The Human CYP1A1 Gene Is Regulated in a Developmental and Tissue-specific Fashion in Transgenic Mice*

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Regulation and expression of human CYP1A1 is demonstrated in transgenic mice. We have developed two transgenic mouse lines. One mouse strain (CYPLucR) carries a functional human CYP1A1 promoter (−1612 to +293)-luciferase reporter gene, and the other strain (CYP1AIN) expresses CYP1A1 under control of the full-length human CYP1A1 gene and 9 kb of flanking regulatory DNA. With CYPLucR+/− mice, 2,3,7,8-tetrachlorodibenzop-p-dioxin (TCDD) and several other aryl hydrocarbon receptor ligands induced hepatocyte-specific luciferase activity. When other tissues were examined, TCDD induced luciferase activity in brain with limited induction in lung and no detectable luciferase activity in kidney. Treatment of CYP1AIN+/− mice with TCDD resulted in induction of human CYP1A1 in liver and lung, while mouse Cyp1a1 was induced in liver, lung, and kidney. Although induced CYP1A1/Cyp1a1 could not be detected by Western blot analysis in brains from CYP1AIN+/− mice, induction in brain was verified by detection of CYP1A1/Cyp1a1 RNA. The administration of TCDD to nursing mothers to examine the effect of lactational exposure via milk demonstrated prominent induction of luciferase activity in livers of CYPLucR+/− newborn pups with limited induction in brain. However, TCDD treatment of adult CYPLucR+/− mice led to a 7-10-fold induction of brain luciferase activity. Combined these results indicate that tissue-specific and developmental factors are controlling aryl hydrocarbon receptor-mediated induction of human CYP1A1.

The cytochrome P450 1A1 (CYP1A1) family of proteins is often linked through metabolism of environmental toxicants to the generation of chemical carcinogens (1). These genes are actively regulated by the aryl hydrocarbon (Ah) receptor (2–4). The Ah receptor is a member of the basic helix loop helix family of proteins (5, 6) and is activated by halogenated hydrocarbons such as 2,3,7,8-tetrachlorodibenzop-p-dioxin (TCDD) and polycyclic aromatic hydrocarbons as well as dietary substances such as bioflavonoids (3, 7, 8). The process of ligand association and Ah receptor activation culminates in nuclear translocation where it partners with the basic helix loop helix region of the aryl hydrocarbon nuclear translocator protein (9–11). The Ah receptor-aryl hydrocarbon nuclear translocator complex serves as a transcriptional unit binding to highly conserved enhancer sequences termed xenobiotic response elements (XREs) (12–14). Those genes that carry conserved XREs in their regulatory region are susceptible to transcriptional activation. While the number of genes that are potential targets for the Ah receptor is extensive (15), the levels of transcriptional activation of Cyp1a1/CYP1A1 exceeds those of other target genes and often serves as a model to investigate the functional properties of the Ah receptor.

Although CYP isoforms have been monitored in human tissues and have been shown to be differentially expressed (16), very little is known regarding the mechanisms associated with Ah receptor control of the CYP1A1 gene in vivo. For the most part, studies undertaken in tissue culture or through animal models have been extrapolated to predict the response in humans. For example, the Ah receptor is functional in early embryogenesis as demonstrated by induction of hepatic rodent Cyp1a1 transplacentally at 15 days gestation following administration of 3-methylcholanthrene to pregnant mice (17, 18). Analysis of Cyp1a1 in rodents has demonstrated that exposure to Ah receptor ligands is a prerequisite for protein expression. Undetectable or minimal Cyp1a1 is found in hepatic as well as most extrathepatic tissues (19–21). These expression profiles change dramatically after the animals have been exposed to Ah receptor ligands as confirmed by transcriptional activation of Cyp1a1 and induction of mRNA and protein. The induced expression of Cyp1a1 is found in many extrathepatic tissues. Studies in various strains and substrains of mice that are genetically compromised in Ah receptor function (22, 23) demonstrate that induction of Cyp1a1 is modulated by the Ah receptor. This has also been verified in mice where the Ah receptor gene has been rendered non-functional by targeted gene knock-out studies (24–26). In addition, it is speculated that circulating humoral factors play an important role in Ah receptor function since the treatment of mice with agents known to alter circulating hormones influences Ah receptor-mediated expression of Cyp1a1 (19, 27). Thus, to understand the regulatory properties associated with Ah receptor function and human CYP1A1 gene expression, it would be an advantage to examine expression patterns of the human gene in transgenic mice.
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While the Ah receptor is conserved between rodents and humans, there are significant structural differences between the rodent Cyp1a1 and human CYP1A1 loci. The CYP1A1 cluster is located on chromosome 15q22-qter with the promoter and luciferase reporter gene. For example, there are 13 XRE core DNA sequences that may participate in transcription of each promoter. Treatment of both CYP1A1 and CYP1A2 with TCDD provides a novel opportunity to study the differential patterns of expression of human CYP1A1 as well as rodent Cyp1a1. As will be demonstrated, the induction of human CYP1A1 by Ah receptor ligands is dependent upon tissue-specific as well as development factors.

