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Neonatal lethality associated with respiratory distress in mice lacking cytochrome P450 1A2

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ABSTRACT **Cytochrome P450 1A2 (CYP1A2) is a constitutively expressed hepatic enzyme that is highly conserved among mammals. This protein is primarily involved in oxidative metabolism of xenobiotics and is capable of metabolically activating numerous procarcinogens including aflatoxin B1, arylamines, heterocyclic amine food mutagens, and polycylic aromatic hydrocarbons. Expression of CYP1A2 is induced after exposure to certain aromatic hydrocarbons (i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin). Direct evidence for a role of CYP1A2 in any physiological or developmental pathway has not been documented. We now demonstrate that mice homozygous for a targeted mutation in the *Cyp1a-2* gene are nonviable. Lethality occurs shortly after birth with symptoms of severe respiratory distress. Mutant neonates display impaired respiratory function associated with histological signs of lung immaturity, lack of air in alveoli at birth, and changes in expression of surfactant apoprotein in alveolar type II cells. The penetrance of the phenotype is not complete (19 mutants survived to adulthood out of 599 mice). Surviving animals, although lacking expression of CYP1A2, appear to be normal and are able to reproduce. These findings establish that CYP1A2 is critical for neonatal survival by influencing the physiology of respiration in neonates, thus offering etiological insights for neonatal respiratory distress syndrome.**

Cytochromes P450 are a superfamily of hemoproteins that are able to metabolize a large number of endogenous and exogenous compounds through oxidative, reductive, and peroxidative mechanisms (1, 2). Twelve families of P450s are found in mammals (3). Eight of these families usually contain a single enzyme that plays a critical role in metabolism or synthesis of endogenous steroid hormones, cholesterol, and fatty acids. These enzymes are usually considered crucial for normal mammalian development and physiological homeostasis. Three, more complex P450 families (designated CYP1, CYP2, and CYP3) function primarily to metabolize foreign compounds such as drugs and dietary and environmental chemicals including procarcinogens. Steroid hormones (4), lipid peroxides, retinoids, and other biologically occurring compounds (5) are metabolized by these enzymes but the precise physiological roles of these reactions are unknown. Based on species differences in substrate specificities, genetic polymorphisms in mammals, and the divergence of these enzymes during the course of evolution, it was argued that xenobiotic-metabolizing P450s evolved to metabolize dietary chemicals with a critical evolutionally selective role in neutralizing phytotoxins (6, 7). However, it remains a possibility that metabolism of endogenous chemicals by these enzymes is important in mammalian development and physiological homeostasis. Among the P450s that have well-conserved expression patterns and catalytic activities are cytochrome P450 2E1 and cytochrome P450 1A2 (CYP1A2). The former enzyme can catalyze oxidation of endogenous ketone bodies in metabolic pathways of gluconeogenesis (8), and CYP1A2 is known to carry out oxidation of estrogens to biologically active metabolites (9). The high conservation in mammals and ability to metabolize endogenous compounds are suggestive of important physiological roles of these P450s. They are also involved in the metabolic activation of a large number of promutagens and procarcinogens (10).

In the present report, we used homologous recombination in embryonic stem (ES) cells (11) to generate a transgenic mouse line that lacks expression of CYP1A2. Homozygous mutant mice display lethality at birth although the penetrance of the defect is not complete. The histological abnormalities in mutant mice were characterized and cytochrome P450 1A1 (CYP1A1) and CYP1A2 expression was analyzed in surviving adult mutants. This work establishes a role for CYP1A2 in development and correlates a null mutation of the gene with lethal lung immaturity at birth.

MATERIAL AND METHODS

Construction of Targeting Vectors. By using a mouse CYP1A2 cDNA probe (12), we isolated a 129/SV mouse genomic DNA clone isogenic to the ES cells used for recombination. A 6.5-kb genomic DNA fragment, containing exons 1–3 of the *Cyp1a-2* gene, was used to generate targeting vectors by inserting a phosphoribosyltransferase II (NEO) gene that confers resistance to the antibiotic G418 at a unique *Hin*dIII site in the second exon (first coding exon). A herpes simplex virus thymidine kinase (HSV-TK) expression cassette was added to allow the use of ganciclovir for negative selection of homologous recombinant clones (13). Four plasmids were produced with various orientations of NEO and HSV-TK. The genomic library and pMC1NeopolyA were obtained from Stratagene. The HSV-TK cassette was under control of the HSV-TK promoter/enhancer. The organization of the targeting vectors is illustrated in Fig. 1*a*.

