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In vitro analysis of Ah receptor domains involved in ligand-activated DNA recognition

KRISTINE M. DOLWICK, HOLLIE I. SWANSON, AND CHRISTOPHER A. BRADFIELD*

Department of Pharmacology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611

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ABSTRACT **The Ah receptor (AHR) is a basic helix–loop–helix protein that mediates the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In this report, we describe a rabbit reticulocyte system that allows functional expression of both the AHR and its dimeric partner, the AHR nuclear translocator protein (ARNT). By using this *in vitro* system, we were able to reconstitute agonist binding to the AHR and agonist-induced AHR–ARNT recognition of a cognate DNA enhancer sequence. Expression of AHR deletion mutants revealed the location of N-terminal domains responsible for ligand and DNA recognition and C-terminal domains that play roles in agonist-induced DNA recognition.**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) serves as the prototype for a number of highly toxic environmental contaminants (1). Genetic studies in TCDD-sensitive and -resistant murine strains and structure–activity analysis of congener potency indicate that the effects of this compound are mediated through its binding to a soluble protein known as the Ah receptor (AHR) (2–4). Although the exact mechanisms underlying many of the receptor-mediated toxic effects are unclear, it has been demonstrated that ligand-activated AHR interacts with dioxin-responsive enhancers (DREs) lying upstream of target promoters to increase the expression of a number of genes involved in xenobiotic metabolism (5–7).

Recent results indicate that at least two other proteins play a role in receptor signaling. The 90-kDa heat shock protein (Hsp90) appears to associate with the AHR, holding it in a conformation able to bind ligand and also repressing the receptor's intrinsic DNA binding properties (8, 9). A second protein, the AHR nuclear translocator (ARNT), was identified and cloned by virtue of its ability to rescue a Hepa 1c1c7 mutant cell line deficient in transducing the signal of receptor agonists. The ARNT protein was named for its suggested role in the translocation of the AHR from the cytosol to the nucleus. Recent evidence indicates that ARNT is a component of the ligand-induced complex that binds to DREs and suggests that it is the AHR's dimeric partner (10–12).

Analysis of the AHR and ARNT cDNAs demonstrated that they are members of a family of proteins that includes the *Drosophila* Sim and Per proteins (10, 13, 14). The most distinctive characteristic of these four proteins is a homologous region of ≈200 amino acids termed the PAS (Per, ARNT, AHR, Sim) domain (15). Adjacent to this domain in Sim, ARNT, and the AHR is a basic helix–loop–helix (bHLH) motif similar to that found in many heterodimeric transcription factors (16, 17). In this report, we describe the role of these two proteins in agonist-dependent DRE recognition and provide a look at the functional domain map of the AHR.

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MATERIALS AND METHODS

Oligonucleotides. Oligonucleotides used in PCR amplification of cDNAs were derived from murine AHR clones as described (ref. 13; GenBank accession no. M94623) and were as follows: OL55, GCTCTAGATGATCACCATGGTGCAGAAGACCGTGAAGCCCATCCCCGCTGAAGGAATTAAGTC (nt 52–95); OL67, GCACTAGTTGATCACCATGGCCAGCCGCAAGCGGCGCAAGCCGGTGCAGAAGACCGTGAAGCC (nt 28–71); OL68, GCACTAGTTGATCACCATGAGCAGCGGCGCCAACATCACCTATGCCAGCCGCAAGCGGCGCAAGC (nt 1–49); OL57, GCAGAGTCTGGGTTTAGAGC (nt 523–542); OL122, CCCAAGCTTACGCGTGGTTCTCTGGAGGAAGCTGGTCTGG (nt 595–618); OL123, CCCAAGCTTACGCGTGGAAGTCTAGCTTGTGTTTGG (nt 848–867); OL124, CCCAAGCTTACGCGTGGTCTTTGAAGTCAACCTCACC (nt 1684–1704); OL125, CCCAAGCTTACGCGTGAAGCCGGAAAACTGTCATGC (nt 1022–1041); OL163, CCCAAGCTTACGCGTGCAGTGGTCTCTGAGTGGCGATGATGTAATCTGG (nt 1108–1140). The nucleotide numbering for these oligonucleotides is from the ATG initiation codon. Oligonucleotides used in gel-shift assays were as follows: DRE, TCGAGTAGATCACGCAATGGGCCCAGC and TCGAGCTGGGCCCATTGCGTGATCTAC (18); mutant DRE, TCGAGTAGATCAATCAATGGGCCCAGC and TCGAGCTGGGCCCATTGATTGATCTAC (19).