MATERIALS AND METHODS

Chemicals and Reagents—TCDD was purchased from Wellington Laboratories (Guelph, Ontario, Canada). Benzo[a]pyrene, benzo[a]anthracene, chrysene, β-naphthoflavone, and 3-methylcholanthrene were obtained from Sigma. Luciferin was obtained from Analytical Luminescence Laboratories (Guelph, Ontario, Canada). TCDD was purchased from Wellington Laboratories. The human CYP1A1, firefly luciferase, and the actin gene. The forward human CYP1A1 primer was 5'-GGTCACCCCACAGTTCCCGG-3', and the reverse Cyp1a1 primer was 5'-GGTCACCCCACAGTTCCCGG-3', and the reverse primer was 5'-GGTCACCCCACAGTTCCCGG-3'. The primers were designed to amplify the region of the human CYP1A1 gene encoding exons 1 and 2 of template DNA which corresponds to the 3'-untranslated region of the gene. The oligonucleotides spanned bases 5558–6551 of the human CYP1A1 gene and encoded bases 7661–7680 (GenBankTM accession number AF253322). The 5'-GGTCACCCCACAGTTCCCGG-3' primer was used in the PCR reaction, 100 ng of template DNA, 0.1% glycerol, 1 mM EDTA, 20 mM Tris- HCl, 0.1% Triton X-100, for 15 min. Control samples were incubated in the absence of NADPH. The incubations were terminated by the addition of 20 μl of nondenatured sample solution (TRIzol, Invitrogen). Three micrograms of total RNA were treated with 10 μl of Qiagen Hotstart Master Mix (following the manufacturer's instructions) to obtain microinjection-quality DNA. The homogenate was centrifuged at 14,000 × g for 30 min. The supernatant was collected, and the protein concentrations were determined using a standard protein assay. The relative light units were normalized to the protein content. Western Blot Analysis—Various tissues were homogenized in 4 volumes of 1.15% potassium chloride solution. The homogenate was centrifuged at 10,000 × g for 30 min in an Eppendorf Microfuge to obtain the supernatant fraction. The supernatant fraction was collected, and the protein concentrations were determined using a standard protein assay. Each supernatant sample was separated by SDS-PAGE and transferred to a nitrocellulose membrane. The nitrocellulose membrane was incubated with a primary antibody directed against the protein of interest, washed, and then incubated with a secondary antibody conjugated to horseradish peroxidase. The membrane was washed, and the bands were visualized using a chemiluminescence detection system.
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Results