Selection of Targeted ES-Cell Clones and Generation of CYP1A2 −/− Mice. The J1 ES cells, developed in the laboratory of R. Jaenisch (14), and mice used to generate the G418-resistant mouse embryo fibroblast (15) were supplied by U. Hochgeschwender (National Institute of Mental Health). Targeting vectors were linearized at a unique *Xho* I site and

Abbreviations: CYP1A2, cytochrome P450 1A2; CYP1A1, cytochrome P450 1A1; ES cell, embryonic stem cell; NEO, phosphoribosyltransferase II gene; HSV-TK, herpes simplex virus thymidine kinase; SAP, surfactant apoprotein.

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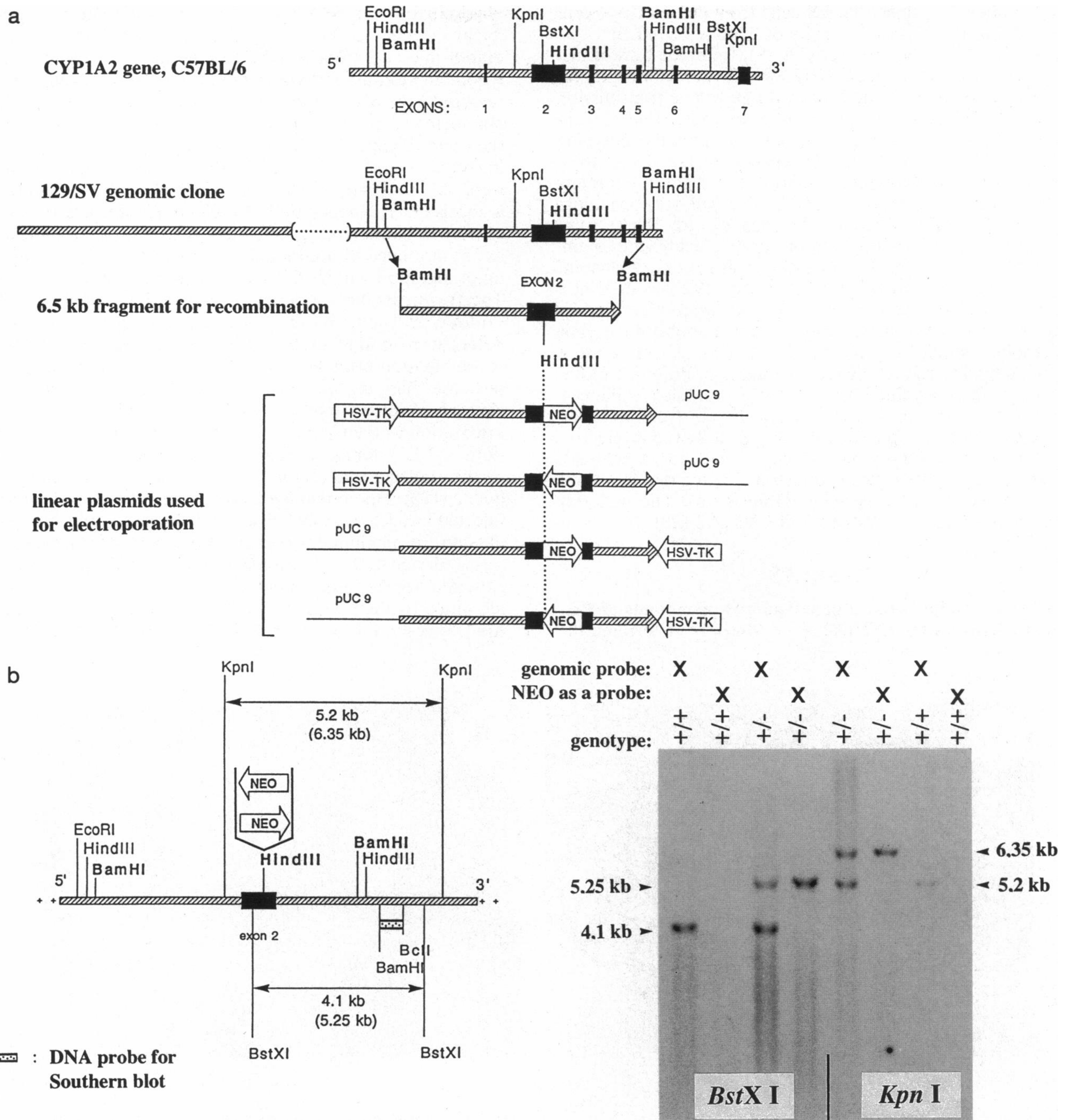

