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Conserved genomic structure of the *Cyp1a1* and *Cyp1a2* loci and their dioxin responsive elements cluster

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Abstract

A thorough DNA sequence analysis reveals that the mouse *Cyp1a1* and *Cyp1a2* loci are located with coding directions opposite to each other. The two genes are separated by approximately 13.9 kb of genomic DNA containing no open reading frames (*mCyp1a1_1a2* junction). Within the *mCyp1a1_1a2* junction, eight consensus dioxin responsive elements (DREs) are present and seven of the eight DREs located less than 1.4 kb upstream from the *Cyp1a1* transcriptional start site. The genomic structure of mouse *Cyp1a1* and *Cyp1a2* loci is similar to that of human *CYP1A1* and *CYP1A2* loci. In the human *CYP1A1* and *CYP1A2* are also arranged in a head to head orientation and separated by a 23 kb genome junction (*hCyp1A1_1A2*). Comparative sequence analysis between these two genomic junctions demonstrated that the 1.4 kb upstream region from the transcriptional start site of mouse *Cyp1a1* was highly conserved with that of human *CYP1A1*. In contrast, there are no conserved DREs in the proximal upstream region of *Cyp1a2*. The “head to head” genomic structure and position of the DREs cluster region near the *Cyp1a1* gene on *Cyp1a1_1a2* were confirmed in cattle, dog and rat genome. These results suggest that the conservation of genomic structure of *Cyp1a1* and *Cyp1a2* genes, and the DREs cluster are important in mammalian biology.

Keywords

Cytochrome P450; *Cyp1a1*; *Cyp1a2*; DRE; AHR; dioxin

1. INTRODUCTION

The Cytochromes P450 (CYP) are membrane-associated hemoproteins that catalyze mono-oxygenation of endogenous and exogenous substrates such as hormones, fatty acids, drugs and xenobiotics [1]. The *Cyp1a1* and *Cyp1a2* loci encode major CYP isozymes in liver important in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs) and procarcinogens [2]. These genes are induced by exposure to halogenated hydrocarbons such as 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (dioxin) or PAHs such as benzo(*a*)pyrene and 3-methylcholanthrene [3–4]. Induction is mediated by the aryl hydrocarbons receptor (AHR). The ligand-bound AHR translocates to nucleus, where it dimerizes with the AHR nuclear translocator (ARNT). The heterodimeric AHR-ARNT complex then binds to dioxin responsive

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element (DRE; 5'-TNGCGTG-3') within the genome, resulting in the transcriptional activation of nearby target gene expression. The reaction leads to transcriptional activation of gene expression [3–4].

The regulation of the *Cyp1a1* gene via the AHR signaling pathway has been well characterized. In the mouse, the upstream enhancer region of *Cyp1a1* has six consensus DRE sequences within –1.4 kb which mediated transcriptional activation of *Cyp1a1* gene by AHR [4–6]. This cluster of DREs in the enhancer region of *Cyp1a1* has been confirmed in several species [7–11]. In contrast, *Cyp1a2* regulation mechanism is poorly understood, because no consensus DREs are located in the nearby upstream region of mouse *Cyp1a2* gene [12]. Although a few AHR response elements, such as X1, X2 and xenobiotics response element II, have been identified in the nearby upstream of human *CYP1A2* or rat *Cyp1a2* [13–14], the position of these potential AHR response elements are not conserved in other species.

The sequence and genomic organization of *CYP1A1* and *CYP1A2* loci on human chromosome 15 determined by J. Corchero *et al.* revealed that the *CYP1A1* and *CYP1A2* genes are located immediately adjacent to each other in a head-to head orientation [15]. The genes are separated by a 23.3 kb genome junction region, designated *CYP1A1_1A2*, which possesses a total of 13 DREs and no open reading frames. In contrast to the human *CYP1A1* and *CYP1A2* loci, there are no reports with regard to genomic structure of the *Cyp1a1* and *Cyp1a2* in other species. We determined sequence and structure of the mouse *Cyp1a1* and *Cyp1a2* loci located on mouse chromosome 9. To compare the sequence with that of human, we identified highly conserved elements which should be important for the regulation of *Cyp1a1* and *Cyp1a2*. In addition, we also inquired into the genomic structure of *Cyp1a1* and *Cyp1a2* loci in cattle, dog and rat for considering the evolutionary and biological meaning of the conservation.

