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# Targeted Knockout of *Cyp1a1* Gene Does Not Alter Hepatic Constitutive Expression of Other Genes in the Mouse [*Ah*] Battery

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**Using the Cre-*lox* system, we have generated a cytochrome P450 1A1 *Cyp1a1(−/−)* knockout mouse by deletion of the translated portions of the *Cyp1a1* gene. These mice are viable and demonstrate no obvious phenotype, compared with wild-type littermates. As a first step toward characterizing genes that might be expected to compensate for loss of CYP1A1, constitutive expression of [*Ah*] gene battery members was examined. In a cultured hepatoma CYP1A1 metabolism-deficient mutant line that does not express *Cyp1a2,* we have previously shown that constitutive transcriptional up-regulation of other [*Ah*] gene battery members occurs; these results are consistent with the elevation of a putative endogenous ligand (EL) for the Ah receptor that is a substrate for CYP1A1. The [*Ah*] battery includes *Cyp1a2,* NAD(P)H:quinone oxidoreductase (*Nqo1*), and three other Phase II genes. Examining mRNA, protein, and enzyme activity, we demonstrate that the absence of CYP1A1 has no effect on the hepatic constitutive expression of *Cyp1a2* or *Nqo1.* We postulate that CYP1A1 and CYP1A2 might have overlapping substrate specificity for metabolism of the EL, such that basal CYP1A2 in the liver can compensate for the loss of CYP1A1.** © 2000 Academic Press

Cytochrome P450 1A1 (CYP1A1) is one of four dozen to six dozen P450 genes of the *CYP* gene superfamily in vertebrates (1, 2). The CYP1A1 enzyme plays a key role in expression of the [*Ah*] gene battery (reviewed in Ref. 3). The mouse [*Ah*] gene battery comprises at least six, and probably many more, genes—two Phase I P450 genes, *Cyp1a1* and *Cyp1a2,* and four Phase II genes: *Nqo1* [NAD(P)H:quinone oxidoreductase]; *Aldh3a1* (cytosolic aldehyde dehydrogenase, ALDH3c; *Ahd4*); *Ugt1a6* (UDP glucuronosyltransferase-1A6); and *Gsta1* (glutathione transferase Ya). All six genes are up-regulated by ligands such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) that bind to the Ah receptor (AHR), and all four [*Ah*] Phase II genes are induced by oxidative stress. The criterion for "membership" in the [*Ah*] battery is the CYP1A1/1A2 metabolism-dependent down-regulation of transcription of each of these six genes (reviewed in Refs. 3, 4).

Dioxin is known to bind to and activate the AHR, which then forms a heterodimer with the Ah receptor nuclear translocator (ARNT) in the nucleus, and the transcription of dioxin-inducible genes is activated through aromatic hydrocarbon response elements (AHREs). The elegant genetic complementation studies of benzo[a]pyrene-resistant mutant lines derived from the mouse hepatoma Hepa-1c1c7 cell culture line (reviewed in Ref. 5) have clearly provided important tools for dissecting the cross-talk among genes in the [*Ah*] battery. Complementation groups include "AHR-receptorless" such as the *c2* line, "ARNT-deficient" such as the *c4* line, and "CYP1A1 metabolism-defective" such as the *c37* line. CYP1A1 is highly inducible by dioxin in the wild-type (*wt*) Hepa-1 parent line. Because CYP1A1 mRNA is negligible in both the control and dioxin-treated *c2* and *c4* lines, it has been concluded that CYP1A1 induction is dependent on both the AHR and the ARNT, respectively. On the other hand, CYP1A1 mRNA is as high in untreated *c37* cells as in dioxin-treated cells; introduction of a functional mouse CYP1A1 cDNA into the *c37* line causes a return to the wild-type phenotype: i.e. CYP1A1 mRNA becomes negligible in untreated cells and highly inducible in dioxin-treated cells (6).