FIG. 1. (*a*) Schematic representation of targeting constructs. In the 6.5-kb genomic fragment used for recombination, exon 2 alone is represented by a solid box. NEO and HSV-TK indicate expression cassettes. Arrows indicate the 5′ → 3′ transcriptional orientation of segments. Gene disruption was generated by inserting NEO at a unique *Hin*dIII site in exon 2. (*b*) Schematic representation of *Cyp1a-2* gene and Southern blot analysis of ES-cell clones after homologous recombination. In a targeted allele, the size of the recombination segment is increased by the size of NEO (1.15 kb). Both orientations are possible. Consequently, a size increase for *Kpn* I and *Bst*XI restriction fragments will be observed (for *Kpn* I from 5.2 to 6.35 kb and for *Bst*XI from 4.1 to 5.25 kb). Southern blot analysis was performed with a *Bam*HI–*Bcl* I 420-bp genomic probe, external to the genomic DNA involved in the recombination event. Wild-type ES-cell clones (+/+) show a single band with the genomic probe and no signal when NEO was used as a probe. Targeted clones (+/−) show two bands with the genomic probe and only the upper one (targeted allele) hybridized to a NEO probe. X indicates the probe used.

used in combination (each at 12.5 μg; 50 μg total) for electroporation into J1 ES cells (20×10^6 cells) by using Bio-Rad Gene Pulser (250 V, 250 μF). Cells (4×10^6 cells per 100-mm dish) were cultured on γ-irradiated G418-resistant mouse primary fibroblasts in medium supplemented with leukemia inhibitory factor [Esgro (Life Technologies Gaithersburg, MD); 1000 units/ml]. Selection in G418 (350 μg/ml) and 5 μM ganciclovir was initiated 24 h after electroporation and resistant ES colonies were picked 6 days later [ganciclovir was a gift from Syntex (Palo Alto, CA)]. After trypsin dissociation, cells were kept under selective conditions for 48 h in fibroblast-coated 24-well dishes. When the cell density reached 60%, a portion of the cells was frozen and a portion was expanded and used to isolate DNA for Southern blot analysis (16). Restriction analysis and PCR were used to further characterize positive clones and determine NEO orientation,

prior to blastocyst injection. ES cells from clone AR99 were injected into the blastocoel cavity of 3.5-day C57BL/6N embryos (12 cells per blastocyst) (17, 18). Embryos were transferred into pseudopregnant NIH Swiss foster mothers. Chimeric males were generated that gave germ-line transmission of the targeted allele. Homozygous mutant mice (−/−) were obtained by heterozygous (+/−) matings. Animal genotyping was carried out by Southern blot analysis of tail DNA (16).

Pathology and Immunochemistry. For light microscopy studies, tissue samples were fixed in 10% (vol/vol) formaldehyde and paraffin-embedded sections (5 μm thick) were stained with hematoxylin/eosin or periodic acid/Schiff stain. For immunohistochemistry, an antibody against rat surfactant apoprotein (SAP), provided by G. Singh (19), was used at a dilution of 1:250. The immune complexes were identified by using the ABC Vectastain kit (Vector Laboratories). For immunoblot studies, microsomes from various organs were prepared by differential microcentrifugation. Protein concentrations were determined by the BCA protein assay (Pierce). Western blot analysis was carried out by electrophoresis on SDS-containing 10% polyacrylamide gels followed by electrotransfer to nitrocellulose membranes (Schleicher & Schuell). The immune complexes were detected by using rabbit anti-rat CYP1A1 and the ECL reagent (Amersham). The antibody crossreacts with both CYP1A1 and CYP1A2 (20).

