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A Transgenic Mouse Expressing Human CYP1A2 in the Pancreas

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ABSTRACT. A transgenic mouse line expressing the human cytochrome P450 CYP1A2 in the pancreas under the control of the mouse elastase promoter was established. The expression of CYP1A2 was specific to the transgenic pancreas and was not found in the control wild-type mouse pancreas. The level of CYP1A2 expressed in pancreatic microsomes from transgenic mice was comparable to that of the endogenously expressed CYP1A2 protein in the liver, as judged by western blotting analyses. Estrone metabolism was used to determine the activity of CYP1A2 expressed in the pancreas of the transgenic mouse. The transgenic pancreas exhibited almost one-third to one-half of the activity of wild-type or CYP1A2 transgenic mouse liver, whereas the wild-type pancreas demonstrated no activity. The addition of NADPH–cytochrome P450 oxidoreductase to the reaction mixture containing pancreatic microsomes from the transgenic mice did not increase the estrone metabolism activity significantly. This transgenic mouse line provides another useful tool to study human CYP1A2 and its relation to chemical toxicity and carcinogenesis. BIOCHEM PHARMACOL **60**;6:857–863, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. CYP1A2; transgenic mouse; pancreas; estrone hydroxylation

Most chemical carcinogens and toxins require metabolic activation by xenobiotic-metabolizing enzymes to exert their harmful effects. This usually involves metabolism to an unstable electrophilic derivative that reacts with nucleophiles in macromolecules such as DNA. Carcinogen–DNA adducts can result in mutations in oncogenes and tumor suppressor genes, leading to cell transformation. Xenobiotic-metabolizing enzymes are responsible for either mediating the toxicity of chemicals or protecting the organism by rapidly detoxifying chemicals to inert derivatives to be eliminated. The most widely studied of these enzymes are the P450s,‡‡ which are responsible for oxidative metabolism.

P450s are usually involved in the oxidation of a vast array of chemicals including therapeutically used drugs and endogenous steroids and fatty acids, and are the principal enzymes responsible for the metabolic activation of carcinogens and toxins. P450s consist of a large superfamily of proteins [1], including four families, CYP1, CYP2, CYP3, and CYP4, that primarily metabolize foreign compounds. Studies of these enzymes are crucial to understanding toxic mechanisms and chemical carcinogenesis [2]. However, there are marked species differences in the expression and catalytic activities of P450s that can have an impact when using rodent models in research and in the testing of chemicals for safety in humans [3, 4]. In particular, P450s in the CYP2 family display considerable differences in expression, regulation, and catalytic activities between humans, rats, and mice. Most P450s in the CYP2 family metabolize drugs to non-electrophilic or stable derivatives. Another rather limited group of P450s catalyze the activation of toxins and carcinogens to electrophilic derivatives such as quinones and epoxides. CYP1A1 and CYP1B1 metabolize and participate in the metabolic activation of polycyclic aromatic hydrocarbons. CYP1A2 carries out the *N*-hydroxylation of arylamine carcinogens and heterocyclic amine food mutagens in a pathway leading to electrophilic derivatives that can cause cell toxicity, death, or transformation [5, 6]. CYP1A2 also metabolically activates aflatoxin B_1 to its ultimate carcinogenic metabolite, the 8,9-epoxide [7].

CYP1A2 is expressed constitutively in the livers of mice, rats, and humans and is inducible by ligands of the aryl hydrocarbon receptor in all mammalian species analyzed. It is well conserved between mice, rats, and humans in its liver-specific expression [8, 9] and catalytic activities [10]. Studies in humans have revealed that interindividual differences in levels of expression of CYP1A2 have been

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‡‡ *Abbreviations*: P450s, cytochromes P450; CYP, cytochrome P450; OR, NADPH–cytochrome P450 oxidoreductase; and B[*a*]P, benzo[*a*]pyrene.