Plasmid Construction. We used the PCR to add the initiation methionine within a synthetic Kozak consensus sequence (30) and the next 24 codons missing from the clone cAH1 (13). This was accomplished with three amplifications using OL55, OL67, and OL68, sequentially, as 5′ primers and OL57 as the 3′ primer. The PCR product was 0.56 kb and contained an *Spe* I site at the 5′ end and the internal *Eco*RI site of this AHR cDNA fragment near the 3′ end. The 0.56-kb product was then subcloned into the *Spe* I and *Eco*RI sites of the pBluescript vector (Stratagene) and sequenced to confirm the fidelity of the PCR. To obtain the entire open reading frame of the cDNA, the downstream 2.6-kb *Eco*RI fragment from a second plasmid containing the fusion of clones cAH1 and cAH3A (pcAHR) was cloned into the *Eco*RI site of the modified cAH1 construct. The resulting full-length murine AHR clone was then subcloned into the *Spe* I and *Hin*dIII sites of the expression vector pSV-Sport1, downstream of the SP6 promoter (pmuAHR) (20). The ARNT expression plasmid was constructed by subcloning the *Bam*HI fragment of pBM5/NEO-M1-1 (10) into PBSK, followed by subcloning the resulting *Xba* I–*Hin*dIII fragment into the corresponding sites of pSV-Sport1, downstream of the SP6 promoter (phuARNT).

Abbreviations: ARNT, Ah receptor nuclear translocator; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AHR, Ah receptor; DRE, dioxin-responsive enhancer; PAS domain, Per, ARNT, AHR, Sim domain; bHLH, basic helix–loop–helix; Hsp90, 90-kDa heat shock protein.

*To whom reprint requests should be addressed.

Construction of AHR Deletion Mutants. The 3′ oligonucleotides used in PCR for the construction of the deletion mutants were OL122 (CΔ599), OL123 (CΔ516), OL124 (CΔ237), OL125 (CΔ458), and OL163 (CΔ425). The 5′ oligonucleotide was OL68 and the template was pmuAHR. The deletion mutants were subcloned into the *Spe* I and *Hin*dIII sites of pSV-Sport1. To minimize PCR-induced mutations, all reactions were carried out using the high-fidelity *Pfu* DNA polymerase (Stratagene). Using this strategy we have found no PCR-induced mutations after sequencing >5.0 kb of amplified clones. The CΔ313 mutant was generated from a *Not* I restriction enzyme fragment of pmuAHR and subcloned into pSV-Sport1. N-terminal deletions were constructed first in the pSG424 vector (21) using the *Eco*RI (NΔ166) and *Kpn* I (NΔ315) fragments from pcAHR and then subcloned into the *Hin*dIII–*Xba* I and *Hin*dIII sites of the pGEM-7Zf vector (Promega), respectively.

***In Vitro* Expression of the AHR and ARNT.** *In vitro* transcription and translation were carried out using the TNT coupled rabbit reticulocyte lysate and wheat germ extract systems (Promega). Briefly, 1 μg of plasmid DNA was added to a 50-μl reaction mixture containing 50% (vol/vol) rabbit reticulocyte lysate or wheat germ extract, reaction buffer, complete amino acid mixture (each amino acid at 20 μM), 40 units of RNasin, and 20 units of SP6 RNA polymerase and incubated at 30°C for 90 min. The efficiency of expression was analyzed in parallel experiments by quantitation of [^{35}S]methionine incorporation present in the corresponding band cut from SDS/polyacrylamide gels. As an additional confirmation of receptor expression, we routinely performed Western blot analysis on all translation reactions (22). By using this protocol, a 50-μl reaction mixture routinely yields ≈10 fmol of AHR, AHR deletion constructs, or ARNT.

Photoaffinity Labeling. Photoaffinity labeling was performed using the ligand 2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin (specific activity = 0.5 μCi/μl; 1 Ci = 37 GBq) and carried out in 50-μl reaction mixtures in MENG buffer (25 mM Mops/1 mM EDTA/0.02% NaN_3/10% glycerol). Samples were incubated with 0.25 μCi of ligand (0.1 pmol) for 30 min at room temperature, cooled on ice, and incubated with 0.2 vol of 3% (wt/vol) charcoal/0.3% gelatin 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°C and the supernatant was irradiated at 310 nm and 0.8 J/cm^2. After irradiation, the reaction was quenched by addition of 300 mM 2-mercaptoethanol. Acetone precipitates were resuspended in 1× Laemmli sample buffer and subjected to SDS/PAGE and autoradiography (23). The specificity of photoaffinity labeling was confirmed by competition experiments with the AHR agonist β-naphthoflavone.

