Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 2662–2666, April 1988
Genetics

Chromosomal organization of the cytochrome *P450-2C* gene family in the mouse: A locus associated with constitutive aryl hydrocarbon hydroxylase

(cytochrome P-450/multigene family/synteny with human/xenobiotic metabolism/carcinogenesis)

R. R. MEEHAN*†, R. M. SPEED*, J. R. GOSDEN*, D. ROUT*, J. J. HUTTON‡, B. A. TAYLOR§, J. HILKENS¶, V. KROEZEN¶, J. HILGERS¶, M. ADESNIK‖, T. FRIEDBERG**, N. D. HASTIE*, AND C. R. WOLF†,††

*Molecular Genetics Section, Medical Research Council Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, Scotland; †Imperial Cancer Research Fund, Laboratory of Molecular Pharmacology and Drug Metabolism, Department of Biochemistry, Hugh Robson Building, George Square, Edinburgh, EH8 9JY, Scotland; ‡Children's Hospital Medical Center, Children's Hospital Research Foundation, Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, OH 45229; §The Jackson Laboratory, Bar Harbor, ME 04609; ¶Department of Tumor Biology, The Netherlands Cancer Institute, Antoni van Lenwenhoek Huis, Plesmanlaan 121, 0166CX, Amsterdam, The Netherlands; ‖Department of Cell Biology and the Kaplan Center, New York University of Medicine, New York, NY 10016; and **Institute of Toxicology, University of Mainz, ObereZahlbacherstrer 67, D6500, Mainz, Federal Republic of Germany

Communicated by Walter F. Bodmer, November 2, 1987 (received for review September 17, 1987)

ABSTRACT **Cytochromes P-450 represent a superfamily of enzymes with a central role in the metabolism of drugs, chemical toxins, and carcinogens. We have used genetic analysis to establish the complexity and catalytic function of a recently identified constitutively expressed murine hepatic cytochrome P-450 encoded by *P450-2C*. Southern blotting analysis shows that there are at least seven or eight genes within this family in the mouse and rat and that DNA restriction fragment length variants between different mouse inbred strains are observed. Analysis of recombinant inbred strains derived from these parent strains shows (*i*) these genes are clustered within 1 centimorgan, (*ii*) this gene family does not correspond to any of the known cytochrome P-450 loci or map near any well-characterized genomic markers, and (*iii*) this gene family segregates to within 1–2 centimorgans of a locus controlling constitutive aryl hydrocarbon hydroxylase activity in mice. With use of Chinese hamster/mouse somatic cell hybrids, the *P450-2C* locus was assigned to a region of mouse chromosome 19 that appears to be syntenic with the previously mapped human *P450C2C* locus on human chromosome 10. By *in situ* hybridization to mitotic mouse chromosomes, we have localized this region to the tip of chromosome 19. These results are discussed in relation to the physiological roles of this P-450 family in foreign compound metabolism and steroid oxidations.**

The mammalian P-450-dependent monooxygenases have a variety of roles ranging from the biosynthesis of steroid hormones, bile acids, and 1,25-dihydroxyvitamin D_3 to the metabolism of foreign compounds (1). Those enzymes involved in the synthesis of hormones and bile acids are specific in their function (2). The enzymes involved in foreign compound metabolism have broad substrate specificities and will also catalyze the metabolism of hormones and fatty acids. Several gene families belonging to the latter group of proteins have been identified (3, 4), and many of these contain a large number of genes. The duplication and expansion of this enzyme system appears to have provided an important selective advantage based on an increased ability to metabolize and excrete potentially harmful chemicals within our environment. The cytochrome P-450 system is complex both because of its multiplicity and also because of the mechanism of regulation of forms within a particular tissue (5).

This system is polymorphic in humans, and a variety of genetically determined differences in cytochrome P-450-mediated metabolism have been implicated in a number of pharmacogenetic responses (6–8). By inference these metabolic differences may also serve as markers for altered susceptibility to environmental toxins and carcinogens (9–12). In epidemiology studies polymorphic P-450 expression has been related to chemical-induced lung and liver cancer (9, 13) and steroid hormone-related cancer(s), which include breast, cervix, and endometrium (14).