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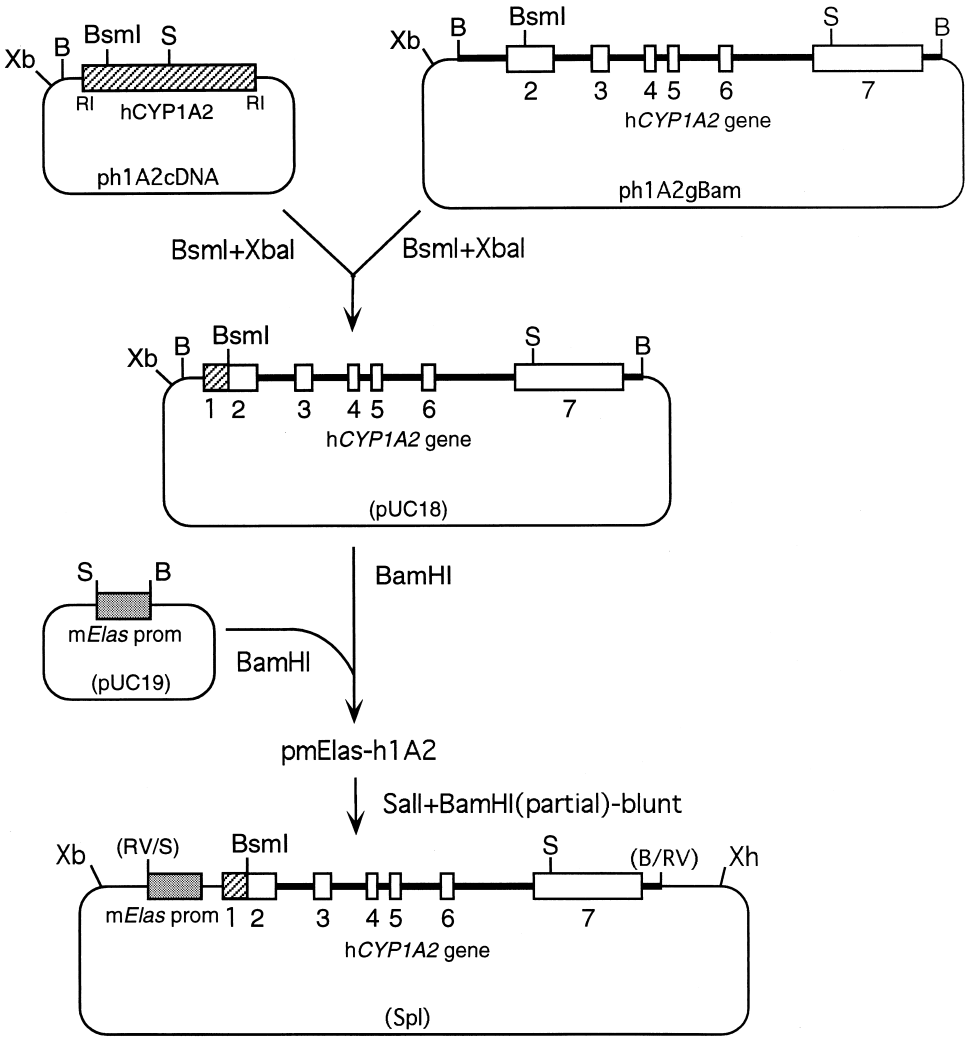

FIG. 1. Schematic strategy used to make the human CYP1A2 transgenic construct. Abbreviations used for restriction enzymes are: B, *Bam*HI; RI, *Eco*RI; Xb, *Xba*I; S, *Sal*I; RV, *Eco*RV; and Xh, *Xho*I.

found to be associated with susceptibility to colon cancer, especially when high dietary intake of food mutagens is considered [11]. The association of CYP1A2 with carcinogenesis is largely assumed, based on *in vitro* metabolic studies with microsomes as well as with purified and cDNA-expressed P450s [10]. However, it is still not clear whether CYP1A2 is required for carcinogenesis induced by arylamine carcinogens in an intact animal. This becomes more complicated once species differences in the expression and catalytic activities of CYP1A2 are taken into account. In the present study, a transgenic mouse line expressing human CYP1A2 in the pancreas is described. The pancreas is one of the organs having a very low detectable level of constitutively expressed endogenous P450s [12–16]. By introducing human CYP1A2 into the null background, we were able to establish a mouse system in which metabolism by human CYP1A2 can be studied *in vivo*.

MATERIALS AND METHODS

Materials

Estrone and 2-hydroxyestrone were purchased from Steraloids Inc. B[*a*]P cis-4,5-dihydrodiol was obtained from the National Cancer Institute Chemical Carcinogen Repository, Midwest Research Institute. Rabbit anti-rat CYP1A1 was obtained from the Gentest Corp. Purified rat OR and rabbit anti-rat OR antibody were provided by Dr. James P. Hardwick (Northeastern Ohio University College of Medicine).

