

The Transactivation Domain of the Ah Receptor Is a Key Determinant of Cellular Localization and Ligand-Independent Nucleocytoplasmic Shuttling Properties[†]

Preeti Ramadoss^{‡,§} and Gary H. Perdew^{*,‡}

Center for Molecular Toxicology and Carcinogenesis, Department of Veterinary Science and Graduate Program in Biochemistry, Microbiology and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

Received May 23, 2005; Revised Manuscript Received June 29, 2005

ABSTRACT: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that regulates transcription of a number of target genes upon binding ligands such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Large intra- and interspecies variations exist with respect to sensitivity to TCDD, and this could, at least in part, be due to a considerable variation in the AhR amino acid sequence between species. The N-terminal half of the AhR is well-conserved across species, whereas the C-terminal half exhibits a considerable degree of degeneracy. It has previously been shown that there are differences between the mouse (mAhR) and human AhR (hAhR) in terms of cellular localization, nucleocytoplasmic shuttling, the effect of chaperone proteins on these properties, and differences in relative ligand affinity. In this study, two chimeras were generated such that each had the N-terminal half of one receptor and the C-terminal half of the other receptor. The C-terminal half of the receptor, containing the transactivation domain, determines the cellular localization of the transiently transfected receptor and regulates the ability of hepatitis B virus X-associated protein 2 (XAP2) to inhibit ligand-independent nuclear import of AhR. In addition, the transactivation domain (TAD) appears to determine the presence of XAP2 in the nuclear ligand-bound AhR/hsp90 complex prior to association with the AhR nuclear translocator protein (ARNT). However, the transactivation domain does not appear to play a role in determining relative ligand affinity of the receptor, and mAHR and hAhR have similar overall transactivation potential in a cell-based reporter system at a saturating dose of ligand. This study demonstrates for the first time that the transactivation domain of the AhR influences important biochemical properties of the N-terminal half of the AhR, and the degeneracy in the transactivation domain between the mAHR and the hAhR results in species-specific differences in receptor properties.

The aryl hydrocarbon receptor (AhR)¹ is a ligand-dependent transcription factor that regulates the transcription of various target genes upon binding to a number of toxic compounds. Many of these compounds are present in the environment and historically were products of industrial processes such as chemical bleaching and pesticide manufacture and resulted from the incineration of municipal and commercial waste. The prototypical AhR ligand is 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) and is the most widely studied AhR ligand. Exposure to TCDD results in a number of adverse health effects including chloracne, blood and

immune disorders, and reproductive and toxicological effects (1).

The AhR is a member of the bHLH-PAS (basic-helix-loop-helix, Per-Arnt-Sim) family of proteins, and it normally exists in the cytoplasm in a complex with the heat shock protein hsp90, the immunophilin homologue hepatitis B virus X-associated protein 2 (XAP2) (also known as AIP and ARA9), and the co-chaperone p23 (as reviewed in ref 2). The AhR shuttles between the nucleus and the cytoplasm in a ligand-independent manner (3–6), but XAP2 can influence this shuttling in the case of the mouse AhR (mAHR) (5, 7, 8). The significance of this shuttling is not yet known, but it is possible that the AhR may serve some function in the nucleus in the absence of ligand through interactions with other proteins. Upon binding ligand, the receptor translocates to the nucleus, dissociates from hsp90 and XAP2, and forms a heterodimer with the Ah receptor nuclear translocator protein (ARNT), which then binds to DNA at dioxin responsive elements (DREs) to regulate the transcription of target genes (9). Some of these target genes include Phase I drug metabolizing enzymes, such as CYP1 family members, and Phase II enzymes such as UGT1A1, GST-Ya subunit, and NADPH-quinone-oxido-reductase (as reviewed in ref 10). The AhR also regulates transcription of other genes that are not metabolizing enzymes such as p27^{Kip1} (11), *N*-

[†] This work was supported by NIH Grants ES04869 and ES011834.

^{*} Corresponding author: Gary H. Perdew, 309 Life Sciences Building, The Pennsylvania State University, University Park, PA 16802. Phone: 814-865-0400. Fax: 814-863-1696. E-mail: ghp2@psu.edu.

[‡] Center for Molecular Toxicology and Carcinogenesis.

[§] Graduate Program in Biochemistry.