One of the outstanding questions is to establish the roles of constitutively expressed cytochromes P-450 relative to those that are expressed only after exposure to chemical-inducing agents in these diseases. Cytochrome P-450 PB-1 is constitutively expressed at high levels in rodent liver and is marginally induced by the xenobiotic phenobarbital (15, 16). An equivalent P-450 that is polymorphic in humans (17) is involved in the metabolism of mephenytoin, an anticonvulsant (18). PB-1 is encoded by a member of the *P450-2C* gene family (4). In this report we have used a genetic approach to establish some of the functions and the chromosomal organization of the *P450-2C* gene family in mice.

MATERIALS AND METHODS

Animals and Cell Lines. Outbred Wistar and DA rats were used in this study. O20, STS, GRS, and AKR/Fu strains were from The Netherlands Cancer Institute (Amsterdam). Except for the CBA/Ca strain from the Western General Animal Unit (Edinburgh), the remaining strains were from both the Western General Animal Unit and The Jackson Laboratory. No subline differences were observed. CD mice were a gift of S. D. M. Brown (St. Mary's Medical School, London). Chinese hamster/mouse somatic cell hybrids were isolated and characterized as described by Hilkens *et al.* (19).

Isolation of DNA and Restriction Endonuclease Analysis. DNA was isolated from progenitor strains as described by Hill *et al.* (20). Chinese hamster/mouse somatic cell hybrid DNA was isolated as described by Hilkens *et al.* (19). Recombinant inbred (RI) mice DNAs from the BXD, AKXL, and BXH series were purchased from The Jackson Laboratory, RI mouse DNAs from the OXA and BXC series were obtained from The Netherlands Cancer Institute. Re-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AHOHase, aryl hydrocarbon hydroxylase; RI, recombinant inbred; cM, centimorgan.

††To whom reprint requests should be addressed.

striction digests were carried out as recommended by the commercial supplier. Digested DNA was fractionated electrophoretically on 0.8–1.0% agarose gels, and the DNA was transferred to nitrocellulose or Hybond-N (Amersham) filter as described by Southern (21).

Preparation of Probes and Hybridization Analysis. The P-450f rat liver cDNA clone pTF-1, coding for a member of the PB-1 family, has been described previously (22). The mouse cDNA clones were isolated by screening a DBA/2J male liver cDNA library (Clontech, Palo Alto, CA) with ^{32}P-labeled pTF-1 insert (23). Three clones were used in the present analysis: pM8-1 encoding the 3′ end of the mouse PB-1 cDNA, as demonstrated by restriction mapping and by probing a set of reference plasmids with the pM8-1 insert, pPB5-21, and pPB3-15. Clones pPB5-21 and pPB3-15 are nearly full-length PB-1 cDNAs. Probes were labeled by random priming (24) and hybridized to Southern blots as described by Hill *et al.* (20). Blots were washed at 68°C in 0.30 M NaCl/0.03 M sodium citrate. In the case of somatic cell hybrid DNA blots, these were washed at 68°C in 0.015 M NaCl/0.0015 M sodium citrate.

Chromosome Preparation. Somatic chromosome preparations from the bone marrow of male and female CD mice were made by a modification of the procedure of Ford and Hamerton (25). G-banded karyotypes were constructed after the trypsin-banding of Gallimore and Richardson (26).

***In Situ* Hybridization.** The preparation and analysis of tritiated pPB3-15 cDNA clones hybridized to mouse metaphase spreads was as described (27).

Aryl Hydrocarbon Hydroxylase (AHOHase) Assays. AHOHase activity was determined fluorimetrically on mouse liver homogenates using benzo[*a*]pyrene as a substrate (28). Six to 10 animals were used per determination.

RESULTS

Genomic Complexity of P-450 PB-1-Related Sequences and Identification of Restriction Fragment Length Variants. Southern blots of mouse and rat genomic DNAs probed with a rat P-450 PB-1 clone, pTF-1, revealed a complex pattern of hybridization with up to 25–30 bands representing 150–200 kilobases (kb) of DNA (Fig. 1). The pattern of hybridization between the rat and mouse DNA was very similar, indicating the presence of several related genes in both species (Fig. 1, lanes A and B versus lanes D–G). Gene counts with 5′ and 3′ PB-1 cDNA probes identified a minimum of seven or eight genes for the PB-1 gene family in rodents (data not shown).

