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MOLECULAR PHARMACOLOGY, 44:911-917

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ACCELERATED COMMUNICATION

Cloning and Expression of a Human Ah Receptor cDNA

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Received July 12, 1993; Accepted August 31, 1993

SUMMARY

In this report, we describe the cloning and expression of a cDNA encoding a human Ah receptor, a basic/helix-loop-helix protein that mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. A comparison of this human cDNA with a murine homologue (*Ah*^{b1} allele) indicates that the molecular mass variation observed between the receptors found in these two species results from hypervariability of amino acid sequences in the carboxyl termini (<60% conserved over 450 amino acids). Differential usage of stop codons generates proteins with molecular masses that differ by 6 kDa. In contrast, the amino-terminal halves of these proteins are highly conserved and show 90% amino acid sequence identity. Northern blot analysis indicates that the human Ah receptor mRNA is expressed at its highest levels in placenta and is also highly expressed in lung, heart, pancreas, and liver, with lower levels of expression found in brain, kidney, and skeletal muscle. Expression of the human cDNA in a rabbit reticulocyte lysate system allowed functional analysis of ligand binding, agonist-induced and Ah receptor nuclear translocator-dependent DNA binding, and receptor stabilization by sodium molybdate.

The AHR is a ligand-activated transcription factor that mediates the biological effects of TCDD (or "dioxin") and related planar aromatic compounds (1). Models to describe the role of this protein in gene expression are derived from studies of the regulation of genes involved in xenobiotic metabolism. TCDD-induced interactions of the AHR with a number of enhancer elements termed DREs can activate the transcription of the *Cyp1a1* and *Ya* genes (2, 3). These receptor-DRE interactions have been shown to lead to DNA bending, chromatin disruption, increased promoter accessibility, and ultimately increased rates of transcriptional initiation (4–7). TCDD-induced AHR/DRE interactions have been shown to be dependent on a second protein, known as ARNT (8–10). Cloning studies have demonstrated that both the AHR and ARNT contain basic/helix-loop-helix domains (8, 11, 12), suggesting that they are heterodimeric partners that act coordinately to activate the expression of downstream promoters, in a manner similar to that of Myc/Max or MyoD/E2A (13, 14).

The AHR and ARNT also share sequence homology with two *Drosophila* proteins, Sim and Per. These four proteins are characterized by a homologous region of approximately 200 amino acids, termed the PAS domain (8, 11, 15). Deletion analysis and receptor fragmentation studies of the AHR indicate that the PAS domain harbors sequences involved in agonist binding (9, 11). In addition, the PAS domain may play a role in the formation of heterodimers between family members, similar to the role of the leucine zipper found in Myc and Max (9, 16). Interestingly, the basic/helix-loop-helix domain is found adjacent to the PAS domain in the AHR, ARNT, and Sim but is absent in Per, suggesting that Per could be a negative regulator of gene expression, in a manner similar to that of the Id protein in the MyoD system (8, 11, 15, 17).

An intriguing aspect of AHR biology is remarkable structural and functional variability of this protein, both within and across species. For example, in the murine system at least four receptor alleles encode the AHR. The *Ahr*$^{b-1}$, *Ahr*$^{b-2}$, and *Ahr*$^{b-3}$ alleles encode proteins of 95, 104, and 105 kDa, respectively (18, 19). The fourth allele, *Ahr*^{d}, encodes a 104-kDa protein that has a 10-fold lower affinity for agonist, compared with the proteins encoded by the *Ahr*^{b} alleles. By virtue of the lower affinity of this receptor, mice harboring the *Ahr*^{d} allele are much less susceptible to the biological/toxic effects of many receptor agonists (20). Structural variability across species is

This work was supported in part by grants from The American Cancer Society (JFRA-303), The Pew Foundation, and The National Institutes of Health (ES05703, T32 CA09560, and ES05589). The sequence of the human AHR cDNA reported in this manuscript has been deposited in the GenBank database (accession number L19872).

ABBREVIATIONS: AHR, Ah receptor; RACE, rapid amplification of cDNA ends; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DRE, dioxin-responsive element; ARNT, Ah receptor nuclear translocator; PAS, Per/Ah receptor nuclear translocator/Ah receptor/Sim; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction; kb, kilobase(s); ORF, open reading frame; UTR, untranslated region; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MOPS, 3-(*N*-morpholino)propanesulfonic acid.

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also remarkable, with receptor molecular masses varying by as much as 50 kDa (18, 21). Differences in receptor function between species are more difficult to characterize, due to the difficulties in controlling for the impact of genetic background on receptor function. With this point in mind, preliminary evidence has suggested that species-specific differences in receptor stability, DNA binding affinity, and protein interactions may exist (22, 23).