¹ Abbreviations: AhR, aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; XAP2, hepatitis B virus X-associated protein 2; hsp90, heat shock protein 90; DRE, dioxin response element; NLS, nuclear localization signal; NES, nuclear export signal; TAD, transactivation domain; mAHR, mouse AhR; hAhR, human AhR; MH, AhR chimera with mouse N-terminal half and human C-terminal half; HM, AhR chimera with human N-terminal half and mouse C-terminal half; LMB, leptomycin B; MENG; 25 mM MOPS/2 mM EDTA/0.02% NaN₃/10% glycerol, TSDS-PAGE: tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis.

myristoyltransferase 2 (12), and Bax (13). It is likely that a large number of AhR target genes remain to be determined, and the identification of these target genes will enable a better understanding of how the AhR modulates a response upon binding its ligands.

The amino (N) terminal half of the AhR contains the bHLH-PAS domain, which includes the hsp90-, XAP2-, and the ligand-binding domains (14, 15). This portion also includes the nuclear localization signal (NLS) and appears to have two nuclear export signals (NES) (3, 16). The carboxy (C) terminal half of the AhR contains a transactivation domain (TAD), which has three subdomains, the acidic, glutamine-rich, and proline-serine-threonine rich domains (17). The AhR shows size variation between different vertebrate species, between 95 and 125 kDa (18), as well as variations in amino acid sequence (19–21). A wide range of sensitivity to TCDD and/or polycyclic aromatic hydrocarbon-induced toxicity also exists among different vertebrate species. For instance, the guinea pig is very sensitive to TCDD (LD_{50} 1 μ g/kg) (22, 23) whereas the hamster shows a 1000-fold greater resistance (24). Although there does not appear to be a correlation between CYP1A1 induction and acute lethality in these species (25), it is still possible that other differences at the molecular level could contribute to the observed variation in sensitivity to TCDD. There are intraspecies variations in sensitivity as well; for instance, C57BL6 mice (Ah^b allele) are sensitive to TCDD, whereas DBA2 mice (Ah^d allele) are about 14-fold more resistant to the same compound (26). While the qualitative response to treatment is similar, in that the same battery of genes is induced, the dose required to activate the AhR^d is 10-fold higher than that for AhR^b (as reviewed in ref 27). DBA2 mice have a critical point mutation in the ligand binding domain of the AhR, which leads to a lower ligand affinity (28). Han-Wistar rats show a 1000-fold greater resistance to TCDD than Long-Evans rats (29), and while the Han-Wistar rats show the same effects of TCDD treatment as Long-Evans rats in terms of enzyme induction, they remain resistant to the acute toxic effects and lethality of TCDD treatment. There are differences between the AhRs in these vertebrates, and may be the cause of differences in sensitivity to TCDD, although this has not yet been shown conclusively. Hamsters have an extended transactivation domain, with an increase in the number of glutamine residues as compared to the AhR from other species, but it has not yet been established whether this is the cause for increased tolerance to TCDD (20). The increase in glutamine residues in the hamster AhR may be important, considering that the glutamine-rich region of the AhR has been shown to be critical for transactivation in the case of the human receptor (30). Interestingly, the Han-Wistar rat also has an altered carboxy-terminal domain due to an insertion/deletion in the AhR that results in a loss of either 38 or 43 amino acids in the carboxy-terminal end, which leads to the production of two types of AhR protein, both of which have a shortened TAD (31). The guinea pig AhR has also been shown to share high homology with the human AhR (hAhR) (21). In general, the N-terminal half of the AhR is well conserved across species, while the C-terminal half, which contains the transactivation domain, is poorly conserved.

The majority of toxicity studies are done with mice as models, and the data are used to estimate risk to humans

upon being exposed to similar compounds. There are significant differences in the amino acid sequences of the mAhR and hAhR, and most of the differences are in the TAD. There is 86% amino acid identity in the N-terminal halves and only 58% identity in the C-terminal halves between mAhR and hAhR. Given that there are several interspecies differences in terms of response to ligand, it is possible that functionally significant differences between mAhR and hAhR may result in differing toxic endpoints. This could limit the extent to which data from mouse models can be extrapolated to estimate risk to humans.

It has previously been demonstrated that mAhR and hAhR show some divergence in biochemical properties, such as nucleocytoplasmic shuttling, modulation of receptor activity by XAP2, and differences in ligand affinity (8, 32–34). To address these differences between mAhR and hAhR and to determine whether the low homology in the C-terminal domain is the cause of some of this variation, we generated two chimeric receptors. Each chimera has the N-terminal half of one receptor (mAhR or hAhR) and the C-terminal half of the other receptor. The N-terminal half of the AhR contains the NLS, NES, XAP2-, hsp90-, and ligand-binding sites. This study found that the TAD influences some properties of the N-terminal portion of the receptor, such as cellular localization and nucleocytoplasmic shuttling. We also found that the TAD appears to regulate the ability of XAP2 to sequester the receptor in the cytoplasm in the absence of ligand, and the TAD may determine whether XAP2 is present in the ligand-bound AhR complex that translocates into the nucleus. However, the TAD does not influence the relative ligand affinity of the AhR or the overall transactivation potential of mAhR and hAhR in a cell-based reporter system. This is the first report examining the ability of the TAD to influence overall AhR function.