2. MATERIALS & METHODS

2.1. Analysis of mouse *Cyp1a1* and *Cyp1a2* loci

The bacterial artificial chromosome (BAC) clone 17278 (BAC17278) carrying intact mouse *Cyp1a1* and *Cyp1a2* genes from 129/Sv strain was employed for sequence analysis (Genome Systems, St. Louis, MO). The sequence was determined by employing both shotgun sequencing and PCR-direct sequencing. Construction of BAC shotgun library was prepared with the CloneSmart® system (Lucigen, Middleton, WI). Plasmids from the shotgun library were isolated and sequenced by DYEnamic™ ET dye terminators and megaBACE technology (GE Healthcare Bio-Science, Piscataway, NJ). Based on the resultant sequence, 49 PCR primer pairs (OL5827–5876, OL5899–5946) were designed to amplify approximately 30 kb of intact *Cyp1a1*, *Cyp1a2* and their junction region (supplementary data). PCR was carried out for 35 cycles (95°C for 30 s, 58°C for 45 s, and 72°C for 1m) in a reaction mixture containing 2.5 units of *Taq* polymerase (Promega, Madison, WI), 50 mM KCl (Sigma-Aldrich, St. Louis, MO), 10 mM Tris-HCl (pH 9.0 at 25°C) (Sigma-Aldrich, St. Louis, MO), 1.5 mM MgCl₂ (Sigma-Aldrich, St. Louis, MO), 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO), 0.2 mM dNTPs (Promega, Madison, WI), and 0.2 μM of each primer. The amplified PCR products were subcloned into pGEM®-T easy vector (Promega, Madison, WI) and sequenced by using Bigdye terminator v3.1 (Applied Biosystems, Foster city, CA).

2.2. Southern blot analysis

Either BAC DNA (100 ng) or genomic DNA (10 μg) were digested by *Afl*III, *Eco*RI, *Kpn*I, *Pst*I, or *Sac*I (Promega, Madison, WI). The digested DNAs were electrophoresed in a 0.8% agarose gel and transferred to Hybond™-N+ membrane (GE Healthcare Life-Science, Piscataway, NJ). The membrane was hybridized with ³²P-labeled cDNA probes specific for *Cyp1a1* and *Cyp1a2*. Probe 1 was a segment of mouse *Cyp1a2* gene (–622 to +298) and probe

2 was that of *Cyp1a1* gene (−246 to +257). Radioactive detection was visualized by Molecular Dynamics Storm® system (GE Healthcare Life-Science, Piscataway, NJ).

2.3. Comparative analysis of genomic sequence

Comparative genomic analysis between *mCyp1a1_1a2* and *hCYP1A1_1A2* (GenBank accession no. [AF253322](#)) was performed by VISTA (<http://genome.lbl.gov/vista/index.shtml>) [16–17]. Conserved elements are defined as above 70% sequence identity over a 50 bp window. Sequences of *Cyp1a1_Cyp1a2* in the cattle, dog and rat genomes were determined by employing genomic contig sequences (GenBank accession no.) [NC007319](#), [NC006612](#) and [NW047799](#), respectively.