Taken together, the data described above suggest that considerable sequence divergence in the AHR exists across species. In this report, we describe the cloning and expression of the human AHR cDNA, provide an analysis of the corresponding mRNA expression across tissues, and present a structural and functional comparison of the encoded protein with the previously characterized murine receptor encoded by the *Ahr*$^{b\text{-}1}$ allele (AHR$^{b\text{-}1}$) (11, 12).

Materials and Methods

cDNA cloning. The 1.4-kb *Eco*RI fragment from the murine AHR$^{b\text{-}1}$ cDNA clone cAh1 (11) was radiolabeled and used as a probe to screen a human cDNA library constructed from oligo(dT)-primed mRNA from the hepatoma cell line HepG2 (Lambda Zap vector; Stratagene). In the first round of screening, 5×10^5 recombinants were screened at 37° in 50% formamide (yielding clones 91A and 71C). To obtain a clone that contained the initiation methionine, an additional 4×10^5 recombinants were screened at 42° in 50% formamide using the 0.92-kb *Bam*HI fragment of 91A as a probe (yielding clone hu14). To complete the 3′ UTR of the human AHR cDNA, the RACE method (24) was carried out using HepG2 RNA. Reverse transcription of poly(A)$^+$ RNA was carried out using an oligo(dT) primer, OL-79, followed by two rounds of PCR using the 91A-specific primers OL-100 and OL-101 sequentially with the adapter primer OL-80. Oligonucleotide primers, 5′ to 3′, were as follows: OL-79, GACTCGAGTCGACATCGATTTTTTTTT-TTTTTTTTT; OL-80, GACTCGAGTCGACATCGA; OL-100 (3739), CCATCGATCTCGAGAGATTGCAGATAGCAAGGTTTG-GTGC; OL-101 (3772), CCATCGATCTCGAGTGTAATG-AGTGAATTGAATGGTGC.[1]

Northern blot analysis. A multiple-tissue Northern blot (Clontech) containing 2 μg of doubly selected poly(A)$^+$ mRNA from eight human tissues was hybridized at 65° in 50% formamide with a riboprobe made to the *Nae*I fragment of hu14 (Fig. 1A). The blot was then stripped and rehybridized at 42° in 50% formamide with a random-primed GAPDH probe.

Cell culture. Hepa 1c1c7 and HeLa cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated bovine calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml fungizone, at 37° in a humidified atmosphere consisting of 95% air/5% CO_2. Cytosolic extracts were prepared from nearly confluent cells. The cells were washed twice and scraped in 10 ml of phosphate-buffered saline, pelleted at 2000 × *g* (at 4°), and resuspended in 25 mM MOPS, 1 mM EDTA, 0.02% NaN_3, 10% glycerol, pH 7.5 (at 4°) (MENG buffer), with or without 10 mM sodium molybdate. The cells were homogenized with 30 strokes in a glass homogenizer and were subjected to centrifugation at 10,000 × *g* for 20 min at 4°. The supernatants were centrifuged at 100,000 × *g* for 1 hr at 4° to remove microsomes.

Plasmid construction. The plasmid phuAHR was constructed by PCR using OL-135, derived from sequence lying 82 nucleotides upstream of the initiation methionine, and OL-136, derived from sequence lying 102 nucleotides downstream of the stop codon of the full length human AHR cDNA clone. The PCR-generated product was subcloned into the *Bgl*II site of the vector pSL1180 (25), subcloned into the *Kpn*I

[1] All nucleotide positions given in the text consider the adenosine of the initiation methionine to be +1. The oligonucleotide positions given in parentheses refer to the 5′ end of the oligonucleotide.

