Human Dioxin-Inducible Cytochrome P_1-450: Complementary DNA and Amino Acid Sequence

Abstract. *Induction of cytochrome P_1-450 has been linked to susceptibility to certain chemically induced cancers in mouse and man. Treatment of the human cell line MCF-7 with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in high levels of aryl hydrocarbon (benzo[a]pyrene) hydroxylase (P_1-450) activity. This cell line was used to isolate a human P_1-450 full-length complementary DNA (cDNA) clone. The cDNA is 2566 nucleotides in length, encodes a polyadenylated messenger RNA (2.8 kilobases in length), and has a continuous reading frame producing a protein with 512 residues (molecular weight, 58,151). The human P_1-450 cDNA and protein are 63 percent and 80 percent similar to mouse P_1-450 cDNA and protein, respectively. Whereas the mouse TCDD-inducible P-450 gene subfamily has two members (P_1-450 and P_3-450), the human TCDD-inducible gene subfamily appears to have only one gene (P_1-450).*

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent inducer of many proteins including drug-metabolizing enzymes such as the cytochrome P-450 proteins (*1–3*). In mice, cytochromes P_1-450 and P_3-450 are dramatically induced by TCDD (*4, 5*). P_1-450 is closely associated with aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) and is responsible for the metabolic activation of polycyclic hydrocarbons such as benzo[*a*]pyrene to form reactive carcinogenic intermediates (*6*). Induction of P_1-450 by TCDD may play a role in the initiation of certain types of environmentally caused malignancies; TCDD is a cocarcinogen in a mouse tumor model system (*7*) and is a very potent promoter of tumorigenesis (*8*). Genetically determined high inducibility of AHH is associated with enhanced risk of certain types of chemically induced cancers in mice (*9*) and in cigarette-smoking humans (*10*). The AHH gene system, like the α_1-antitrypsin gene system (*11*), may be important in relating morbidity and mortality to environmental chemical exposure via cigarettes or industry (*12*).

As a first step in developing an assay for genetic susceptibility to chemically induced cancers we determined the complementary DNA (cDNA) and amino acid sequence of human P_1-450. When the human breast carcinoma cell line MCF-7 is treated with TCDD, AHH activity is induced at a specific activity of >20 units per milligram of protein, which is a high value for a cell line derived from nonhepatic tissue (Fig. 1). Similar kinetics of AHH induction were observed when cells were treated with benzo[*a*]anthracene. Polyadenylate-enriched RNA (*13*) was isolated from 10^9 MCF-7 cells that had been treated with 50 n*M* TCDD for 24 hours. Double-stranded cDNA was synthesized after reverse-transcription of RNA; the DNA was methylated with Eco RI methylase, and inserted into the bacteriophage cloning vector λgt11 by use of Eco RI linkers (*14*). Clones that hybridized to a mixture of ^{32}P-labeled nick-translated full-length cDNA inserts of mouse P_1-450 and P_3-450 (*5*) were plaque-purified, digested with Eco RI, and subcloned into the Eco RI site of pBR322. Two Eco RI inserts from the largest clone were separately purified by agarose gel electrophoresis and used to prepare a library of DNA fragments (*15*) in M13 mp11. Sequencing was carried out by standard M13 cloning protocols and the dideoxynucleotide sequencing method (*5, 16*). Each stretch of DNA was sequenced at least once on both strands and usually 5 to 20 times. A Stu I fragment of 470 base pairs straddled the one Eco RI site in the cDNA; this fragment was sequenced to ensure that there was not a second Eco RI site. Nucleotide alignment and analysis of nucleotide and protein data were examined by standard computer programs (*17*).

