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REGIONAL LINKAGE ANALYSIS OF THE DIOXIN-INDUCIBLE P-450 GENE FAMILY ON MOUSE CHROMOSOME 9

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SUMMARY: The dioxin-inducible P-450 gene family in the C57BL/6N mouse comprises two genes, P_1-450 and P_3-450. Restriction endonuclease-digested genomic DNA was probed with P_1-450 and P_3-450 full-length cDNA clones in an attempt to find species-specific fragment length differences between mouse and hamster cell lines and any restriction fragment length polymorphism among four inbred mouse strains. With this Southern blot hybridization technique, PstI fragments were used to distinguish between the mouse and hamster P_1-450/P_3-450 genes, and PvuII fragments were used to distinguish P_3-450 differences between the AKR/J and C57L/J inbred strains. Analysis of nineteen mouse x hamster somatic cell hybrid lines and sixteen AKXL (AKR/J x C57L/J) recombinant inbred lines showed that the P_1-450/P_3-450 genes are located near the Mpi-1 locus, between the Thy-1 and Pk-3 loci, in the middle portion of mouse chromosome 9.

The cytochrome P-450 enzymes are NAD(P)H-dependent multisubstrate monooxygenases present in every eukaryotic cell and in certain prokaryotes [1-5]. The multicomponent enzyme system in most eukaryotes is predominantly located in the endoplasmic reticulum, but also in the mitochondria and nuclear envelope; the enzyme system in Pseudomonadeae and certain primitive fungi is located in the cell sap [6, 7]. These enzymes are responsible for the biosynthesis and degradation of steroids, fatty acids, prostaglandins, leukotrienes, thyroxine, biogenic amines, pheromones and phytoalexins. Many of these same enzymes also oxygenate drugs, chemical carcinogens and other

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environmental pollutants. Certain forms of inducible P-450 therefore play a central role in chemical mutagenesis and carcinogenesis [1-8].

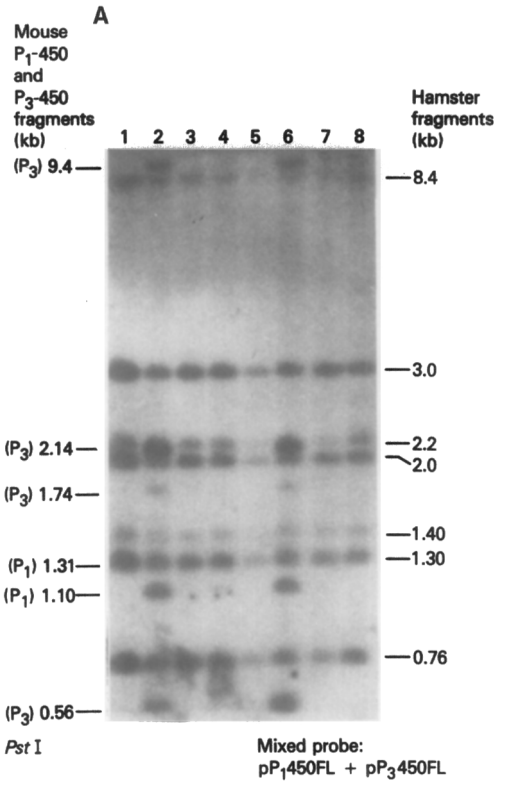
Several members of the phenobarbital-inducible P-450 gene family [9-13] and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-inducible P-450 gene family [14-18] have been cloned and assigned to mouse chromosomes 7 [19] and 9 [20], respectively. Recently a TCDD-inducible glutathione transferase gene has also been localized to mouse chromosome 9 [21]. The TCDD-inducible P-450 gene family is known to be controlled by the Ah receptor [22], whose gene (or some other major regulatory gene) has been mapped to the distal half of mouse chromosome 17 [23]. The purpose of this report is to determine the regional localization of the TCDD-inducible P-450 gene family.