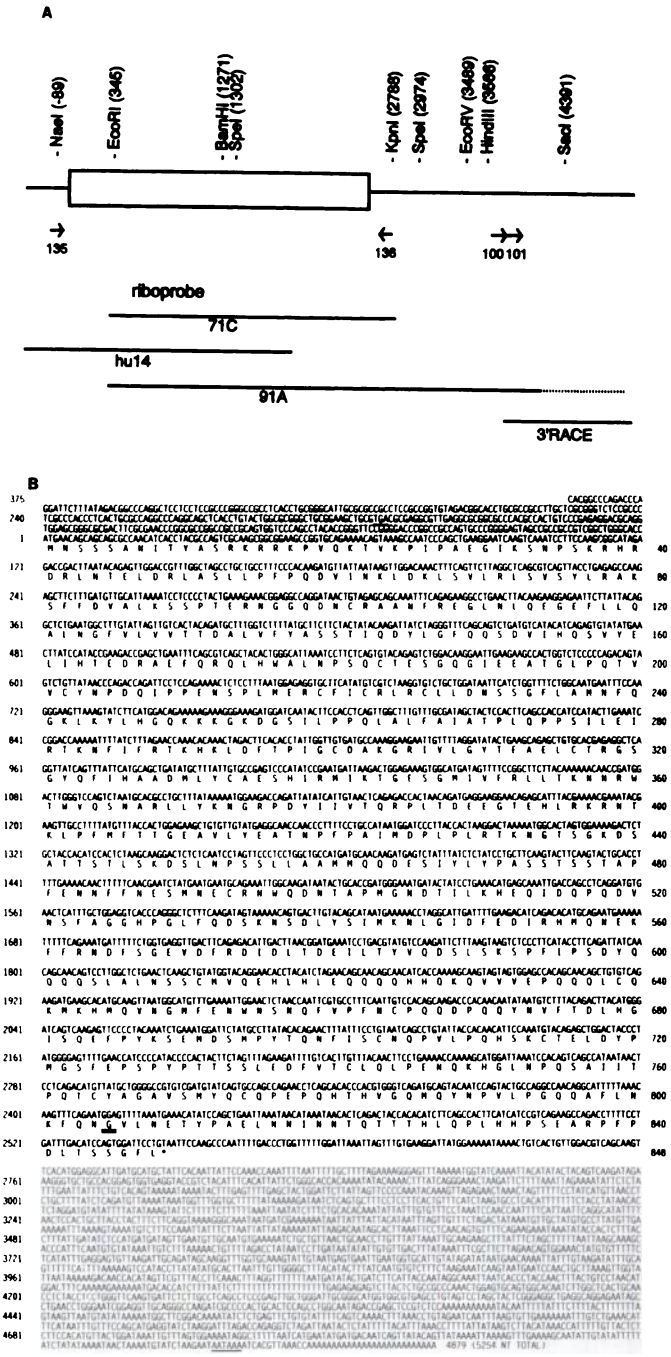

Fig. 1. A, Partial restriction map and location of human AHR cDNA clones. Restriction enzyme sites are numbered with the adenosine of the initiation methionine as +1. *Arrows*, positions of oligonucleotides used in the PCR for human expression vector construction (OL-135 and OL-136) and 3′ RACE (OL-100 and OL-101). *Arrowheads*, positions of oligonucleotides (arrows not drawn to scale). *Dashed line*, position of riboprobe used in Northern blot analysis. *Broken line*, region of discrepancy between clones 91A and 3′ RACE (see Discussion). B, Nucleotide sequence and deduced amino acid sequence of the human AHR cDNA. *Numbers on the left side*, nucleotide numbering with the initiation ATG as +1. *Numbers on the right side*, amino acid numbering. *, Termination codon. Position of the codon (GGA) that corresponds to the murine (AHR^{b1}) AHR termination codon (TGA) is *double-underlined*. Positions of the in-frame termination codon upstream of the initiation methionine and the polyadenylation signal are *single-underlined* in the 5′ and 3′ ends, respectively.

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and *SalI* sites of the expression vector pSV-Sport1 (26), and confirmed by DNA sequence analysis. The construction of the plasmid pmuAHR was described previously (9). The ARNT expression plasmid phuARNT was constructed by subcloning the *Bam*HI fragment of pBM5/NEO-M1-1 (8) into pBluescript (Stratagene), followed by subcloning of the resulting *Xba*I/*Hind*III fragment into the corresponding sites of pSV-Sport1 (26). Oligonucleotide primers were as follows: OL-135 (−82), GAAGATCTTCCAGTGGTCCCAGCCTACACC; OL-136 (2646), GAAGATCTTCATGTGAACTTGCTGACGTCC.

***In vitro* expression.** *In vitro* transcription and translation were carried out using the TNT-coupled rabbit reticulocyte lysate (Promega). Briefly, 1 μg of plasmid DNA was added to a 50-μl reaction containing 50% TNT rabbit reticulocyte lysate, reaction buffer, 20 μM complete amino acid mixture, 40 units of RNasin, and 20 units of SP6 RNA polymerase and was incubated at 30° for 90 min. For some experiments sodium molybdate was added to a final concentration of 10 mM after the reactions were completed. The efficiency of expression was analyzed in parallel experiments by both [^{35}S]methionine labeling and Western blot analysis using an affinity-purified antibody raised against an amino-terminal peptide derived from the murine AHR (27).

Photoaffinity labeling. Photoaffinity labeling of the AHR was performed using the radioligand 2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin (specific activity, 0.5 μCi/μl) and was carried out in 50-μl reactions in MENG buffer. Samples were incubated with 0.25 μCi of ligand (0.1 pmol) for 30 min at room temperature, cooled on ice, and incubated with 1/5 volume of charcoal/gelatin (3%/0.3%, w/v) for 30 min on ice. To remove unincorporated radioligand, the charcoal/gelatin slurry was subjected to centrifugation at 10,000 × *g* for 5 min at 4° and the supernatant was irradiated at 310-nm wavelength (0.8 J/cm^2). After irradiation, the reaction was quenched by addition of 300 mM β-mercaptoethanol. Acetone precipitates were resuspended in 1× Laemmli sample buffer (0.06 M Tris, pH 6.8, 0.002% bromphenol blue, 0.2% LDS, 0.14 M β-mercaptoethanol) and subjected to SDS-PAGE and autoradiography (28).

