Cytochrome P450 Family 1 Genes in *Xenopus tropicalis*

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(Received 14 April 2010; accepted 29 May 2010)

Abstract—Cytochrome P450 family 1 (CYP1) genes are involved in the metabolism of chemical pollutants including halogenated aromatic hydrocarbons such as 2,3,7,8-terachlorodibenzo-p-dioxin (TCDD). Whereas the molecular characterization of CYP1 genes has been well investigated in various vertebrates, information on CYP1 genes in amphibians is relatively scarce. In the present study, we attempt to characterize CYP1 genes in *Xenopus tropicalis*, the only amphibian species whose genome has been sequenced. A novel CYP1 gene, CYP1D was identified in the *X. tropicalis* genome sequence, besides the genes of CYP1A, 1B, and 1C subfamilies that have been so far reported. Moreover, by a bioinformatics approach, three putative dioxin responsive elements (DREs) were found in the 5′-flanking regions of the *X. tropicalis* CYP1 genes, but the number of DREs and their localization within the 5 kb upstream sequences were different from those of other vertebrates. These findings provide new insights into the evolutionary and functional diversity of CYP1 genes in vertebrates and less susceptibility of amphibians to TCDD.

Keywords: aryl hydrocarbon receptor, cytochrome P450 family 1, TCDD, *Xenopus tropicalis*

INTRODUCTION

Genes of cytochrome P450 (CYP) superfamily are responsible for the metabolism of endogenous and xenobiotic compounds. Among them, CYP1 family (CYP1) consists of four subfamilies, CYP1A, CYP1B, CYP1C, and CYP1D (Nelson, 1999, 2009; Godard *et al*., 2005; Goldstone and Stegeman, 2006; Goldstone *et al*., 2007, 2009). In most of the vertebrates, CYP1A genes are strongly induced by polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons (HAHs), in particular, 2,3,7,8-terachlorodibenzo-p-dioxin (TCDD), through the activation of aryl hydrocarbon receptor (AHR), and the induced CYP1A protein can specifically metabolize some of these compounds. AHR forms a heterodimer with AHR nuclear translocator (ARNT) and binds to dioxin response elements (DREs) on the regulatory regions of CYP1A genes in the presence of PAHs and HAHs (Fujii-Kuriyama and Kawajiri, 2010). Mammalian CYP1B genes are also regulated via the AHR signaling pathway (Zhang *et al*., 1998, 2003). Both CYP1C and 1D subfamilies have been recently identified in several fishes...
Fig. 1. Alignment of deduced amino acid sequences of CYP1A, CYP1B1, CYP1C, and CYP1D genes in *Xenopus tropicalis*. The CYP1 genes were searched from Ensembl database, and their sequences were aligned by MAFFT program. Substrate recognition sites (SRSs) and heme-binding domain (Heme) are shown as gray and white boxes, respectively.
Cytochrome P450 Family 1 Genes in *Xenopus tropicalis* (Godard et al., 2005; Goldstone et al., 2007, 2009; Goldstone and Stegeman, 2008). CYP1C1 and 1C2 genes are activated by TCDD and 3,3′,4,4′,5-pentachlorobiphenyl (PCB126), whereas CYP1D gene is not induced by these HAHs (Jönsson et al., 2007; Goldstone et al., 2009). Although large number of studies focused on CYP1A genes, much attention has been paid to a limited number of fish and mammalian species. On the other hand, much less information on CYP1 genes in amphibians is available.

In this study, we searched CYP1 genes in *Xenopus tropicalis* on a whole genome basis. The genome of this species is known to be diploid and has been sequenced thoroughly. We confirmed four CYP1 genes, CYP1A, 1B, 1C, and 1D in *X. tropicalis* genome. Phylogenetic analysis showed the orthologous relationships of these CYP1 genes among various vertebrates. Results of the number of putative DREs and their localization in the 5′-flanking sequences of

![Phylogenetic tree](image.png)

Fig. 2. Phylogenetic analysis of CYP1 amino acid sequences in vertebrates. *X. tropicalis* CYP1 genes were boxed. Amino acid sequences of CYP1 genes from primate (human, *H. sapiens*), rodent (mouse, *M. musculus*), bird (chicken, *G. gallus*), frog (*X. tropicalis*), and fish (zebrafish, *D. rerio*) were deduced using MAFFT program. Human CYP2A6 sequence was used as an outgroup. All CYP1 genes are divided into two clades, 1A/1D and 1B/1C clades.
the CYP1 genes in *X. tropicalis* support a hypothesis that amphibians are not susceptible to TCDD.