Characterization of Hepatic CYP1A1-Luciferase Expression in CYP1A1−/− Mice—The human CYP1A1 gene is regulated in part through activation of the Ah receptor, but other important transcriptional factor activators such as Sp1 (33), NF1, NF-YB (34), and protein kinase C (35, 36) play important roles in modifying gene expression in the presence of Ah receptor ligands. In HepG2 cells, a −1612 bp fragment of the CYP1A1 promoter (Fig. 1) containing these DNA binding sequences as well as the XRE sequences has been shown to serve as a template for transcriptional activation of luciferase reporter gene activity in the presence of Ah receptor ligands (30). To examine whether this CYP1A1-luciferase gene can function as a reporter gene in vivo, CYP1A1-luciferase (CYP1LucR)-positive mice (see “Materials and Methods”) were generated. Characterization of luciferase induction was examined in CYP1LucR−/− mice after 24 h following a single intraperitoneal injection of TCDD. Examining a range of TCDD treatments between 0.0125 and 16 μg/kg, induction of luciferase activity was observed with a minimal dose of 0.32 μg/kg (Fig. 2). Detection of microsomal Cyp1a1 by Western blot analysis was also confirmed with a minimal dose of 0.32 μg/kg, demonstrating that the cellular and molecular properties that influence gene expression of the CYP1A1-luciferase and the endogenous Cyp1a1 gene are similar.

In tissue culture cells, the human CYP1A1-luciferase gene is responsive to halogenated hydrocarbons and polycyclic aromatic hydrocarbons (30, 36) as well as to bioflavonoids such as chrysin (8) and certain substituted benzimidazoles such as omeprazole (38). Along with TCDD, 3-methylcholanthrene, benzo[a]pyrene, and benz[a]anthracene induced hepatic luciferase activity as well as microsomal Cyp1a1 in CYP1LucR−/− mice (Fig. 3). The compound 5,6-benzoflavone (β-naphthoflavone) was a good inducer of Cyp1a1 in liver microsomes and also induced luciferase activity, but the induction of luciferase activity was less than that observed for 3-methylcholanthrene, benzo[a]pyrene, and benz[a]anthracene. Omeprazole and chrysin, which induce human CYP1A1 in human tissue culture cells as well as CYP1A1-luciferase in TV101 cells (8, 38), were not able to induce luciferase activity in CYP1LucR−/− mice. This result would indicate that chrysin and omeprazole are being...
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TABLE I

| Treatment       | Liver* | Hepatocytesb | Hepatocytes in culturec |
|-----------------|--------|--------------|-------------------------|
| Control (Me₂SO) | 1.23 ± 0.22 | 2.70 ± 1.92 | Not detectable          |
| TCDD            | 906.7 ± 17.7 | 159.4 ± 1.7  | 179.5 ± 21.7           |

*Luciferase activity measured in cytosolic fractions from CYPLucR mice treated for 24 h with 3.2 µg/kg TCDD.

'b' CYPLucR mice were treated for 24 h with TCDD, and luciferase activity was measured in isolated hepatocytes.

'c' Hepatocytes were isolated from CYPLucR mice and placed into culture for 24 h. TCDD (10 nM) was then added to the isolated hepatocytes, and after a 24-h exposure luciferase activity was determined.

* RLU, relative light units.

Extrahepatic Expression of CYP1A1-Luciferase—Analysis of luciferase activity in different tissues both in the absence and presence of Ah receptor ligands would be considered a good indicator of the actions of ligand toward activation of the Ah receptor and induction of the CYP1A1 gene. To examine the expression of CYP1A1-luciferase in different tissues, 8-week-old CYPLucR mice were treated with 3.2 µg/kg TCDD for 24 h, the tissues were isolated, and luciferase activity was monitored (Fig. 4). As expected, the most significant level of induced expression was noted in liver. No induction was noted in small or large intestine, which differs from previous reports on the induction of Cyp1a1 in rat and mouse gastrointestinal tract (39). To our surprise, there was no induction of luciferase activity in kidney and only limited induction in lung, two tissues that have been studied extensively and shown to elicit induction of Cyp1a1 (19), as shown in Fig. 5.