Plasmid for Microinjection

The *Bam*HI fragment containing a part of intron 1 through the 3′ flanking sequence of the human *CYP1A2* gene [17] was subcloned into pUC 18 to generate ph1A2gBam (Fig. 1). The human CYP1A2 cDNA [18] was subcloned into the *Eco*RI site of pUC 18 to yield ph1A2cDNA, with *Bam*HI and *Xba*I sites of the vector located at the 5′ end of the cDNA. The *Xba*I–*Bsm*I fragment from ph1A2gBam and ph1A2cDNA was switched after digesting both plasmids with *Bam*HI and *Bsm*I; the *Bsm*I site is unique and is located in exon 2. This results in fusion of exons 1 and 2 with intron 1 removed. The mouse elastase I gene promoter (m*Elas* prom) [19] (obtained from Dr. R. J. MacDonald) was subcloned at the *Bam*HI and *Sal*I site in pUC 19. The *Bam*HI fragment containing the entire human *CYP1A2*

gene except intron 1 was inserted at the *Bam*HI site of this plasmid. The construct thus obtained (pmElas-h1A2) was digested partially with *Sal*I and *Bam*HI, and was then inserted into the vector Spl [20] at the *Eco*RV site after being made blunt-ended. The entire transgenic construct was released from the plasmid by digesting with *Xba*I and *Xho*I. The construct was then purified through sucrose gradient centrifugation.

Production and Analyses of Transgenic Mice

The pmElas-h1A2 10-kb DNA fragment was microinjected into the pronuclei of B6/SJL F_1 1-cell eggs [21] at a DNA concentration of 2.5 ng/μL. Injected eggs were transferred into pseudopregnant CB6 F_1 females [22], and offspring were tested for the presence of the transgene by Southern analysis. At 3 to 4 weeks of age, tail biopsies (~1.5 cm) were incubated in 2 mL of 50 mM Tris–HCl, pH 8.0, 100 mM NaCl, 50 mM EDTA, 0.5% SDS, and 40 μL proteinase K (20 mg/mL) overnight at 37° with shaking. DNA was extracted with phenol/chloroform and resuspended in Tris–EDTA buffer. Ten micrograms of genomic DNA was digested with *Pst*I, run on 0.8% agarose gels, and transferred to a GeneScreen Plus membrane (New England Nuclear). Filters were probed with [α-^{32}P]dCTP-labeled 2.3-kbp *Pst*I fragment obtained from the human CYP1A2 cDNA [18], which contains a part of the coding and the 3′ noncoding sequences. Hybridization was performed at 42° in a solution containing 50% formamide, 5 × SSPE (5 × SSPE is 0.75 M NaCl, 5 mM EDTA, 50 mM sodium phosphate, pH 7.2), 5 × Denhardt's solution, 10% dextran sulfate, 200 μg salmon sperm DNA, and 0.1% SDS. The filters were washed in 2 × SSC (2 × SSC is 0.3 M sodium chloride and 30 mM sodium citrate, pH 7.0) and 0.5% SDS at 65°.

Microsomal Preparation

Microsomes were prepared from pancreata and livers of transgenic and C57BL/6 mice as a wild-type control. Briefly, tissues were homogenized in 0.5 M KCl, 100 mM EDTA, 20 mM Tris–HCl, pH 7.5, containing 200 μM phenylmethylsulfonyl fluoride, 2 μg/mL of antipain, 2 μg/mL of chymostatin, and 2 μg/mL of pepstatin. The homogenate was centrifuged at 10,000 *g*, and then the supernatant was centrifuged at 100,000 *g*. The microsomal pellet was suspended in 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 0.1 mM dithiothreitol containing 20% glycerol, and kept frozen until used. Protein concentration was determined by use of the bicinchoninic acid (BCA) protein assay reagent (Pierce Chemical Co.) using bovine serum albumin as a standard.

Western Blotting

Proteins were run on 8% SDS–polyacrylamide gels and were transferred to nitrocellulose membranes for western blotting (Schleicher & Schuell). The blots were developed using rabbit anti-rat CYP1A1 or OR antibodies and secondary antibody coupled with horseradish peroxidase for enhanced chemiluminescence detection (ECL, Amersham) or with alkaline phosphatase for color detection using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium as substrates (KPL Laboratories). Anti-rat CYP1A1 antibody cross-reacts with mouse and human CYP1A2. Vaccinia virus-expressed human CYP1A2 was used to determine the amount of human CYP1A2 expressed in the pancreata of transgenic mice. The amount (pmol/mg protein) of vaccinia virus-expressed CYP1A2 was determined spectrophotometrically [23].