Intriguingly, the same pattern found for CYP1A1 mRNA in all these cell lines is seen for NQO1 mRNA (6), ALDH3A1 mRNA (7), UGT1A6 mRNA and GSTA1 mRNA (8). These findings can be explained by our

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hypothesis that the CYP1A1 enzyme might degrade a putative endogenous ligand (EL) for the AHR; absence of CYP1A1 activity in *c37* cells would lead to a build-up of EL, which would activate the *Cyp1a1* gene (and other [*Ah*] battery members). Recent experiments (9) are consistent with our hypothesis for the existence of an EL. Hence, from Hepa-1 cell culture studies, we conclude that the active CYP1A1 enzyme is critical in controlling AHR-mediated functions (reviewed in Ref. 3).

Although the endogenous ligand for the AHR is not yet known, we believe that evolution of this gene battery during the past 400 million years or longer is principally due to the importance of this battery in a critical life function(s) rather than its secondary role in the metabolic detoxification of foreign chemicals. At least one critical life function is believed to be the participation of [*Ah*] battery genes in the cell's decision between oxidative stress-induced apoptosis and progression with the cell cycle (reviewed in Ref. 3).

What would happen in the liver of intact mice having an ablation of the *Cyp1a1* gene? Based on the hepatoma cell culture studies described above, we posed the question whether other hepatic genes in the [*Ah*] battery might be up-regulated, as seen in the *c37* mutant cell line. We found, however, that hepatic CYP1A2 and NQO1 expression is unaffected in the intact *Cyp1a1(−/−)* mouse.

## MATERIALS AND METHODS

*Construction of the targeting vector and electroporation into embryonic stem (ES) cells.* An *Eag*I-*Eco*RV fragment, spanning nucleotides (nt) −588 to +6997 (relative to the transcription start site) of the *Cyp1a1* gene, was subcloned into Bluescript (Stratagene; La Jolla, CA) from a 129Sv/J γ-phage clone (Stratagene). An *Hprt* minicassette (10), flanked by direct-repeat *loxP* sites (11), was inserted into the *Cyp1a1* intron 1 unique *Sac*I site (nt +2269), and a third direct-repeat *loxP* site was inserted into the *Bgl*II site (nt +5456) in the 3′-untranslated region (UTR) of the *Cyp1a1* gene. To finalize the targeting construct, we cloned in the HSV-*tk* gene (12) at the 3′ end of the floxed *Cyp1a1* gene. For more details, please contact the first author (TPD) at tim.dalton@uc.edu.

The targeting construct was linearized with *Cla*I and electroporated into E14tg2a ES cells, which are *hprt*$^-$ (13). Selection in hypoxanthine-aminopterin-thymidine (HAT) supplement (Sigma; St. Louis, MO) was initiated 24 h later. Further selection with 2 μM ganciclovir (Syntex; Palo Alto, CA) was begun 48 h following electroporation. Resistant ES colonies were selected 14 days after electroporation, treated with trypsin, and transferred to 24-well plates. After 2 or 3 days in culture, half the cells from each well were frozen, and the other half were transferred to gelatin-coated 6-well plates.

*Southern hybridization analysis.* DNA was isolated from each ES cell colony, digested with *Eco*RV (10 μg/clone), and Southern blotted (14). Following prehybridization, the membranes were hybridized with a $^{32}$P-labeled random-primed DNA probe that was generated by PCR and encompassed nt −763 to −643 in the *Cyp1a1* 5′-flanking region; this probe, outside the targeted region, will identify all targeted clones—including those that do not contain the 3′-nonselected *loxP* site. To subselect for clones that contained the 3′-*loxP* site, we stripped the membranes and rehybridized with a 3′ probe encompassing nt +6857 to +6997.

*Generation of the Cyp1a1(−/−) mouse.* Targeted ES cells (agouti, gray) were microinjected into the blastocoele cavity of C57BL/6J embryos (nonagouti, black), and surviving blastocysts were transferred into pseudopregnant CD-1 foster dams by uterine implantation (15). Identification of chimeric pups was determined by the presence of nonagouti or agouti coat color at 10 days of age. Male chimeric mice having partial or complete agouti coat-color were then bred to C57BL/6J females. Agouti-colored offspring were screened by Southern blot analysis for germline transmission of the floxed *Cyp1a1* gene.

