Chromosomal Assignments of Genes Coding for Components of the Mixed-function Oxidase System in Mice

GENETIC LOCALIZATION OF THE CYTOCHROME P-450PCN AND P-450PB GENE FAMILIES AND THE NADPH-CYTOCHROME P-450 OXIDOREDUCTASE AND EPOXIDE HYDRATASE GENES*

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Filter-hybridization studies show that major phenobarbital and pregnenolone-16α-carbonitrile-inducible cytochrome P-450 isozymes in rats were encoded by members of separate, distinct gene families. These gene families are genetically divergent from each and show no cross-hybridization, even under low-stringency conditions. Furthermore, sequences contained in the P-450PB and P-450PCN gene families map to separate chromosomes of the mouse genome. Using mouse × Chinese hamster somatic cell hybrids (EBS cell lines), all distinguishable P-450PCN sequences were found to map to chromosome 6, whereas all P-450PB sequences were located on chromosome 7. Our data support the notion that the region of the Coh locus on chromosome 7 is the site of the cytochrome P-450PB gene family. The presence of gene families for the cytochromes P-450 occurs in many mammalian species and is likely an important part of the mechanism by which the mixed-function oxidase system is capable of recognizing and metabolizing such a wide array of endogenous and foreign compounds. Conversely, NADPH-cytochrome P-450 oxidoreductase appears to be encoded in many vertebrate species by a single gene and is located on chromosome 6 of the mouse. Corroborative data are presented to show that the Eph-1 locus on chromosome 1 is the site of at least one microsomal epoxide hydratase gene.

The mixed-function oxidase system, comprising the cytochromes P-450 and the electron-donating NADPH-cytochrome P-450 oxidoreductase, has been the focus of intensive research by many laboratories. The ability of these proteins to catalyze the oxidation of a broad spectrum of xenobiotics, as well as to participate in the metabolism of various endogenous substrates such as steroids and fatty acids, makes them enzymatically important. Recent studies (1–7) have demonstrated that the diverse substrate specificities are attributable to the existence of numerous isozymic forms of cytochrome P-450. Characterization of individual cytochromes P-450, such as the major forms induced by phenobarbital, 3-methylcholanthrene, and pregnenolone-16α-carbonitrile, indicates differences in antigenic and catalytic properties as well as amino-terminal sequence and peptide maps (1–9). On the other hand, research directed at both the protein and nucleic acid levels clearly demonstrates the existence of other closely related isozymes which show extensive homology to previously known forms (3, 5, 10, 11). The basis for this microheterogeneity is thought to be primarily genetic, with each slightly variant cytochrome P-450 being encoded by a separate gene, or an allelic form of a gene, which is itself a member of a gene family.

This study presents data regarding the genetic localization of two cytochrome P-450 gene families in mice, one detected by a cDNA to a major phenobarbital-induced cytochrome P-450 mRNA in rats (P-450b) and the other detected by a cDNA to a major pregnenolone-16α-carbonitrile-induced species also in rats (P-450PCN-10). The chromosomal assignments of the NADPH-cytochrome P-450 oxidoreductase and epoxide hydratase genes are also reported, and the significance of the genetic arrangement of the mixed-function oxidase system to its function is discussed.

EXPERIMENTAL PROCEDURES

Somatic Cell Hybrids—Cell hybrids (ERS) between Chinese hamster cells (clone ES6) deficient in hypoxanthine phosphoribosyltransferase (HPRT−) and mouse spleen cells from BALB/c mice were generated and maintained as described (12). Under the conditions employed, hybrid clones retain all the Chinese hamster chromosomes but randomly segregate mouse chromosomes, resulting in the isolation of Chinese hamster × mouse cell hybrids that contain different numbers and combinations of mouse chromosomes. The mouse chromosome content of the hybrid clones was determined enzymatically and cytogenetically. Each hybrid clone was analyzed for the expression of 25 enzymes representing linkage groups assigned to 16 of the 19 autosomes and the X chromosome as described (13–15). Trypsin-Giemsa banding was used to identify each of the mouse chromosomes as described (15). The enzymatic, cytogenetic, and Southern blotting analyses were carried out on parallel cultures of each hybrid clone so that all the data were correlated. Individual clones were considered positive for a given chromosome if greater than 15% of the metaphases examined contained the chromosome; the clones were scored negative for that chromosome if less than 6% of the metaphases contained the chromosome. If chromosomes were retained with a frequency between 5 and 15%, the score was considered indeterminant and not included in the segregation analysis of that chromosome.