Most of the inbred strains examined could be classified as possessing one of three DNA haplotypes designated *P450-2C^a*, *P450-2C^b*, and *P450-2C^c* (Table 1, Fig. 1). Using the pTF-1 probe *Sst* I gave the largest number of variant fragments between the three haplotypes, the most common haplotype being *P450-2C^c*. The four strains of haplotype

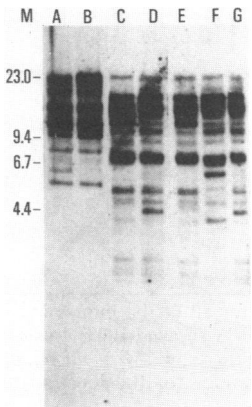

FIG. 1. Southern blot analysis of *Sst* I digests of genomic DNA (5 μg) from inbred mouse strains. The blot was probed with ^{32}P-labeled rat PB-1 cDNA (pTF-1 plasmid DNA). Lanes: M, sizes in kb of marker fragments (λ phage *Hin*dIII fragments); A, DA rat; B, Wistar rat; C, 129 mouse; D, C3H/He mouse; E, AKR mouse; F, C57/L mouse; and G, DBA/2 mouse.

Table 1. Distribution of *P450-2C* haplotypes in inbred mouse strains

Haplotype	Inbred strains
a	C57BL/6J, C57BL/10, C57L
b	DBA/2J, C3H/HeJ, O20, CBA/Ca
c	AKR, AKR/Fu, BALB/c, 129, STS, GRS

P450-2C^b differed from strains of haplotype *P450-2C^c* in two variant fragments (Fig. 1, lane D versus lane C). *P450-2C^a* haplotype differed from *P450-2C^c* and *P450-2C^b* haplotypes with respect to a minimum of nine different fragments (Fig. 1, lane F versus lanes G and E). Additional restriction fragment length variants were detected between the haplotypes when shorter 3′ mouse and rat 5′ PB-1 P-450 cDNA clones were used as probes to *Bam*HI, *Hin*dIII, and *Msp* I digests of mouse strain DNAs (data not shown). None of the *P450-2C^c* variants were shared by *P450-2C^a* or *P450-2C^b*, suggesting that these haplotypes are not recombinants. No substrain differences were observed for mouse strains from the different mouse facilities.

Genomic Organization of P450-2C. RI mouse strains were used to determine the relative chromosomal location and organization of the members of the PB-1 gene family. The BXD series of mouse strains was initially used, as it has been extensively characterized with chromosomal markers and contains the largest number of independent strains. The 26 strains analyzed showed the inheritance of either the C57BL/6J (*P450-2C^a*) or DBA/2J (*P450-2C^b*) progenitor haplotype. No intermediate patterns were observed (Table 2), which demonstrates that the PB-1 genes map to a single chromosome and are present as a large cluster, the outermost variant fragments of which are within 4.1 centimorgans (cM) of each other (95% confidence limit). This genetic locus was designated *P450-2C* in accordance with the new cytochrome P-450 nomenclature (4). Comparison of the strain distribution pattern obtained with those markers previously mapped showed that *P450-2C* was not tightly linked to any of the known genomic loci including the *Pcn* and *Coh* P-450 loci. Loose linkage (20 of 26 strains) was however observed to the *Xmmv-25* locus on chromosome 12 (29).

Table 2. RI strains inheritance of *P450-2C* restriction fragment variants

RI strain	Series no.	Progenitor DNA pattern
AKXL	5, 9, 12, 21, 24, 29, 37, 38	*A*
	6, 7, 8, 13, 14, 16, 17, 19, 25, 28	*L*
BXD	2, 5, 9, 12, 13, 14, 15, 16, 18, 22, 24, 25, 27, 28, 29, 30, 31	*B*
	1, 6, 8, 11, 19, 20, 21, 23, 32	*D*
BXH	3, 4, 6, 9, 12, 14	*B*
	2, 7, 8, 10, 11, 19	*H*
CXB	D, J, K, N, O, P	*C*
	E, G, I, L, Q, R	*B*
OXA	A, B, F, G, I, M, N	*O*
	C, D, E, J, K, L	*A*