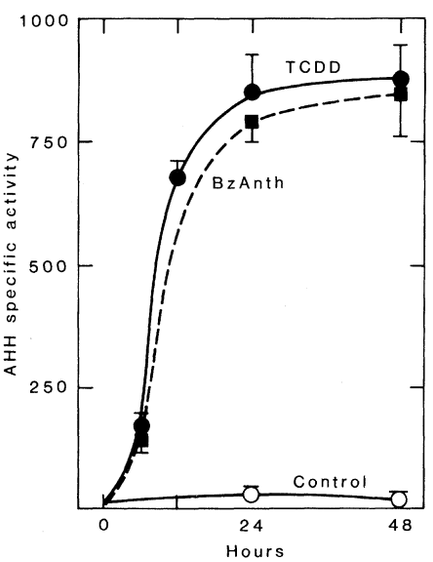

Fig. 1. Human AHH induction kinetics in MCF-7 cells treated with 50 n*M* TCDD, 50 μ*M* benzo[*a*]anthracene (BzAnth), or control medium. Specific activity denotes units (picomoles of phenolic benzo[*a*]pyrene products formed in 1 minute at 37°C) per milligram of cellular homogenate protein (*27*). The origin and development of the MCF-7 line (*28*), growth and treatment of the cells in culture (*29*), and preparation of the cells for AHH assay (*27, 29*) have been described.

The human P_1-450 cDNA was 2566 nucleotides long and had a continuous reading frame (from nucleotide 87 to 1625) that could produce a protein of 512 residues (Figs. 2 and 3). The termination codon for both human and mouse P_1-450 messenger RNA (mRNA) was UAG (U, uracil; A, adenine; G, guanine; T, thymine; C, cytosine) (Fig. 2), although only UGA has been reported as the termination codon in the more than 280 published sequences of human and mouse mRNA's. UAA is the termination codon for hamster vimentin mRNA (*18*), however, providing further evidence that UGA is not the only termination codon in mammals. There were several termination codons (including two UGA codons) in two of the three possible reading frames in the 25 bases after the occurrence of the human UAG codon (Fig. 2); this is seen near the translation termination site of many eukaryotic and prokaryotic genes (*19*).

The human and mouse cDNA nucleotide sequences (Fig. 2) had 63 percent similarity overall, with much greater similarity (83 percent) in the translating regions than in the 5′ nontranslating and the 3′ nontranslating regions (34 percent similarity). Of 270 base changes in the translating region (17 percent divergence), 29, 16, and 55 percent occur in the first, second, and third codon positions, respectively. The large divergence in the nontranslating regions, as compared with that in the translating regions of human and mouse P_1-450 cDNA, is consistent with the finding that the region of cDNA encoding an important protein remains much more conserved than regions of cDNA that do not (*19*).

There was an 80 percent similarity between the human and mouse P_1-450 deduced amino acid sequences (Fig. 3). In contrast, the human P_1-450 protein sequence was 68, 79, 68, 35, and 27 percent similar to mouse P_3-450, rat P-450c, rat P-450d, rat P-450e, and rat P-450scc proteins, respectively (*5, 20*). Our sequencing data thus indicate that we have isolated the human P-450 cDNA that is equivalent to mouse P_1-450 and rat P-450c cDNA. The amino acid composition of human and mouse P_1-450 proteins was strikingly similar, including the presence of nine cysteine residues in both proteins. The mouse P_3-450 protein has six cysteine residues (*5*). The molec-

ular weight of the human P_1-450 protein (58,151) is about 1080 less than that of the mouse P_1-450 protein (59,230).

When mouse genomic DNA is probed with mouse P_1-450 or P_3-450 full-length cDNA clones, the Southern blot hybridizations are consistent with the existence of only two genes in this P-450 family (*5*). In addition, when liver RNA from TCDD-treated mice is probed with these two full-length clones, mRNA 2.9 kilobases (kb) and 2.1 kb in length are found (*21*). When a probe derived from the 5′ end of human cDNA (Fig. 4) was used for DNA or RNA blot hybridizations, however, all the data were consistent with the presence of a single TCDD-inducible human P-450 gene. Digestion with Xba I and Hind III resulted in fragments 5.2 and 8.8 kb in size, respectively. These bands did not appear to differ among HeLa, human placenta, and MCF-7 genomic DNA's (Fig. 4A). Digestion with Kpn I resulted in two fragments (5.9 and 2.2 kb) in all three human genomic DNA preparations; these data are consistent with a known Kpn I site in the human P_1-450 cDNA. The same result was obtained under lower hybridization stringencies (*22*).