RESULTS

Gene Targeting: Lethal Phenotype with Symptoms of Respiratory Distress for CYP1A2 −/− Mice. To determine the physiological relevance of CYP1A2 and potentially investigate its involvement in chemically induced carcinogenesis, we generated mice deficient in CYP1A2 by means of homologous recombination in embryonic stem cells. As shown in Fig. 1*a*, targeted gene disruption was generated by insertion of NEO, conferring G418 resistance, in exon 2, the first coding exon of the *Cyp1a-2* gene (21). By using a combination of four targeting vectors and a positive/negative selection approach, we were able to generate ES homologous recombinant clones at a consistent frequency of 3.5%. Genomic blot analysis demonstrated that heterozygous clones were obtained as indicated by the presence of additional 5.25-kb and 6.35-kb fragments upon digestion with *Bst*XI and *Kpn* I, respectively (Fig. 1*b*). The fragments corresponding to the targeted allele also hybridized with the NEO probe and no other bands were detected with this probe, indicating a single homologous recombination event in the *Cyp1A-2* gene. Restriction endonuclease mapping demonstrated that the majority of these clones contained the NEO cassette in the same transcriptional orientation as the *Cyp1a-2*, thus suggesting that both orientations of NEO were not equally efficient and that the structure of the replacement vector may influence the targeting frequency (22). One homologous recombinant clone was microinjected into C57BL/6N blastocysts and gave germ-line transmission for all chimeras created. Resultant heterozygous animals, designated −/+, appear normal and were used to generate homozygous mutants, designated −/−. These were identified by the presence of only the 6.25-kb fragment upon *Kpn* I digestion. Most animals with this genotype died within

