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Assignment of the human 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible cytochrome P_1-450 gene to chromosome 15

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ABSTRACT

The human 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible cytochrome P_1-450 full-length cDNA has been recently isolated and sequenced [Jaiswal, A.K., Gonzalez, F.J. and Nebert, D.W. (1985) Science, in press]. A 1521-bp 5' DNA fragment representing almost all of the translating region was used to probe DNA from human, mouse, hamster, 53 human x mouse somatic cell hybrids, and 36 human x hamster somatic cell hybrids. These data indicate that the P_1-450 gene resides on human chromosome 15. Knowledge of the chromosomal assignment of this gene should help in our understanding of its regulation and role in development and disease.

INTRODUCTION

The environmental impact of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a deleterious health hazard to man (1) and the mechanism(s) of TCDD toxicity (2) are not resolved. Studied in various laboratory animal settings and experimental model systems, TCDD has been shown to be a very potent cause of birth defects and embryotoxicity (3-5), to be genotoxic in animals (6) but not mutagenic in the Salmonella/liver in vitro assay (7), to influence markedly total body lipids (8) and the immune system (2, 9), to stimulate epithelial proliferation (2, 10), and to be an extremely potent promoter of tumorigenesis (11). Most types of TCDD toxicity in experimental model systems appear to require the Ah receptor (2, 12-15).

Cytochrome P-450 enzymes are important in the biosynthesis and degradation of steroids, fatty acids, prostaglandins, leukotrienes, biogenic amines, thyroxine, pheromones and phytoalexins (16, 17). These monooxygenases are also responsible for the metabolism (usually detoxification, but sometimes formation of reactive intermediates) of countless drugs, chemical carcinogens, and other environmental pollutants (16, 17). The TCDD-inducible P-450 gene family represents a small subset of the overall number of P-450 genes (reviewed in Ref. 18); this family is controlled by the Ah receptor and comprises at least two genes (P_1-450 and

P_3-450) in the mouse (19-21) and probably only a single gene (P_1-450) in the human (22).

P_1-450 is responsible for polycyclic hydrocarbon-inducible aryl hydrocarbon (benzo[a]pyrene) hydroxylase (AHH) activity. The genetically regulated induction of AHH in the mouse (23, 24) and human (24, 25) appears to be correlated with genetic differences in risk of environmentally caused cancers and toxicity. Although P_1-450 induction by TCDD is not believed to be directly the cause of TCDD toxicity (2), this induction process clearly proceeds via the Ah receptor and is important in the initiation of tumorigenesis by numerous environmental carcinogens (16, 17, 23-25). The mouse P_1-450 gene is transcriptionally activated by TCDD (26, 27) and 3-methylcholanthrene (26). As first steps in examining the role of P_1-450 in human carcinogenesis and in screening various clinical populations to determine who is at increased risk for polycyclic hydrocarbon-caused cancer and toxicity, we have sequenced the human P_1-450 cDNA (22) and show in the present report the chromosomal location of this human gene.

EXPERIMENTAL PROCEDURES

We studied the DNA of 53 human x mouse and 36 human x hamster somatic cell hybrids that have retained varying numbers of human chromosomes after segregation. Construction of these hybrids, karyotypic analysis of banded mitotic chromosomes from these hybrids, and electrophoretic analysis of human biochemical markers in these hybrids have been detailed elsewhere (28-30). Briefly described, the strategy for chromosomal assignment (31, 32) involves the use of a cDNA corresponding to a well characterized gene to probe restriction endonuclease-digested DNA from a battery of these hybrids. The presence or absence of the human gene is then correlated with the presence or absence of each human chromosome. We used the probe ph$P_1$450-5', which represents the 1521-bp 5' EcoRI fragment of the human P_1-450 full-length cDNA (Fig. 1). We also used p$P_1$450FL and p$P_3$450FL, full-length cDNA probes corresponding to the mouse P_1-450 and P_3-450 genes, respectively (19), to analyze many of the same hybrid cell DNA preparations; the results were in complete agreement.

RESULTS

Nucleotide Sequence Data

TCDD treatment of the human breast carcinoma cell line MCF-7 induces

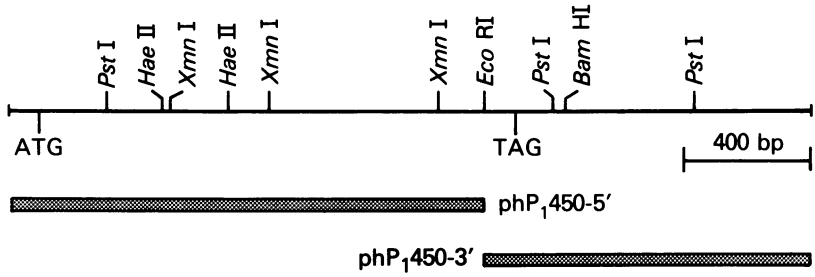

Fig. 1. Restriction map of the human P_1-450 full-length cDNA. A single EcoRI site afforded a convenient 1521-bp 5' probe, phP_1450-5', and a 1045-bp 3' probe. The human P_1-450 coding region is terminated by UAG, the same as that found in the mouse P_1-450 gene (21), whereas the mouse P_3-450 coding region ends in UGA (20).

remarkably high AHH (P_1-450) activity (22). This cell line was thus used as a source for isolating a human P_1-450 full-length cDNA clone via the phage cloning vector λgt11 (33). The cDNA was found to be 2,566 nucleotides in length, to encode a 2.8-kb mRNA which includes the poly(A) tract, and to have a continuous reading frame (87-1625) producing a protein with 512 residues (M_r = 58,151) (22). The human P_1-450 protein is 80% and 68% similar to mouse P_1-450 and P_3-450 protein, respectively (22).