Males determined to be heterozygous for the floxed *Cyp1a1* were mated with superovulated C57BL/6J dams; the resulting zygotes were collected and then injected with RNA transcribed from the IRES-Cre expression plasmid (16). DNA, prepared from the injected offspring (17), was analyzed for CRE-mediated recombination by PCR analysis using the following primers:

A. 5′-CTGTCTCTGAATCTTACTGCAGCC-3′
B. 5′-GAGCTGCCACTGTGTGCTGCAG-3′
C. 5′-GTCAAAGTAACCAGACACATCCTGC-3′

The rationale for using these primers is discussed under Results. The PCR conditions used were 1 μl of the template DNA in a 20-μl reaction containing 0.5 μM of each primer, 1.5 mM $MgCl_2$, 1× PCR buffer (Life Technologies; Rockville, MD), 150 μM of each dNTP, and 0.25 μl *Taq* DNA polymerase (Life Technologies). Cycling conditions included an initial 4-min denaturation step at 94°C, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. PCR products were separated on a 2% agarose gel.

Southern blot analysis was also employed to confirm CRE-mediated recombination of the floxed *Cyp1a1* gene. DNA (20 μg) was digested for 12 h with 50 U of restriction enzyme (New England Biolabs; Beverly, MA) using the appropriate buffer and bovine serum albumin (BSA, 10 mg/ml). The digested samples were separated by electrophoresis on a 0.7% agarose gel, nicked with 0.25 M HCl, denatured with 0.5 M NaOH, neutralized with 0.5 M Tris-HCl, and capillary-transferred to Nytran SuperCharge membrane (Schleicher & Schuell; Keene, NH). Membranes were prehybridized at 65°C for 4 h in 6× SSC, 10× Denhardt's, 1% sodium dodecyl sulfate (SDS), and hybridized with $2 \times 10^6$ cpm/ml of $^{32}$P-labeled 3′ probe (described above) overnight in the same solution. Membranes were washed in 1× SSC, 0.1% SDS at 65°C for 1 h, then 0.1× SSC, 0.1% SDS at 65°C for an additional hour. Membranes were exposed for 24 h to Kodak X-OMAT film (Rochester, NY).

*Treatment of animals.* All experiments involving mice were conducted in accordance with the National Institutes of Health (NIH) standards for the care and use of experimental animals and the University of Cincinnati Medical Center (UCMC) Institutional Animal Care and Use Committee (IACUC). Mice of the C57BL/6J inbred strain were purchased from The Jackson Laboratory (Bar Harbor, ME). Some mice were treated with intraperitoneal TCDD (5 μg/kg) in dimethylsulfoxide (0.5 ml/kg) for 24 h before Northern hybridization and Western immunoblot analysis, and were handled in accordance with the UCMC Committee on Chemical Safety.

*RNA isolation and analysis.* Total RNA was isolated using an initial acid guanidinium thiocyanate/phenol/chloroform extraction (18), followed by an alkaline buffer/phenol/chloroform extraction and an ammonium acetate precipitation (19). RNA was denatured, size-separated and blotted, as described; the RNA was then prehybridized and hybridized (19). For Northern blot analysis using RNA probes, the hybridization solutions contained yeast tRNA (50 μg/ml). For Northern analysis using DNA probes, hybridization solutions contained salmon sperm DNA (50 μg/ml). Blots were washed as described (19) and exposed to Kodak X-OMAT film and then to a phosphorimager screen (Molecular Dynamics; Sunnyvale, CA). Specific hybridization was quantitated using a Storm Phosphorimager and ImageQuant software (Molecular Dymanics).

*Hybridization probes.* RNA probes were used to hybridize to CYP1A1, NQO1 and Cu,Zn-superoxide dismutase (SOD) mRNAs. For CYP1A1, a *Stu*I fragment from the 3′-untranslated region of the mouse cDNA (20) was used. For NQO1 (21) and SOD (22), full-length cDNAs were used. RNA probes were labeled, as described (23). cDNA probes were used to hybridize to CYP1A2 and CYP1B1 mRNA; probes were prepared from PCR fragments amplified from the 3′-UTR of the respective mouse gene. Primers used to amplify a CYP1A2 probe were nt +7462 and +8010 (20), and a CYP1B1 probe were nt +1998 and +2528 (24). DNA probes were labeled, as described (19).