MATERIALS AND METHODS

Cell Culture and Transfections. COS-1 and BP8 cells were routinely maintained in α -minimum essential medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) at 37 °C in 95% air, 5% CO₂. Transfections in COS-1 cells were performed using LipofectAMINE-PLUS (Invitrogen, Carlsbad, CA), while transfections in BP8 cells were done using GenePorter Transfection reagent (Gene Therapy Systems, San Diego, CA) according to the manufacturer's protocol.

Constructs. The pcDNA3/mAhR/FLAG, pCI/hAhR/FLAG, pCI/XAP2, pEFV5/hAhR/FLAG, pEYFP/mAhR, pEYFP/hAhR, and pCI/XAP2-NLS constructs were generated previously (5, 8, 30, 35). The pcDNA3/MH construct was generated by first subcloning hAhR/FLAG from pEFV5/hAhR/FLAG into pcDNA3, and then digesting both pcDNA3/hAhR/FLAG and pcDNA3/mAhR/FLAG with Bam HI and Apa I, and then ligating the insert generated from the pcDNA3/hAhR/FLAG digest into the pcDNA3/mAhR/FLAG (Apa I/Bam HI) backbone. The pEFV5/HM/FLAG construct was generated by digesting both pEFV5/hAhR/FLAG and pcDNA3/mAhR/FLAG with Xba I and Bam HI and ligating the fragment from the pcDNA3/mAhR/FLAG into the pEFV5/hAhR (Bam HI/Xba I) backbone (See Figure 1). pEYFP/HM and pEYFP/MH were generated by digesting pEYFP/hAhR and pEYFP/mAhR with Bam HI and then

swapping and ligating the inserts. pEYFP/hAhR CΔ45 was generated using the QuickChange Site Directed Mutagenesis kit (Stratagene, La Jolla, CA) according to a previously described method (36). All chimeric constructs were completely sequenced to ensure that they had been generated correctly.

Immunoprecipitation Experiments. COS-1 cells in 100-mm dishes were transfected with 6 μ g of pcDNA3/mAhR/FLAG, pCI/hAhR/FLAG, pEFV5/HM/FLAG or pcDNA3/MH/FLAG and 3 μ g of pCI-XAP2 using LipofectAMINE-PLUS. Approximately 20 h after transfections, cells were lysed in lysis buffer (MENG plus 20 mM NaMoO₄, 1% NP40, protease inhibitor cocktail (Sigma)). The lysates were centrifuged to remove debris, and supernatants were used to perform immunoprecipitations using Anti-FLAG M2 agarose (Sigma) in binding buffer (MENG plus 20 mM NaMoO₄, 2 mg/mL of bovine serum albumin and 50 mM NaCl). Immunoprecipitated complexes were washed in wash buffer (MENG, 20 mM NaMoO₄, and 50 mM NaCl) three times, and complexes were resolved by tricine SDS-PAGE (8% acrylamide). For anti-GFP immunoprecipitations, COS-1 cells were transfected with 2 μ g of each receptor construct and 2 μ g of pCI-XAP2-NLS and an anti-GFP antibody (BD Biosciences, Palo Alto, CA) coupled to goat-anti Rabbit agarose (Sigma) was used.

Fluorescence Microscopy. COS-1 cells in six-well plates or 35-mm microscope dishes (MatTek Corporation, Ashland, MA) were transfected with 0.75 μ g of each receptor construct, with or without cotransfected pCI/XAP2 or pCI/XAP2-NLS. Approximately 20 h post-transfection, cells were visualized using SPOT RT Color model 2.2.0 Cooled CCD camera fitted to a Nikon Eclipse TE300 inverted microscope with a Nikon Plan Fluor 60 \times objective or a Nikon Plan Apo 60 \times oil immersion lens. Leptomycin B and TCDD were used at a concentration of 10 nM for 1 h to block nuclear export or induce nuclear translocation, respectively.

Western Blotting. Primary antibodies against the AhR, XAP2, and hsp90 were RPT1 mAb (37), anti-ARA9 (Novus Biologicals, Littleton, CO), and anti-hsp84 and anti-hsp86 (Affinity Bioreagents, Golden, CO), respectively. Goat anti-mouse and Donkey anti-rabbit horseradish peroxidase conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, Westgrove, PA) and a chemiluminescent substrate (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Rockford, IL) were used to detect the primary antibodies.