Animals—Outbred Holtzmann rats and New Zealand White rabbits were used in this study. Recombinant inbred mouse strains (RI strains) as well as inbred strains came from the Jackson Laboratory (Bar Harbor, ME).

Materials—Proteinase K and agarose were purchased from Curtin-Matheson Scientific, Inc. and Bio-Rad, respectively, and [32P]CTP (800 Ci/mMol) was purchased from Amersham Corp. Restriction
endonucleases were obtained from Promega Biotec and New England Biolabs. Schleicher and Schuell BA85 nitrocellulose was used for blotting. All other reagents were purchased from Sigma.

**DNA Preparation**—DNA extraction and purification were patterned after the method of Lin and Stafford (16). Human DNA was obtained from a diploid lymphoid line maintained by Dr. Bill Sugden (McArline Laboratory, University of Wisconsin). In the isolation of DNA from this cell line, as well as from the hamster E36 and hamster × mouse (EBS) lines, cells were washed several times in ice-cold phosphate-buffered saline (17) and then lysed by gently suspending them in 100 mM Tris (pH 8.0), 200 mM EDTA, and 0.5% sodium dodecyl sulfate. They were then processed as previously described (18).

**Blotting and Hybridization**—DNA preparations were digested 10-20-fold using the restriction buffers recommended by the manufacturer and were electrophoresed in a 1% agarose gels containing Tris acetate buffer (40 mM Tris (pH 8.1), 20 mM sodium acetate, 2 mM EDTA). Blotting, hybridization, and washing conditions were generally done as previously described (18). These hybridization conditions (i.e. 6 × SSC, 10 × Denhardt’s solution (19), 0.2 mg/ml denatured salmon sperm DNA, 0.5% sodium dodecyl sulfate, 50 μg/ml poly(A), 65 °C) are defined as “aqueous” conditions in the remainder of this paper. In some instances, as indicated in the figure legends, hybridizations were done in deionized formamide solutions containing 10 mM Hapes (pH 7.0), 3 × SSC, 2 mM EDTA (pH 7.0), 50 μg/ml poly(A), 0.2 mg/ml denatured salmon sperm DNA, and 10 × Denhardt’s solution. Stringency of hybridization was determined by the temperature, the amount of formamide and salts included in the hybridization solution, as well as the washing conditions. High-stringency conditions utilized 50% formamide in hybridization and multiple 1-h washes in 2 × SSC followed by 0.2 × SSC at 50 °C. Low-stringency conditions employed a hybridization solution containing 30% formamide and 6 × SSC, and the blots were hybridized and washed at 42 °C.

**Preparation of the cDNA Probes**—Construction and characterization of the pEH-1 (20), pP-450PCN-10 (21), pP-450b-5 (22), and pOR-7 (22) cDNAs have been described. pP-450PCN-10 hybridizes with a mRNA that is inducible by both pregnenolone-16α-carbonitrile (11-fold) and dexamethasone (18-fold) and in this regard is detectable by an mRNA exhibiting an induction response similar to the cytochrome P-450PCN described by Guzelian and co-workers (23, 24). At this stage, however, the precise relationship of the cDNA to this cytochrome P-450PCN remains to be established. The rat serum albumin clone (pRSA-1) was prepared from poly(A)+ mRNA isolated from endoplasmic reticulum-bound polypeptides and was identified by its co-hybridization at restriction sites with the cDNA published by Sargent et al. (25). pRSA-1 represents approximately 1600 base pairs of the 3’ end of the rat serum albumin mRNA.