Determined with pM8-1 only. DNAs from five sets of RI strains were digested with restriction enzymes, Southern blotted, and probed with pTF-1 and pM8-1. The progenitor of the various sets are as follows. AKXL (AKR × C57L), BXD (C57BL/6J × DBA/2J), BXH (C57BL/6J × C3H/HeJ), CXB (BALB/c × C57BL/6J), and OXA (O20 × AKR/Fu). Italic letters *A*, *B*, *C*, *D*, *H*, *L*, and *O* denote the inherited progenitor DNA pattern from AKR, C57BL/6J, BALB/c, DBA/2J, C3H/HeJ, C57L, and O20/A, respectively. The restriction enzymes used to type the various RI strains were as follows. *Sst* I (AKXL, BXD, BXH, CXB, and OXA), *Hin*dIII (AKXL, BXD, BXH, CXB, and OXA), *Bam*HI (BXH), *Msp* I (BXH), and *Taq* I (BXD).

Possible linkage for the *P450-2C* locus was further investigated by using the RI series of strains AKXL, BXH, CXB, and OXA. In the AKXL RI strains (Fig. 2), as well as the BXH, OXA, and CXB series, no intermediate haplotype pattern was observed. This provides further evidence that *P450-2C* exists as a clustered array. Again no tight linkage was found with any known genomic markers including the *P450-1* locus on chromosome 9 (30).

Linkage Between *P450-2C* and Constitutive AHOHase Activity. The AKR/J progenitor strain has a higher constitutive hepatic AHOHase activity than those of the C57L and C57BL/6 strains (28). This difference in AHOHase activity is heritable, and a strain distribution pattern was obtained for the AKXL series (Table 3). This segregation pattern showed tight linkage (17 of 18) with the *P450-2C* locus, the only discordance being in the AKXL38 strain. In spite of the use of a number of restriction enzymes as well as different PB-1 cDNA clones, no recombination within the *P450-2C* locus in the AKXL38 strain was observed. The genetic distance between the *P450-2C* and the constitutive AHOHase activity is 1.5 cM, and the 95% confidence limits are 0.04 to 11.6 cM (31).

Nonlinkage Between *P450-2C* and Candidate Functions. The oxidative metabolism of estradiol is mediated by cytochrome P-450 (1). A correlation between high circulating 16α-hydroxyestradiol levels *in vivo* and the incidence of breast tumors has been reported in humans (14) and mice (10). In C3H/HeJ mice the extent of estrogen 16α-hydroxylation is increased relative to C57BL/6J mice. This difference has been shown to be heritable, and a segregation difference pattern was established for the BXH series of RI strains (10). No linkage was observed between the *P450-2C* locus and the reported segregation difference pattern for this activity. The *P450-2C* locus is not linked with either the epoxidation of the hepatocarcinogen aflatoxin B_1 (32) or the induction of liver tumors by *N*-ethyl-*N*-nitrosourea (33).

Chromosomal Localization of *P450-2C* Locus. Somatic cell hybrid DNAs from Chinese hamster mouse fusions were used to determine the chromosomal location of *P450-2C*. To facilitate these studies, two mouse PB-1 cDNA clones were isolated from a male DBA/2J liver library to improve detection of mouse sequences on a Chinese hamster background. Partial sequencing and mapping to a set of PB-1 cDNA plasmids showed that the first probe, pM8-1, contains 650 base pairs corresponding to the 3′ end of the PB-1 mRNA. This clone was shown to be part of the *P450-2C* locus by mapping it in the RI strains. This analysis gave 100% concordance with *P450-2C*. A comparison of the segregation of *P450-2C* in the mouse–Chinese hamster hybrids (n = 24) with a set of chromosomal markers shows concordance with *Got-1* on chromosome 19 (Table 4). Further studies with the

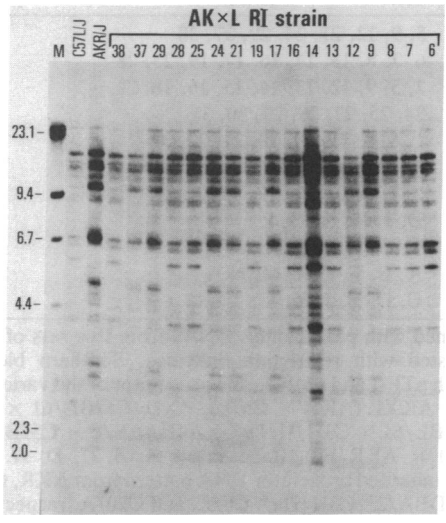

FIG. 2. Southern blot analysis of *Sst* I digests of genomic DNA (5 μg) from AKXL mouse strains and their progenitors. The blot was probed as in Fig. 1.