3. RESULTS & DISCUSSION

To determine the sequence of the mouse *Cyp1a1* and *Cyp1a2* loci, we carried out multiple sequence analyses of the BAC17278 clone which contained approximately 192 kb of genomic DNA derived from 129/Sv strain. From analysis of the sequences of approximately 4,000 clones in BAC shotgun library, we determined the sequence for 30 kb of the *Cyp1a1* and *Cyp1a2* loci. The sequence of the *Cyp1a1* and *Cyp1a2* loci was reconfirmed by the sequencing of specific PCR fragments using primers designed from the sequence determined by the BAC shotgun sequencing. Fig. 1A presents a schematic diagram and restriction enzyme digestion map of *Cyp1a1* and *Cyp1a2* loci, showing that the *Cyp1a1* gene is located next to the *Cyp1a2* gene and the coding directions are opposite to each other. The two genes are separated by 13,927 bp of genomic junction region, designated *mCyp1a1_1a2*, which contains no known or putative open reading frames. The genomic map was verified by Southern blot analysis employing cDNA probes corresponding to the first exon and proximal promoter regions of *Cyp1a2* (probe1) or *Cyp1a1* (Probe2) (Fig. 1A–B, C). The size of digested BAC and genomic DNA fragments by restriction enzymes, *KpnI*, *AflIII*, *EcoRI*, *SacI* and *PstI*, corresponded with predicted sizes estimated from the sequences of the *Cyp1a1* and *Cyp1a2* loci. The results demonstrate that the sequence of *Cyp1a1* and *Cyp1a2* loci in native mouse genome would be the same in BAC clone.

The *Cyp1a1* and *Cyp1a2* genes are known as AHR inducible genes. Since it is generally considered that induction of the AHR targeted gene is regulated through dioxin responsive elements (5'-TNGCGTC-3') [3–4], we screened the sequence of *mCyp1a1_1a2* to determine the precise number and location of potential DREs (Fig. 2). A total of eight DREs were identified and seven out of eight DREs were located 1.4 kb upstream region from the transcriptional start site of *Cyp1a1* (Table 1). In contrast, there is no DRE up to 4.2 kb upstream region from the transcriptional start site of *Cyp1a2* gene.

The genomic sequence of human *CYP1A1* and *CYP1A2* loci was determined by Corchero *et al* [15]. Interestingly, genomic structure of human *CYP1A1* and *CYP1A2* loci is also in a head to head orientation and separated by approximately 23.3 kb of genomic junction region (*hCYP1A1_1A2*). This fact suggests that the genomic structure of *Cyp1a1* and *Cyp1a2* genes is conserved between human and mouse, although the size of *mCyp1a1_Cyp1a2* junction is 9.4 kb smaller than that of the *hCYP1A1_1A2* junction. Since induction of *Cyp1a1* and *Cyp1a2* mediated by AHR are observed in both species [3–4,18], we predict that the AHR regulatory elements of *Cyp1a1* and *Cyp1a2* should be conserved between human and mouse. To identify the conserved AHR regulatory elements between *mCyp1a1_Cyp1a2* and *hCYP1A1_CYP1A2*, we compared to the sequences by employing VISTA as comparative pairwise alignment analysis tool (Fig. 3). Overall, the conserved sequences, defined above 70% sequence identity over 50 bp length, of *mCyp1a1_1a2* was only 13% compared to that of *hCYP1A1_1A2*. However, the sequence conservation of 1.5 kb upstream region immediately proximal the mouse *Cyp1a1* gene was 55% compared to that of human *CYP1A1* gene. This

region has seven DREs in mouse and five DREs in human, respectively. It has been reported that two DREs (mouse DRE1 and DRE4) of them are fully conserved in the position and sequence with the cluster of human DREs (human DRE1 and DRE3), and all of them show AHR binding activity *in vitro* [19]. The observation suggests that the two conserved DREs could represent significant and universal AHR regulatory elements for *Cyp1a1*. In contrast, examination of upstream region of *Cyp1a2* gene shows little conservation with the exception of the proximal promoter region of *Cyp1a2* (+1 to -147 from *Cyp1a2*) and a part of the upstream regions (-4720 to -5158 from *Cyp1a2*). Although one consensus DRE is found at -4184 from *Cyp1a2* gene, this DRE is not conserved with no counterpart in *hCYP1A1_1A2*. Interestingly, the conserved upstream region of mouse *Cyp1a2* gene from -4720 to -5158 corresponds to that of human *CYP1A2* gene from -2,138 to -2,602. Although this conserved region shows AHR-mediated enhancer activity and contains another type of AHR regulatory element, X1 and X2, in humans [13], no counterpart of X1 or X2 is present in the mouse. We assume three possibilities responsible for the AHR regulatory elements of *Cyp1a2*. First, the non-conserved DRE could act as mouse-specific AHR regulatory element of *Cyp1a2*. Second, another AHR regulatory element could exist in the conserved upstream region (-4720 to -5158 from *Cyp1a2*). Finally, *Cyp1a1* and *Cyp1a2* genes could share the DREs cluster proximal to *Cyp1a1* as a common AHR regulatory element.