Interestingly significant levels of induced luciferase activity occurred in brain. This large increase in luciferase activity may in part be a result of basal luciferase activity that is nearly 100-fold higher than that observed in livers from untreated mice. Elevated levels of constitutive luciferase activity are in agreement with reports that Cyp1a1 is constitutively expressed in rodent brain (39, 40). These results are also in agreement with previously published observations indicating that induced levels of Cyp1a1 accumulate in rat cerebral cortex and cerebellum (39). Localization of Cyp1a1 mRNA by in situ hybridization has also been identified in the choroid plexus and distributed in the lateral third and fourth ventricles. Since expression of CYP1A1-luciferase is Ah receptor-dependent, this finding suggests that one possibility of the higher basal levels of luciferase activity may result from exposure to brain-specific factors that serve as endogenous Ah receptor ligands.

Expression of the Human CYP1A1 Gene—To determine whether the hepatic and extrahepatic expression patterns of the CYP1A1-luciferase gene mimic expression patterns found with the full-length human CYP1A1 gene, CYP1A1N mice were developed that express the human CYP1A1 gene (Fig. 1). To examine expression of human CYP1A1 in CYP1A1N mice, Western blots were conducted with an anti-human CYP1A1 antibody (32) with specificity toward mouse Cyp1a1 and human CYP1A1. Using microsomes from wild type mice, it was seen that a dose of 3.2 µg/kg TCDD induces liver Cyp1a1 (Fig. 5). In contrast, when TCDD was administered to CYP1A1N mice and liver microsomal proteins were identified by Western blot analysis, two prominent induced proteins were identified. One migrated as Cyp1a1, while the other migrated slightly faster with an Rf value that is identical to TCDD-induced CYP1A1 from human HepG2 cells. TCDD-induced liver microsomal ethoxyresorufin O-deethylase activity in CYP1A1N mice was nearly twice the value detected in wild type mice from the same litter (Table II).

FIG. 2. Induction by TCDD of CYP1A1-luciferase activity and Cyp1a1 in liver of CYPLucR mice as a function of dose. Adult CYPLucR mice were treated with differing concentrations of TCDD by intraperitoneal injection for 24 h. Liver tissue was homogenized, and microsomal membranes were prepared. A sample of the 9000 × g supernatant fraction was saved for analysis of luciferase activity. A, relative luciferase values in cytosolic fractions as a function of the dose of TCDD. B, Western blot analysis using microsomes to detect the induction of mouse Cyp1a1. DMSO, Me₂SO; RLU, relative light units.

FIG. 3. Induction of luciferase activity in CYPLucR mice using several standard CYP1A1 inducers. A, transgenic CYPLucR mice were treated by intraperitoneal injection every 24 h with 100 mg/kg β-naphthoflavone (β-NF), benz[a]anthracene (B[a]A), benzo[a]pyrene (B[a]P), chrysene, omeprazole, and 50 mg/kg 3-methylcholanthrene (3-MC) for 3 days. Each bar is an average of luciferase activity from three different mice. B, Western blot analysis of liver microsomes using the anti-human CYP1A1 antibody. An equal portion of liver from each of three mice representing the treated groups was pooled for preparation of the microsomes used in this experiment. DMSO, Me₂SO; RLU, relative light units.

influenced by in vivo factors in a pattern that differs from their ability to induce CYP1A1 in tissue culture cells.

Microsomal P450 expression in liver is localized to the hepatocytes. To examine whether induced luciferase activity is expressed in hepatocytes, CYPLucR mice were treated for 24 h with 3.2 µg/kg TCDD, and luciferase activity was assayed in isolated hepatocytes. When we compared luciferase activity in hepatocytes isolated from untreated mice, CYP1A1-luciferase activity was induced in hepatocytes from the TCDD-treated mice (Table I). A similar induction profile was observed when hepatocytes from untreated CYPLucR mice were first cultured for 24 h and then treated with 10 nM TCDD. These results suggest that integration of the CYP1A1-luciferase gene into the mouse genome can be regulated in a tissue-specific and inducible fashion comparable to what is observed for expression of the mouse Cyp1a1 gene.
When we compared the induction profiles in extrahepatic tissues from TCDD-treated CYP1A1N−/− mice, Cyp1a1 was detectable in lung and kidney (Fig. 4B) with minor identification in brain (data not shown). Unlike other reports, we did not observe any induced Cyp1a1 in small or large intestine. In contrast, human CYP1A1 was identified in lung, but there was no detectable induction in kidney or brain.