*Western immunoblot analysis.* Small pieces of liver were homogenized on ice in 50 mM potassium phosphate buffer containing 0.1 mM EDTA and 1.15% KCl. Following centrifugation at 9000*g* for 15 min, the resulting supernatant (S9 fraction) was subjected to electrophoresis on a 12% SDS polyacrylamide mini-gel under denaturing conditions. Proteins (1 $\mu$g/lane) were transferred to nitrocellulose. After staining with Ponceau red to confirm equivalent loading across lanes, the membrane was blocked with 3% BSA in PBST (0.1 M phosphate buffer containing 0.1% Tween 20). The membrane was then incubated with anti-CYP1A1/1A2 primary goat antiserum (Gentest; Woburn, MA; Cat. 210105; 1:1000, diluted in PBST) for 90 min at room temperature. After triplicate washes in PBST, the membrane was incubated in a horseradish peroxidase (HRP)-conjugated secondary (Dako; Carpinteria, CA; 1:1000) for 45 min. After triplicate washes, protein bands were visualized using enhanced chemiluminescence (ECL, Amersham; Piscataway, NJ). The membrane was stripped (by shaking in a solution of 62.5 mM Tris, pH 6.8, 2% SDS, 0.7% (v/v) 2-mercaptoethanol) and re-probed with an anti-CYP3A antiserum (Gentest; Cat. 242496; 1:2000).

*Enzyme assays.* Aryl hydrocarbon hydroxylase (AHH) activity was assayed by the usual spectrophotofluorometric method (25). Acetanilide 4-hydroxylase (A4H) activity was determined as before (26), with modifications as described (27). Ethoxyresorufin- and methoxyresorufin-*O*-dealkylase (EROD, MROD) activities were measured using a microtiter plate modification of a previous method (28), in a final reaction volume of 200 $\mu$l. Although not absolutely specific, AHH and EROD activities generally represent the CYP1A1 enzyme, and A4H and MROD activities generally reflect the CYP1A2 enzyme (29, 30). Cytosolic NQO1 (DT-diaphorase) activity was assayed as dicoumarol-inhibited 2,6,-dichlorophenolindophenol reduction (31).

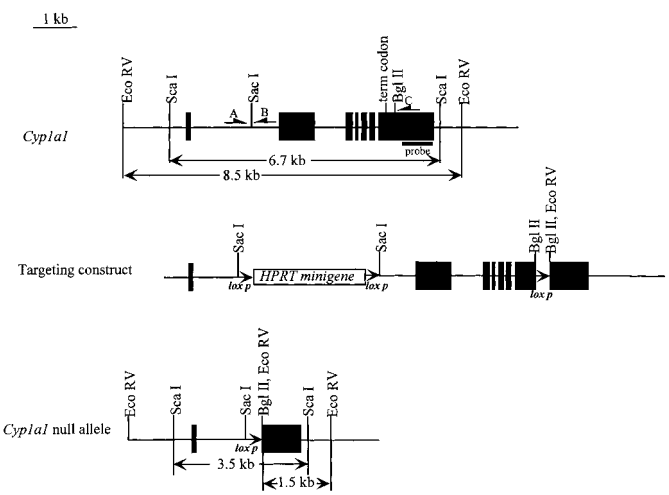


**FIG. 1.** Diagram of the targeting construct and the *Cyp1a1* null allele. Top: The mouse *Cyp1a1* gene, with its seven exons, showing the restriction endonuclease sites that are discussed in the text and the exon 7 probe used in Fig. 2. Positions and directions of primers A, B and C, used for PCR in Fig. 2, are depicted. Middle: The targeting construct, having the *loxP*-flanked *Hprt* mini-cassette inserted at the *Sac*I site and the third *loxP* sequence inserted at the *Bgl*II site in the 3′ UTR. The HSV-*tk* mini-gene cloned at the 3′ end of the targeting construct is not shown. Bottom: *Cyp1a1* null allele (conventional knockout) in which all translated (coding) sequences are deleted. The sizes of restriction fragments that are pertinent to the Southern analysis in Fig. 2 are shown.