Gel-Shift Assay. A complementary pair of synthetic oligonucleotides containing a consensus DRE was annealed and end-labeled with [γ-^{32}P]ATP as described (24). Nonspecific competitor, poly(dI-dC), was added to the cytosolic or *in vitro* AHR preparations and incubated 15 min at room temperature. The radiolabeled probe (1 × 10^6 cpm; 0.5 ng) was then added and incubated 15 min at room temperature followed by nondenaturing gel electrophoresis and autoradiography (25). Gel-shift analysis of the AHR deletion mutants was quantitated on a Fuji bas 1000 phosphor imaging system or by densitometric scanning. The intensity of the AHR–ARNT–DRE complexes was expressed relative to the DRE binding of the full-length AHR in the presence of TCDD and all values were normalized to the level of expression of the full-length AHR construct.

RESULTS AND DISCUSSION

***In Vitro* Expression of the AHR and ARNT.** To determine whether *in vitro* models could be developed that faithfully reproduced *in vivo* signaling events, we attempted to recover both AHR and ARNT function from cDNAs that were expressed in a rabbit reticulocyte lysate. In preliminary studies, we were able to recover both ligand and DRE binding activities using AHR and ARNT that were either coexpressed in the same tube or expressed independently and then mixed. Since independent translation allowed greater control over the relative amounts of the two proteins, we chose to use mixing protocols in all experiments that required both proteins. Interestingly, our preliminary experiments indicated that neither ligand nor DRE binding could be obtained when the AHR and/or ARNT were translated from a wheat germ extract system (data not shown). Although numerous differences exist in these two expression systems, reticulocyte lysates contain significant amounts of Hsp90 and wheat germ extracts are deficient in this protein (26, 27). This observation is consistent with, but does not prove, a role for Hsp90 in receptor folding and function and may suggest that it plays a similar role for the structurally related ARNT protein.

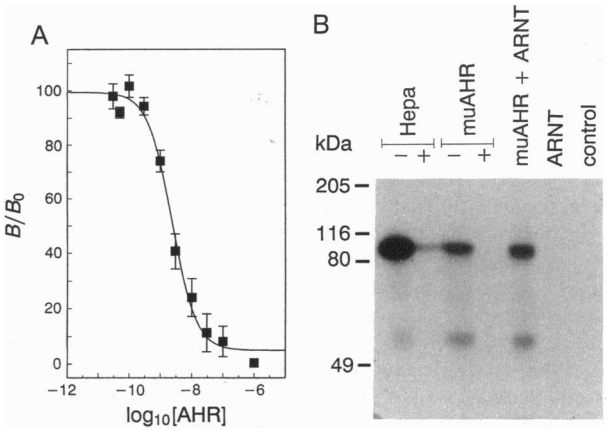

FIG. 1. Ligand binding of the murine AHR. (*A*) Competitive binding curve of the *in vitro*-expressed AHR. Photoaffinity labeling was carried out with increasing concentrations of β-naphthoflavone (in 0.5 μl of dimethyl sulfoxide) added immediately prior to addition of the photoaffinity ligand. The results were obtained by determining the radioactivity in the 95-kDa band and were quantitated on a Fuji bas 1000 phosphor imaging system. The curve was generated using the LIGAND program (28) and is the average of three experiments. (*B*) Photoaffinity labeling of the expressed AHR. Hepa cytosol (10 μg) was photoaffinity labeled. The pmuAHR and phuARNT plasmids were expressed by *in vitro* transcription/translation and 0.2 vol of the *in vitro* reaction mixture was used in the corresponding photoaffinity-labeling reactions. pSV-Sport1 was used as a labeling control. Labeling reactions were carried out in the absence (−) or presence (+) of 100 nM β-naphthoflavone to demonstrate the specificity of labeling of the 95-kDa band.