**RESULTS AND DISCUSSION**

**Chromosomal Assignment of the P-450PCN Gene Family**—Although only a single pregnenolone-16α-carbonitrile-induced cytochrome P-450 has so far been purified (6), Southern blots (Fig. 1) suggest that like the phenobarbital-induced forms, other related isozymes exist. This conclusion is based on the observation that the cytochrome P-450PCN probe hybridizes with approximately 50–60 kb of rat genomic DNA. By comparison, cytochrome P-450b cDNA, which is known to recognize approximately eight genes (26, 27), detects in the range of 90–120 kb of DNA. These data are taken as being a strong indication that multiple genes do exist for cytochrome P-450PCN rather than a single large gene. We have termed the genes for these putative proteins the P-450PCN gene family because of the homology of these gene sequences to the Pp-450PCN-10 probe. However, it is important to note that all these genes may not be functional and, if functional, may not be induced by pregnenolone-16α-carbonitrile. Indeed, such is the case in the P-450PB gene family where only a subset of genes shows marked phenobarbital induction (26). Similarly, genes from other cytochrome P-450 gene families may be induced by pregnenolone-16α-carbonitrile. Hence, the nomenclature used is based on sequence similarity and not inducibility.

Digression of DNA from the EBS somatic cell hybrids with the restriction enzyme PvuII generates seven fragments, of which four can be discriminated from hamster sequences on Southern blots with the P-450PCN-10 probe (Fig. 2). Comparison of the autoradiographic pattern with the chromosomal complement of each hybrid (Table I) indicates that all four fragments map to chromosome 6 of the mouse. An identical chromosomal assignment has also been obtained for all discernible fragments of DNA from these cell lines digested with EcoRI and MspI.2 When DNA from DBA/2J and C57BL/6J mice was digested with several 6-base-cutting restriction enzymes and probed with pP-450PCN-10, a single restriction fragment-length polymorphism was detected. The polymorphism (PCN-10HindIII-1) was identified as an extra 1.4 ± 0.15-kb fragment in HindIII-digested DNA from C57BL/6J mice when compared to that of DBA/2J mice. A strain-distribution pattern for this restriction fragment-length polymorphism generated in B6D recombinant inbred mice showed strains 15, 18, 21, 22, 25, and 28 to exhibit the C57BL/6J allele and 2, 5, 9, 11, 12, 13, 14, 16, 19, 20, 23, and 24 to exhibit the DBA/2J allele. No close association of this polymorphism with any other marker in these recombinant inbred strains, including the Coh locus, was found.3 Since all markers on chromosome 6 which have been typed in B6D mice are positioned at the central region of the chromosome (e.g. Mtu14, Lep-1, Lyt-2, and Gigg), combining the somatic cell hybrid data with these results would place this gene complex either very near the centromere or on the telomeric end of chromo-

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1 The abbreviations used are: Hapes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobase pairs.

2 D. L. Simons, P. A. Lalley, and C. B. Kasper, unpublished data.

3 B. A. Taylor, personal communication.
on a 0.9% agarose gel. Following transfer to nitrocellulose, the filter was hybridized to nick-translated pP-450PCN-10 (BALB/cByJ is the mouse strain closely related to the BALB/c strain for 48 h at hybrid (EBS) DNAs with pP-450PCN-10.

Arars are HindIII-digested DNA from each cell line were digested with some 6. We have named the locus for this gene complex the Pcn locus.