Estrone Metabolism

One milliliter of an incubation mixture containing liver or pancreatic microsomes obtained from C57BL/6 mice as a wild-type control or CYP1A2 transgenic mice, 50 mM potassium phosphate buffer (pH 7.4), and 150 μM estrone was preincubated for 2 min at 37° in a shaking water bath, and the reaction was initiated by the addition of 1 mM NADPH. The reaction was terminated after 25 min by the addition of 6 vol. methylene chloride; 0.5 nmol B[*a*]P *cis*-4,5-dihydrodiol was added as an internal standard. After vortexing, the mixture was centrifuged for 10 min (2000 *g*) to extract unreacted substrate and metabolites formed in the organic phase. The organic phase was evaporated to dryness at 40° under a nitrogen stream, and the residue was dissolved in methanol and analyzed immediately by reversed-phase HPLC. For some of the samples, purified OR (200 pmol) was added to the reaction mixture to see the effect of exogenous OR on the metabolism of estrone.

HPLC

Separation of estrone metabolites was carried out as described [24]. Briefly, metabolites formed were separated on an Ultrasphere ODS column (5 μm, 4.6 mm × 25 cm, Beckman Instruments, Inc.) with a 30-min linear gradient of water:acetonitrile (0.5% acetic acid):methanol from 85:15:0 (by vol.) to 30:45:25 (by vol.) and then in 5 min to 0:50:50 (by vol.) at a flow rate of 1 mL/min. The retention times of 2-hydroxyestrone, internal standard, and estrone were 22.5, 26.5, and 27.8 min, respectively. 2-Hydroxyestrone was identified by comparing the retention time and UV–visible spectrum (Diode Array Detector on HP1050 HPLC) of the HPLC chromatographic peaks with authentic 2-hydroxyestrone. Specific activity was determined using the ratio of metabolite formed to internal standard and was expressed as nanomoles of metabolite formed per minute per milligram of microsomal protein.

RESULTS

Generation of Transgenic Mice

The pmElas-h1A2 fusion gene contained the mouse elastase I gene promoter connected to the entire human