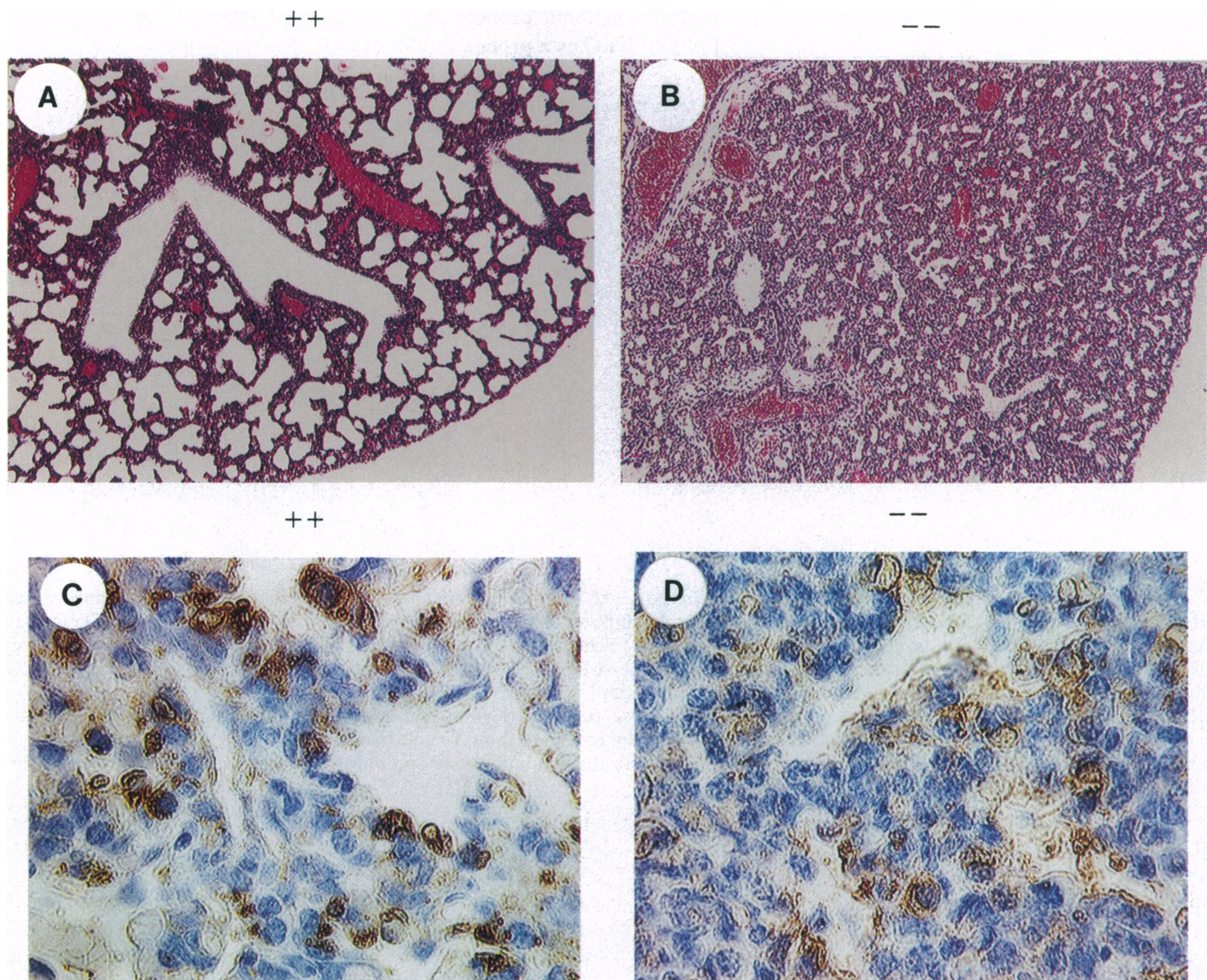

FIG. 2. Histological analysis of newborn mice lacking CYP1A2. Transversal cut of wild-type (+/+) (*A*) and mutant (−/−) (*B*) lungs. (×135.) Wild-type (*C*) and mutant (*D*) type II alveolar cells stained for SAP. (×270.)

15–45 min after birth and displayed early-onset symptoms of respiratory distress including arrhythmic breathing, spasms of the chest, hypothermia, and cyanosis. They never fed prior to death as indicated by the lack of gastric milk. However, the penetrance of this neonatal mortality phenotype is not complete. Among 599 animals analyzed as of February 1, 1995, a total of 19 homozygous mutants have survived. This survival rate (3.2%) is far below the expected Mendelian distribution of 25% = 150 mice. In two additional cases, the death of a mutant mouse occurred at 48 h and at 3 weeks after birth, but the causes of death could not be determined. The adult mutant survivors exhibit no apparent abnormality and were successfully bred with heterozygotes.

Necropsy: Lungs Do Not Inflate at Birth and the Gallbladder Is of Reduced Size in CYP1A2 −/− Mice. Anatomical observations of 51 mutant newborns after necropsy suggest that their lungs never inflated despite their vain attempt to breath after delivery. Macroscopic presence of air in the lungs was usually not seen in mutants compared to control littermates. In addition, we observed a decreased size of the gallbladder in mutants, which appears to be the result of a reduced volume of its contents. Nevertheless, the liver and the extrahepatic bile ducts appear not to be affected. After histological examination, no other organ was found to display morphological abnormality.

Histology and Immunohistochemistry of Lungs and Thyroid: Atelectasis of the Lungs in CYP1A2 −/− Mice. Histological studies of the mutant lungs revealed a severe atelectasis, consistent with anatomical observations (Fig. 2 *A* and *B*). The alveolar compartment of these animals is not expanded and debris and macrophages are present in the centroacinar spaces, which evidently result from incomplete clearance of amniotic fluid at birth without its replacement by air. No hyaline membranes were observed. The presence of surfactant-associated proteins in the lungs was investigated by immunohistochemistry studies of mutant and wild-type lungs by using antibodies against SAP (Fig. 2 *C* and *D*). Immunoreactive alveolar type II cells were found in both samples but cells in wild-type lungs were more intensely stained. SAP was also found in mutant mice, but the staining pattern was more diffuse, suggesting that it was decreased in these animals. Since the association between hypothyroidism and respiratory distress syndrome at birth has been documented (23, 24), the thyroid was examined and no difference was found between mutant and wild-type neonates. Immunostaining of the thyroid with anti-thyroglobulin antibodies also uncovered no abnormality (data not shown). No other histologic differences between −/− and −/+ mice were observed in any other organ.