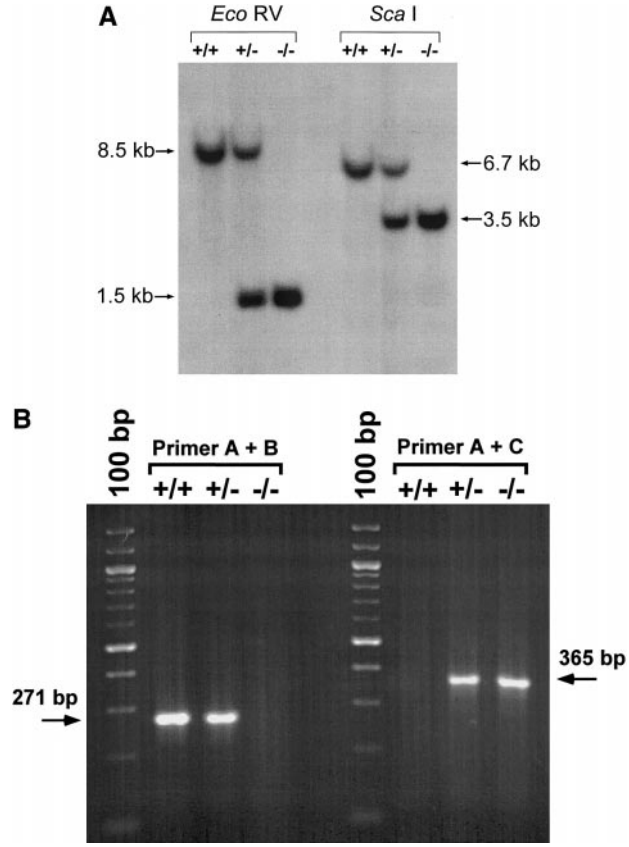


**FIG. 2.** Analysis of the *Cyp1a1* null allele: Southern and PCR analysis on DNA from *Cyp1a1(+/+)* and *Cyp1a1(−/−)* mice. (A) DNA from animals of the indicated genotype was cut with the indicated restriction enzyme, blotted, and hybridized with the probe depicted in Fig. 1. Fragment sizes are shown diagrammatically in Fig. 1. (B) PCR was performed on DNA from mice of the indicated genotype. The primers used in PCR analysis are illustrated in Fig. 1.

## RESULTS AND DISCUSSION

*Generation of mice having the loxP-flanked Cyp1a1 gene.* With our eventual goal of understanding the role of CYP1A1 in the tissue-specific toxicity of certain environmental pollutants, and to avoid the possible lethality associated with the targeted ablation of all *Cyp1* genes, we have generated mice with a floxed *Cyp1a1* gene (Fig. 1). Deletion of the sequence between the outermost *loxP* sites will remove all *Cyp1a1* translated sequence, thus producing a null allele. The 3′-most *loxP* site is not associated with the antibiotic-resistance gene, and so is not selected for by the antibiotic. To aid in diagnosing inclusion of the 3′-most

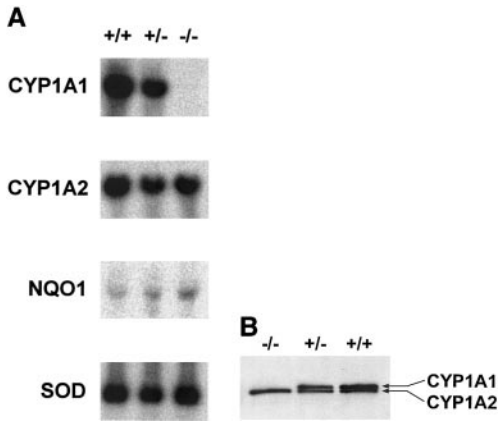


**FIG. 3.** Analysis of CYP1A1 mRNA and protein in liver from TCDD-treated *Cyp1a1(+/+), Cyp1a1(+/−)* and *Cyp1a1(−/−)* mice. Animals were treated with TCDD, the livers from mice of the indicated genotype (3 per group) were pooled, and total RNA and an S9 fraction were prepared. (A) RNA was blotted and hybridized with the indicated probe. Hybridization for SOD mRNA was included as a loading control. (B) Protein (S9 fraction) from the mice of indicated genotype was blotted and probed with an antibody that recognizes both CYP1A1 (56 kDa) and CYP1A2 (54 kDa).