Gel shift assay. A complementary pair of synthetic oligonucleotides containing a consensus enhancer element (DRE) (OL-73 and OL-74) were annealed and end-labeled with [γ-^{32}P]ATP as described (29). Nonspecific competitor, poly(dIdC) (4.5 ng), was added to the *in vitro* expressed human AHR and ARNT proteins (approximately 1 fmol of each) and incubated for 15 min at 30°. The radiolabeled probe (1 × 10^5 cpm; 0.5 ng) was then added and incubated for 15 min at room temperature (final volume, 25 μl), followed by nondenaturing gel electrophoresis and autoradiography (30). A complementary pair of oligonucleotides containing a mutated core sequence (OL-113 and OL-114) were used in competition experiments to demonstrate specificity (31). Oligonucleotides were as follows: OL-73, TCGAGTAGATCACGCA-ATGGGCCCAGC; OL-74, TCGAGCTGGGCCCATTGCGTGAT-CTAC; OL-113, TCGAGTAGATCAATCAATGGGCCCAGC; OL-114, TCGAGCTGGGCCCATTGATTGATCTAC.

Results

Cloning of the human AHR. The 1.4-kb *Eco*RI fragment from the murine AHR^{b-1} cDNA clone, cAh1 (11), was used as a probe to screen a human cDNA library constructed from mRNA of the hepatoma cell line HepG2. Three overlapping cDNA