The human 5′ cDNA clone hybridized to both 2.1- and 2.9-kb mRNA from 3-methylcholanthrene–treated C57BL/6J inbred mice (Fig. 4B). No hybridization to untreated MCF-7 cultures was observed, but in TCDD-treated cultures, a single size of mRNA (2.8 kb) hybridized strongly to the probe. A length of 2566 nucleotides for the cDNA, plus a poly(A) region of about 250 nucleotides, would produce the human P_1-450 mRNA of about 2.8 kb. These findings suggest a species difference in this TCDD-inducible P-450 gene subfamily; man probably has only P_1-450, whereas mouse has both P_1-450 and P_3-450. The functions of human P_1-450 thus might be divided between rodent P_1-450 and P_3-450. Alternatively, new functions may have evolved in P_3-450.

From the sequence data, it has been estimated that the TCDD-inducible P-450 gene family diverged from the phenobarbital-inducible P-450 gene family more than 200 million years ago (*5, 20*) and that the P_1-450 and P_3-450 genes separated (probably via gene duplication) about 65 million years ago (*5*). This estimate is consistent with the finding of at least two major TCDD-inducible P-450 proteins in mouse, rat, and rabbit (*3, 5*). Moreover, this calculation predicts that human P-450 proteins might not exhibit this pattern, because divergence of human from rodent would have occurred prior to the split of P_3-450 from P_1-450