*loxP* site in targeting events, we included an *Eco*RV site in the flanking sequence just outside the canonical *loxP* sequence. The construct shown in Fig. 1 was used to target ES cells; of 231 clones initially selected, 33 clones were targeted and, of these, eight clones contained the 3′-*loxP* site. Targeted ES cell clones were then used to generate chimeric mice; of three chimeric mice produced, one showed germline trasmission as concluded from the birth of agouti offspring. PCR analysis of DNA from these offspring (data not shown) confirmed germline trasmission of the floxed *Cyp1a1* gene.

*Generation of Cyp1a1(−/−) homozygous knockout mice.* Male mice heterozygous for the floxed *Cyp1a1* gene were mated with superovulated C57BL/6J females. Twenty-two resulting zygotes were injected with IRES-Cre RNA (16) and transferred to pseudopregnant dams, and seven pups were born—three of which were transgenic. PCR analysis of DNA from the transgenic mice revealed that the floxed *Cyp1a1* gene from two animals had undergone Cre-mediated recombination between the distal two *loxP* sites (data not shown), such that the *Cyp1a1* null allele was generated. These mice were then bred to C57BL/6J to increase the number of mice harboring the *Cyp1a1* null allele. Finally, *Cyp1a1(+/−)* heterozygotes were bred: of 47 offspring there were nine *Cyp1a1(+/+)* wild-type, 29 *Cyp1a1(+/−)* heterozygotes, and nine healthy *Cyp1a1(−/−)* null-allele mice. We thus conclude that disruption of the *Cyp1a1* gene is not lethal.

When the *Cyp1a1* gene is excised, Southern blot analysis (Fig. 2A) revealed the expected changes in size of fragments following restriction enzyme digestion. In particular, the decreased size of the *Cyp1a1* restriction fragment upon *Sca*I digestion shows a deletion of 3.1 kb, indicating the Cre-mediated removal of the translated region (exons 2 through beginning of 7) of the *Cyp1a1* gene and of the *Hprt* mini-cassette. In addition, the *Eco*RV digestion pattern confirms the presence of the *Eco*RV site in the 3′-most *loxP* site.

PCR analysis of DNA from *Cyp1a1(+/+), Cyp1a1(+/−)* and *Cyp1a1(−/−)* mice using primers A and C (Fig. 2B) validated the loss of the *Cyp1a1* translated region in mice containing one or two null alleles. In addition, using the A and B primer pair, the deletion of sequence between the distal *loxP* sites was verified (Fig. 2B).

*Absence of gene product in the Cyp1a1(−/−) mice.* Following 24 h of TCDD treatment, liver of the three genotypes was examined for CYP1A1 mRNA, protein and enzyme activity. A CYP1A1 inducer must be administered before analysis, because CYP1A1 is expressed at extremely low levels in untreated animals (4). Figure 3A shows a gene–dose relationship: hepatic CYP1A1 mRNA was strongly induced in *Cyp1a1(+/+)*, not detected in *Cyp1a1(−/−)*, and intermediate in