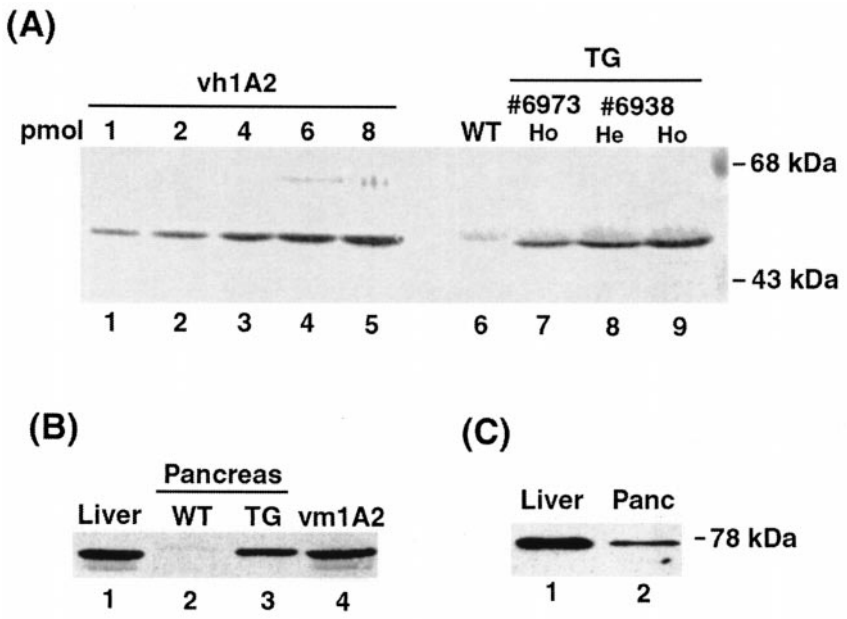

FIG. 2. Western blotting analyses of transgenic mouse pancreas. (A) Determination of the amount of CYP1A2 expressed in pancreatic microsomes (100 μg/lane, lanes 6–9). Lanes 1–5: various amounts of vaccinia virus-expressed recombinant human CYP1A2 were used as a standard, the concentration of which was determined spectrophotometrically [23], and the amount (in picomoles) loaded per lane is shown on the top; lane 6, wild-type (WT) mouse pancreas; lane 7, pancreas from homozygous (Ho) transgenic mouse line No. 6973; lane 8, pancreas from hemizygous (He) transgenic mouse line No. 6938; lane 9, pancreas from homozygous (Ho) transgenic mouse line No. 6938. The molecular weight markers are shown on the right. The alkaline phosphatase color detection method was used. (B) CYP1A2 expression in transgenic mouse microsomes (15 μg/lane). Lane 1, transgenic mouse liver microsomes; lane 2, wild-type (WT) mouse pancreatic microsomes; lane 3, transgenic (TG) mouse pancreatic microsomes; lane 4, vaccinia virus-expressed (vm1A2) recombinant mouse CYP1A2 as a control. Note that human CYP1A2 expressed in transgenic mouse pancreas (lane 3) had a slightly larger molecular weight than mouse CYP1A2 (lanes 1 and 4). (C) OR expression in transgenic mouse microsomes (30 μg/lane). Lane 1, transgenic mouse liver microsomes; lane 2, transgenic mouse pancreatic microsomes. The molecular weight marker is shown on the right. In B and C, the enhanced chemiluminescence detection method was used.

CYP1A2 structural gene except intron 1 as described in Materials and Methods (Fig. 1). This construct was microinjected into fertilized oocytes, and three transgenic founder mice were obtained. In all three founder mice, the transgene was stably integrated and propagated. Two of the transgenic lines (No. 6938 and 6973) produced a protein in their pancreata as judged by western blotting (see below). One transgenic mouse line (No. 6938), especially, expressed an equivalent level of CYP1A2 in the pancreas as compared with the endogenous mouse gene in the liver. The copy number of the transgene in this line was approximately 50, as estimated by comparing the intensity of the hybridization signal to that obtained with known amounts of human DNA (data not shown). Transgenic mouse line No. 6938 was chosen for further analyses, and the other lines of mice were not studied further.

CYP1A2 Protein Expression in the Pancreas

Western blot analysis was performed to determine whether human CYP1A2 protein is expressed in the pancreas (Fig. 2). A standard curve was obtained by using vaccinia virus-expressed human CYP1A2 (Fig. 2A). Based on this analysis, the amount of CYP1A2 expressed in the transgenic pancreas was calculated to be 85 pmol/mg protein for the homozygous high-expresser line (No. 6938), 42 pmol/mg protein for hemizygous No. 6938, and 33 pmol/mg protein for the homozygous low-expresser line (No. 6973). Microsomes obtained from the pancreas of transgenic mouse line No. 6938 had a level of human CYP1A2 expression similar to the endogenously expressed mouse hepatic CYP1A2 (Fig. 2B). Neither mouse nor human CYP1A2 was detected in pancreatic microsomes from a control mouse, as expected (Fig. 2, A and B). Further, human CYP1A2 expression was not found in liver microsomes of the CYP1A2 transgenic mouse, which could happen if the transgene is not tissue-specifically expressed (Fig. 2B). Pancreatic microsomes were also examined for the presence of OR (Fig. 2C). Western blotting results clearly demonstrated that OR is present in the pancreas, albeit at levels much lower than that of the liver. This explains the estrone metabolism results wherein the addition of exogenous OR did not increase the activity significantly (see below).

Estrone Metabolism and CYP1A2 Activity

Metabolites formed in the reaction of estrone with liver or pancreas microsomes were separated efficiently by reversed-phase HPLC (data not shown). Since estrone-2-hydroxylation is specific for CYP1A2 [24], activity of CYP1A2 can be monitored by the formation of 2-hydroxyestrone, which