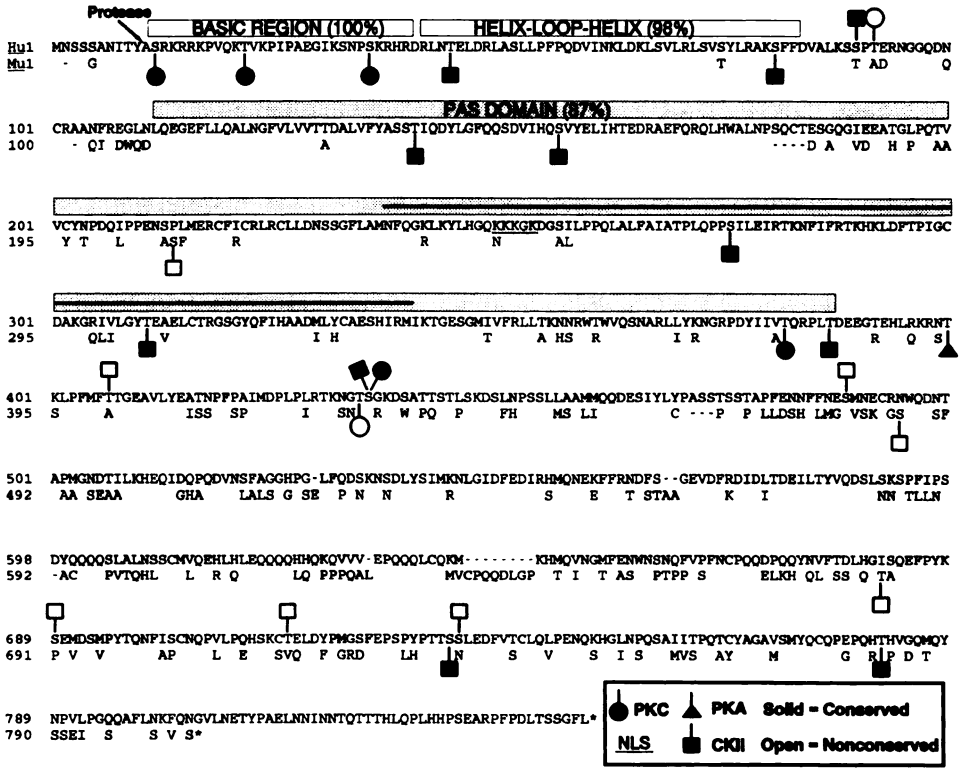

Fig. 2. Amino acid sequence comparison of the human (*Hu*) and murine (*Mu*) AHRs. Only the amino acids that differ in the murine AHR^{b-1} are shown; –, Gaps inserted to improve the alignment. Functional domains are drawn as *shaded boxes* above the corresponding sequences, with percentage identity indicated. *Solid line within the PAS domain*, ligand binding domain, as mapped by photoaffinity labeling of the murine protein; *arrow*, proteolytic cleavage site, as determined by amino-terminal amino acid sequencing of the murine AHR^{b-1} (11, 49). Potential phosphorylation sites for protein kinase C (*PKC*), cAMP-dependent protein kinase (*PKA*), and casein kinase II (*CKII*) are indicated as *solid symbols* when conserved in human and murine AHRs and as *open symbols* when not conserved. Consensus nuclear localization signal (*NLS*) in the PAS domain is *underlined*. *, Termination codons. The sequence alignment was done using the PALIGN program of Myers and Miller (34), with the following values: comparison matrix = genetic code matrix, open gap cost = 10, and unit gap cost = 10. Identification of protein motifs was done using the PROSITE analysis program of Bairoch (35). The consensus phosphorylation recognition sequences used were as follows: PKC, S/T-x-R/K; PKA, R/K-R/K-x-S/T; CKII, S/T-x-x-D/E; x is any amino acid.

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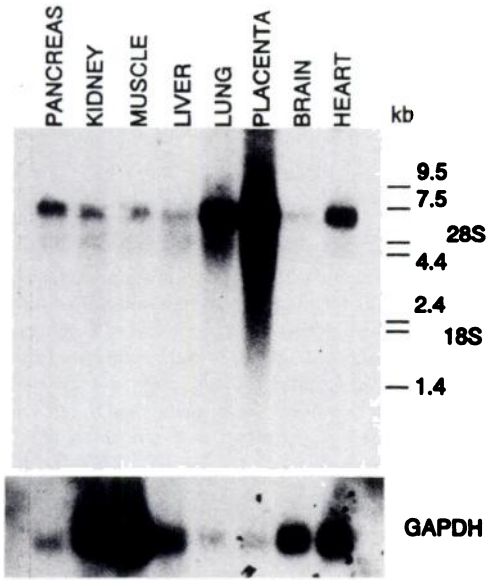

Fig. 3. Northern blot analysis of the human AHR. Each lane contains 2 μg of doubly selected poly(A)$^+$ mRNA from the indicated human tissues. *Top*, the blot was hybridized with a riboprobe made to the *Nae*I fragment of hu14 (Fig. 1). The bands were quantitated on a Fuji BAS 1000 Phosphoimager and the relative intensities were as follows: pancreas, 552; kidney, 468; skeletal muscle, 373; liver, 341; lung, 1765; placenta, 4344; brain, 371; heart, 1178. The film was exposed at −70° with intensifier screens for 72 hr. *Bottom*, the blot was then rehybridized with a random-primed GAPDH probe. The bands reflect the expected abundance of GAPDH in each of the tissues (36). The bands were quantitated on a Fuji BAS 1000 Phosphoimager and the relative intensities were as follows: pancreas, 105; kidney, 366; skeletal muscle, 4901; liver, 270; lung, 87; placenta, 95; brain, 415; heart, 610. The film was exposed at −70° with intensifier screens for 18 hr.

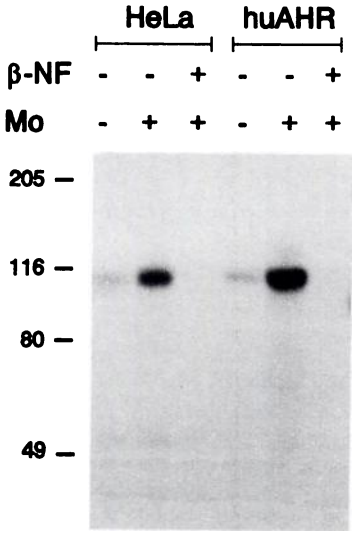

Fig. 4. Ligand binding of the human AHR. Photoaffinity labeling of the human AHR isolated from HeLa cells (*HeLa*) and encoded by the human cDNA (*huAHR*). Sixty micrograms of HeLa cytosol were photoaffinity labeled. The human AHR cDNA was expressed by *in vitro* transcription/translation and 1/5 volume of the *in vitro* reaction was used in the photoaffinity labeling reactions. pSV-Sport1 was used as a negative control. Labeling reactions were carried out in the absence and presence of sodium molybdate. Specificity of ligand binding was demonstrated by competition with 100 nM β-naphthoflavone.

clones, 91A, 71C, and hu14, were isolated and sequenced (Fig. 1A). Clone 91A contained a 4.47-kb insert that began with a continuous ORF coding for 732 amino acids before reaching an in-frame termination codon (TAA). Clone 71C contained a 2.45-kb insert that began at the same site as 91A and perfectly matched the sequence of 91A, confirming the ORF and position of the termination codon. Clone hu14 contained a 2.28-kb insert that overlapped by 1.56 kb with the 5′ end of 91A and extended the ORF an additional 116 amino acids at the amino terminus to a proposed initiation methionine. This methionine lies within the context of the Kozak consensus sequence (32) and aligns with the initiation methionine previously described for the murine AHR$^{b\text{-}1}$. An in-frame stop codon is found 171 nucleotides immediately upstream (Fig. 1B).