To characterize this reticulocyte lysate expression system, we photoaffinity labeled the translation product of the murine AHR cDNA with 2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin (23). Competitive binding experiments using the receptor agonist β-naphthoflavone demonstrated that the ligand binding properties of the *in vitro*-translated receptor were similar to the binding properties of the receptor isolated from Hepa1c1c7 cells (Fig. 1*A*). The dose–response curves for β-naphthoflavone using the *in vitro*-expressed AHR were highly reproducible in three experiments yielding IC_{50} values of 2.2 ± 0.4 nM and slopes of 0.94 ± 0.13 (mean ± SD). Analysis of receptor from Hepa1c1c7 cytosol yielded an IC_{50} value of 4.3 nM and a slope of 0.96.† The glucocorticoid

†Previous data from this laboratory (29) have indicated that background protein concentration is an important determinant of the concentration of ligand, which is actually free in solution. Therefore, we suspect that although this slight difference in IC_{50} values could be due to subtle differences in receptor function, differences in free ligand solubility in these two receptor preparations is probably a more important factor.

dexamethasone was unable to compete for AHR binding in this system (data not shown). Photoaffinity labeling experiments also demonstrated that the receptor generated *in vitro* migrated with a molecular mass identical to that observed for the receptor produced *in vivo* (i.e., 95 kDa) (Fig. 1*B*). Despite its structural similarity to the AHR, ARNT does not bind the photoaffinity ligand nor is its presence required for the receptor to bind ligand (Fig. 1*B*).

Experiments were then performed to demonstrate that this *in vitro* system could reproduce agonist-induced AHR–ARNT interactions and their specific binding to target DRE sequences. To examine these properties, we employed gel-shift assays using synthetic oligonucleotides corresponding to a well-characterized DRE and a nontarget sequence that contains mutations in the core recognition sequence (18, 19). These experiments provided further support for the idea that both the AHR and ARNT are required for DNA binding, since neither protein was able to bind to the DRE alone (11) (Fig. 2*B*). To more directly prove that both proteins were part of the DRE binding complex, we demonstrated that antibodies directed against each of the translated proteins could supershift the DRE binding complex in gel-shift assays (Fig. 2*C*). More importantly, DRE binding of the *in vitro*-expressed full-length proteins was induced by the presence of agonist, demonstrating that the ligand-induced activation of the AHR could be reproduced in this system (Fig. 2*B*). The specificity of DRE binding was demonstrated by competition experiments. An excess of unlabeled DRE oligonucleotide could efficiently compete for AHR–ARNT binding, whereas an oligonucleotide containing a mutated DRE was relatively inefficient at competing.

Deletion Analysis and Domain Map of the AHR. Once we had characterized the *in vitro* expression system, we focused our attention on mapping functional domains of the AHR. To this end, a series of deletion mutants were constructed. Autoradiography and Western blot analysis demonstrated

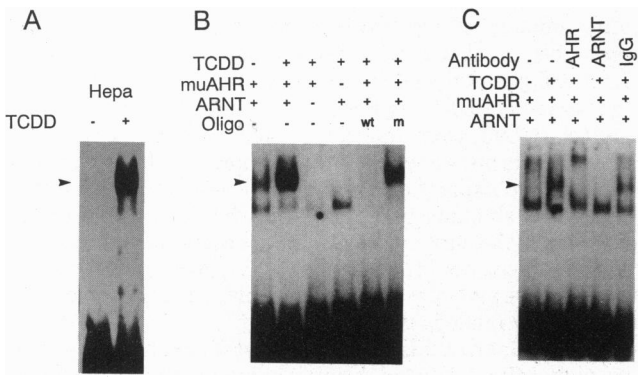

FIG. 2. Gel-shift assays demonstrating binding of AHR–ARNT heterodimers to a DRE. (*A*) Cytosolic extracts (35 μg of protein) obtained from Hepa1c1c7 cells were incubated in the presence of dimethyl sulfoxide (−) or 20 nM TCDD (+) for 2 hr at 30°C followed by gel-shift analysis (25). (*B*) Murine AHR (muAHR, 7 μl) and human ARNT (5 μl) *in vitro*-translated proteins (an ≈1:1 ratio of expressed proteins) were incubated with either dimethyl sulfoxide (−) or 20 nM TCDD (+) for 2 hr at 30°C followed by gel-shift assays. The arrowhead indicates the location of the AHR–ARNT–DRE complex. Addition of excess competitor wild-type DRE (wt) or mutant DRE (m), containing two nucleotide substitutions in the core region (19), demonstrates specificity of complex formation. (*C*) Supershift analysis of DRE binding complex was carried out as described above using *in vitro*-translated AHR and ARNT proteins incubated with 20 nM TCDD. Reaction mixtures were incubated for 15 min at room temperature with 1 μg of affinity-purified AHR-specific or ARNT-specific antibodies prior to nondenaturing gel electrophoresis (ref. 22 and R. Pollenz and A. Poland, personal communication). Control reaction mixtures were incubated with 1 μg of purified IgG (preimmune serum).

