold type. Consensus transcription factor binding sites are underlined (see text). These were determined using MacDNASIS program.

Acknowledgements

This work was funded by a KRF Grant (KRF-2002-005-C00019) to Y.-D.Y. We thank Dr. Tim D. Williams (School of Biosciences, University of Birmingham, United Kingdom) for improving the earlier manuscript.

References

Aoki, J., Itakura, T., Kato, H., & Sato, M. (1999). *Marine Biotechnology, 1*, 371–375.

Cousinou, M., Nilsen, B., Lopez-Barea, J., & Dorado, G. (2000). *Science of the Total Environment, 247*, 213–225.

Fent, K. (2003). *Toxicological Letters, 140/141*, 353–365.

Lee, J.-S., Park, E.-H., Choe, J., & Chipman, J. K. (2000). *Teratogenesis, Carcinogenesis, and Mutagenesis, 20*, 1–9.

Lee, J.-S., Chang, S. Y., Kim, I.-C., Han, M.-S., Lee, Y.-S., & Lee, Y.-S. (2002). *Teratogenesis, Carcinogenesis, and Mutagenesis, 22*, 363–367.

Moore, M. J., Mitrofanov, I. V., Valentini, S. S., Volkov, V. V., Kurbskiy, A. V., Zhimbey, E. N., Eglinton, L. B., & Stegeman, J. J. (2003). *Marine Pollution Bulletin, 46*, 107–119.

Morrison, H. G., Weil, E. J., Karchner, S. I., Sogin, M. L., & Stegeman, J. J. (1998). *Comparative Biochemistry and Physiology, 121C*, 231–240.

Meyer, J. N., Nacci, D. E., & Di Giulio, R. T. (2002). *Toxicological Sciences, 68*, 69–81.

Rotchell, J. M., Lee, J.-S., Chipman, J. K., & Ostrander, G. K. (2001). *Aquatic Toxicology, 55*, 1–21.

Schlezinger, C., & Stegeman, J. J. (2001). *Aquatic Toxicology, 52*, 101–115.

Williams, D. E., Lech, J. J., & Buhler, D. R. (1998). *Mutation Research, 399*, 179–192.

Williams, T. D., Lee, J.-S., Sheader, D. L., & Chipman, J. K. (2000). *Marine Environmental Research, 50*, 1–6.

Source : Marine Environmental Research, (2004), 58: 125-129