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Genetics

Localization of cytochrome P_1-450 and P_3-450 genes to mouse chromosome 9

(hamster–mouse somatic cell fused hybrids/gene mapping/Southern blotting)

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ABSTRACT **Treatment of mice with polycyclic aromatic hydrocarbons results in the induction of P_1-450 and P_3-450 forms of cytochrome P-450. The genes for both cytochromes have recently been cloned and shown to be coordinately regulated by the *Ah* receptor. The mouse analogues of P_1-450 and P_3-450 can be distinguished from their hamster counterparts by Southern blot analysis with *Kpn I*-digested DNA fragments. DNA from hamster–mouse somatic cell hybrids that have selectively lost mouse chromosomes was used in Southern blots to map the location of the two mouse genes. Chromosome segregation analysis of 12 hybrid clones demonstrated that the structural genes for both P_1-450 and P_3-450 can be assigned to mouse chromosome 9.**

The microsomal monooxygenase system contains cytochrome P-450 as the terminal oxidase.¶ The P-450s are a family of enzymes that have the capacity to oxidize a wide variety of structurally unrelated compounds ranging from endogenous substrates such as steroids (1), bile acids (2, 3), and prostaglandins (4, 5) to exogenous substrates including drugs, insecticides, hydrocarbons, and other chemical carcinogens (6). This diversity in substrate specificity has been attributed to the presence of multiple forms of P-450. Several of these specific forms are inducible, depending on the type of inducing agent that is administered. Purification and characterization of the respective forms indicate that each protein is most likely a distinct gene product (7).

In C57BL/6N mice, the administration of polycyclic aromatic hydrocarbons results in the induction of at least two forms of P-450 (8), termed P_1-450 and P_3-450. Mouse genetic studies have established that the induction of both cytochromes is controlled by a regulatory gene product of the *Ah* locus, a cytosolic receptor (9). In C57BL/B6 inbred mice, the cytosolic *Ah* receptor specifically binds these inducing chemicals, whereas in DBA/2N inbred mice this binding is defective and induction of P_1-450 and P_3-450 proceeds only with much higher doses of inducer. Recombinant DNA and genetic studies have shown a strict stoichiometric relationship between the translocation of the inducer–receptor complex into the nucleus and the accumulation of P_1-450 mRNA (10, 11), suggesting that the receptor interacts with chromatin to initiate transcription. A similar conclusion was reached, with use of the same probe in selective cell lines defective in the cytosolic receptor (12). Recently, clones to P_1-450 cDNA and P_3-450 cDNA have been used in combination with extensive mouse genetic studies to show that the accumulation of P_1-450 and P_3-450 mRNA is coordinately regulated by the *Ah* locus (13). Increases in the specific mRNAs are a direct result of transcriptional activation, as determined from *in vitro* nuclear transcription studies (14).

Chromosomal localization of the individual genes concerned with the various P-450s is important for understanding in detail the mechanisms of gene expression and enzyme induction. In this report, evidence is presented that the *P_1-450* and *P_3-450* structural genes are localized on the same mouse chromosome.

MATERIALS AND METHODS

Somatic Cell Hybrids. Cell hybrids between Chinese hamster cells (clone E36) deficient in hypoxanthine phosphoribosyltransferase (*hprt*$^-$) and mouse spleen cells from BALB/cJ mice were generated and maintained as described (15). Under the conditions employed, hybrid clones retain all of 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 (15–17). Trypsin-Giemsa banding was used to identify each of the mouse chromosomes as described (17). The enzymatic, cytogenetic, and Southern blotting analyses were carried out on parallel cultures of each hybrid clone so that all the data were correlated.

DNA Isolation and Restriction Enzyme Digestion. DNAs from the parent hamster and mouse cell lines and 12 hamster–mouse hybrids were isolated by the method of Blin and Stafford (18). The DNA was digested with a number of restriction enzymes (New England Biolabs) as recommended by the vendor. Digested DNA was electrophoresed in 0.8% agarose gels.

Construction of P_1-450 and P_3-450 Probes. Double-stranded cDNA to P_1-450 and P_3-450 was cloned into pBR322 (13). The P_1-450 probe is a 1770-base-pair (bp) insert termed pP_1450-57, and the P_3-450 is a 1710-bp insert termed pP_3450-21.