EXPERIMENTAL PROCEDURES

The development of the mouse x hamster cell lines used in this study has been previously described [24-26]. The C57BL/6J, C57L/J, DBA/2J, AKR/J and AKXL inbred lines were purchased from The Jackson Laboratory (Bar Harbor, ME). Following digestion with PstI, the DNA samples were electrophoresed on 0.7% agarose gel and transferred to Zetabind® membrane filters (AMF Cuno, Meriden, CT). DNA from the somatic cell hybrids was hybridized with a mixture of full-length P_1-450 and P_3-450 cDNA (pP_1450FL and pP_3450FL, respectively [15, 17]). Mouse DNA was probed with either pP_1450FL or pP_3450FL. These [α-^{32}P]dCTP-labeled probes were radiolabeled by nick translation (BRL Nick Translation Kit, Gaithersburg, MD) to a specific activity of $\geq 10^8$ dpm/μg DNA. Hybridization and filter washing conditions were performed according to those recommended by the vendor of Zetabind®

RESULTS AND DISCUSSION

Mouse x Hamster Somatic Cell Hybrids. The TCDD-inducible P-450 gene family has two members in the C57BL/6N mouse: P_1-450 and P_3-450 [15]. The P_1-450 and P_3-450 full-length cDNA clones are designated pP_1450FL and pP_3450FL, respectively [15, 17]. The complete genes and flanking regions for mouse P_1-450 and P_3-450 have been sequenced [27], thus affording the opportunity to assign specific restriction fragments to known locations in either gene. Among 16 restriction endonucleases tested, PstI was chosen (Fig. 1) because it produces species-specific restriction fragment lengths that are easiest for distinguishing both P_1-450 and P_3-450. Hence, with a mixture of pP_1450FL plus pP_3450FL as the probe, it was possible to demonstrate the cosegregation of both the P_1-450 and P_3-450 genes in the hybrids



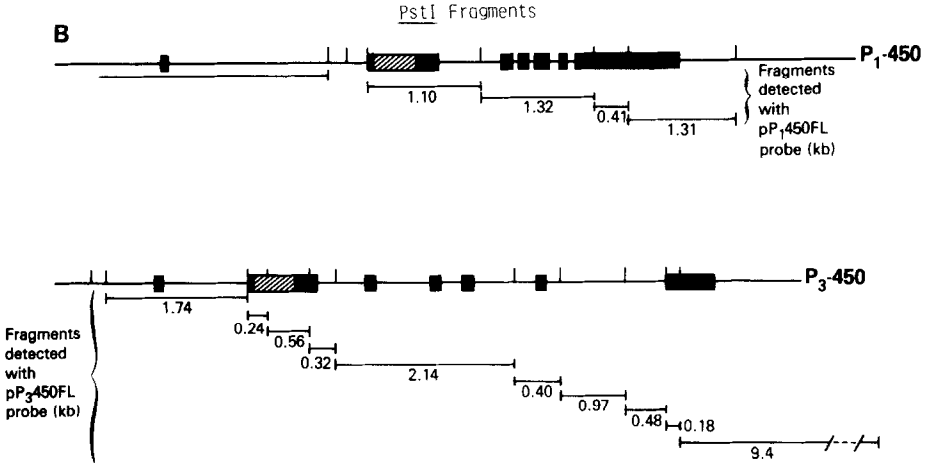


Fig. 1. Detection of mouse P_1-450 and P_3-450 PstI fragments in mouse x hamster somatic cell hybrids. A, Southern blot of PstI-digested genomic DNA from parental hamster and seven mouse x hamster hybrid lines. The DNA was probed with a mixture of pP_1450FL and pP_3450FL. The PstI fragments of mouse P_1-450 and P_3-450 are denoted at left; those of hamster are denoted at right. Lanes 1 through 4 represent mouse x hamster hybrid HM35, HM37, HM38 and HM39, respectively. Lane 5 represents the parental hamster cell line HM40. Lanes 6 through 8 represent MH42, HM47 and HM56, respectively. B, Diagram of the mouse P_1-450 and P_3-450 genes and their PstI sites. The seven exons are indicated by the solid boxes, the 0.5-kb region of high homology (96% simi-