### Linkage of the Cytochrome P-450PB Gene Family on Chromosome 7

We have previously reported the mapping of five restriction fragment-length polymorphisms, detected by hybridization to the pP-450b-5 probe, to the Coh locus on chromosome 7 in mice (18). This locus was shown by Wood and Taylor (28) to code for coumarin hydroxylase, a phenobarbital-inducible, cytochrome P-450-dependent, enzyme activity. When the pP-450b-5 restriction fragment-length and coumarin hydroxylase polymorphisms were compared among 24 recombinant inbred and 15 inbred mouse strains, a single discordance (in the CBA/CaJ mouse) was noted. From these observations, we postulated that the coumarin hydroxylase gene was very closely linked to other similar cytochrome P-450 gene sequences which collectively were designated the cytochrome P-450PB gene family. The autoradiographic pattern, observed among DNAs obtained from the EBS cell lines digested with MspI and probed with pP-450b-5, demonstrates that 12 of 19 mouse MspI restriction fragments can be discriminated (Fig. 3). All 12 fragments map to chromosome 7, and similar results have been obtained with PvuII and EcoRI digests of genomic DNA. Therefore, in conjunction with the previously reported data, these results strongly suggest clustering of the P-450PB gene family on chromosome 7, probably at the Coh locus (complex).

Another factor which substantially increases the complexity of this gene complex is the relatively high degree of genetic polymorphism evidenced by the cytochromes P-450 in such species as mice (18, 28), rabbits (29), rats (30, 31), and humans (32–34). Vaslux et al. (35) demonstrated using two-dimensional gel electrophoresis that at least four variant cytochromes P-450 were induced by phenobarbital in rats. Outbred rat strains and even individual colonies of the same strain exhibited different combinations of the variant forms. Recently, Rampersaud and Walz (30), using the same electrophoretic technique, showed that at least six variants were detectable, and appropriate crosses between outbred rats having different phenotypes suggested that these six forms were encoded by two very closely linked autosomal loci. These loci, designated P-450b and P-450e, have multiple alleles, with the P-450b locus having four allelic forms and the P-450e locus having two. The genetic mechanism for this relatively large number of polymorphisms has yet to be explored. Certainly,

### Table I

**Segregation of epoxide hydratase, oxidoreductase, P-450PB, P-450PCN, and mouse chromosomes in mouse × hamster cell hybrids**

Restriction-endonuclease digestion was performed on DNA extracts of mouse × Chinese hamster hybrid clones; duplicate cultures of the same passage were used for enzyme and karyotype analyses. Whether a particular chromosome was scored, + or − was determined as described under "Experimental Procedures."

| Hybrid | Markers | Chromosomes |
|--------|---------|-------------|
| EBS-1  | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 2      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 3      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 4      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 5      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 6      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 7      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 8      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 9      | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 10     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 11     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 12     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 13     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 14     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 15     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 16     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 17     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 18     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 19     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 20     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 21     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 22     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 23     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |
| 24     | +       | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           | +           |

EH, epoxide hydratase; ( ), a fragment, but not the complete chromosome was present; *, indeterminant, i.e. less than 15% of the cells in this clone contained this chromosome.
these observations raise important questions regarding the experimental design of pharmacological studies involving outbred animals, since individual colonies and even individual animals within colonies may possess different combinations of allelic forms of these proteins and, therefore, may give variant responses to a particular xenohiost. Such responses could be incorrectly attributed to a new selective induction by the xenobiotic or to the discovery of new cytochrome P-450 forms. The experiments of Rampersaud and Walz (30) are in agreement with our conclusions that members of the P-450PB gene family are tightly linked, with the Coh locus (complex) being the site of the clustered genes in mice and the P-450b and P-450e loci representing the homologous complex in rats.

Analysis of Homology between Cytochromes P-450b and P-450PCN—Major differences have been noted at the cDNA and mRNA levels for cytochromes P-450b and P-450PCN (21). Comparison of the respective cDNAs by restriction mapping revealed no similarities (21), and Northern blot analysis demonstrated that P-450b and P-450PCN mRNAs were 2150 and 2500 nucleotides in length, respectively (21). Furthermore, Southern blot experiments (Fig. 1) indicate that the genes encoding the P-450b and P-450PCN-like cytochromes are either unrelated or possess a very low level of homology.