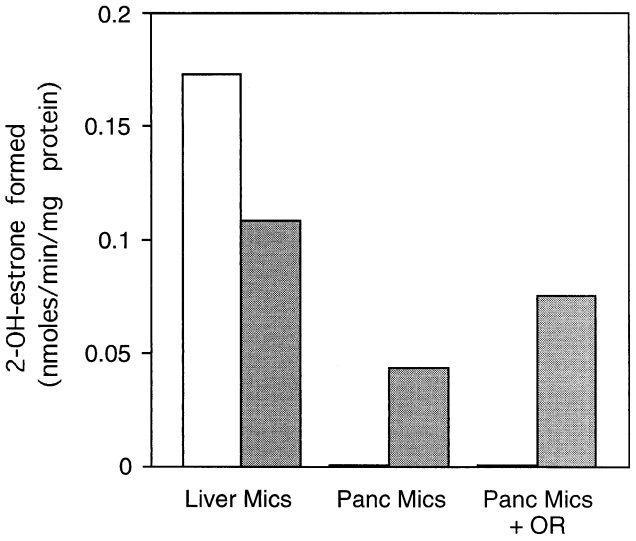

FIG. 3. Estrone metabolism by CYP1A2. Liver microsomes (Liver Mics), pancreatic microsomes (Panc Mics), or pancreatic microsomes with the addition of exogenous OR (Panc Mics + OR) were subjected to estrone metabolism assays, which were obtained from the wild-type mouse (open bar) or the transgenic mouse (shaded bar). The activity is indicated as nanomoles of 2-OH-estrone formed per minute per milligram protein. This is a representative experiment of three experiments; each was performed in duplicate.

was identified readily by comparing the UV–visible absorption spectrum and retention time of the HPLC peaks with the authentic standard. The CYP1A2 activities present in the liver and pancreas were compared between wild-type and transgenic mice (Fig. 3). Activities of CYP1A2 in liver microsomes from wild-type and CYP1A2 transgenic mice were 0.172 and 0.108 nmol product formed/min/mg of microsomal protein, respectively, suggesting that the activities of CYP1A2 present in the liver are comparable between the two lines. In contrast, CYP1A2 activity in pancreatic microsomes from wild-type mice was negligible (0.0002), whereas that of the CYP1A2 transgenic mouse was 0.0432. These results indicate that CYP1A2 is not present at a detectable level in the pancreata of wild-type mice, but is significant in CYP1A2 transgenic mice. The addition of purified OR to the reaction mixture slightly enhanced CYP1A2 activity in the pancreas of the CYP1A2 transgenic mouse (0.0432 to 0.0748), whereas the wild-type mouse pancreas showed no increase in activity. These estrone metabolism results are consistent with the western blotting observations, in which wild-type pancreas showed no CYP1A2 expression. Further, pancreata of CYP1A2 transgenic mice exhibited an OR level that supports sufficiently the CYP1A2-mediated estrone 2-hydroxylation.

DISCUSSION

A transgenic mouse line that expresses human CYP1A2 in the pancreas was generated using a construct containing the human *CYP1A2* gene connected to the mouse elastase I promoter. The pancreas-specific promoter was chosen to express CYP1A2 because P450 is not expressed naturally in the pancreas. Among three founder mouse lines obtained, one exhibited an equivalent CYP1A2 expression in the pancreas as compared with the liver, as judged by western blotting. By using known amounts of vaccinia virus-expressed human CYP1A2 as a standard, the amount of CYP1A2 expressed in the pancreas was calculated to be 85 pmol/mg protein. The total amount of P450s expressed in liver microsomes when animals were treated with phenobarbital was thought to be 3–4 nmol/mg protein, which represents at least 15–20% of the protein content of these liver microsomes [25]. It was also shown that phenobarbital induces 2- to 3-fold the amount of P450 in liver microsomes as compared with untreated liver. In the human case, CYP1A2 may constitute 13% of the total liver P450s [26]. Thus, the expression level of CYP1A2 in the pancreas of the transgenic mouse, 85 pmol/mg protein, is reasonably high.

Estrone metabolism has been studied extensively by using individual human P450s [24, 27], and the roles of individual human P450s responsible for the oxidation of estrone at several positions have been defined. Of all P450s, CYP1A2 is the most active in catalyzing the formation of 2-hydroxyestrone. Thus, estrone-2-hydroxylation can be used precisely as a marker for identifying CYP1A2 activity in tissue preparations.

Estrone metabolism was used to determine the activity of CYP1A2 expressed in the pancreas of a transgenic mouse. The activity of CYP1A2 in liver microsomes was comparable between wild-type and transgenic mice. In contrast, the pancreas of the wild-type mouse exhibited no activity, whereas the pancreas of the CYP1A2 transgenic mouse had almost one-third to one-half of the activity of wild-type or CYP1A2 transgenic mouse liver. The facts that the estrone metabolite was detected in transgenic pancreas and that the addition of OR did not increase CYP1A2 activity significantly suggest that there is a sufficient amount of OR present in the pancreas. In fact, western blotting results demonstrated that OR is expressed in the pancreas, although the expression level is considerably lower than in the liver.