Immunochemical Study: Normal Neonates Do Not Express CYP1A2, and CYP1A2 −/− Adult Lack the CYP1A2 Protein. We investigated the pattern of expression of CYP1A2 in liver, lung, and kidney by Western blot analysis with an antibody that crossreacts with CYP1A1 and CYP1A2. In mouse, these proteins can be distinguished by different SDS/PAGE mobilities (20). Littermates were killed at birth and microsome preparations from homozygous mutants and wild-type animals were analyzed. CYP1A2 was not detected in liver or lung microsomes of neonates of either the −/− or +/+ genotype. Expression of CYP1A2 was also not revealed in these tissues using Northern blot analysis of 3 μg of polyadenylated mRNA. In wild-type adult microsomes, CYP1A2 was constitutively expressed in liver and CYP1A1 was found in lung (Fig. 3) in accordance with the known sites of synthesis of these enzymes (25). In three surviving adult littermates of the −/− genotype, CYP1A2 was not detectable in liver, thus confirming the complete destruction of gene function. In lung, CYP1A1 was found to be overexpressed in these mice compared to +/− littermates, although the extent of the increase was variable among different animals. CYP1A1 and CYP1A2 were not detected in the kidney of any of these animals as noted (25).

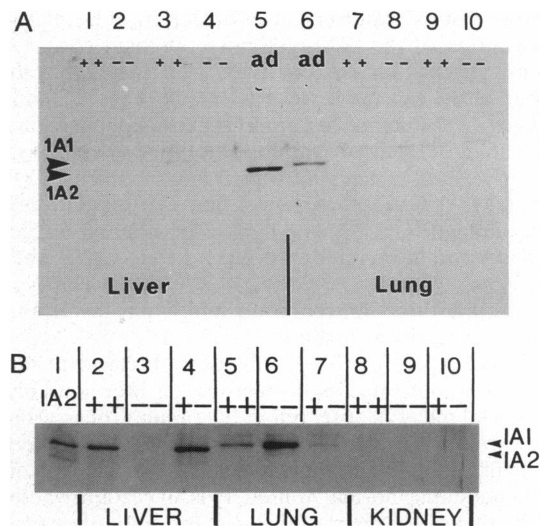

FIG. 3. Western blot analysis of liver, lung, and kidney microsomes. (*A*) Lanes; 1–5, liver microsomes; 6–10, lung microsomes; 1–4 and 7–10, samples from neonates sacrificed at birth (genotype is indicated for each lane); 5 and 6, samples from an adult (ad) wild-type (+/+) mouse of the same strain. Each well contains 20 μg of protein except lanes 5 (4 μg) and 6 (10 μg). The antibody reacts with CYP1A2 (lower band) and CYP1A1 (upper band). (*B*) Liver, lung, and kidney microsomes from three adult female littermates. The genotype of the animal is indicated for each lane. Liver samples, 5 μg of protein per well; lung, 10 μg; kidney, 7 μg. Lane 1 contains recombinant CYP1A2 expressed in HepG2 cells. CYP1A2 is not detected in mutant mouse liver (−/−, lane 3). CYP1A1 is detected in all lung microsomes and overexpressed in mutant mouse (−/−, lane 6).

DISCUSSION

The results presented herein demonstrate a role for CYP1A2 during development that appears critical for the acquisition of lung functionality and, therefore, for neonatal survival. A reduction of the size of the gallbladder is also observed in most mutants. CYP1A2 is not expressed in liver or lungs of neonates, indicating that the lethality cannot be related to a lack of expression of CYP1A2 in mutant lungs, rendering the phenotype paradoxical. A limited number of homozygous mutants survived the absence of the protein, suggesting incomplete penetrance of the mutant phenotype. In surviving adult −/− animals, CYP1A2 was not expressed, thus, demonstrating that the gene was disrupted. These animals appear healthy and some mice from both sexes were found to be fertile, suggesting that CYP1A2 is not required for normal physiological homeostasis.

One hundred percent of the animals found dead in the cages within 30–40 min after delivery had the mutant genotype. The lungs of these mice display an atelectasis with variable intensities. The organ failed to fully evacuate its fluid content and replace it by air. These observations were supported by the biochemical finding of decreased and diffuse expression of SAP in type II alveolar cells. SAP expression normally starts in late fetal development so that less expression in −/− neonates also suggests immaturity of the lungs. Clinical symptoms and histological/immunohistochemical observations indicate that mutant individuals died from respiratory distress. Nevertheless, hyaline membranes, a pathological feature associated with some forms of respiratory distress syndrome at birth, were not observed. Since these membranes are seldom found before 4–10 h after birth (26, 27), the early death of the mutants may occur before their formation. Identical observations are made in immature infants who do not survive