**MATERIAL AND METHODS**

Details of *X. tropicalis* CYP1 genes and their 5 kb upstream sequences were obtained from Ensembl database (http://www.ensembl.org/), which is based on the JGI *Xenopus tropicalis* genome assembly ver. 4.1. Ensembl ID numbers of the CYP1 genes are as follows: CYP1A, ENSXETG00000021140; CYP1B, ENSXETG00000025303; CYP1C, ENSXETG00000015938; CYP1D, ENSXETG00000019609. As for CYP1 genes in other vertebrates, the accession numbers are given by Goldstone et al. (2007, 2009).

Sequence alignment and phylogenetic analysis were performed with MAFFT program (http://align.genome.jp/mafft/). Searching of putative dioxin response elements (DREs) was performed utilizing JASPAR database (http://jaspar.cgb.ki.se/).

**RESULTS AND DISCUSSION**

*Structural and sequence characteristics of X. tropicalis CYP1 genes*

CYP1A, CYP1B, and CYP1C genes in *X. tropicalis* have been reported in other studies (Goldstone and Stegeman, 2006; Goldstone et al., 2007). Hence, we...
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Further searched other CYP1 genes using the latest *X. tropicalis* genome data. Consequently, we confirmed four CYP1 genes, CYP1A, CYP1B, CYP1C, and a novel CYP1 gene in *X. tropicalis* with diploid genome. Deduced amino acid sequences of the CYP1 genes are shown in Fig. 1. Six substrate recognition sites (SRS) and one heme-binding domain were found in the amino acid sequences of the CYP1 genes.

Moreover, we performed phylogenetic analysis of CYP1 genes among the representative model vertebrates, including *Homo sapiens* (human), *Mus musculus* (mouse), *Gallus gallus* (chicken), *Danio rerio* (zebrafish), and *X. tropicalis* (clawed frog), whose genome sequences are well annotated. The results clearly show that the orthologous relationship of each CYP1 gene among the species of interest (Fig. 2). *D. rerio* and *X. tropicalis* have only one CYP1A gene, whereas other vertebrates, birds and mammals possess two CYP1A genes, suggesting that duplication of CYP1A gene occurs in a common ancestor of bird and mammal lineages during evolution (Goldstone and Stegeman, 2006). *D. rerio* and other fishes have two paralogues of CYP1C genes, whereas *X. tropicalis* and *G. gallus* have only one CYP1C gene (Goldstone *et al.*, 2007). Interestingly, *X. tropicalis* CYP1C gene is an intronless gene as have been observed in fishes (Jönsson *et al.*, 2007). In mammals, CYP1C genes have not been identified yet. Thus, single or multiple genes of CYP1C are likely to be lost during the evolutionary process of amphibian and other tetrapod lineages. The identity of the amino acid sequences between the novel *X. tropicalis* CYP1 gene and *D. rerio* CYP1D is 50%. Moreover, in the phylogenetic tree the novel CYP1 gene clustered with *D. rerio* CYP1D gene in CYP1A/1D clade. Consequently, the novel gene was defined as an orthologue to CYP1D gene. In *H. sapiens*, CYP1D gene was found to be a pseudogene (described in Nelson’s homepage, http://drnelson.uthsc.edu/CytochromeP450.html; Nelson, 2009).

**Putative DREs on the upstream regions of *X. tropicalis* CYP1 genes**

We found only three DREs (5′-GCGTG-3′) within the 5 kb upstream sequences from the putative transcription start sites of CYP1A gene (Fig. 3). As compared with CYP1A genes in other vertebrates, a small number of DREs in *X. tropicalis* was identified. In addition, the putative DREs were found only in the distal site (from −5.0 to −4.0 kb) of *X. tropicalis* CYP1A upstream sequence. It has been reported that functional DREs exist within the −2.0 kb sequences of CYP1A genes in human, mouse, chicken, and zebrafish (ZeRuth and Pollenz, 2007; Lee *et al.*, 2009; Nukaya and Bradfield, 2009). In *X. laevis*, a closely related species to *X. tropicalis*, AHR exhibits a low binding ability to TCDD and much higher requirement of TCDD dosage for the induction of CYP1A gene than those of other vertebrates (Lavine *et al.*, 2005). Our results suggest that differences in the number of DREs and their localization in the upstream regions of CYP1 genes may account for the resistance to TCDD in amphibians.

**Acknowledgments**—We thank Dr. John J. Stegeman, Woods Hole Oceanographic Institution for excellent advices and also thank Prof. Annamalai Subramanian in Ehime
University for critical reading this manuscript. This research was supported by Grants-in-Aid for Scientific Research (S) (21221004, to H.I.) and (B) (21710067, to K.S.) from Japan Society for the Promotion of Science, and “Global COE Program” from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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