TABLE 1
CORRELATION BETWEEN MOUSE CHROMOSOME 9 AND THE MOUSE P_1-450 AND P_3-450 GENES AMONG NINETEEN MOUSE X HAMSTER SOMATIC CELL HYBRIDS[a]

Mouse chromosome	Number of hybrids with P_1-450 and P_3-450 versus chromosome retention[b]				Percent discordant
	+/+	-/-	+/-	-/+	
1	3	4	2	5	50
2	4	5	0	5	36
3	3	8	0	1	8
4	3	8	3	3	35
5	0	9	8	0	47
6	4	6	0	3	23
7	8	3	0	8	42
8	3	9	1	1	14
9	6	11	0	0	0
10	3	10	3	2	28
11	0	11	7	0	39
12	4	2	1	6	54
13	3	6	1	3	31
14	3	10	2	0	13
15	5	0	0	9	64
16	3	5	2	5	47
17	6	5	0	4	27
18	4	7	1	3	27
19	5	7	1	3	25
X	3	8	0	1	8

[a]The development of these mouse x hamster somatic cell hybrids has been previously described [24-26]. Thirteen hybrids were characterized for mouse chromosomes by direct karyotyping with the use of Giemsa-trypsin banding and staining with Hoechst 33258; the other hybrids were typed for the presence or absence of specific chromosomes with the use of isoenzyme markers.

[b]+/+, containing the P_1-450/P_3-450 genes and the indicated chromosome; -/-, lacking the P_1-450/P_3-450 genes and the indicated chromosome; +/-, containing the P_1-450/P_3-450 genes but lacking the indicated chromosome; -/+, lacking the P_1-450/P_3-450 genes but containing the indicated chromosome.

(lanes 2 and 6 of Fig. 1). Among nineteen mouse x hamster somatic cell hybrids (Table 1), mouse chromosome 9 was the only chromosome without any discordancy. These data confirm the recent report [20] assigning these two

larity between the two genes [17]) in exon 2 is denoted by stripes, and the vertical lines represent the seven and eleven known PstI sites in the genes and flanking regions of P_1-450 and P_3-450, respectively. The sizes of the mouse P_1-450 and P_3-450 PstI fragments (in kilobases) noted in [A] were verified by separate blot hybridizations probed individually with pP_1450FL and pP_3450FL (data not included). Fragments larger than 10 kb or smaller than 0.5 kb (and of course those representing only intron DNA) are not visualized in [A].

genes to chromosome 9, although in that study different somatic cell hybrids, a different restriction endonuclease, and different P_1-450 and P_3-450 probes (short 3'-specific probes) were used.

One of the mouse x hamster somatic cell hybrid lines, HM56, which has lost the proximal half of mouse chromosome 9 (including the centromere and Mpi-1 but not Mod-1), does not contain either the mouse P_1-450 or P_3-450 gene (lane 8 of Fig. 1). This line has remained remarkably stable and contains the distal half of mouse chromosome 9 in more than 80% of the cells. The results in Fig. 1 and Table 1 thus implicate the proximal half of mouse chromosome 9 as the location of the P_1-450 and P_3-450 genes.

AKXL Recombinant Inbred Lines. Detailed linkage analyses can be gained by strain distribution patterns among recombinant inbred lines [reviewed in Ref. 28]. This gene-mapping procedure requires (i) uncovering a polymorphism between any two parental inbred mouse strains that have been used to establish a defined set of recombinant inbred lines, (ii) determining the segregation of the parental alleles among all the recombinant inbred lines, (iii) comparing the strain distribution pattern of the polymorphism under study with the strain distribution patterns of all other known loci on the chromosome of interest, and (iv) determining by mathematical calculations the degree of linkage and therefore the relative location of the new gene under study.