Table 3. Inbred strains distribution of constitutive liver AHOHase activities and *P450-2C* in AKXL RI series

AKXL RI series no.	AHOHase activity,* nmol/g of tissue per min	Genotypes†		Progenitor *P450-2C* DNA pattern
5	42.5 ± 3.9	*A*		*A*
6	21.1 ± 1.8	*L*		*L*
7	24.4 ± 1.6	*L*		*L*
8	21.8 ± 2.0	*L*		*L*
9	30.4 ± 1.9	*A*		*A*
12	32.0 ± 2.9	*A*		*A*
13	24.4 ± 1.5	*L*		*L*
14	22.7 ± 0.9	*L*		*L*
16	19.0 ± 1.1	*L*		*L*
17	23.0 ± 1.3	*L*		*L*
19	21.6 ± 1.0	*L*		*L*
21	28.0 ± 2.6	*A*		*A*
24	34.8 ± 4.4	*A*		*A*
25	22.2 ± 1.7	*L*		*L*
28	19.9 ± 1.6	*L*		*L*
29	29.8 ± 2.4	*A*		*A*
37	34.4 ± 2.1	*A*		*A*
38	30.4 ± 1.4	*A*	X	*L*

*Mean ± SEM.
†Determined by concentration of AHOHase. *A* and *L* refer to the pattern observed for the parental AKR/J and C57L/J strains, respectively. The "X" represents an apparent recombination event that occurred between the two loci.

Table 4. Segregation of *P450-2C* with *Got-1* in mouse–hamster hybrids

Hybrid	*P450-2C*	*Got-1*
EGR2/1	−	−
EGR7F/1	−	−
EGR13/1	+	+
EGR17BL/1	+	+
EGR23F/1	−	−
EGR25K/1	−	−
EGR30K/1	+	+
EMT3C/2	−	−
EMT3F/1	+	+
EMT6C1/1	+	+
EMT12C/0	+	+
EMT13D1/0	−	−
EMT18B1A/1	−	−
EMT31F/1	+	+
EMT32C2/1	+	+
EMT33D3A/1	+	+
EMT36F/1	+	+
EMT37A1/0	−	−
EMT37A4/0	−	−
EMT40F/1	+	+
EMT40F2/1	+	+
EMT25E1B/1	−	−
EMT6E4C/1	−	−
EMT6E6B/1	+	+

Chromosomal markers were available for all of the mouse autosomes; only *Got-1* on mouse chromosome 19 showed 100% concordance. Backcross analysis of *Mus spretus* and *Mus musculus domesticus* ruled out X and Y chromosomes for the location of *P450-2C*.

more complex *P450-2C* probe pPB5-21 mapped extra fragments that also showed concordance with *Got-1* (Fig. 3).

In situ hybridization was used as an additional approach to map the *P450-2C* locus. To identify unequivocally the mouse 19 chromosome, we used a wild strain of mouse (*Mus musculus*), CD (34), in which all of the chromosomes apart from the 19,X, and Y chromosomes are fused as metacentrics. The 19,X, and Y chromosomes are readily distinguishable as acrocentrics. In female CD mice, chromosome 19 is smaller and therefore distinguishable from the X chromosome (35). The cDNA probe, pPB3-15, was tritium-labeled by random priming, prehybridized with total mouse DNA to eliminate hybridization of a repeat sequence, and used as a probe to metaphase spreads of CD mouse bone marrow cells. Ten cells were analyzed with a total of 88 grains. Of these, 21 (23.9%) were located on mouse chromosome 19 and of these 21, 19 (91.5% of total) were localized to within the 19D1/D2 region (Fig. 4).