that all mutants were efficiently expressed and that their observed molecular masses were in agreement with those calculated from the primary amino acid sequence (Fig. 3 *Left*). In an effort to make comparisons between the deletion mutants, all photoaffinity-labeling and gel-shift results were normalized to the relative expression of the full-length AHR as determined by [^{35}S]methionine incorporation. In addition, the corresponding deletion mutants were also constructed for the human AHR and yielded identical results (ref. 31 and data not shown).

In AHR, the covalently bound photoaffinity ligand has been found to be between residues 232 and 334 (13). As a result, we suggested that this region might correspond to the ligand binding site of the AHR. We considered this assignment tentative since a lack of reactive sites within the ligand binding pocket and/or secondary structure may have led to a preference for the labeling of amino acid residues distant from those residues actually involved in the formation of a ligand binding pocket. Therefore, we investigated the location of the ligand binding domain by characterizing the ability of our deletion constructs to bind the photoaffinity ligand (Fig. 3 *Right*). Our experiments revealed that C-terminal deletions of up to 313 aa (CΔ313) did not affect ligand binding function. However, the CΔ425 mutant displayed ligand binding activity that was ≈3% of the full-length protein. Since this minimal ligand binding activity was highly reproducible and since the truncation of an additional 33 aa (CΔ458) resulted in undetectable ligand binding activity, we use CΔ425 to define the approximate C-terminal boundary of the ligand binding domain. To define the N-terminal boundary of this domain, N-terminal deletion mutants/chimeras containing the DNA binding domain of the Gal4 protein proved useful. A fusion protein missing 166 aa from the N terminus of the receptor (NΔ166) retained the capacity to bind ligand, whereas the deletion of 315 aa from the N terminus (NΔ315) abolished ligand binding; thus, NΔ166 defines the approximate N-terminal boundary of the ligand binding domain. Importantly, the ligand binding domain, defined functionally by mutants NΔ166 and CΔ425, describes essentially the same region of the receptor as that determined (13) by photoaffinity labeling, CNBr cleavage, and amino acid sequencing (Fig. 4).

Once the ligand binding domain was identified, we focused our attention on the characterization of receptor domains required for agonist-induced DRE binding by the AHR–ARNT complex. Our deletion analysis suggests that multiple independent domains play a role in this process. The observation that the Gal4–AHR chimera that was missing the bHLH domain (NΔ166) did not bind to the DRE was consistent with the well-described role of bHLH domains in heterodimer formation and in positioning the adjacent basic regions for proper DNA sequence recognition (32). In support of this functional assignment is the observation that C-terminal deletions of up to 516 aa (CΔ516) still had DRE binding activity in the presence of ARNT (Fig. 3 *Right*). The observation that the deletion mutant CΔ516 appeared to define the C-terminal boundary of a domain required for DRE binding suggests that residues in the PAS domain as far as 245 aa from the N terminus may play a role in AHR–ARNT–DRE complex formation (Fig. 4). We have previously proposed (13) that the PAS domain may serve as a secondary dimerization motif, similar to the leucine zippers in Myc and Max (33, 34). This idea has gained support from a recent report (35) demonstrating that the PAS domain is sufficient for the formation of Per–Per homodimers and Per–Sim heterodimers. An alternate and equally tenable explanation for the lack of DRE binding activity by the CΔ599 mutant may be that this protein is improperly folded and is, therefore, unable to obtain the conformation necessary for dimerization with ARNT and thus cannot bind to the DRE. In this regard, we may have deleted PAS sequences required for interaction