Initially, genomic DNA from C57BL/6J, C57L/J, DBA/2J and AKR/J was digested with each of 16 restriction endonucleases, and the genomic digests were probed with either pP_1450FL or pP_3450FL. The only distinct restriction fragment length polymorphism (RFLP) uncovered by this screening procedure involved a PvuII fragment of P_3-450: the fragment was 4.6 kb in AKR/J and 4.8 kb in C57BL/6J, C57L/J and DBA/2J. Because of the well-established set of recombinant inbred lines derived from AKR/J and C57L/J [29], this RFLP was studied in these AKXL lines (Fig. 2). Again, knowledge of the entire sequence of the P_1-450 and P_3-450 genes and flanking regions [17, 27] enabled us to account for three P_3-450 fragments hybridizing to pP_3450FL and for two P_1-450 fragments cross-

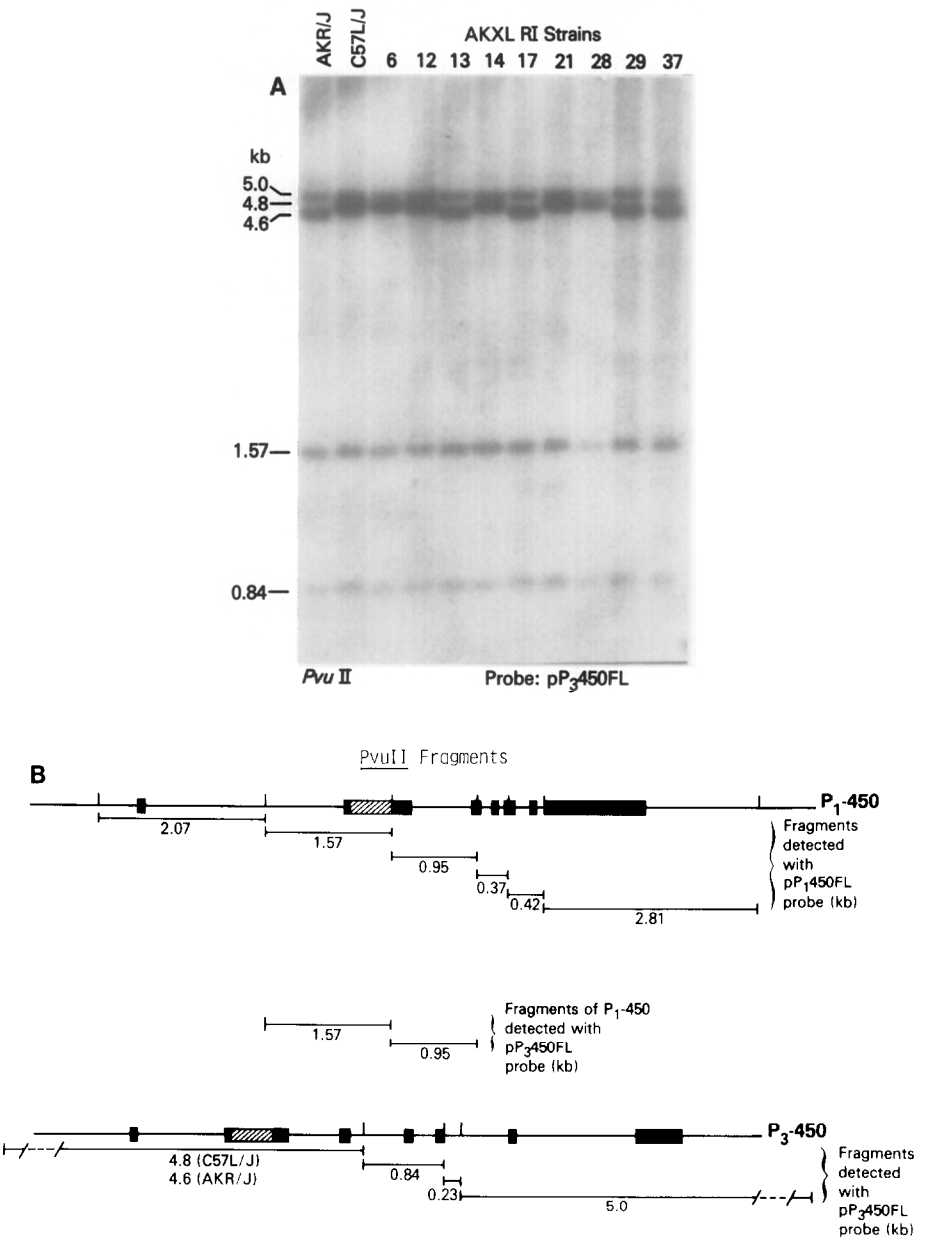


Fig. 2. Segregation of allelic PvuII restriction fragments specific for the P_3-450 gene in AKXL recombinant inbred lines. A, Southern blot of PvuII-digested genomic DNA from the parental AKR/J and C57L/J strains and nine representative AKXL recombinant inbred lines: AKXL-6, -12, -13, -14, -17, -21, -28, -29 and -37. Following digestion of hepatic genomic DNA with PvuII, the experimental protocol was identical to that described in Figure 1, except that the nick-translated probe was $pP_3$450FL alone. B, Diagram of the mouse P_1-450 and P_3-450 genes and their PvuII sites. The seven exons, the region of high homology in exon 2, and the restriction sites are depicted in the same manner as shown in Figure 1. The P_3-450 fragments (5.0, 4.8 or 4.6, and 0.84 kb) and the P_1-450 fragment (1.57 kb) illustrated in [B] are shown at left in [A]. The 0.95-kb fragment of P_1-450 is weakly visible on the x-ray film, but this band is difficult to see during photographic reproduction. This 0.95-kb fragment is more easily detected by $pP_3$450FL under relaxed stringency of blot washing conditions (data not included).