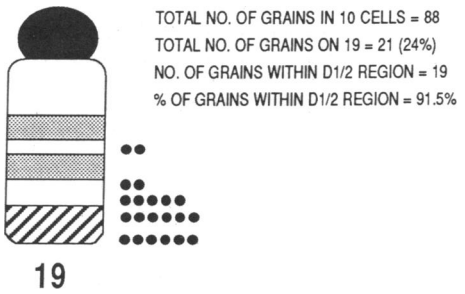

FIG. 4. Autoradiographic grain distribution of pPB3-15 on mouse chromosome 19. Hybridization was carried out *in situ* with pPB3-15 to a mouse chromosome from CD female mice. Chromosome 19 is easily identifiable as a small acrocentric chromosome in metaphase preparations from cells of these mice (see refs. 33 and 34 for the karyotype).

DISCUSSION

The power of mouse genetics for analysis of a multiple gene family as complex as cytochrome P-450 is evidenced by its dual ability to determine chromosomal organization and gene function. Genetics can provide the best evidence for the *in vivo* function of specific P-450 gene families (36–38). We have shown that in mice *P450-2C* is a multigene family that, by RI strain mapping, is clustered; it is not linked to any other known P-450 loci and is associated with constitutive AHOHase metabolism.

P450-2C was mapped to the tip of chromosome 19 by mapping in somatic cell hybrids and by *in situ* hybridization to mouse metaphase chromosomes. This mapping data suggests that *P450-2C* in mice and its equivalent *P450C2C* in man map to regions of homology. *Got-1, Pgam, Lip-1,* and *Tdt* all map to chromosome 19 in mice, with the corresponding human genes mapping to chromosome 10 in man (39, 40). *P450C2C, GOT1, PGAM,* and *TDT* have been localized to the region 10q23–10q25 in humans (27, 39, 40). The *in situ* hybridization cannot distinguish the relative order of markers; in man *GOT1* is more distal than *P450C2C* (27). No recombination between any *P450-2C* haplotypes could be observed in 81 RI strains, corresponding to a genetic locus of <0.96 cM (95% confidence limits), equivalent to ≈1920 kb of mouse DNA.

The apparent recombination between *P450-2C* and constitutive AHOHase might suggest that these are different but closely linked loci. However, we think it is likely that constitutive AHOHase activity is encoded by *P450-2C*, as purified PB-1 (*P450-2C*) proteins from rat, rabbit, and human have this activity (15, 18, 41). The constitutive AHOHase activity was determined several years ago; given the modest difference between AKR/J and C57L/J progenitors, a single discordant RI strain might be due to statistical variation. Antibodies to PB-1 will inhibit this function in certain microsomal preparations (42). A third possibility is that *P450-2C* has a closely linked regulator of constitutive AHOHase metabolism. Therefore, proteins within the PB-1 family have the potential to play a role in the deactivation or activation of polycyclic hydrocarbons. Their role in carcinogenesis remains to be established.

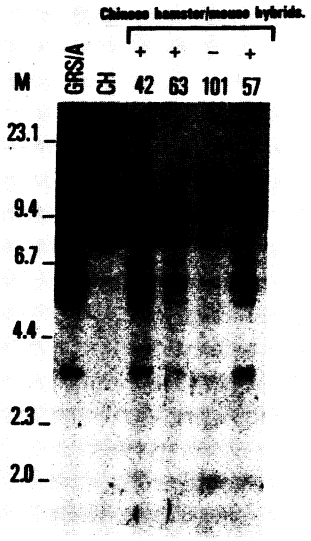

FIG. 3. Southern blot analysis of *Bgl* II digests of Chinese hamster–mouse somatic cell hybrid DNA (5 μg) and their parental cell lines. The blot was probed with ^{32}P-labeled mouse PB-1 cDNA (pPB5-21 plasmid DNA). A hybrid positive for mouse DNA in that particular somatic cell hybrid is indicated by "+"; "−" is negative. Hybrids 42(EMT3F/1), 63(EMT-40F2/1), and 57(EMT36F/1) were all positive for *Got-1*, whereas hybrid 101(EMT18-B1A/1) was negative (see Table 4).

PB-1 proteins also have activity towards steroids at the 2, 15β, and 16α positions and in the 25-hydroxylation of vitamin D (1). A male-specific form of PB-1 has estrogen 16α-hydroxylation activity in rats (1) and probably in mice (43). This sexual dimorphism is not seen in mice *in vivo* (10); taken together with our BXH RI data, this would suggest that circulating levels of 16α-hydroxyestrogen are not determined by *P450-2C*.