Southern Blot Analysis. Restriction enzyme-digested DNA was size-fractionated by gel electrophoresis and transferred to nitrocellulose paper (19). The filter was washed in 0.3 M NaCl/0.03 M sodium citrate (Cit) for 10 min, then air-dried, and baked for 2 hr. Filters were prehybridized overnight at 42°C in 10 ml of 0.6 M NaCl/0.06 M Cit, 5× concentrated Denhardt's solution (20), 0.1% $NaDodSO_4$, 20 mM sodium

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¶The term "P-450" is used to designate any or all forms of microsomal P-450. P_1-450 and P_3-450 in C57BL/6N mice are defined as those forms of 3-methylcholanthrene-induced P-450 having the highest turnover number for induced aryl hydrocarbon hydroxylase activity and acetanilide 4-hydroxylase activity, respectively.
Abbreviations: bp, base pair(s); kb, kilobase(s).
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phosphate (pH 6.4), denatured salmon sperm DNA (200 μg/ml), tRNA (50 μg/ml), and 50% formamide. Each filter was hybridized overnight at 42°C in 10 ml of the same solution containing 5×10^7 cpm of nick-translated insert (1×10^8 cpm/μg of DNA) from either the pP_1450-57 or the pP_3450-21 plasmid. After hybridization, the filters were washed at room temperature in 0.3 M NaCl/0.03 M Cit and 0.1% $NaDodSO_4$ for 15 min and then at 60°C for ≈2 hr with 15 mM NaCl/1.5 mM Cit and 0.1% $NaDodSO_4$. When the filters were rehybridized after the first exposure, they were prehybridized and then hybridized as outlined above, without any attempt to remove the residual probes. Autoradiographs were developed after exposure of Kodak XAR-5 film with an intensifying screen at −70°C.

RESULTS

Restriction Enzyme Analysis. In preliminary experiments, DNA from parent hamster E36 and mouse cells was digested with a series of restriction enzymes, electrophoresed, and transferred to nitrocellulose paper. Filters were hybridized with inserts from pP_1450-57 and pP_3450-21 to determine the hybridization patterns for each probe against E36 and mouse DNA. From 10 restriction enzymes, *Kpn* I and *Bgl* II generated hybridization patterns that were different between E36 and mouse DNA when hybridized with both probes (data not shown). *Kpn* I was chosen because it generates large DNA fragments more easily distinguishable between the two species.

Mapping *P_1-450* and *P_3-450* Genes at the Chromosomal Level with Somatic Cell Hybrids. Among hamster–mouse somatic cell-fused hybrids, hamster chromosomes are retained, whereas many mouse chromosomes are lost. Swan *et al.* (21) applied the Southern technique with hamster–mouse hybrids to show that the variable and constant region of the mouse immunoglobulin κ light chain genes both map to chromosome 6. The DNA from those hybrids that contain mouse chromosome 6 hybridizes to a specific genomic DNA fragment not seen in those hybrids in which mouse chromosome 6 is absent. The same strategy was used in these experiments to map the *P_1-450* and *P_3-450* genes.

Southern blot experiments with the pP_1450-57 probe showed a major 23-kilobase (kb) hybridization band in *Kpn* I-digested mouse DNA (Fig. 1). A *Kpn* I digestion of E36 DNA did not produce any hybridization in this region of the filter. This same band was present in hybrid cell lines EBS-1, EBS-5, EBS-9, EBS-17, and EBS-9AZ. A second mouse fragment, ≈9 kb, hybridized very lightly to pP_1450-57 but was not visible in any of the hybrids under the conditions used in this experiment. When hybridization and washing conditions were performed under less stringent conditions, this band was present in the same hybrids in which the 23-kb band was found. Not present in the mouse DNA was a common 15-kb hamster fragment, which hybridized in E36 and all of the hybrids. Thus, by *Kpn* I digestion of DNA from the hybrid panel, it was possible to determine which hybrids contained the mouse *P_1-450* gene. When the chromosome content of the hybrid clones was compared with the pres-