CYP1A2 is constitutively expressed in the livers of mice, rats, and humans and is inducible by ligands of the aryl hydrocarbon receptor in all mammalian species analyzed. Neither CYP1A2 nor any other P450s are known to be expressed constitutively at detectable levels in the pancreas [12–16]. The observation that the microsomes obtained from the pancreas of the transgenic mouse had almost as high an estrone metabolism activity as liver microsomes suggests that all the necessary components required for P450 activity are present in the pancreas. These results suggest that a microsomal mixed-function oxidoreductase system may be present in the pancreas and be too subtle to be detected by regular methods. In fact, its presence in the pancreas has been suggested based on immunohistochemical analysis [28] or by activity assays after induction [14, 16].

Our CYP1A2 transgenic mouse provides a unique system to explore the role of CYP1A2 in chemical toxicity and carcinogenesis. There are an increasing number of instances where marked species differences are noted in the expression and catalytic activities of P450s. These differences can have a significant impact when rodent model systems are used in research testing chemicals for safety in humans [4]. In this regard, our transgenic mouse is an ideal model in which to study human CYP1A2 in an organ where endogenous mouse CYP1A2 expression is negligible. Microsomes obtained from the transgenic mouse pancreas can be used *in vitro* to study the metabolism of chemicals by human CYP1A2. Potential differences present between mouse and human enzymes in catalytic activities towards several CYP1A2 substrates can be examined by comparing liver and pancreatic microsomal activities. The CYP1A2 transgenic mice can also be used as an *in vivo* system to study human CYP1A2-mediated chemical toxicity and carcinogenesis, for instance by analyzing DNA adducts produced *in vivo* by CYP1A2 and their possible connections to pancreatic carcinogenesis. Thus, the CYP1A2 transgenic mice would provide another useful system to study human CYP1A2 metabolism and chemical carcinogenesis.