In rat six distinct forms of PB-1 isoenzymes have been isolated, termed P450-PB_{1a}, -PB_{1b}, -f, -g, -h, and -i (44, 45). Sequence analysis of P450-PB_{1a}, -f, and -h (M) cDNA clones show them to have 75% amino acid identity (22, 46, 47). The estimate of divergence time between these forms is 65–130 million years ago (ref. 46 and unpublished data), which is prior to the mouse/rat divergence time of 25 million years ago (48). The locus we have defined has the potential to code for all of these PB-1 isoenzyme forms, so orthologous forms might be expected in both species. In contrast, man contains fewer members of the PB-1 family (27). Sequence comparison and estimates from substitution rates indicate that the human PB-1 cDNA clones isolated are not orthologous to any of the sequenced rat forms. This raises an important question in relation to the rodent as a model for human P-450 function. Our genetic analysis opens new avenues with which to pursue these questions.

We thank R. E. Hill and D. J. Porteous for critical advice and discussion and P. Monaghan for advice on sequence comparisons. R.R.M. thanks Imperial Chemical Industries Corporate Bioscience Division for financial support and Prof. H. J. Evans for laboratory support and encouragement. We thank N. Davidson and S. Bruce for photographic work and K. Browne for typing the manuscript. This work was supported in part by Grant GM18684 from the National Institute of General Medical Sciences to B.A.T.

1. Aström, A. & De Pierre, J. W. (1986) *Biochim. Biophys. Acta* **853,** 1–27.
2. Jefcoate, C. R. (1986) in *Cytochrome P-450: Structure, Mechanism, and Biochemistry*, ed. Ortiz de Montellano, P. R. (Plenum, New York), pp. 387–428.