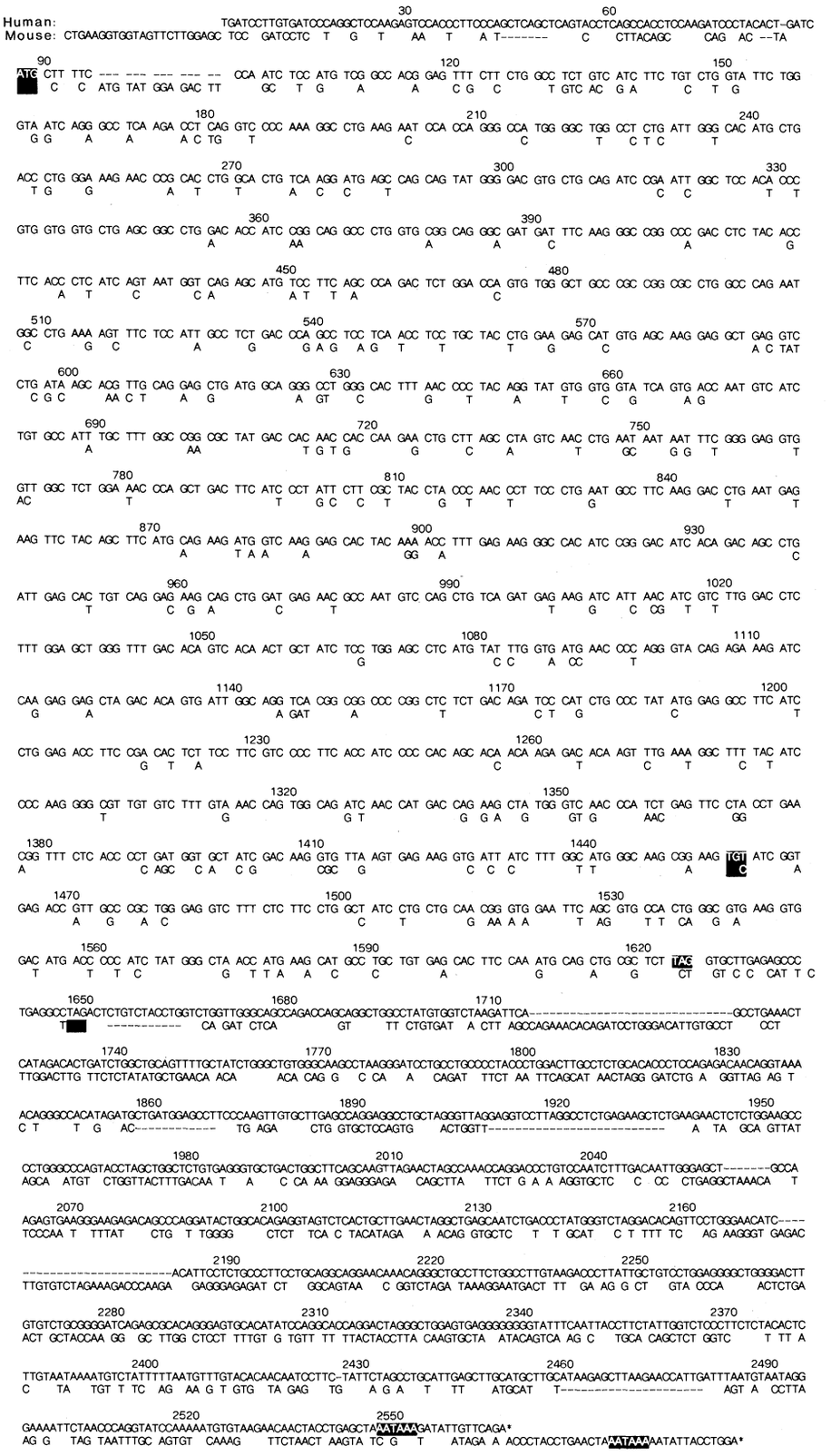

Fig. 2. Comparison of human and mouse P_1-450 cDNA sequences. The initiation codons, the cysteine codons believed to participate in the enzyme active-sites (*5, 20*), the termination codons, and the putative poly(A) addition signal (AATAAA) are enclosed in blackened boxes. The human sequence is numbered; the mouse sequence is shown only at those positions where there was no correlation.

(*23*). The data in Fig. 4 support this prediction.

The time required for 1 percent divergence in amino acid sequence [unit evolutionary period (UEP) (*24*)] was estimated to be 2.1 and 2.4 million years by means of comparisons between rabbit and rat P-450 proteins (*25*) and between mouse and rat P-450 proteins (*5*), respectively. If human predecessors separated from the rodent line approximately 80 million years ago (*24*), the human and mouse P_1-450 proteins would have diverged between 33 and 38 percent. We were surprised to find only 20 percent divergence (Fig. 3). These results suggest the existence of an additional force or forces conserving the P_1-450 protein in these two species. The metabolic activation of foreign chemicals or detoxification may contribute to this conservation. In any event, there is greater similarity between human and mouse P_1-450 proteins (80 percent) than between the mouse P_1-450 and P_3-450 proteins (73 percent); P_1-450 and P_3-450 are in the same TCDD-inducible P-450 subfamily.

Knowledge of the cDNA sequence should make it possible to look for restriction-fragment length polymorphisms (RFLP's), which are probes for diagnosis and prediction in a growing number of clinical disorders and human family studies (*11, 26*). As has been shown in mice (*9*), the human population exhibits a wide range of AHH (P_1-450) inducibility (*10*). The finding of human RFLP's representing high and low AHH inducibility would be of importance in predicting the degree of risk for persons exposed to various environmental pollutants.

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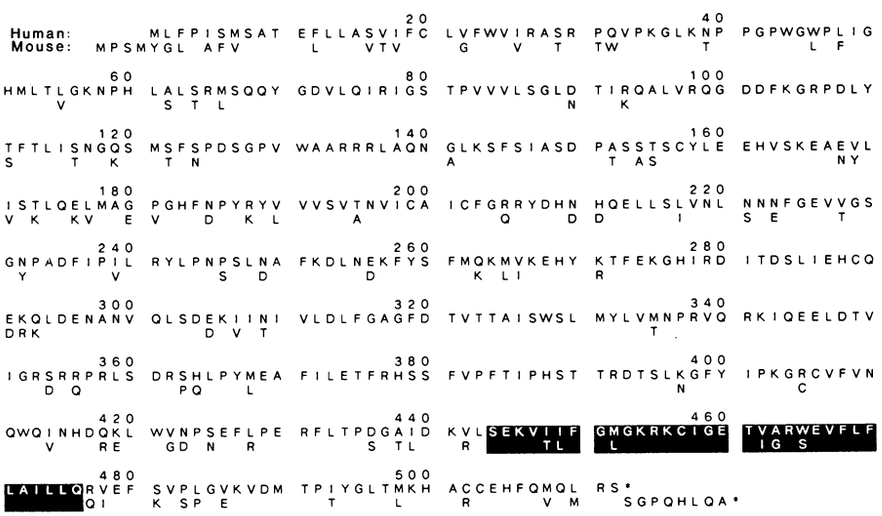