respiratory distress syndrome after birth (26). It is noteworthy that disruption of the LIM-type homeodomain gene, *Gsh-4*, results in a very similar phenotype (28). This gene is specifically expressed in the central nervous system between 9.5 and 12.5 days of gestation, and its disruption generates a morphological and biochemical delay of maturation of a normal respiratory epithelium. As observed for *Gsh-4* mutant mice, CYP1A2 mutant lungs are morphologically and cytologically normal and only immature in development. This example suggests a direct answer to the paradox that we noticed earlier: the lack of a temporary expression of CYP1A2 during gestation, even in organs other than the embryonic lungs, may be responsible for their immaturity at birth.

The reduced size of the gallbladder, observed in mutant neonates, was confirmed at the necropsy of three adult mutant survivors and bile was observed in the organ. Considering the normal extrahepatic bile ducts of these mice, the reduced size of the gallbladder suggests an impaired excretion of biliary components along normal routes. It is uncertain whether it should be regarded as part of the primary cause of death or as consequence of the neonatal stress in mutant mice.

Liver (25) and olfactory epithelia (29) are the primary sites of constitutive expression of CYP1A2 in adult mammals. It was suggested that the hepatic enzyme may be involved in an alternate disposal pathway of bilirubin (30). CYP1A1 mRNA and protein are not detectable in neonate livers of any genotype (+/+, +/−, −/−). Mutant livers appear normal and bilirubin deposits were not found in liver, brain, or kidney. Evaluation of plasma total bilirubin in mutant mice, minutes after birth, shows no pathological levels and no elevation compared to control littermates (data not shown). This excludes hyperbilirubinemia and bilirubin-specific toxicity as the primary cause for lethality. Therefore, it is questionable whether the abnormal phenotype is related to hepatic metabolic function.

CYP1A1 is an enzyme closely related to CYP1A2 and displays limited overlaping substrate specificities (20, 31). It is also induced *in utero* in various tissues by treatment with polycyclic aromatic hydrocarbon compounds (32). In an attempt to rescue the lethal phenotype by overexpressing the CYP1A1 protein, we administered 3-methylcholanthrene to pregnant heterozygous mice during their last week of pregnancy. This strategy was unsuccessful, as the rate of death among these litters was unaffected and no homozygous mutant survived delivery, suggesting that an overexpression of CYP1A1 cannot enzymatically override the absence of CYP1A2 in fetuses or that the timing of this induction (last third of gestation) was inappropriate to influence lung maturation.

This study demonstrates an unexpected and critical role of CYP1A2 for mammalian neonatal survival. The mechanism of this association remains unclear. Of particular importance is the absence of expression of CYP1A2 in the liver of normal neonates and absence of expression of this enzyme in lungs even in mature animals. Thus, the possibility remains that CYP1A2 is expressed at low levels either before or just after birth in an extrahepatic tissue such as brain and transiently functions to metabolize and endogenous hormone that is required for lung maturation. Indeed, CYP1A2 can produce catechol estrogens through 2-hydroxylation of estradiol (9). Further studies should establish the biological link between CYP1A2 and the process of lung maturation.

Finally, CYP1A2 participates in pathways of metabolic activation of aflatoxin B1, arylamines, and heterocyclic amine carcinogens. The availability of CYP1A2 −/− mice should allow the design of experiments to determine whether this enzyme is responsible for the carcinogenicity of these agents in an intact animal model.