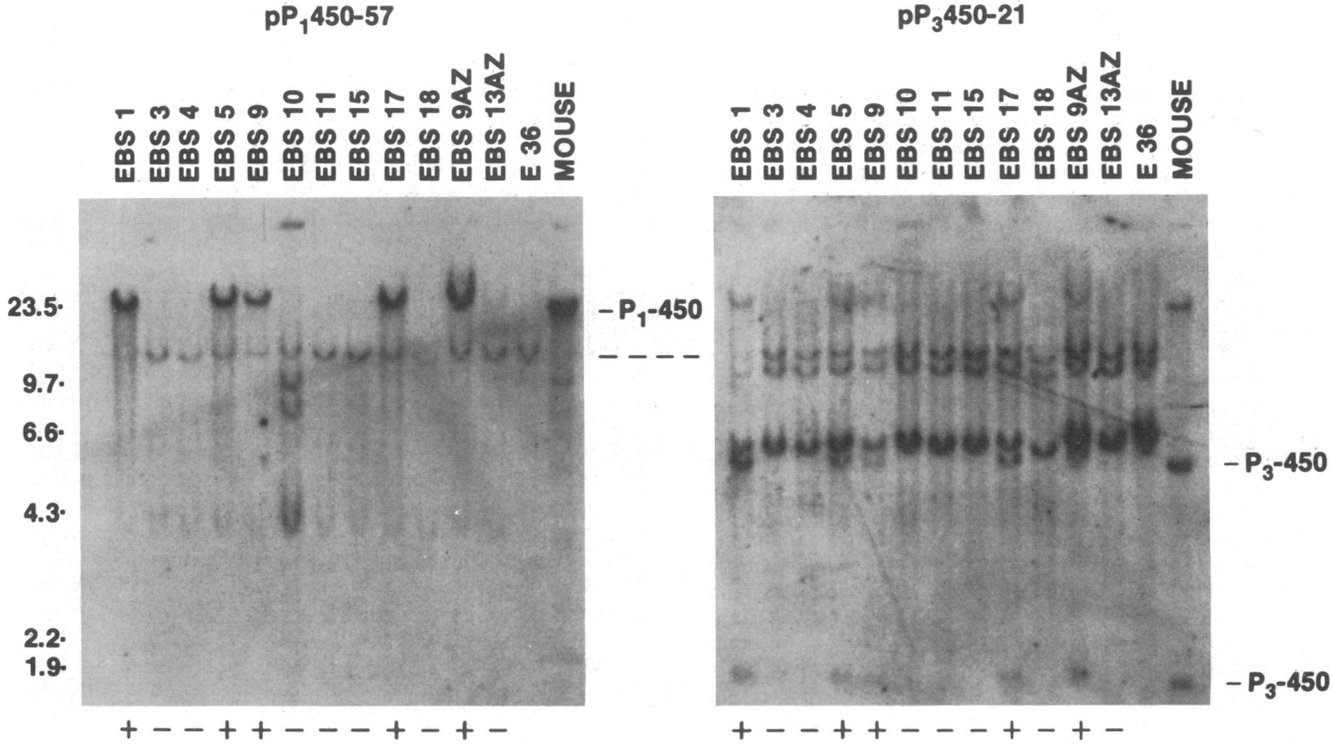

FIG. 1. Hybridization of the mouse cDNA probes pP_1450-57 (*Left*) and pP_3450-21 (*Right*) to *Kpn* I-digested DNA of hamster E36, mouse, and hamster–mouse hybrid DNA. E36 is a Chinese hamster cell line; the mouse DNA was taken from a BALB/cJ-derived cell line (RAG). Restriction digests containing 10 μg of DNA were electrophoresed in 0.8% agarose gels, transferred to nitrocellulose filters, and hybridized. The EBS series of hybrids are derived from hamster E36 and BALB/cJ mouse spleen cells. EBS-9AZ and EBS-13AZ are hybrids that have been back-selected in the presence of 8-azaguanine. After autoradiography of the hybridization with pP_1450-57, the filter was subjected to prehybridization conditions and then hybridized with pP_3450-21. Fragment sizes, given in kb (*Left*), were estimated by comparison with co-electrophoresed *Hin*dIII-digested λ phage DNA. Indicated at the right of either gel are the migration patterns for the mouse *P_1-450* or *P_3-450* gene fragments. The 6-kb *P_3-450* gene fragment (*Right*) appears smeared in EBS-9AZ, yet it is clearly present in other experiments. The four dashes between the gels denote the migratory distance of a 15-kb fragment present in the hamster parent line and in all of the hybrids. At the bottom *Left*, + denotes the presence and − denotes the absence of the mouse *P_1-450* hybridizable fragment in the 12 somatic cell hybrids. At the bottom *Right*, + denotes the presence and − denotes the absence of the mouse *P_3-450* hybridizable fragments in the 12 somatic cell hybrids.