Fig. 3. Comparison of human and mouse P_1-450 protein sequences. The conserved COOH-terminal cysteinyl fragment (*5, 20*) is enclosed in a blackened box. Residues of mouse that do not match those of human are shown (*30*). Abbreviations for amino acids: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

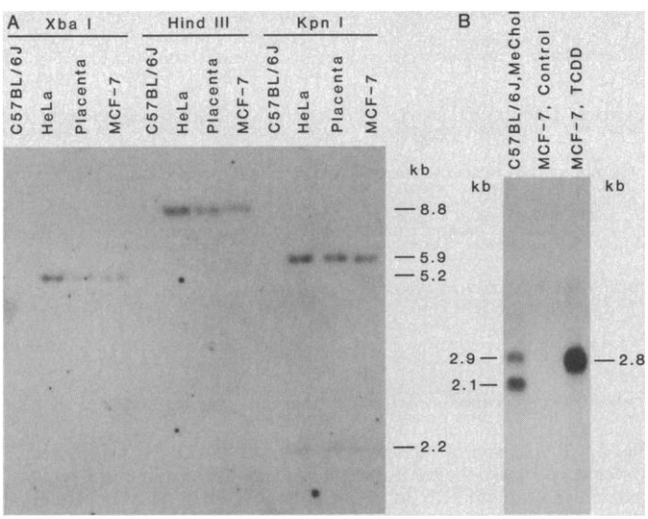

Fig. 4. Hybridization analyses with the human P_1-450 cDNA 5′ probe $phP_1450\text{-}5'$. This probe is the Eco RI fragment from base 1 to base 1521 (Fig. 2). (A) Southern blot hybridization. Conditions were sufficiently stringent and exposure of the filter to the x-ray film was short enough to exclude almost completely the cross-hybridization of the human probe with C57BL/6J mouse DNA (lanes 1, 5, and 9). (B) Northern blot hybridization. Poly(A)-enriched RNA was prepared from the liver of C57BL/6J inbred mice that had received 3-methylcholanthrene (MeChol) (200 mg per kilogram of body weight) 18 hours before killing and from MCF-7 cells that had been exposed to control medium or 50 n*M* TCDD for 24 hours. Hybridization conditions and nick-translation of the probe were as described (*5*).

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30. The mouse P_1-450 NH_2-terminal sequence determined on detergent-solubilized protein (M. Negishi, D. W. Nebert, J. E. Shively, unpublished data) was Y-G-L-P-A-F-V- . . . , whereas the corresponding sequence determined on immunoaffinity-purified protein [K.-C. Cheng *et al.*, *Biochem. Biophys. Res. Commun.* **123**, 1201 (1984)] was P-S-M-Y-G-L-P-A-F-V. The original estimation of P_1-450 molecular weight (58,914) and 521 residues (*5*) was therefore based on translation from the second of two "in-frame" ATG codons and the detergent-solubilized protein data. It now seems certain that P_1-450 has 524 residues and that the molecular weight is 59,230, based on the immunoaffinity-purified protein data. A similar observation of one or two NH_2-terminal residues being lost during detergent solubilization of rabbit P-450 form 4 has been reported [V. S. Fujita, S. D. Black, G. E. Tarr, D. R. Koop, M. J. Coon, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4260 (1984)].
31. HeLa cell DNA and human placental DNA were provided by O. Wesley McBride. The Southern blot was provided by C. Edgar Hildebrand.