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1. Gonzalez, F. J. (1989) *Pharmacal. Rev.* **40,** 243–288.
2. Guengerich, F. P. (1993) in *Cytochrome P450*, eds. Schenkman, J. B. & Greim, H., (Springer, Berlin), pp. 89–103.
3. Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C., Gotoh, O., Okuda, K. & Nebert, D. W., (1993) *DNA Cell Biol.* **12,** 1–51.
4. Zimiac, P. & Waxman, D. J. (1993) in *Cytochrome P450*, eds. Schenkman, J. B. & Greim, H. (Springer, Berlin), pp. 123–144.
5. Coon, M. J., Ding, X., Pernecky, S. J. & Vaz, A. D. N. (1992) *FASEB J.* **6,** 669–673.
6. Gonzalez, F. J. & Nebert, D. W. (1990) *Trends Genet.* **6,** 182–187.
7. Nelson, D. R. & Strobel, H. W. (1987) *Mol. Biol. Evol.* **4,** 572–593.
8. Koop, D. R. & Casazza, J. P. (1985) *J. Biol. Chem.* **260,** 13607–13612.
9. Aoyama, T., Korzekwa, K. R., Nagata, K., Gillette, J., Gelboin, H. V. & Gonzalez, F. J. (1990) *Endocrinology* **126,** 3101–3106.
10. Guengerich, F. P. (1992) *Drug Metab. Dispos.* **21,** 1–6.
11. Thomas, K. R. & Cappecchi, M. R. (1987) *Cell* **51,** 503–512.
12. Kimura, S., Gonzalez, F. J. & Nebert, D. W. (1984) *J. Biol. Chem.* **259,** 10705–10713.
13. Mansour, S. L., Thomas, K. R. & Capecchi, M. R. (1988) *Nature (London)* **336,** 348–352.
14. Li, E., Bestor, E. H. & Jaenisch, R. (1992) *Cell* **69,** 915–926.
15. Tybulewicz, V. L. J., Tremblay, M. L., LaMarca, M. E., Willemsen, R., Stubblefield, B. K., Winfield, S., Zablocka, B., Sidranski, E., Martin, B. M., Huang, S. P., Mintzer, K. A., Westphal, H., Mulligan, R. C. & Ginns, E. I. (1992) *Nature (London)* **357,** 407–410.
16. Laird, P. W., Zijderveld, A., Linders, K., Rudnicki, M. A., Jaenisch, R. & Berns, A. (1991) *Nucleic Acids Res.* **19,** 4293–4295.
17. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 247–277.
18. Bradley, A. (1987) in *Terotocarcinomas and Embryonic Stem Cell: A Practical Approach*, ed. Robertson, E. J. (IRL, Oxford), pp. 113–151.
19. Singh, G., Katyal, S. L., Ward, J. M., Gottron, S. A., Wong-Chong, M. & Riley, E. J. (1995) *J. Histochem. Cytochem.* **33,** 564–568.
20. Aoyama, T., Gonzalez, F. J. & Gelboin, H. V. (1989) *Mol. Carcinogen.* **1,** 253–259.
21. Gonzalez, F. J., Kimura, S. & Nebert, D. W. (1985) *J. Biol. Chem.* **260,** 5040–5049.
22. Zhang, H., Hasty, P. & Bradley, A. (1994) *Mol. Cell. Biol.* **14,** 2404–2410.
23. Redding, R. A. & Peirera, C. (1974) *Pediatrics* **54,** 423–428.
24. Cunningham, M. D., Hollingsworth, D. R. & Belin, R. P. (1980) *Obstet. Gynecol.* **55,** 439–443.
25. Kimura, S., Gonzalez, F. J. & Nebert, D. W. (1986) *Mol. Cell. Biol.* **6,** 1451–1477.
26. Robertson, A. F. (1985) in *Pulmonary Development*, ed. Nelson, G. H. (Dekker, New York), pp. 349–383.
27. Singer, D. B. (1984) in *Hyaline Membrane Disease*, ed. Stern, L. (Grune & Stratton, Orlando, FL), pp. 63–96.
28. Li, H., Witte, D. P., Branford, W. W., Aronow, B. J., Weinstein, M., Kaur, S., Wert, S., Singh, G., Schreiner, C. M., Whitsett, J. A., Scott, W. J. & Potter, S. S. (1994) *EMBO J.* **13,** 2876–2885.
29. Ding, X., Peng, H. M. & Coon, M. J. (1992) *Mol. Pharmacol.* **42,** 1027–1032.
30. Kapitulnik, J. & Gonzalez, F. J. (1993) *Mol. Pharmacol.* **43,** 722–725.
31. Shimada, T., Yun, C.-H, Yamazaki, H., Gautier, J.-C, Beaune, P. & Guengerich, F. P. (1992) *Mol. Pharmacol.* **41,** 856–864.
32. Borlakoglu, J. T., Scott, A., Henderson, C. J., Jenke, H. J. & Wolf, C. R. (1993) *Biochem. Pharmacol.* **45,** 1373–1386.