Direct hybridization between P-450b-5 and P-450PCN-10 under conditions of low stringency (30% formamide, 6 X SSC, 42°C) failed to detect any sequence homology (Fig. 4). No hybridization of nick-translated P-450PCN-10 probe to the filter-bound 1.8-kb P-450b-5 insert was observed (Fig. 4C), even on long exposures of the autoradiogram. Similarly, the isotopically labeled P-450b-5 probe did not hybridize to the PstI restriction fragments (1.1 and 0.9 kb) derived from P-450PCN-10 (Fig. 4B). Furthermore, no hybridization was noted between the respective probes and epoxide hydratase, oxidoreductase, and serum albumin cDNAs. Taken in total, the data indicate that marked genetic divergence has occurred to produce these significantly different cytochrome P-450 species. Physicochemical and immunological studies have also illustrated clear differences in the properties of cytochromes P-450b and P-450PCN (6).

Chromosomal Assignment of the NADPH Oxido-reductase Gene (Por)—The pattern obtained on a Southern blot analysis of restricted genomic DNA using pOR-7 cDNA as a probe is illustrated in Fig. 5. A single fragment in both mouse (15 kb) and hamster (9.4 kb) DNA was generated upon EcoRI digestion. Comparison of the electrophoretic pattern with the chromosomal complement of each hybrid clone indicates that the locus for the NADPH-cytochrome P-450 oxidoreductase gene, designated Por (NADPH-cytochrome P-450 oxidoreductase), maps to chromosome 6 of the mouse (Table I). Identical results have been obtained with PvuII digests of these DNAs.

Chromosomal Assignment of the Epoxide Hydratase Gene (Eph-I)—The autoradiographic pattern resulting from the hybridization of epoxide hydratase cDNA (pEH-1) and EcoRI-digested EBS somatic cell hybrid and control DNA is presented in Fig. 6. Several hybridizable fragments are noted in hamster (E36), while a single restriction fragment (>23 kb) capable of hybridizing with epoxide hydratase cDNA was found in the BALB/cByJ mouse genome. The mouse specific epoxide hydratase gene fragment mapped to chromosome 1 (Table I). This result is in agreement with the assignment made by Lyman et al. (36) who used an enzyme-activity polymorphism in C57BL/6J and DBA/2J mice to map epoxide hydratase using BXD recombinant inbred mice. Recent evidence suggests that in hamsters at least two related mesosomal epoxide hydratase-like genes are expressed (37). This is consistent with the multiple hybridizing sequences observed on Southern blots for this species (Fig. 6). Mice, however, consistently show fewer hybridizing DNA fragments in genomic blots and may possess fewer genes coding for this protein.

Genetic Arrangement of the Mixed-function Oxidase System—Somatic cell hybrids in combination with cloned cDNA probes are powerful tools to analyze the genetic arrangement of the mixed-function oxidase system and to clarify the relationship of individual cytochromes P-450 to one another. Our studies have shown that the major phenobarbital- and pregnenolone-16α-carbonitrile-induced forms of cytochrome P-450 arise from distinct gene families, which are not syntenic, located on chromosomes 7 and 6 respectively. In addition, it is likely that these gene families are clustered on these chromosomes, since all restriction fragments, regardless of the enzyme utilized, map to chromosome 7 in the case of P-450b-5 and chromosome 6 in the case of P-450PCN-10. Based on earlier mapping studies (18), we identified the Coh locus as the site of the P-450PB gene family, and we now recommend that the locus on chromosome 6 representing the site of the P-450PCN gene family be termed the Pen locus.