**TABLE 1**

Hepatic Enzyme Activities in the Three Mouse Genotypes Characterized in this Study

| | *Cyp1a1(−/−)* | | *Cyp1a1(+/−)* | | *Cyp1a1(+/+)* | |
|---|---|---|---|---|---|---|
| | Control | TCDD | Control | TCDD | Control | TCDD |
| AHH | 0.2 ± 0.1[a] | 0.5 ± 0.2[a] | 0.1 ± 0.05 | 2.8 ± 0.4 | 0.2 ± 0.1 | 4.6 ± 0.5 |
| EROD | 1.7 ± 0.7[a] | 41 ± 4[a] | 1.2 ± 0.3 | 350 ± 46 | 1.1 ± 0.4 | 510 ± 55 |
| A4H | 0.2 ± 0.1 | 1.2 ± 0.4 | 0.1 ± 0.03 | 0.9 ± 0.2 | 0.1 ± 0.02 | 0.9 ± 0.2 |
| MROD | 3.2 ± 0.6 | 180 ± 25 | 2.3 ± 0.4 | 170 ± 24 | 2.1 ± 0.8 | 240 ± 26 |
| NQO1 | 3.5 ± 0.4 | 13 ± 1.8 | 3.5 ± 0.5 | 14 ± 1.3 | 3.8 ± 0.5 | 14 ± 1.2 |

*Note.* Activities of AHH, A4H, and NQO1 are expressed as nmol/min/mg protein, and activities of EROD and MROD are expressed as pmol/min/mg protein (means ± standard deviations). $N = 3$ mice per group.

[a] The presence of some constitutive and dioxin-inducible AHH, and especially EROD, activity presumably reflects the contribution of small amounts of CYP1B1 in endothelial cells of the liver vasculature (32) and slight overlapping substrate specificity with hepatic CYP1A2.

*Cyp1a1(+/−)* mice. In contrast, CYP1A2 mRNA and NQO1 mRNA levels were induced 8-fold and 4-fold, respectively, in TCDD-treated animals of all three genotypes, as compared with control levels. Western immunoblots (Fig. 3B) demonstrate the complete loss of the hepatic CYP1A1 protein in *Cyp1a1(−/−)* mice. To the contrary, CYP1A2 protein levels were similarly induced in TCDD-treated animals of all three genotypes. The enzyme activities reflect a gene-dose relationship (Table 1): hepatic AHH and EROD activities (reflecting CYP1A1) were increased in *Cyp1a1(+/+)*, low in *Cyp1a1(−/−)*, and intermediate in *Cyp1a1(+/−)* mice. In contrast, A4H and MROD activities (reflecting CYP1A2)—as well as NQO1 activities—were similarly increased in TCDD-treated animals of all three genotypes.

Taken together, these data conclusively demonstrate the generation of a *Cyp1a1(−/−)* mouse line having a Cre-mediated deletion of the gene. Despite the complete absence of CYP1A1, these mice develop normally and display no obvious phenotypic differences, as compared with *Cyp1a1(+/+)* and *Cyp1a1(+/−)* littermates, through 4 months of age. There is no change in the sex ratio of *Cyp1a1(−/−)* pups born. In addition, *in utero* lethality does not occur, because the frequency of viable *Cyp1a1(−/−)* mice from heterozygous parents does not deviate from the expected Mendelian distribution ($P > 0.30$ by chi-square analysis).

*No effect of the absent hepatic CYP1A1 enzyme on other genes in the [Ah] battery.* As discussed above, when the CYP1A1 enzyme is mutated and inactive in the mouse hepatoma Hepa-1 untreated *c37* cell culture line, other genes transcriptionally regulated by the AHR are constitutively up-regulated—perhaps due to the build-up of a putative EL. To determine whether this occurs in the liver of *Cyp1a1(−/−)* mice, we examined CYP1A2 and NQO1 mRNA and CYP1A2 protein in untreated *Cyp1a1(+/−)* and *Cyp1a1(−/−)* animals (Fig. 4). No differences were found. Finally, A4H and MROD (reflecting CYP1A2) and NQO1 activities (Table 1) were not different among untreated *Cyp1a1(+/+)*, *Cyp1a1(+/−)* and *Cyp1a1(−/−)* animals.