Clone 91A contained 2.27 kb of the 3′ UTR of the human AHR cDNA. To obtain the complete 3′ UTR, we used the RACE method (24) and amplified a single species of 1.1 kb (3′ RACE) that ended with a polyadenylate tract immediately downstream of a consensus polyadenylation site (Fig. 1). The 5′ end of this clone aligned with 91A for 0.48 kb to nucleotide 4258, where the two sequences diverged. The remaining 0.6 kb of the 3′ RACE clone retained good homology with the murine AHR$^{b\text{-}1}$ cDNA, whereas the corresponding sequence of 91A did not (data not shown). Because the 0.21-kb sequence at the 3′ end of 91A was not amplified by the 3′ RACE method, was not homologous to this region of the murine AHR$^{b\text{-}1}$ cDNA, and did not diverge at splice sites known to occur in the murine gene (33), this region of 91A may represent an independent cDNA that was ligated onto the AHR cDNA during the construction of the library (i.e., a cloning artifact).[2]

Comparison of the human and murine receptors. We used the PALIGN program (34) to compare the amino acid sequences encoded by the human and murine AHR cDNAs (Fig. 2). This analysis revealed that the amino-terminal halves of the two proteins are highly conserved, with 100% sequence identity in the basic region, 98% in the helix-loop-helix domain, and 87% in the PAS domain. In contrast, the carboxyl termini of the two proteins exhibit <60% sequence identity. One of the most prominent differences involves the position of the translational stop codon (nucleotide 2545 in the human AHR and 2416 in the murine AHR$^{b\text{-}1}$) (Fig. 1B) (11). The resulting human and murine receptors have calculated molecular masses of 96 and 90 kDa, respectively. These calculated molecular masses differ by <10%, compared with the apparent molecular masses predicted from SDS-PAGE for the two receptors (105 and 95 kDa, respectively) (27). This difference in apparent and calculated molecular mass may be due to post-translational modification or aberrant mobility upon SDS-PAGE. Comparison of the nucleotide sequences in the UTRs indicated 80% homology in the 3′ UTR but little if any homology in the 5′ UTR (although both 5′ UTR regions are G/C-rich) (data not shown). It is not known whether the 5′ UTR sequence of the human AHR cDNA is complete, but its length of 382 nucleotides is very close to the 422-nucleotide length of the murine AHR$^{b\text{-}1}$ cDNA 5′ UTR (33).

In an effort to identify possible sites for modification of the AHR protein, the deduced primary amino acid sequences of the cDNAs for the human and murine receptors were analyzed

[2] The sequence of the 3′ end of clone 91A that appears to be a cloning artifact has been deposited in the GenBank database (accession number U00791).

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using the PROSITE analysis program (35). This analysis revealed consensus phosphorylation sites for protein kinase C, protein kinase A, and casein kinase II (Fig. 2). A number of these sites are conserved between the human and murine AHRs. In the human protein, a consensus nuclear localization signal sequence (KKKGK) was identified within the PAS domain. In the murine protein, this sequence was somewhat degenerate (NKKGK) and thus was not identified by the computer algorithm.

Northern analysis of human AHR expression. Northern blot analysis of poly(A)$^+$ RNA from eight different human tissues using a 2.0-kb riboprobe from the human AHR cDNA revealed the presence, in all tissues, of a major transcript of approximately 6.6 kb (Fig. 3). The levels of AHR mRNA expression varied greatly between the different tissues, with the highest levels being found in placenta and lung and the lowest levels in kidney, brain, and skeletal muscle. The use of GAPDH as a standard internal control is problematic, in that this message also has considerable tissue variation (36). Its use here supports the contention that lanes were loaded equally. This conclusion is based upon the observations that our description of the relative expression of GAPDH across tissues is essentially identical to that reported by others and that RNA degradation appears to be minimal in all samples (37).

Functional expression of the human AHR cDNA. To prove the identity of the human clone, the cDNA was expressed in a rabbit reticulocyte lysate system and analyzed for its agonist and DRE-binding properties. To demonstrate ligand-binding activity, we photoaffinity labeled the translation product with 2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin. To determine the fidelity of the translated protein, we performed a number of functional comparisons with receptor isolated from the human-derived HeLa cell line (Fig. 4). We observed that the human AHR expressed *in vitro* exhibited the same mobility on SDS-PAGE as did the receptor isolated from whole cells (both migrate with an apparent molecular mass of 105 kDa) (Fig. 4). In addition, ligand binding analysis indicated that the *in vitro* translated and cellular receptors displayed similar binding characteristics when the receptor agonist β-napthoflavone was used as a competitor (Fig. 4). As we have shown previously for the murine AHR^{b-1}, the human AHR did not require the ARNT protein for its ligand-binding activity (9). Unlike the murine AHR^{b-1}, ligand binding to the human receptor was markedly enhanced by the addition of sodium molybdate to the binding buffer (Fig. 4; Table 1). This differential effect of sodium molybdate on the murine AHR^{b-1} and human AHR has also been shown for receptors isolated from whole cells (22, 38).