References

1. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC and Nebert DW, P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6:** 1–42, 1996.
2. Nebert DW, McKinnon RA and Puga A, Human drug-metabolizing enzyme polymorphisms: Effects on risk of toxicity and cancer. *DNA Cell Biol* **15:** 273–280, 1996.
3. Gonzalez FJ and Nebert DW, Evolution of the P450 gene superfamily: Animal-plant 'warfare', molecular drive and human genetic differences in drug oxidation. *Trends Genet* **6:** 182–186, 1990.
4. Guengerich FP, Comparisons of catalytic selectivity of cytochrome P450 subfamily enzymes from different species. *Chem Biol Interact* **106:** 161–182, 1997.
5. Guengerich FP, Humphreys WG, Yun CH, Hammons GJ, Kadlubar FF, Seto Y, Okazaki O and Martin MV, Mechanisms of cytochrome P450 1A2-mediated formation of *N*-hydroxy arylamines and heterocyclic amines and their reaction with guanyl residues. *Princess Takamatsu Symp* **23:** 78–84, 1995.
6. Windmill KF, McKinnon RA, Zhu X, Gaedigk A, Grant DM and McManus ME, The role of xenobiotic metabolizing enzymes in arylamine toxicity and carcinogenesis: Functional and localization studies. *Mutat Res* **376:** 153–160, 1997.
7. Eaton DL, Gallagher EP, Bammler TK and Kunze KL, Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. *Pharmacogenetics* **5:** 259–274, 1995.
8. Kimura S, Gonzalez FJ and Nebert DW, Tissue-specific expression of the mouse dioxin-inducible $P_1$450 and $P_3$450 genes: Differential transcriptional activation and mRNA stability in liver and extrahepatic tissues. *Mol Cell Biol* **6:** 1471–1477, 1986.
9. Shimada T, Mimura M, Inoue K, Nakamura S, Oda H, Ohmori S and Yamazaki H, Cytochrome P450-dependent drug oxidation activities in liver microsomes of various animal species including rats, guinea pigs, dogs, monkeys, and humans. *Arch Toxicol* **71:** 401–408, 1997.
10. Aoyama T, Gonzalez FJ and Gelboin HV, Mutagen activation by cDNA-expressed $P_1$450, $P_3$450, and P450a [published erratum appears in *Mol Carcinog* **3:** 319, 1990]. Mol *Carcinog* **1:** 253–259, 1989.
11. Lang NP, Butler MA, Massengill J, Lawson M, Stotts RC, Hauer-Jensen M and Kadlubar FF, Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiol Biomarkers Prev* **3:** 675–682, 1994.
12. Anderson KE, Hammons GJ, Kadlubar FF, Potter JD, Kaderlik KR, Ilett KF, Minchin RF, Teitel CH, Chou HC, Martin MV, Guengerich FP, Barone GW, Lang NP and Peterson LA, Metabolic activation of aromatic amines by human pancreas. *Carcinogenesis* **18:** 1085–1092, 1997.
13. Bell DR, Bars RG and Elcombe CR, Differential tissue-specific expression and induction of cytochrome P450IVA1 and acyl-CoA oxidase. *Eur J Biochem* **206:** 979–986, 1992.
14. Kessova IG, DeCarli LM and Lieber CS, Inducibility of cytochromes P-4502E1 and P-4501A1 in the rat pancreas. *Alcohol Clin Exp Res* **22:** 501–504, 1998.
15. Rich KJ, Sesardic D, Foster JR, Davies DS and Boobis AR, Immunohistochemical localization of cytochrome P450b/e in hepatic and extrahepatic tissues of the rat. *Biochem Pharmacol* **38:** 3305–3322, 1989.
16. Wilson JD, Miller H, Gelboin HV and Friedman FK, Variation in inducibility of cytochrome P-450c and aryl hydrocarbon hydroxylase in rat liver, lung, kidney, pancreas and nasopharynx. *Pharmacology* **41:** 256–262, 1990.
17. Ikeya K, Jaiswal AK, Owens RA, Jones JE, Nebert DW and Kimura S, Human CYP1A2: Sequence, gene structure, comparison with the mouse and rat orthologous gene, and differences in liver 1A2 mRNA expression. *Mol Endocrinol* **3:** 1399–1408, 1989.
18. Jaiswal AK, Nebert DW, McBride OW and Gonzalez FJ, Human $P_3$450, cDNA and complete protein sequence, repetitive Alu sequences in the 3′ nontranslated region, and localization of gene to chromosome 15. *J Exp Pathol* **3:** 1–17, 1987.
19. Ornitz DM, Hammer RE, Davison BL, Brinster RL and Palmiter RD, Promoter and enhancer elements from the rat elastase I gene function independently of each other and of heterologous enhancers. *Mol Cell Biol* **7:** 3466–3472, 1987.
20. Heilig R, Lemaire C and Mandel JL, A 230kb cosmid walk in the Duchenne muscular dystrophy gene: Detection of a conserved sequence and of a possible deletion prone region. *Nucleic Acids Res* **15:** 9129–9142, 1987.
21. Palmiter RD, Brinster RL, Hammer RE, Trumbauer ME, Rosenfeld MG, Birnberg NC and Evans RM, Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* **300:** 611–615, 1982.
22. Hogan B, Beddington R, Constantini F and Lacy E, *Manipulating the Mouse Embryo, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994.
23. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J Biol Chem* **239:** 2379–2385, 1964.
24. Shou M, Korzekwa KR, Brooks EN, Krausz KW, Gonzalez FJ and Gelboin HV, Role of human hepatic cytochrome P450 1A2 and 3A4 in the metabolic activation of estrone. *Carcinogenesis* **18:** 207–214, 1997.
25. Estabrook RW, Cytochrome P450, From a single protein to a

family of proteins—with some personal reflections. In: *Cytochromes P450. Metabolic and Toxicological Aspects* (Ed. Ioannides C), pp. 3–28. CRC Press, New York, 1996.
26. Vermeulen NPE, Role of metabolism in chemical toxicity. In: *Cytochromes P450. Metabolic and Toxicological Aspects* (Ed. Ioannides C), pp. 29–53. CRC Press, New York, 1996.
27. Yamazaki H, Shaw PM, Guengerich FP and Shimada T, Roles of cytochromes P450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. *Chem Res Toxicol* **11:** 659–665, 1998.
28. Hall PM, Stupans I, Burgess W, Birkett DJ and McManus ME, Immunohistochemical localization of NADPH-cytochrome P450 reductase in human tissues. *Carcinogenesis* **10:** 521–530, 1989.