These data demonstrate that the constitutive up-regulation of other [*Ah*] battery genes in the untreated *c37* mutant hepatoma line lacking a functional CYP1A1 enzyme (6) is not seen in liver of the intact untreated *Cyp1a1(−/−)* mouse. As described above, CYP1A1 mRNA is as high in untreated *c37* cells as in dioxin-treated cells; actually, introduction of a functional human CYP1A2 cDNA—as well as mouse CYP1A1 cDNA—into the *c37* line (6) causes a return to the wild-type phenotype (i.e., endogenous CYP1A1 mRNA becomes negligible in untreated *c37* cells and highly inducible in dioxin-treated cells). It is therefore possible that this constitutive up-regulation occurs in the CYP1A1 metabolism-deficient *c37* cell line because there is no CYP1A1 or CYP1A2 activity, whereas constitutive CYP1A2 is abundant in liver of the *Cyp1a1(−/−)* mouse (Figs. 3 and 4; Table 1). Future experiments in other tissues of the *Cyp1a1(−/−)* mouse, of the *Cyp1a2(−/−)* mouse (33), and in the planned *Cyp1a1(−/−)*/

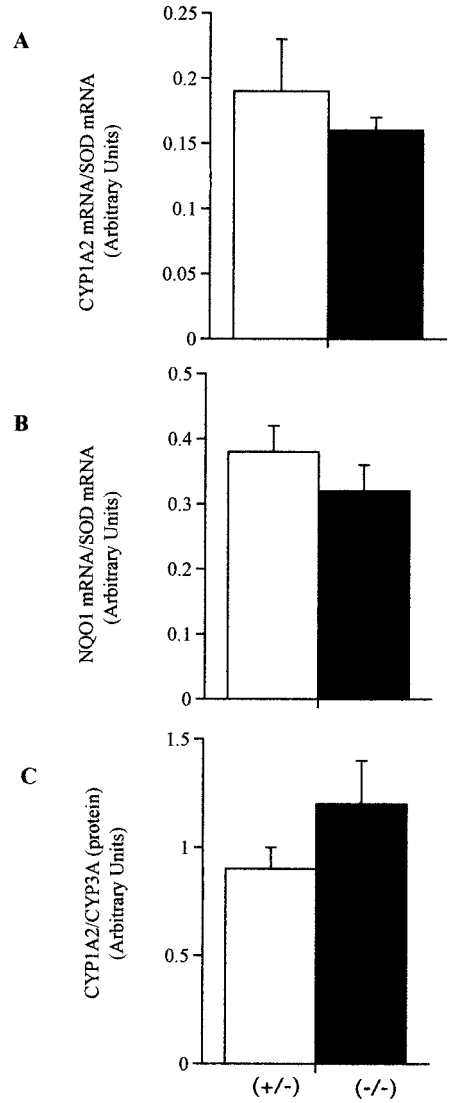


**FIG. 4.** Semiquantitative densitometric analysis of CYP1A2 and NQO1 levels in untreated *Cyp1a1(+/−)* and *Cyp1a1(−/−)* animals. Livers from mice of the indicated genotype (3 per group) were harvested for the preparation of total RNA or S9 protein. (A and B) RNA from each liver was blotted and hybridized with a probe specific for CYP1A2, NQO1, or SOD (CYP1A1 mRNA was below the level of detection in samples from these untreated mice). Specific hybridization signals were quantified using phosphorimage analysis, and the CYP1A2 and NQO1 signals were normalized to SOD from the same sample. (C) S9 protein from each sample was blotted and probed with an antibody that recognizes CYP1A1/1A2 or CYP3A family members (CYP1A1 was below the level of detection in these samples). Blots were scanned and quantified. The CYP1A2 hybridization signal was normalized to the CYP3A signal. Data are presented as means ± standard deviations.

*Cyp1a2(−/−)* double-knockout mouse should answer this question.

*Conclusions.* In summary, we have generated a *Cyp1a1(−/−)* knockout mouse line that appears to show no phenotypic differences from that of *Cyp1a1(+/+)* mice. Because CYP1A1 and AHR activity appear to go hand-in-hand, we had entertained the possibility that the *Cyp1a1(−/−)* knockout mouse might be less viable than normal, as has been observed for the *Ahr(−/−)* knockout mouse (34–36). Finally, since CYP1A1 plays a role in the metabolic potentiation of many environmental carcinogens, mutagens and toxicants, this mouse line should be invaluable for innumerable studies.

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