Table 1. Concordance rates of both pP_1450-57 and pP_3450-21 hybridization with the presence of each mouse chromosome in the 12 hamster–mouse somatic cell hybrids

Mouse chromosome	Concordant		Discordant		Total	
	+/+	−/−	+/−	−/+	Concordant	Discordant
1	3	4	2	3	7	5
2	5	3	0	4	8	4
3	3	4	2	3	7	5
4	4	6	1	1	10	2
5	3	6	2	1	9	3
6	3	5	2	2	8	4
7	5	2	0	5	7	5
8	4	5	1	2	9	3
9	5	7	0	0	12	0
10	3	3	3	2	6	5*
11	0	7	5	0	7	5
12	4	2	1	5	6	6
13	5	2	0	5	7	5
14	3	5	2	2	8	4
15	5	0	0	7	5	7
16	4	4	1	3	8	4
17	5	3	0	4	8	4
18	3	6	1	2	9	3
19	4	4	1	3	8	4
X	4	1	1	6	5	7

Kpn I-digested DNA from the 12 somatic cell hybrids was used (Fig. 1). Concordant: +/+, probe hybridizes to the mouse chromosome and chromosome is present, or −/−, probe does not hybridize and chromosome is not present. Discordant: +/−, probe hybridizes to the mouse chromosome and chromosome is not present, or −/+, probe does not hybridize and chromosome is present. Data are summarized for pP_1450-57 and pP_3450-21 from hybridization to DNA from the hybrids previously analyzed for mouse chromosome content by karyotype and isozyme analysis.
*Enzyme marker data from EBS-13AZ are not available.

ence of the P_1-450 sequence, it was found that the mouse *P_1-450* gene segregated concordantly with mouse chromosome 9 but discordantly with all other mouse chromosomes (Tables 1 and 2). These data demonstrate that the *P_1-450* gene is located on mouse chromosome 9.

When the same filter was probed with pP_3450-21 (Fig. 1), 6-kb and 2-kb fragments were present in mouse DNA and in those same hybrids that the *P_1-450* gene was detected. The hamster bands seen in E36 are easily distinguishable from the 6-kb and 2-kb mouse bands present in these hybrids. Since the filter was not stringently washed between hybridizations (to avoid shearing the DNA off the filter), the *P_1-450* sequence was faintly visible. Like the *P_1-450* gene, the *P_3-450* sequence mapped concordantly with mouse chromosome 9 (Tables 1 and 2). Thus, the *P_1-450* and *P_3-450* genes are both located on chromosome 9.

DISCUSSION

The observation that the mouse *P_1-450* and *P_3-450* genes are located on the same chromosome has interesting implications from the standpoint of gene regulation of this family of enzymes. Induction of both cytochromes by polycyclic aromatic compounds is controlled by the cytosolic *Ah* receptor (9). The appearance of the *Ah* receptor in the nucleus is closely associated with dramatic increases in transcriptionally mature P_1-450 mRNA (11) and P_3-450 mRNA (14), and both are coordinately regulated genetically in mice having the high-affinity form (such as C57BL/6N) and the poor-affinity form (such as DBA/2N) of the *Ah* receptor. The *Ah* receptor is therefore an integral component in controlling the induction process. Recently, it was shown that both P_1-450 mRNA and the P_3-450 mRNA exhibit a high degree of 5′ homology (13). Hybridization analysis of pP_3450-21 with about 6 kb in the 5′ direction and about 13 kb in the 3′ direction of the *P_1-450* gene has demonstrated no evidence for these genes lying close in tandem. Because these genes are governed by the same receptor, however, it is tempting to speculate that the genes are arranged in tandem and are derived from a common ancestral gene. Proof of this concept will require further work.