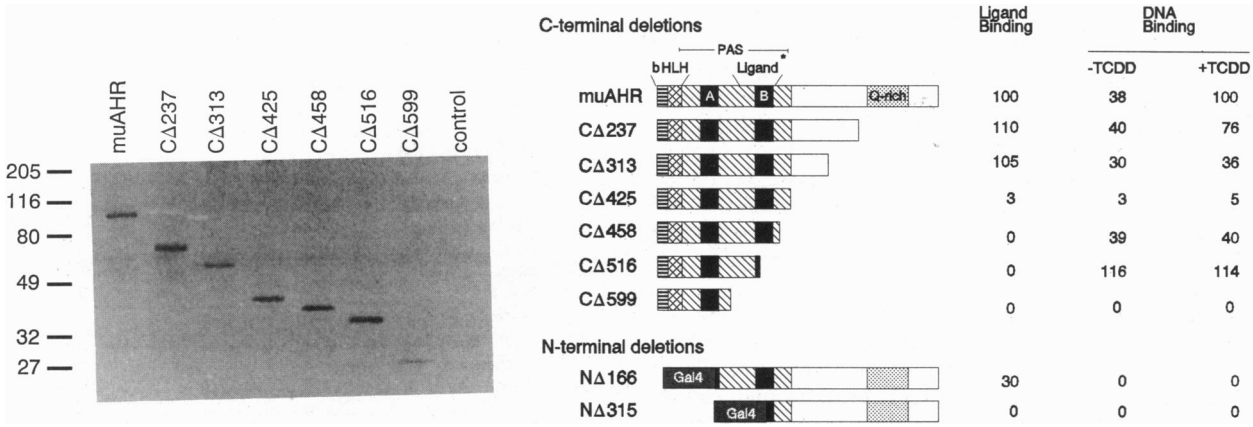

FIG. 3. Deletion analysis of the murine AHR. (*Left*) Western blot analysis of AHR C-terminal deletions. *In vitro* transcription/translation reaction products (5 μl) were subjected to SDS/PAGE, transferred to nitrocellulose, and incubated with an affinity-purified antibody raised against an N-terminal peptide derived from the murine AHR (muAHR) (22). Control lane represents 5 μl of the reticulocyte lysate incubated with pSV-Sport1. (*Center*) Schematic diagram of deletions. Hatched box represents the PAS domain. Solid boxes within the PAS domain indicate the position of the A/B repeats. The position of the helix–loop–helix (HLH) domain is indicated by a cross-hatched box; the basic (b) region is indicated by horizontal bars; the glutamine-rich (Q-rich) region is indicated by a stippled box. Ligand* indicates the position of the ligand binding domain as determined by photoaffinity labeling (13). Deletion nomenclature indicates the number of amino acids truncated from the C terminus (CΔ) or N terminus (NΔ). (*Right*) Ligand binding and DNA binding of AHR deletion mutants. Deletions were expressed by *in vitro* transcription/translation. Photoaffinity labeling was carried out as described in Fig. 1. DRE binding was analyzed by gel-shift assays in the absence (−) or presence (+) of 20 nM TCDD. Results were quantitated on a Fuji bas 1000 phosphor imaging system or by densitometric scanning. The amount of receptor protein was determined by ^{35}S labeling and all results were normalized to receptor quantity and expressed as a percentage relative to the ligand binding or DRE binding (+ TCDD) of the full-length AHR. Expression ratio is fmol of mutant/fmol of AHR. Ligand binding is the amount of photoaffinity labeling/expression ratio, which equals the normalized labeling/photoaffinity labeling of AHR or the percent of ligand binding. DNA binding is the amount of specific DRE binding/expression ratio, which is the normalized binding/DRE binding of AHR (+ TCDD) or the percent of DRE binding. All experiments were carried out at least two times. With triplicate samples, standard deviations were <20%. DRE binding of all deletion mutants required ARNT and was sequence-specific as determined by competition with the DRE and the mutant DRE as described in Fig. 2.

with accessory protein(s) required for proper receptor folding and function, such as Hsp90. Finally, it is important to note that ARNT dependency and DRE binding specificity (as measured by DRE competition) were maintained in all active deletion constructs, strongly supporting the integrity of these AHR mutants.