From the available data, it may be hypothesized that mammalian cytochrome P-450 gene families arose from a primordial gene through a series of duplications. These events could have given rise to genetic divergence to generally nonsyntenic, distantly related clusters of cytochrome P-450 genes of which the P-450PB and P-450PCN gene families are representative. A similar scenario is thought to have created such nonsyntenic, but homologous, gene families as the α- and β-globins (38) as well as the genetic superfamilies comprising the class I and II histocompatibility, IgG, β-globulin, and Thy 1 genes (39). Hybridization of both the P-450b-5 and P-450PCN-10 probes to filter bound DNA from diverse vertebrate species (Table II) is intense in mammals which are evolutionarily closely related to the rat, such as mouse and hamster. In contrast, much weaker hybridization signals are
Genetic Analysis of the Mixed-function Oxidase System

Fig. 4. Filter hybridization of pP-450b-5 and pP-450PCN-10. Aliquots (1 µg) of pRSA-1, pEH-1, pP-450PCN-10, pOR-7, and pP-450b-5, and pBR322 were digested with PstI to liberate the insert from the vector. HindIII-digested λ DNA (marker 1) and HindIII-digested pBR322 (marker 2) were used as size standards. Digests were electrophoresed in 0.9% agarose gels, blotted, and hybridized under low-stringency conditions (see “Experimental Procedures”). A is the ethidium bromide-stained gel, and B and C are autoradiographs of filter lifts hybridized with nick-translated pP-450b-5 and pP-450PCN-10, respectively. No isotopically labeled λ was included in the hybridization solution; therefore, only the HindIII-digested pBR322 marker appears.

Fig. 5. Filter hybridization of Chinese hamster × mouse (EBS) cell lines to pOR-7. Aliquots were digested with EcoRI and treated as described in the legend to Fig. 2. The black dots bracket the restriction fragment used to map the NADPH-cytochrome P-450 oxidoreductase gene to chromosome 6.

detected in more distantly related species such as rabbit (18) and human, and hybridization has not been detected to non-mammalian DNAs such as from turkey, yeast, or slime mold even under low-stringency conditions (Table II). These data indicate that the cytochrome P-450 genes are not conserved among species to the same extent as genes coding for such highly conserved proteins as tubulins (40, 41), actin (41, 42), and certain oncogenes (43, 44) which are conserved not only among mammals, but among other vertebrate classes and nonvertebrates as well.

In contrast to the cytochromes P-450, NADPH-cytochrome P-450 oxidoreductase is encoded by a single gene (Por) located on chromosome 6. Fig. 7 shows the hybridization of pOR-7 cDNA with EcoRI-digested DNA from a collection of vertebrate and nonvertebrate species. Two salient features differentiate the patterns observed for the cytochromes P-450 and that seen for NADPH-cytochrome P-450 oxidoreductase: 1) oxidoreductase cDNA detects one to two fragments as opposed to multiple fragments detected by the cytochrome P-450 cDNAs, and 2) homologous sequences are detected in a non-mammalian species (turkey) by pOR-7. These observations are consistent with oxidoreductase being encoded by a single
gene which is more highly evolutionarily conserved than the cytochromes P-450. Physical and functional similarity of microsomal NADPH-cytochrome P-450 oxidoreductase in such distantly related vertebrates as rabbits (45), elasmobranchs (46), and teleosts (47, 48) further indicates evolutionary preservation of this protein. This high degree of conservation is important in light of the diversity of forms of cytochrome P-450, since it is thought that all microsomal cytochrome P-450s directly interact with NADPH-cytochrome P-450 oxidoreductase as terminal electron acceptors. It appears that oxidoreductase has been structurally conserved, whereas the cytochromes P-450 have retained only those structural regions essential for heme binding and correct interaction with the oxidoreductase, thus preserving the functional relationship in the electron-transport chain. The relatively high degree of evolutionary conservation of the oxidoreductase gene presumably reflects the essential nature of this protein.

In conclusion, the evidence suggests that gene duplication, possibly in conjunction with a high degree of genetic polymorphism (18, 30), is a critical part of the mechanism by which the cytochromes P-450 as a group manifest such a broad range of catalytic abilities.

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