TABLE 1

Effects of sodium molybdate on ligand binding of the human and murine AHRs

Photoaffinity labeling of the AHR encoded by the human and murine cDNAs was performed in the absence (−Mo) or presence (+Mo) of 2 mM sodium molybdate. Results were quantitated with a Fuji BAS 1000 Phosphoimager or by densitometric scanning. Results are expressed as a percentage of ligand binding in the presence of sodium molybdate and represent the average ± standard deviation of three independent experiments. See Materials and Methods and Fig. 4.

	Binding	
	−Mo	+Mo
	%	
Human AHR	30 ± 11	100
Murine AHR	95 ± 0.6	100

To examine the agonist-induced DNA-binding properties of the expressed human AHR, we employed gel shift assays using a synthetic oligonucleotide corresponding to a well characterized DRE (39). The *in vitro* expressed human receptor bound to the DRE in an agonist-dependent manner but was unable to bind to the DRE in the absence of the ARNT protein (Fig. 5). The specificity of DRE binding was demonstrated by competition experiments using an excess of unlabeled DRE oligonucleotide or an oligonucleotide containing a mutated DRE. The addition of sodium molybdate inhibited the ability of the AHR-ARNT complex to bind to the DRE even in the presence of agonist. Finally, the presence of both the AHR and ARNT in the DRE-binding complex was demonstrated by supershift experiments using AHR- and ARNT-specific antibodies (9) (data not shown).

Discussion

Cloning the human AHR cDNA from HepG2 cells allowed a structural and functional comparison with the murine AHR^{b-1} homologue. Alignment of the amino acid sequences encoded by these cDNAs allowed identification of those domains that are either highly or poorly conserved between species. The highly conserved nature of the amino-terminal halves of the two AHR forms (90%) is consistent with the importance of this region in ligand binding, DRE recognition, and ARNT dimerization (9). Comparison of the carboxyl termini of the two proteins indicated that this region of the protein is poorly conserved at the amino acid level (60%), leading us to characterize this region as "hypervariable." This hypervariable domain contains a high concentration of glutamine residues ("Q-rich"), as well as do-

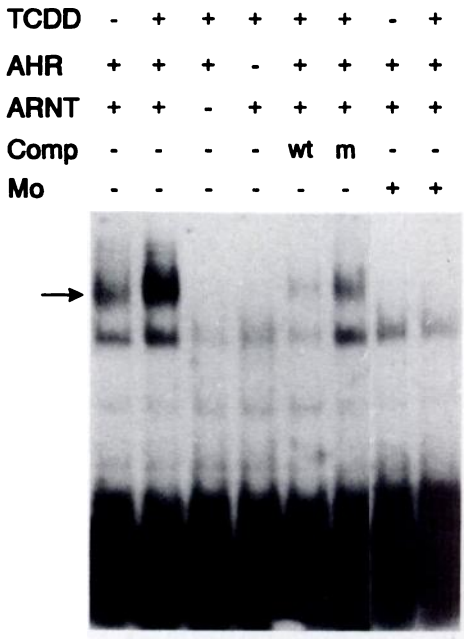

Fig. 5. DNA binding of the human AHR. *In vitro* translated human AHR and ARNT proteins were incubated with either dimethylsulfoxide (−) or 20 nM TCDD (+) for 2 hr at room temperature, followed by gel shift assays as described in Materials and Methods. *Arrow*, AHR-ARNT-DRE complex formation. Addition of excess competitor (*Comp*) wild-type DRE (*wt*) or mutant DRE (*m*), containing two nucleotide substitutions in the core region (31), demonstrates specificity of complex formation. The lower shifted band represents a nonspecific binding acitivity, and its migration corresponds to that of the single-stranded binding protein complex described previously (30).

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mains that play roles in receptor transformation to a DRE-binding state (9). In addition, the differential usage of termination codons within the carboxyl terminus provides an explanation for the molecular mass difference between these two receptor forms. The full length human AHR cDNA encodes an 848-amino acid protein with a calculated molecular mass of 96 kDa that is approximately 6 kDa larger than the receptor encoded by the murine AHR^{b-1} cDNA (calculated molecular mass of 90 kDa) (11). The data described above, as well as other results from our laboratory, suggest that the hypervariability and alternative usage of termination codons are the primary mechanisms underlying the molecular mass differences between the two AHR species (33).