While we clearly identified TCDD-inducible CYP1A1-luciferase activity in CYPLucR−/− mice by TCDD, adult CYPLucR mice were treated with a single intraperitoneal injection of MeSO (100 μl) or TCDD (3.2 μg/kg), and cytosolic fractions were prepared after 24 h. Tissues from three MeSO- or TCDD-treated mice were pooled, and relative luciferase activity was determined. Each determination was carried out three times. RLU, relative light units. Statistically significant differences are noted by an asterisk.

Fig. 4. Tissue-specific induction of CYP1A1-luciferase activity in CYPLucR−/− mice by TCDD. Adult CYPLucR mice were treated with a single intraperitoneal injection of MeSO (100 μl) or TCDD (3.2 μg/kg), and cytosolic fractions were prepared after 24 h. Tissues from three MeSO- or TCDD-treated mice were pooled, and relative luciferase activity was determined. Each determination was carried out three times. RLU, relative light units. Statistically significant differences are noted by an asterisk.

Fig. 5. Induction of CYP1A1 and Cyp1a1 in CYP1A1N−/− and CYP1AIN−/− mice by TCDD. Western blots were prepared using microsomal preparations, and TCDD-induced CYP1A1 or Cyp1a1 was monitored by detection with the anti-human CYP1A1 antibody. A, three CYP1AIN-negative (F1-1A1−/−) and three CYP1AIN-positive (F1-1A1+/−) mice derived from the same litter were treated with 3.2 μg/kg TCDD (T) for 24 h, and microsomal preparations from liver were analyzed for induction of human CYP1A1 and mouse Cyp1a1. Microsomes from an MeSO (D)-treated CYP1AIN-positive (F1-1A1+/−) mouse is included. To monitor for induction of human CYP1A1, microsomal preparations from human HepG2 cells treated with 10 nM TCDD for 24 h were included. B, liver, lung, and kidney microsomes were prepared from adult CYP1AIN−/− (IA1−/−) and CYP1AIN+/− (IA1+/−) mice that were treated with either MeSO (D) or TCDD (T). Tissues were pooled for two animals, and microsomal preparations were generated. Each lane contains 20 μg of microsomal protein. C, RNA from liver and brain tissue from MeSO (D)-treated and TCDD (T)-treated CYP1AIN−/− (IA1−/−) and CYP1AIN−/− (IA1+/−) mice was used in a reverse transcription-PCR. Primers specific for the detection of human CYP1A1, mouse Cyp1a1, and actin were included in the same reaction. Std, standard.
ase activity in brain and this corresponds to previous reports that Cyp1a1 is inducible in brain, the inability of Western blot analysis to identify induced CYP1A1 in brain indicates that the expression patterns are very low in this tissue. However, when we analyzed CYP1A1/Cyp1a1 expression patterns in brain by reverse transcription-PCR, inducible RNA that encoded both Cyp1a1 and CYP1A1 was detected.

**Effect of Lactational Exposure to TCDD on Neonatal Liver and Brain CYP1A1-Luciferase**—To examine whether TCDD is capable of modifying neonatal gene expression patterns in CYP1LucR−/− mice, lactating maternal mice were given a single injection of TCDD, and activity in liver and brain from CYP1LucR−/− mice was monitored in feeding neonates. In this experiment, a single dose of 3.2 μg/kg TCDD was injected into maternal mice the 1st day postpartum. Analysis of luciferase activity in liver and brain was assessed after 24 h in nursing neonatal CYP1LucR+/− mice.

In CYP1LucR−/− neonatal mice, luciferase activity in liver was barely detectable from feeding with the TCDD-treated maternal mouse whose mothers received a single injection of MeSO (Fig. 6). In litters where the maternal mouse received a single intraperitoneal injection of TCDD, luciferase activity in CYP1LucR−/− neonatal mice was induced. The levels of luciferase activity were more than double those observed in livers from 2-month-old mice that received a single intraperitoneal injection of TCDD.