With the two mouse homologous genes, there is 96% similarity among a 492-bp stretch in exon 2 (21). Consequently, a full-length probe or a 5' probe of either cDNA detects two genes in this P-450 family (19). If this P-450 gene family in human has two corresponding members, one would expect to detect evidence for two genes by probing Southern blots with phP_1450-5' or phP_1450-3' (Fig. 1). With XbaI, HindIII and KpnI digestions of human DNA from three sources, however, only bands representative of the human P_1-450 gene are found with the phP_1450-5' or the phP_1450-3' probe (22). Although these data do not rule out the possibility of one (or more) additional members of the human TCDD-inducible P-450 gene family that do not share extensive sequence homology, these results are strongly suggestive that P_1-450 represents the only member of this human gene family.

The mouse P_1-450 and P_3-450 genes were estimated to have arisen by gene duplication about 65 million years ago (21). Divergence of human from rodent species is estimated to have occurred at least 80 million years ago (34, 35). It is therefore reasonable to expect that the number

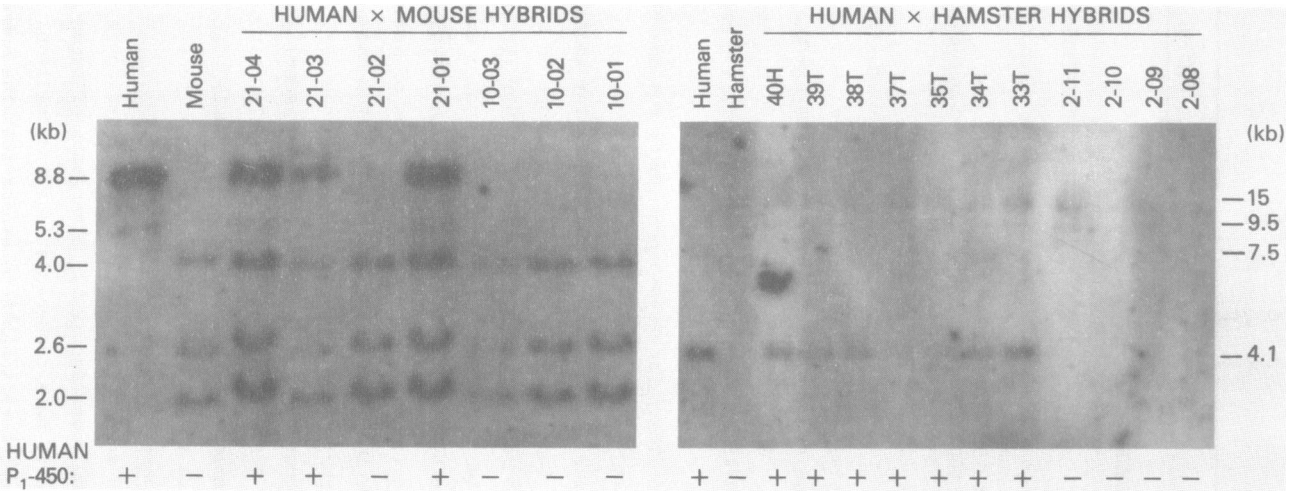

Fig. 2. Southern blot hybridization analyses of genomic DNA from hybrid cell lines and subclones, and from the parent human, mouse and hamster cells. Seven representative human x mouse (left) and eleven representative human x hamster (right) somatic cell hybrids are shown. Each lane contains 20 µg of DNA, digested with HindIII (left) or BamHI (right). Following agarose gel electrophoresis (19) and transfer to Zetabind® membrane filters (AMF Cuno, Meriden, CT), the DNA was probed with ^{32}P-labeled nick-translated phP$_1$450-5' (Fig. 1). Hybridization procedures were performed according to recommendations by the Zetabind® vendor. Autoradiographic exposures were 10 days (left) and 8 days (right). The mouse bands are 4.0, 2.6 and 2.0 kb. The hamster bands are 15 and 9.5 kb.