Observations from a number of laboratories have led to the suggestion that phosphorylation is an important step in receptor signaling (40, 41). With this in mind, we analyzed the human and murine AHRs to identify putative sites for phosphorylation by protein kinase C, protein kinase A, and casein kinase II. This analysis revealed a number of conserved phosphorylation sites, primarily in the amino-terminal half of the receptor. These data provide a starting point for future site-directed mutagenesis studies to determine the role of protein kinases/phosphatases in receptor tranformation, dimerization, ligand binding, and DNA binding. Sequence analysis also revealed the location of a consensus nuclear localization sequence in the center of the PAS domain of the human AHR (KKKGK). This sequence has homology to the nuclear localization sequences found in a number of transcription factors, including SV40 large T antigen and the steroid receptors (42, 43). The presence of such a sequence in the AHR is predicted by evidence that the AHR undergoes an agonist-induced cytosolic to nuclear translocation event (44, 45). Interestingly, this sequence is slightly different in the murine homologue (NKKGK), suggesting either that asparagine or lysine can yield a functional signal sequence or that a second unique sequence also plays a role in nuclear localization (43).

Northern blot analysis using the human AHR cDNA as a probe revealed that a major transcript of approximately 6.6 kb is expressed in varying amounts in eight different human tissues. These results provide a preliminary description of the differential tissue distribution of the AHR and may possibly help to identify those tissues that are more susceptible to TCDD-induced toxicity. Importantly, the high levels of AHR mRNA in human placenta may explain the sensitivity of this tissue to receptor agonists, such as those found in cigarette smoke, and the relative ease with which the human receptor protein is isolated from this tissue (38, 46). However, it is important to note that the ability to accurately quantitate this expression is greatly limited by the differential, tissue-specific expression of the GAPDH control probe (see above) and by the fact that, although all tissues were free of disease, the tissues used in the multiple-tissue Northern blot were obtained from different human donors, who varied in sex, age, race, and cause of death.

To further characterize the human AHR cDNA, we used an *in vitro* transcription/translation system to express the human AHR. Photoaffinity labeling experiments demonstrated that the protein encoded by the human AHR cDNA is able to bind ligand in a manner similar to that of the receptor isolated from HeLa cells. That is, ligand binding of the *in vitro* expressed AHR is enhanced by the presence of sodium molybdate, is blocked by an excess of the receptor agonist β-naphthoflavone, and displays mobility upon SDS-PAGE that is identical to that of receptor isolated from whole cells. Gel shift analysis demonstrated that the AHR encoded by the human cDNA binds specifically to a consensus DRE oligonucleotide after activation by TCDD. The DRE-binding properties of this clone are almost identical to those observed for receptor isolated from mouse- and human-derived cell lines and receptor expressed from the murine AHR^{b-1} cDNA. These properties include the requirement for ARNT in DRE binding and efficient competition for DRE binding by excess unlabeled DRE oligonucleotide, but not by a mutated DRE oligonucleotide. In addition, the DRE binding of the *in vitro* expressed human AHR is inhibited by sodium molybdate in a manner similar to that seen for the glucocorticoid receptor (47). This inhibition is indicative of the proposed stabilizing effect of sodium molybdate on the interaction of Hsp90 with these receptors. This stabilization not only enhances the ability of the receptors to bind their ligands (see above) but also prevents the dissociation of Hsp90 and thus prevents the ligand-induced transformation of the receptors to their DNA-binding forms (48). In contrast, sodium molybdate has no effect on the ligand- and DRE-binding properties of the murine AHR^{b-1}, reflecting the differences in the transformation of these two receptors to their DRE-binding forms (22) (Table 1).

The cloning of the human AHR cDNA has provided the first opportunity to make comparisons between two receptor forms at the molecular level. This comparison has demonstrated both conserved and nonconserved regions of the receptor protein. The hypervariable domain identified in the carboxyl terminus of the AHR explains the mechanism underlying the molecular mass variability that exists for this receptor and may yield insights into aspects of AHR biology that may contribute to differences in receptor function within and across species. Finally, the demonstration that the AHR expressed from the human cDNA retains *in vitro* properties identical to those of the receptor isolated from a human-derived cell line provides the initial basis for further investigation into the nature of human AHR function, the risks associated with exposure to AHR agonists, and potential receptor polymorphism within the human population.

Acknowledgments

Thanks to Alan Poland and Ed Glover of the McArdle Laboratory for Cancer Research for the gift of 2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin. Thanks to Oliver Hankinson of the University of California, Los Angeles, for the gift of the human ARNT cDNA plasmid pBM5/NEO-M1-1. Thanks to James P. Whitlock, Jr., of Stanford University for the Hepa 1c1c7 cells.

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