In comparison to liver, brain CYP1A1-luciferase activity in neonatal mice that were breastfeeding from the TCDD-treated maternal mouse was only 2-fold that of constitutive luciferase activity. In contrast, brain CYP1A1-luciferase activity following TCDD treatment in adult mice was nearly 7-fold that observed from untreated adult mice. In addition, the constitutive levels of brain CYP1A1-luciferase activity in adult mice were nearly 3-fold higher than those in neonatal mice. The higher levels of constitutive luciferase activity as well as inducible activity in adult brain indicate that specific developmental factors are maturing in adult animals to aid in the expression of CYP1A1.

**DISCUSSION**

The development of transgenic animal models expressing human genes offers an opportunity to investigate the contribution of regulatory factors that modulate gene expression as well as structural determinants of the gene that may influence expression patterns in vivo. These studies provide an advantage over classical tissue culture model systems since expression patterns can now be linked to the role of circulating humoral factors as well as the contribution of tissue-specific regulatory factors. In this report, we have evaluated the tissue-specific expression patterns of a luciferase reporter gene under control of 1600 bases of the CYP1A1 promoter in CYP1LucR mice and compared luciferase induction to expression patterns of human CYP1A1 in CYP1AIN mice that carry the entire human CYP1A1 gene. Both the CYP1LucR and CYP1AIN genes were expressed in a similar fashion in response to TCDD induction in liver. Expression of CYP1LucR in liver was localized to hepatocytes and corresponded with induction of mouse Cyp1a1 as well as induction of CYP1A1 in CYP1AIN mice.

Since both transgenes are induced in liver by TCDD, gene expression might be controlled entirely through activation of the Ah receptor. However, this does not appear to be the case. TCDD treatment induced mouse Cyp1a1 in lung and kidney tissue as previously reported (21, 39). However, transgenic gene expression in kidney CYP1LucR and CYP1AIN mice was not observed. Since CYP1A1 has been detected in human kidney (41), the dichotomy in expression of rodent Cyp1a1 and human CYP1A1 in kidney might indicate that the organization of the chromatin around the transgenic sequences is not conducive to activation by the Ah receptor. Alternatively the human CYP1A1 gene contains sequence elements that require tissue-specific regulatory factors that are only available in human kidney. Interestingly the human CYP1A1 gene contains a negative regulatory domain within −800 bases from the transcriptional start site that is not present in the mouse Cyp1a1. It is predicted that transcriptional factors associating with the negative regulatory domain sequences suppress transcriptional activation (34). Although Cyp1a1 was induced in mouse kidney, regulation of human CYP1A1 in this tissue may be tightly controlled through the negative regulatory domain sequence. We can only speculate that the differences in expression of Cyp1a1 and CYP1A1 in kidney result from evolutionary differences in the organization of the mouse and human regulatory regions flanking the Cyp1a1/CYP1A1 genes. However, it cannot be ruled out that positioning of the transgene at or near
the rodent Cyp1a1 locus is necessary for appropriate expression in selective tissues.

There are several dramatic differences in regulation of the transgenes in CYPLucR and CYP1AINv mice. Human CYP1A1 was induced in lung microsomes from CYP1AINv mice but was not observed in brain where dramatic inducibility of luciferase activity occurred in CYPLucR mice. While expression of CYP1A1 in CYP1AINv liver and lung paralleled closely the patterns observed for induction of Cyp1a1 and best mimicked the regulatory pattern expected for expression of CYP1A1 gene, the high levels of inducible luciferase activity in CYPLucR brain is unusual since the human CYP1A1 and mouse Cyp1a1 gene do not appear to be dynamically regulated in mouse brain. However, this may be a fortuitous finding by providing an animal model that will allow for analysis of Ah receptor ligands that are permeable to the blood-brain barrier.