hybridizing to pP_3450FL. Additional double-digestion restriction endonuclease analyses of AKR/J, C57L/J and C57BL/6J genomic DNA probed with pP_3450FL indicated that the RFLP between AKR/J and either C57L/J or C57BL/6J is located around the 5' end of the P_3-450 gene, within approximately 1.7 kb upstream from the cap site.

The strain distribution pattern of the P_3-450 RFLP in 16 AKXL recombinant inbred lines was compared with the strain distribution patterns of four other markers located on chromosome 9 (Table 2). Recombination frequencies (r) between P_3-450 and Lap-1, Thy-1, Pgm-3 and Bgl were 0.10 ± 0.0048,

TABLE 2

COMPARISON OF THE STRAIN DISTRIBUTION PATTERN OF P_3-450 WITH THAT OF FOUR OTHER LOCI ON MOUSE CHROMOSOME 9 AMONG SIXTEEN AKXL RECOMBINANT INBRED LINES[a]

	P_3-450[b]	Lap-1	Thy-1	Pgm-3	Bgl
AKXL-6	L	L	L	L	K
AKXL-8	K	K	K	K	K
AKXL-9	K	L	L	K	K
AKXL-12	L	L	L	K	K
AKXL-13	K	K	K	K	K
AKXL-14	L	L	L	L	L
AKXL-16	K	K	L	L	L
AKXL-17	K	K	K	L	K
AKXL-19	L	L	L	L	L
AKXL-21	L	L	L	K	L
AKXL-24	K	K	K	K	K
AKXL-25	L	L	L	K	K
AKXL-28	L	L	L	K	K
AKXL-29	K	L	K	L	K
AKXL-37	K	L	L	K	L
AKXL-38	K	L	K	K	L

[a]Entries in this table indicate the parental origin (K = AKR/J; L = C57L/J) of the phenotype or genotype of the five loci. These data permit estimation of map distances among each of the loci with the use of relationships originally defined by Haldane and Waddington [30]. Up-to-date strain distribution patterns for Lap-1, Thy-1, Pgm-3 and Bgl were provided by Benjamin A. Taylor of The Jackson Laboratory (Bar Harbor, ME).

[b]Presence of the AKR/J-specific 4.6-kb fragment is denoted by K, the C57L/J-specific 4.8-kb fragment by L.