Analysis of the genomic structures of the *Cyp1a1* and *Cyp1a2* loci in cattle, dog and rat (GenBank; [NC007319](#), [NC00612](#), [NW047799](#)) showed that the three species also had the “head to head” genomic structure of *Cyp1a1* and *Cyp1a2* loci. The size of genomic junction region between *Cyp1a1* and *Cyp1a2* in cattle, dog and rat was 19.1 kb, 18.9 kb and 13.8 kb, and the conservation percentage was 49%, 38% and 11%, respectively, compared to *hCYP1A1_1A2*. In these species, several DREs were present in 1.5 kb upstream region of *Cyp1a1* gene. Although the total number of DREs in the genomic junction region was different, the two of highly conserved DREs confirmed in mouse and human are conserved in the position and sequence among these species (Table 2). Our finding suggest that the genomic structure of *Cyp1a1* and *Cyp1a2* loci, and the DREs cluster are highly conserved across mammals, although the size of genomic junction region between the *Cyp1a1* and *Cyp1a2* genes are different.

We speculate that the structural conservation of *Cyp1a1* and *Cyp1a2* loci, and different lengths of *Cyp1a1_1a2* might be related to differential evolutionary stresses. During the course of evolution, the organism(s) might have experienced selective pressure for a broad range of regulatory elements for *Cyp1a1* and *Cyp1a2* genes as a response to diverse environments. As a result, the length of *Cyp1a1_Cyp1a2* could be extended or reduced in this process. In contrast to the alternation of length of the *Cyp1a1_Cyp1a2*, the DREs cluster is highly conserved among species. This suggests that the conservation of the DREs cluster in *Cyp1a1_Cyp1a2* would be important in biology. It is understood that the AHR plays important roles in dioxin or PAHs-induced toxicity, hepatic vascular development and up-regulation of the adaptive battery of xenobiotics metabolism enzymes [3–4,18,20–21]. Our experiments with *Ahr* and *Arnt* mutant mouse models demonstrate that the AHR carries out these functions through the binding to DRE [22–23]. Therefore, we prospect that the DREs cluster might be essential to control these AHR functions in all mammalian species. We believe that the analysis of *Cyp1a1* and *Cyp1a2* loci would help to clarify the regulatory mechanism(s) of *Cyp1a1* and *Cyp1a2* genes, the role of DREs cluster in AHR biology, evolutionary and biological meaning of these conservations among species.

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The abbreviations

AHR	aryl hydrocarbon receptor
ARNT	AHR nuclear translocator
BAC	bacterial artificial chromosome
DRE	dioxin responsive element
CYP	cytochrome P450
dioxin	2, 3, 7, 8-tetrachlorodibenzo- <i>p</i> -dioxin
<i>Cyp1a1_1a2</i>	Genomic junction region of <i>Cyp1a1</i> and <i>Cyp1a2</i>
kb	kilo basepair(s)
PAHs	polycyclic aromatic hydrocarbons

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Fig. 1. Genomic structure of mouse *Cyp1a1* and *Cyp1a2* loci

(A) Schematic diagram and R.E. digestion map of *Cyp1a1* and *Cyp1a2*. Probe 1; -622 to +298 of *Cyp1a2* gene, Probe 2; -246 to +257 of *Cyp1a1* gene. A; *Afl*III, E; *Eco*RI, K; *Kpn*I, P; *Pst*I, S; *Sac*I site. Open box; untranslated exon, Solid box; translated exon. (B) Determination of physical distance between *Cyp1a1* and *Cyp1a2* in BAC17278 by Southern blot analysis. (C) Comparison of restriction enzymes digestion pattern between BAC clone and Genomic DNA (C57BL/6J and CBA/2 mice).