The sensitive induction profiles in CYPLucR mice also served as an in vivo model system to examine developmental regulation of CYP1A1. Mouse Cyp1a1 is expressed constitutively and induced in adult brain (39, 40), and we demonstrated that TCDD was capable of inducing CYP1A1-luciferase activity in adult brain from CYPLucR mice. In contrast, induction of CYP1A1-luciferase in brains from neonatal mice that were receiving TCDD through lactational exposure showed minimal induction. The lack of CYP1A1-luciferase induction in neonatal brain from CYPLucR mice was not observed in liver tissue where induction of luciferase activity in liver was nearly double that found in livers from adult CYPLucR mice. The progressive induction of CYP1A1-luciferase in brains from CYPLucR mice as a result of age indicates that late stage neonatal or adult-specific regulatory factors are necessary in brain tissue for the activated Ah receptor to participate in CYP1A1 induction. The induction pattern of CYP1A1-luciferase in adult brain was also concordant with induction of brain Cyp1a1 mRNA by TCDD, indicating that brain-specific regulatory factors work in concert with the Ah receptor to modulate transcriptional activation of the Cyp1a1 gene.

For the most part, agents that are capable of activating the mouse Ah receptor in tissue culture cells have been demonstrated to activate the human Ah receptor. However, species differences in Ah receptor response have been reported. For example, omeprazole, which is a substituted benzimidazole used clinically to suppress gastric acid secretion by inhibiting the H+/K-ATPase in gastric parietal cells, transcriptionally activates CYP1A1 in human cells in an Ah receptor-dependent mechanism (38). However, the treatment of mouse hepatoma cells fails to initiate transcriptional activation of Cyp1a1 (42). This difference in response has been reported to result from a species-specific difference in the generation of an omeprazole metabolite that serves as a unique ligand for activation of the Ah receptor. Indeed, when we administer omeprazole to CYPLucR mice, there was no induction of CYP1A1-luciferase activity, supporting findings that unique metabolic profiles may influence the actions of Ah receptor activation in a species-specific fashion.

Chrysin, on the other hand, is a dietary flavonoid that has been shown to induce human CYP1A1 (8) as well as mouse Cyp1a1 in vitro (43). When chrysin was administered to CYPLucR mice, there was no detectable induction of hepatic CYP1A1-luciferase activity even after repeated administrations up to 72 h (data not shown). Although chrysin is an active inducer in mouse and human cells, the in vivo environment can dramatically influence the biological fate of specific substances. It might be anticipated that absorptive properties or extensive metabolism may alter the ability of chrysin to activate the Ah receptor in vivo. Thus, while analysis of Cyp1a1/CYP1A1 gene induction profiles in tissue culture is an excellent predictor that agents are potential Ah receptor ligands, the use of an animal model is more informative in predicting in vivo outcomes of events that regulate CYP1A1 gene transcription.

In developing a CYP1A1 transgenic model, we have attempted to clone a gene fragment that contains all of the regulatory elements. It has been emphasized by Corchero et al. (28) that regulatory elements on the CYP1A2 gene may participate in CYP1A1 gene expression. This is possible, and the lack of expression of CYP1A1 in kidney tissue may reflect an absence of an element that is needed for tissue-specific regulation in kidney tissue. However, our results do indicate that transgenic expression of the human CYP1A1 gene by Ah receptor agonists requires the involvement of unique tissue-specific and developmental factors, some of which may be independent of those required to induce expression of the mouse Cyp1a1 gene. This work differs slightly from previous reports describing the transgenic expression of the mouse Cyp1a1 (44) or rat CYP1A1 promoter (37) in that reporter gene expression of these constructs closely mimicked the expression patterns of the endogenous Cyp1a1 gene. It might be argued that the lack of expression of the CYPLuc and CYP1A1 transgenes in certain extrahepatic tissues results from a chromosomal integration site that prevents access of the activated Ah receptor to XRE sequences in these tissues. However, other founders carrying the CYP1A1 gene fragment generated similar induction profiles in liver, kidney and lung (data not shown). These data might indicate that induction of human CYP1A1 by Ah receptor ligands in vivo requires the presence of additional regulatory elements or transcriptional factors that are not essential for the induction of the mouse Cyp1a1.

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The Human CYP1A1 Gene Is Regulated in a Developmental and Tissue-specific Fashion in Transgenic Mice

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