A major regulatory gene controlling P_1-450 induction has recently been localized to mouse chromosome 17 (22). It is common for a regulatory gene encoding a receptor not to be linked with a structural gene controlled by that receptor. For example, testosterone binds to the androgen receptor, whose gene exists on the X chromosome; testosterone induces the β-glucuronidase structural gene (*Gus*) on mouse chromosome 5 (23).

Several different classes of P-450 have been characterized. Two major families are those induced by polycyclic aromatic compounds, as presented in this communication, and those that are inducible by phenobarbital. Each cytochrome is different from each other with respect to antigenic properties, substrate specificities, and primary structure (24–29). The *P_1-450* gene from the mouse MOPC 41 plasmacytoma is <5 kb in length and has at least five exons (30). The rat phenobarbital-inducible *P-450e* gene spans >14 kb and has at

Table 2. Segregation of *P_1-450* and *P_3-450* genes with mouse chromosomes in hamster–mouse cell hybrids

Cell hybrid	Marker				Chromosome																		
	P_1-450	*P_3-450*	*Mod-1*	*Mpi-1*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
EBS-1	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
EBS-3	−	−	−	−	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	+	+	−	+	+
EBS-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+
EBS-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+
EBS-9	+	+	+	+	−	+	+	+	−	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+
EBS-10	−	−	−	−	+	+	+	−	−	−	+	−	−	+	−	+	+	+	+	+	−	+	−	+
EBS-11	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	+	−	−	+	+	−	−	−	+
EBS-15	−	−	−	−	−	+	+	+	−	+	+	+	−	+	−	+	+	−	+	−	+	−	+	+
EBS-17	+	+	+	+	+	+	−	−	+	−	+	−	+	−	−	−	+	+	+	−	+	−	−	+
EBS-18	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+*	−	−	−	−	+
EBS-9AZ	+	+	+	+	−	+	+	+	−	−	+	+	+	+	−	+	+	−	+	+	+	+	+	−
EBS-13AZ	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	+	−	−	+	−	+	−	+	−

Malic enzyme (*Mod-1*) and mannose phosphate isomerase (*Mpi-1*) represent genes located on mouse chromosome 9. Restriction enzyme analyses were performed on DNA extracts of hamster–mouse hybrid clones; duplicate cultures of the same passage were used for enzyme and karyotypic analyses. Twenty-five metaphase spreads were analyzed per hybrid clone. The presence or absence of mouse enzyme markers assigned to 16 of the 19 mouse autosomes and the X chromosome agreed with the presence or absence, respectively, of the particular mouse chromosome. EBS-9AZ and EBS-13AZ were back-selected in 8-azaguanine in order to isolate clones that had lost the X chromosome.
*A fragment, but not the complete chromosome, was present.

least nine exons (31, 32). At the level of Southern or RNA transfer blot hybridizations, no homology appears to exist between polycyclic hydrocarbon-inducible and phenobarbital-inducible *P-450* genes. The former class of genes is governed by the *Ah* receptor; the mechanism of regulation by the latter class of genes is unknown. Moreover, Simmons and Kasper (33) have recently used recombinant inbred mice to demonstrate that multiple sequences homologous to phenobarbital-inducible P-450b cDNA (34) map to chromosome 7. *P-450b* also shares homology with *P-450e* (35), which therefore also may be present on chromosome 7. The *P-450b* gene(s) are clustered around the *Coh* locus (36), which encodes a phenobarbital-inducible P-450 that has coumarin 7-hydroxylase activity. Hence, polycyclic aromatic hydrocarbon-inducible and phenobarbital-inducible P-450 proteins both have monooxygenative functions, yet these two classes of genes are very different in size, number of exons, lack of homology at the DNA level, regulatory mechanism of gene expression, and chromosomal location.

In summary, two polycyclic aromatic hydrocarbon-inducible *P-450* genes are localized on mouse chromosome 9. We report here the localization of structural genes that are regulated by the *Ah* locus. Other enzyme activities shown to be correlated with the *Ah* locus include microsomal UDP-glucuronosyltransferase activity with 4-methylumbilliferone as substrate (37) and cytosolic reduced NAD(P):menadione oxidoreductase (38). As the structural genes for these proteins are cloned, it will certainly be of interest to determine their chromosomal assignment. It is important to understand not only how the P-450 induction process is controlled and expressed but also how these genes are organized with respect to one another and to other genes associated with the *Ah* locus.

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