Fig. 2. Location of DREs on *mCyp1a1_1a2* region
Bar line; DRE consensus sequence.

Fig. 3. Comparative analysis of genomic sequences between *mCyp1a1_1a2* and *hCYP1A1_1A2* by VISTA

Curve indicates the conserved percentage of sequences between *mCyp1a1_1a2* and *hCYP1A1_1A2*, (A) *mCyp1a1_1a2* vs *hCYP1A1_1A2*, (B) *hCYP1A1_1A2* vs *mCyp1a1_1a2*. The red area represents conserved sequences (above 70% identity over 50 bp length). The schematic diagrams of (A) *mCyp1a1_1a2* and (B) *hCYP1A1_1A2* (Upper), and distribution map of conserved sequences (lower) correspond to each other. Open box; exon 1, Bar line; DRE.

Table 1 Sequences and positions of DREs on *mCyp1a1_1a2* region

DRE (No.)	Location from <i>Cyp1a1</i>	Sequence	Location from <i>Cyp1a2</i>	Reference
1	-488	cTcGCGTGaga	-13434	[6,19]
2	-821	cTcGCGTGgat	-13101	[5-6,19]
3	-892	cTaGCGTGcgt	-13030	[5-6,19]
4	-981	gTtGCGTGaga	-12941	[5-6,19]
5	-1058	cTaGCGTGaca	-12864	[5-6,19]
6	-1203	tTtGCGTGcga	-12719	[5-6,19]
7	-1379	tTgGCGTGtct	-12543	[19]
8	-9738	tTgGCGTGgga	-4184	

Table 2 Sequences and positions of DREs on *Cyp1a1_1a2* in human, cattle, dog and rat

Organism	DRE (No.)	Location from <i>Cyp1a1</i>	Sequence
Human	1 ^a	-497	cTcGCGTGaga
	2	-892	cTtGCGTGcgc
	3 ^b	-980	gTtGCGTGaga
	4	-1061	cTcGCGTGact
	5	-1373	tTtGCGTGcct
	6	-2116	gTgGCGTGatc
	7	-5604	gTgGCGTGatc
	8	-12213	tTtGCGTGaga
	9	-12384	cTgGCGTGagc
	10	-15061	aTgGCGTGaac
	11	-18617	aTaGCGTGcct
	12	-20417	aTgGCGTGatc
Cattle	1 ^a	-490	cTcGCGTGaga
	2	-949	cTaGCGTGcct
	3 ^b	-1040	gTtGCGTGaga
	4	-1118	cTcGCGTGact
	5	-1273	tTtGCGTGcag
	6	-4276	tTtGCGTGaga
Dog	1 ^a	-506	cTcGCGTGaga
	2 ^b	-972	gTtGCGTGaga
	3	-1036	tTcGCGTGaca
	4	-1115	tTtGCGTGcgg
	5	-4332	tTgGCGTGgag
	6	-4379	tTtGCGTGaga
	7	-5152	tTtGCGTGccc
	8	-15797	aTtGCGTGagc
	9	-17856	cTgGCGTGcca
Rat	1 ^a	-538	cTaGCGTGaga
	2	-898	tTgGCGTGcac
	3	-923	cTgGCGTGcgt
	4 ^b	-1012	gTtGCGTGaga
	5	-1089	cTaGCGTGaca
	6	-1235	tTtGCGTGcaa
	7	-1409	tTgGCGTGtct

Sequences and positions of DREs on human *CYP1A1_CYP1A2* were referred to [AF253322](#) (GenBank) and [15]. (XRE13 was not included.) Those of DREs on cattle, dog and rat *Cyp1a1_Cyp1a2* were determined by analyzing genomic contig sequences [NC007319](#), [NC006612](#) and [NW047799](#), respectively.

^{a, b} Highly conserved DREs in the position and sequence among these organisms