In addition to the bHLH and PAS domains, our deletion analysis indicated that domains within the C terminus of the AHR can have an impact on the agonist-dependent formation of AHR–ARNT–DRE complexes. Our results suggest that amino acid sequences located within the C-terminal 313 aa of the AHR play a role in the efficiency of agonist-induced transformation of the AHR to a species capable of forming AHR–ARNT–DRE complexes. This domain is defined by the observation that the CΔ237 and CΔ313 mutants displayed

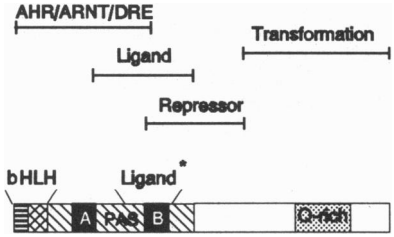

FIG. 4. Domain map of the AHR (see *Discussion*). DRE/AHR/ARNT (DRE recognition complex) corresponds to the region defined by the observation that NΔ166 and CΔ599 did not bind the DRE in gel-shift assays (aa 1–289). Ligand corresponds to the region defined by the observation that NΔ166 and CΔ458 were not photoaffinity labeled (aa 166–380). Repressor corresponds to the region defined by the observation that CΔ425, CΔ458, and CΔ516 provide constitutively active DRE binding forms of the AHR–ARNT complex (aa 289–492). Transformation corresponds to the region defined by the observation that CΔ313 and CΔ237 retain full ligand binding activity but are no longer as efficiently activated to a DRE binding form (aa 492–805).

decreasing ligand-induced DRE binding when compared to the full-length receptor (Fig. 3 *Right*). This can be seen as both a decrease in ligand activation to a DRE binding form and a decrease in total DRE binding of the mutants. Although we cannot entirely rule out the possibility that inappropriate protein folding by these mutants has corrupted the conformation of domains involved in ARNT and DRE interactions, we consider this a less likely possibility since the function of the ligand binding domain has been unaffected in these mutants and even larger C-terminal deletions maintain high levels of AHR–ARNT–DRE complex formation (see CΔ516).

A second C-terminal domain that has an impact on agonist-induced AHR–ARNT–DRE complex formation is defined by the CΔ425, CΔ458, and CΔ516 mutants. These mutants exhibited increasing DRE binding activity that did not require the presence of ligand. Interestingly, deletion of the 516 C-terminal amino acids led to a slightly greater level of DRE binding activity compared to agonist stimulation of the full-length receptor. This suggests that the region defined by the CΔ516 and CΔ313 mutants contains a domain with a role in attenuating or repressing the intrinsic dimerization or DRE binding properties of the receptor. We suggest that receptor transformation may be mediated by an agonist-induced "derepression" of this domain. Given the proximity of this domain to the ligand binding domain and a domain potentially involved in ARNT heterodimer formation (PAS), it is tempting to speculate that this repressor domain represents a site where, in the absence of agonist, an inhibitory protein binds or is maintained in a conformation that prevents AHR–ARNT heterodimer formation. In response to binding ligand, the proximity of these domains might then allow subtle conformational changes to be transduced over a short distance to derepress this domain and allow ARNT dimerization and DRE binding. Since association of the AHR with Hsp90 has been demonstrated to repress DRE binding activity (9), it is tempting to speculate that this region is required for Hsp90–

receptor association. Alternatively agonist binding could simply induce a conformational change that switches the receptor from a latent to a dimerizing species in a manner that is independent of any associated proteins. The presence of this domain in the AHR is similar to what is seen for the glucocorticoid receptor where activities such as nuclear localization and DNA binding require derepression of the receptor via hormone binding. In a manner similar to the results demonstrated here for the AHR, the glucocorticoid receptor is also constitutively transformed by large C-terminal deletions (36).

Conclusion. These studies have led us to the following conclusions. (*i*) *In vitro* translation of the AHR cDNA provides an expression system that can reproduce ligand binding, interaction with the ARNT protein, and ligand-induced DRE binding, three important steps in receptor signaling. (*ii*) Deletion analysis of the AHR has allowed the localization of previously undescribed domains involved in receptor transformation to a DRE binding form and repression of DRE binding activity. (*iii*) Deletion analysis also indicated that the PAS region appears to contain a number of important functions, including domains required for ligand binding and possibly AHR–ARNT–DRE complex formation.

Notes. (*i*) While this manuscript was in review, Whitelaw *et al.* (37) published results also demonstrating that ARNT is required for AHR–DRE binding activity and that *in vitro*-translated ARNT is functional.
(*ii*) The full-length murine AHR expression construct, pmuAHR, used in this work differs in the context of the initiation methionine and should be distinguished from other full-length murine AHR constructs (pSportAHR and pcDNAAHR) previously distributed from this laboratory (CACCATGA vs. GCTTATGA).

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