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Human T-Cell Receptor α-Chain Genes: Location on Chromosome 14

Abstract. *The genes encoding the α chain of the human T-cell receptor have been mapped to chromosome 14, the chromosome on which the human immunoglobulin heavy chain locus resides. Thus, genes encoding two different classes of antigen receptor are present on the same chromosome. Furthermore, breaks involving chromosome 14 are frequently seen in tumors of T-cell origin. The potential relation of these chromosome abnormalities to α-chain genes is discussed.*

The major histocompatibility complex (MHC) restricted antigen receptor on T lymphocytes is a disulfide-linked heterodimer and is composed of an α and a β chain (*1*). The complementary DNA (cDNA) clones encoding the β chain have been isolated and described in mice and man (*2*). The β-chain locus consists of several gene segments that encode variable (V), diversity (D), joining (J), and constant (C) regions of the β-chain protein, which is itself related to immunoglobulins. The β chain is encoded on chromosome 7 in man (*3*) and on chromosome 6 in mouse (*4*). The locus for murine immunoglobulin (Ig) kappa light chains is encoded on chromosome 6 as well (*5*), although the evolutionary significance of this linkage is not understood. The α-chain cDNA clones have been isolated and characterized in humans (*6*) and in mice (*7*). The sequence of these α-chain cDNA's suggest that, like the β chain and the Ig's, the α chain is encoded in separate, noncontiguous gene segments (V, J, and C). We have now mapped the genes encoding the α chain and have assigned these genes to human chromosome 14.

We used a series of human-rodent somatic cell hybrids, containing characterizing combinations of human chromosomes (see legend to Fig. 1 for description of hybrids); the isolation and characterization of most of these hybrids have been described (*8*), and those not previously described are discussed in the legend to Fig. 1. Hybrid cells were grown in culture, and DNA was prepared from each hybrid (*9*). The presence or absence of human α-chain genes in each hybrid was determined by probing Southern blots of genomic DNA with a labeled α-chain cDNA probe isolated from the human T-cell tumor, HPB-MLT. This human α-chain cDNA has been characterized by one of us (E.P.) and is described in (*6*).

Analysis of hybrid DNA's with an α-chain cDNA probe by the Southern blot method (Fig. 1) shows three Hind III fragments (2.6, 4.4, and 9.6 kb) in human DNA. A cDNA fragment specific for the V region of this particular α chain hybridizes to the 9.6-kb Hind III fragment, whereas a cDNA fragment specific for the α-chain C region hybridizes to the 2.6- and 4.4-kb Hind III fragments (*10*).

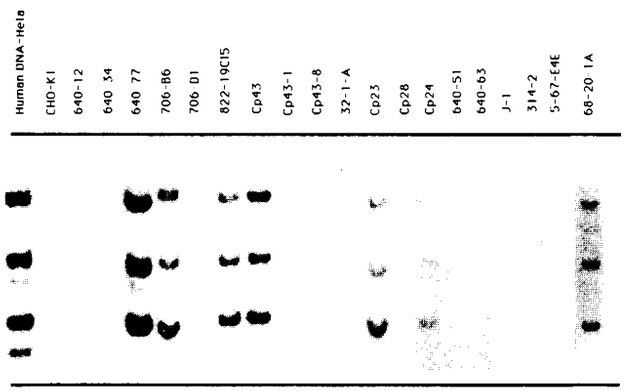

Fig. 1. Southern analysis of human-rodent cell hybrids for human α-chain genes. Hybrid or tumor cell DNA was digested with Hind III and subjected to electrophoresis through 0.7 percent agarose gels. Samples of DNA were blotted and hybridized by the method of Wahl *et al.* (*14*), except that the acid depurination step was omitted and the gel was irradiated with shortwave ultraviolet for 8 minutes prior to denaturation. The blots were hybridized with the 1.3-kb insert of pGA5, a cDNA clone that encodes the α chain from the human T-cell tumor HPB-MLT. This insert contains variable joining and constant region sequences and has been described (*6*). The upper, middle, and lower bands are 9.6, 4.4, and 2.6 kb, respectively. The faint bands seen in human DNA are apparently unrelated to the α-chain locus since they do not segregate with the major bands. They may represent α-chain pseudogenes, which are unlinked to the α-chain locus. The hybrid cell lines in (*8*) were used except for 32-1-A, a human lymphoblast-mouse L-cell hybrid; Cp23, a human fibroblast–Chinese hamster ade$^-$B hybrid; Cp24, a human fibrosarcoma–Chinese hamster ade$^-$B hybrid; 314-2, a human lymphocyte–Chinese hamster uri$^-$C, ade$^-$G hybrid; 706-D1, a human lymphocyte–Chinese hamster ade$^-$F hybrid.