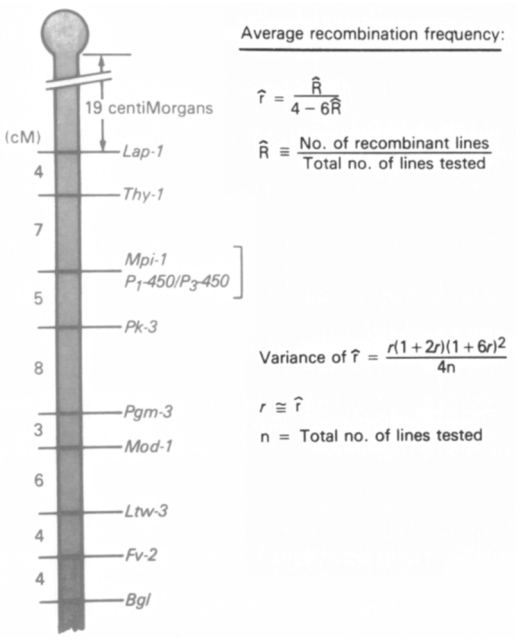


Fig. 3. Diagram of mouse chromosome 9, with the locations of several key loci and the distances between these loci in centiMorgans (cM). The circle at top represents the centromere. According to the sixteen AKXL recombinant inbred lines studied and the relationships originally described by Haldane and Waddington [30] and illustrated at right, the P_1-450/P_3-450 genes are distal to Thy-1 and near Mpi-1. Lap-1, intestinal leucine arylaminopeptidase; Thy-1, thymic antigen; Mpi-1, mannose phosphate isomerase; Pk-3, pyruvate kinase; Pgm-3, phosphoglucomutase; Mod-1, malate dehydrogenase; Ltw-3, liver protein tw-3; Fv-2, murine viral leukemogenesis; and Bgl, ß-galactosidase.

0.066 ± 0.0023, 0.32 ± 0.070 and 0.32 ± 0.070 Morgans, respectively. These recombination frequencies indicate that the P_1-450/P_3-450 genes map about 7 cM distal to the Thy-1 locus (Fig. 3).

It should be noted that, despite our use of a reasonably large number (sixteen) of recombinant inbred lines and despite the established strain distribution patterns among these sixteen AKXL lines for four reasonably close markers on mouse chromosome 9, Pgm-3 and Bgl were both mapped 32 ± 7 cM from P_1-450/P_3-450; however, all available data to date (Fig. 3) have mapped Pgm-3 and Bgl 17 cM from each other. Such problems have previously been recognized and discussed [28, 30]. These kinds of difficulties will be resolved with time, as increasingly larger numbers of strain distribution patterns for different genes become established and the entire system thus becomes more finely tuned.

Unfortunately, no RFLP for P_1-450 was uncovered during our screening procedure. Sequence analysis and the positions at which each exon is split [17, 27], however, are consistent with the mechanism of gene duplication about 65 million years ago and then divergence as a means of explaining the two homologous genes in the TCDD-inducible P-450 gene family. These data [17, 27] thus strongly suggest, but do not prove, that these two genes will be found in tandem.

Linkage Conservation. Certain clusters of genes have remained together during the evolutionary divergence of several species; this phenomenon has been termed autosomal linkage conservation. For example, the human genes ME1 and PGM3 (corresponding to mouse Mod-1 and Pgm-3, respectively) are found in the q12→q15 and q12→qter regions of human chromosome 6 [31]. The human genes MPI and PKM2 (corresponding to mouse Mpi-1 and Pk-3, respectively) are found in the q22→qter region of human chromosome 15 [31]. The hamster genes MPI and PKM2 have been localized to hamster chromosome 4 [32]. The close linkage of the TCDD-inducible P-450 gene family to the Mpi-1 locus on mouse chromosome 9 would suggest that this P-450 gene family is located on human chromosome 15 and hamster chromosome 4. These hypotheses have recently been confirmed for both human [33] and hamster [34]. It can therefore be concluded that during evolution the TCDD-inducible P-450 gene family has segregated with the Mpi-1 - Pk-3 linkage group. Because the Mpi-1 - Pk-3 linkage group has segregated with human chromosome 15 and the Pgm-3 - Mod-1 linkage group has segregated with human chromosome 6, assignment of the human TCDD-inducible P-450 gene family to human chromosome 15 is consistent with our regional linkage analysis data with the AKXL recombinant inbred lines (Figs. 2 and 3; Table 2).

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