Table 1. Assignment of the cytochrome P_1-450 gene to human chromosome 15

Series	Total hybrids	P_1-450 positive	Percent discordancy	
			Chromosome 15	Other chromosomes
a	17	5	0	≥24
b	15	11	0	≥33
c	32	14	0	≥16
d	25	12	4	≥24
a - d	89	42	1	26-66

This table represents a summary of all four series of hybrid cell lines and subclones that were analyzed. Primary mouse x human (a) and Chinese hamster x human (b) hybrid lines, as well as subclones of four human x mouse and two human x hamster lines (c), were examined (28-30). The results from eleven additional primary and fourteen subcloned hybrid lines (d) are also presented. Discordancy with a given chromosome occurs when a hybrid either (i) retains that chromosome but lacks the human P_1-450 gene, or (ii) lacks the human chromosome but has the human P_1-450 gene. Percent discordancy equals 100 times the sum of these two types of discordancy, divided by the total number of hybrids examined. The human P_1-450 gene is assigned to that chromosome having the lowest percent discordancy, in this case, chromosome 15. One hybrid containing isoenzyme markers for chromosome 15 lacked a detectable P_1-450 gene (series d); this observation resulted from either (i) a difference in sensitivity between Southern hybridization analysis and isoenzyme analysis or (ii) a break or translocation involving chromosome 15 in the hybrid. The minimum discordancy between all other human chromosomes and the P_1-450 gene is shown with the following exceptions: series a, 12% and 18% discordancy with chromosomes 7 and 9, respectively; series b, 20% discordancy with chromosome 6.

of genes in the TCDD-inducible P-450 gene family may differ between human and mouse.

Somatic Cell Hybrid Data

From initial experiments in which human, mouse and hamster DNAs were cleaved with BamHI, BglII, EcoRI, HindIII, KpnI, PstI, SacI and XbaI and probed with ph$P_1$450-5', we chose HindIII and BamHI for human x mouse and human x hamster hybrids, respectively, because these endonucleases afforded the optimal resolution of human P_1-450 fragments from the rodent P_1-450 fragments. Representative autoradiograms of the human x mouse and human x hamster hybrids are shown in Fig. 2. The major and minor human bands are 8.8 and 5.3 kb, respectively (left), and 4.1 and 7.5 kb, respectively (right). The mouse HindIII restriction fragments (4.0, 2.6 and 2.0 kb) detected by the human P_1-450 probe can be predicted from considerations of nucleotide sequence similarities between human P_1-450 cDNA and both mouse P_1-450 (83%) and P_3-450 (72%) in the translating

regions (22) and from the known HindIII sites in and near the mouse P_1-450 and P_3-450 genes (36). In this context, it is noteworthy that multiple mouse restriction fragments (representing both TCDD-inducible P-450 genes) are detected by the heterologous human cDNA probe under stringent hybridization and filter-washing conditions, whereas only a single major human fragment is detected. This observation is suggestive of the presence of only one member in the human TCDD-inducible P-450 gene family, an interpretation supported by several other independent lines of evidence (Ref. 22 and described above).

Southern blot hybridizations of 53 human x mouse and 36 human x hamster somatic cell hybrids (Fig. 1; Table 1) demonstrate 99% concordance of the human P_1-450 5' cDNA probe with human chromosome 15. In contrast, all of the other human chromosomes exhibit at least 26% discordance with the P_1-450 probe in the entire group of 89 hybrids. These data permit the unambiguous assignment of the human P_1-450 gene to chromosome 15. Both the major and minor hybridization bands cosegregate with this chromosome, suggesting again a single human P_1-450 gene and the presence of at least one HindIII and one BamHI site in the 5' two-thirds of the human P_1-450 gene.

DISCUSSION

This study represents the first unequivocal chromosomal assignment for a human gene whose expression is regulated by TCDD. Earlier human x mouse somatic cell studies have assigned AHH induction by polycyclic hydrocarbons to human chromosome 2, segregating with the malate dehydrogenase (MDH-1) and isocitrate dehydrogenase (IDH-1) loci (37, 38). These studies relied only on catalytic activity, however, and did not take into account the possibility that the receptor derived from one parent cell line might regulate the P_1-450 gene from the other parent cell line. In fact, this effect was postulated to have occurred with benzo[a]anthracene-induced AHH activity in mouse x rat somatic cell hybrids (39).

It is now known that the murine P_1-450/P_3-450 structural genes are located on chromosome 9 (40), probably in tandem and lying between the Mpi-1 and Pkm-3 loci (41). In contrast, a major regulatory gene controlling AHH induction by benzo[a]anthracene has been localized to the distal end of mouse chromosome 17 (42). Based on these observations and the known linkage conservations between mouse and human genes (43), the meaning of the earlier assignment (37, 38) of AHH inducibility to human chromosome 2 is thus unclear.

Since the present report localizes the human P_1-450 structural gene to chromosome 15, the AHH locus previously assigned to human chromosome 2 must represent a regulatory gene. However, the putative human (37, 38) and mouse (42) regulatory genes do not appear to be located on homologous chromosomes. Further work on localization of AHH inducibility gene(s) and the Ah receptor gene(s) is therefore necessary.

In summary, the human P_1-450 gene has been unambiguously assigned to chromosome 15. Knowledge of this chromosomal location may provide possible linkage markers to aid in screening human populations and family studies for high versus low risk of certain types of environmentally caused cancers and toxicity. Differences in genetic expression of P_1-450 inducibility (24, 25), however, are more likely to reside in or near the (regulatory) Ah receptor gene(s) than the P_1-450 structural gene.

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