3. Wolf, C. R. (1986) *Trends Genet.* **8,** 209–214.
4. Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1987) *DNA* **6,** 1–11.
5. Adesnik, M. & Atchison, M. (1986) *CRC Crit. Rev. Biochem.* **19,** 247–309.
6. Mahgoub, A., Idle, J. R., Dring, L. G., Lancaster, R. & Smith, R. L. (1977) *Lancet* **ii,** 584–586.
7. Küpfer, A. & Preisig, R. (1984) *Eur. J. Clin. Pharmacol.* **26,** 753–759.
8. Lush, I. E. & Andrews, K. M. (1978) *Genet. Res.* **31,** 177–186.
9. Ayesh, R., Idle, J. R., Ritchie, J. C., Crothers, M. J. & Hetzel, M. R. (1985) *Nature (London)* **311,** 169–170.
10. Bradlow, H. L., Hershcopf, R. E., Martucci, C. P. & Fishman, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6295–6299.
11. Beaune, P., Dansette, P. M., Mansuy, D., Keffel, L., Finck, M., Amar, C., Lenoux, J. P. & Homberg, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 551–555.
12. Stout, D. L. & Becker, F. F. (1986) *Cancer Res.* **46,** 2693–2696.
13. Idle, J. R. & Ritchie, J. C. (1983) in *Human Carcinogenesis*, eds. Harris, C. C. & Autrup, H. N. (Academic, New York), pp. 857–881.
14. Schneider, J., Kinne, D., Fracchia, A., Pierce, V., Anderson, K. E., Bradlow, H. L. & Fishman, J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3047–3051.
15. Waxman, D. J. & Walsh, C. (1983) *Biochemistry* **22,** 4846–4855.
16. Wolf, C. R., Moll, E., Friedberg, T., Oesch, F., Buchmann, A., Kuhlmann, W. D. & Kunz, H. W. (1984) *Carcinogenesis* **5,** 993–1001.
17. Inaba, T., Jurima, H. & Kalow, W. (1986) *Am. J. Hum. Genet.* **38,** 768–772.
18. Shimada, T., Misono, K. S. & Guengerich, F. P. (1986) *J. Biol. Chem.* **261,** 909–921.
19. Hilkens, J., Cuypers, H. T., Selten, G., Kroezen, V., Hilgers, J. & Berns, A. (1986) *Somatic Cell Mol. Genet.* **12,** 81–88.
20. Hill, R. E., Shaw, P. H., Barth, R. K. & Hastie, N. D. (1985) *Mol. Cell. Biol.* **5,** 2114–2122.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
22. Friedberg, T., Waxman, D. J., Atchison, M., Kuman, A., Haaparanta, T., Raphael, C. & Adesnik, M. (1986) *Biochemistry* **25,** 7975–7983.
23. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.
24. Feinberg, D. P. & Vogelstein, B. (1983) *Anal. Biochem.* **136,** 6–13.
25. Ford, C. E. & Hamerton, J. L. (1956) *Stain Technol.* **31,** 247–251.
26. Gallimore, P. H. & Richardson, C. R. (1973) *Chromosoma* **41,** 259–263.
27. Meehan, R. R., Gosden, J. R., Rout, D., Hastie, N. D., Friedberg, T., Adesnik, M., Buckland, R., van Heyningen, V., Fletcher, J., Spurr, N. K., Sweeney, J. & Wolf, C. R. (1988) *Am. J. Hum. Genet.* **42,** 26–37.
28. Hutton, J. J., Meier, J. & Hackney, C. (1979) *Mutat. Res.* **66,** 75–94.
29. Blatt, C., Mileham, K., Haas, M., Nesbitt, M. N., Harper, M. E. & Simon, M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6298–6302.
30. Hildebrand, C. E., Gonzalez, F. J., Kozak, C. A. & Nebert, D. W. (1985) *Biochem. Biophys. Res. Commun.* **130,** 396–406.
31. Silver, J. (1985) *J. Hered.* **76,** 436–448.
32. Gurtoo, H. L., Dahms, R. P., Kanter, P. & Vaught, J. B. (1978) *J. Biol. Chem.* **253,** 3952–3961.
33. Drinkwater, N. R. & Ginsler, J. J. (1986) *Carcinogenesis* **7,** 1701–1707.
34. Capanna, E., Cristaldi, M., Perticone, P. & Rizzoni, M. (1975) *Experimentia* **31,** 294–297.
35. Fisher, E. M. C., Cavanna, J. S. & Brown, S. D. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5846–5849.
36. Simmons, D. L. & Kasper, C. B. (1983) *J. Biol. Chem.* **258,** 9585–9588.
37. Nebert, D. W., Eisen, H. J., Negishi, M., Lang, M. A. & Hjelmeland, L. M. (1981) *Annu. Rev. Pharmacol. Toxicol.* **21,** 431–462.
38. Taylor, B. A. (1984) *Banbury Rep.* **18,** 97–108.
39. Grzeschik, K. H. & Kazazian, H. H. (1985) *Cytogenet. Cell Genet.* **40,** 179–205.
40. Yang-Feng, T. L., Landan, N. R., Baltimore, D. & Franke, U. (1986) *Cytogenet. Cell Genet.* **43,** 121–126.
41. Raucy, J. L. & Johnson, E. F. (1985) *Mol. Pharmacol.* **27,** 296–301.
42. Adams, D. J., Seilman, S., Amelizad, S., Oesch, F. & Wolf, C. R. (1985) *Biochem. J.* **232,** 869–876.
43. Harada, H. & Negishi, M. (1984) *J. Biol. Chem.* **259,** 12285–12290.
44. Haniu, M., Ryan, D. E., Iida, S., Lieber, C. S., Levin, W. & Shively, J. E. (1984) *Arch. Biochem. Biophys.* **235,** 304–311.
45. Wolf, C. R., Seilman, S., Oesch, F., Mayer, R. T. & Burke, M. D. (1986) *Biochem. J.* **240,** 27–33.
46. Gonzalez, F. J., Kimura, S., Song, B.-J., Pastewka, J., Gelboin, H. V. & Hardwick, J. P. (1986) *J. Biol. Chem.* **251,** 10667–10672.
47. Yoshioka, H., Morohashi, K.-I., Sugawa, K., Miyata, T., Kawajiri, K., Hirose, T., Inayama, S., Fuji-Kuriyama, Y. & Omura, T. (1987) *J. Biol. Chem.* **262,** 1706–1711.
48. Sarich, N. M. (1985) in *Evolutionary Relationships Among Rodents*, eds. Pluckett, N. & Hartenberger, J.-L. (Plenum, New York), pp. 423–452.