Ligand Binding Assays. 2-Azido-3-[¹²⁵I]iodo-dibromodibenzo-*p*-dioxin ([¹²⁵I] N₃Br₂DpD) was synthesized, and ligand binding assays were performed as described previously (34, 38). The specific activity of the ligand was 2176 Ci/mmol, and the sample was stored in methanol. Briefly, COS-1 cells were transfected with each receptor construct, and cytosol was prepared by dounce homogenization in MENG buffer + 20 mM NaMoO₄ and diluted to a final concentration of 0.5 mg/mL. Cytosol (150 μ L at 0.5 mg/mL) was incubated with 0.104 pmol of radioligand for 30 min at room temperature. A 200-fold molar excess of nonradioactive 2,3,7,8-tetrachlorodibenzofuran (TCDBF) was then added to the tubes and incubated for the specified time. The samples were placed on ice for 5 min, charcoal-dextran was added to the samples to a final concentration of 1%/0.1%, and the samples were incubated on ice for 20 min.

The cytosolic extracts were then centrifuged at 3000g for 10 min at 4 °C. The samples were photolyzed at > 302 nm, at a distance of 8 cm, for 4 min using two 15-watt UV lamps (Dazor Mfg. Corp. St. Louis, MO). The supernatant was transferred into a microfuge tube containing SDS-sample buffer, heated for 5 min at 95 °C followed by TSDS-PAGE (8% acrylamide). Gels were dried and exposed to film, and bands were subsequently excised and counted in a gamma counter (LKB Wallac, Gaithersburg, MD).

Luciferase Reporter Assay. COS-1 and BP8 cells in 12-well plates were transfected using LipofectAmine-PLUS or Gene Porter transfection reagents according to the manufacturers protocol. pcDNA3/mAhR/FLAG, pCI/hAhR/FLAG, pEFV5/HM/FLAG or pcDNA3/MH/FLAG (7.5 or 20 ng), pGUDLUC6.1 (20 or 100 ng) and pCMV- β gal (20 or 100 ng) were transfected into COS-1 and BP8 cells, respectively, and 20 h post-transfection, cells were treated with 10 nM TCDD, an equal volume of DMSO, or no treatment for 8 h. Media was removed after 8 h, and cells were washed twice with PBS and lysed. Luciferase activity was measured using the Promega Luciferase Assay kit (Promega, Madison, WI) and a Turner TD-20e luminometer and normalized for β -gal activity.

RESULTS

Chimeric Receptors Form a hsp90/XAP2 Tetrameric Complex. To test the influence of the TAD on functional properties localized in the N-terminal half of the AhR, chimeric constructs were made. Chimera cDNA constructs were made as described in the methods section and are referred to as HM (human N-terminus, mouse C-terminus) and MH (mouse N-terminus and human C-terminus) (Figure 1A,B). A Bam HI site (amino acid 418 in mAhR and amino acid 424 in hAhR) was used to swap the TADs of mAhR and hAhR. This Bam HI site was used to generate these constructs because this restriction enzyme site in the AhR is unique and conserved in mAhR and hAhR. In addition, it is present at an appropriate location that divides the AhR into the C-terminal half with the TAD and the N-terminal half that contains the NLS, NES, hsp90-, XAP2- and ligand-binding domains. The amino acids at this junction site are conserved between mAhR and hAhR and thus should preclude the introduction of folding problems at this junction site. FLAG-tagged wild type and chimeric receptors were coexpressed in COS-1 cells with XAP2 and cytosol was prepared. Immunoprecipitations were carried out using anti-FLAG M2 resin and complexes were separated by TSDS-PAGE. Proteins were transferred to PVDF membrane and visualized by Western blot analysis. All four receptors are capable of binding to XAP2 and hsp90; therefore, switching the TADs of the two receptors did not affect the ability of the receptor to form a heterotetrameric complex (Figure 1C).

The Transactivation Domain of the AhR Influences the Cellular Localization and Nucleocytoplasmic Shuttling of the Receptor. COS-1 cells in six-well plates were transfected with pEYFP-mAhR, pEYFP-hAhR, pEYFP-HM, or pEYFP-MH, with or without pCI-XAP2 and observed using a fluorescence microscope after 24 h. Subsequently, cells were treated with 10 nM leptomycin B or 10 nM TCDD for 1 h and observed. As reported previously, mAhR-YFP is predominantly nuclear, whereas hAhR-YFP is cytoplasmic in

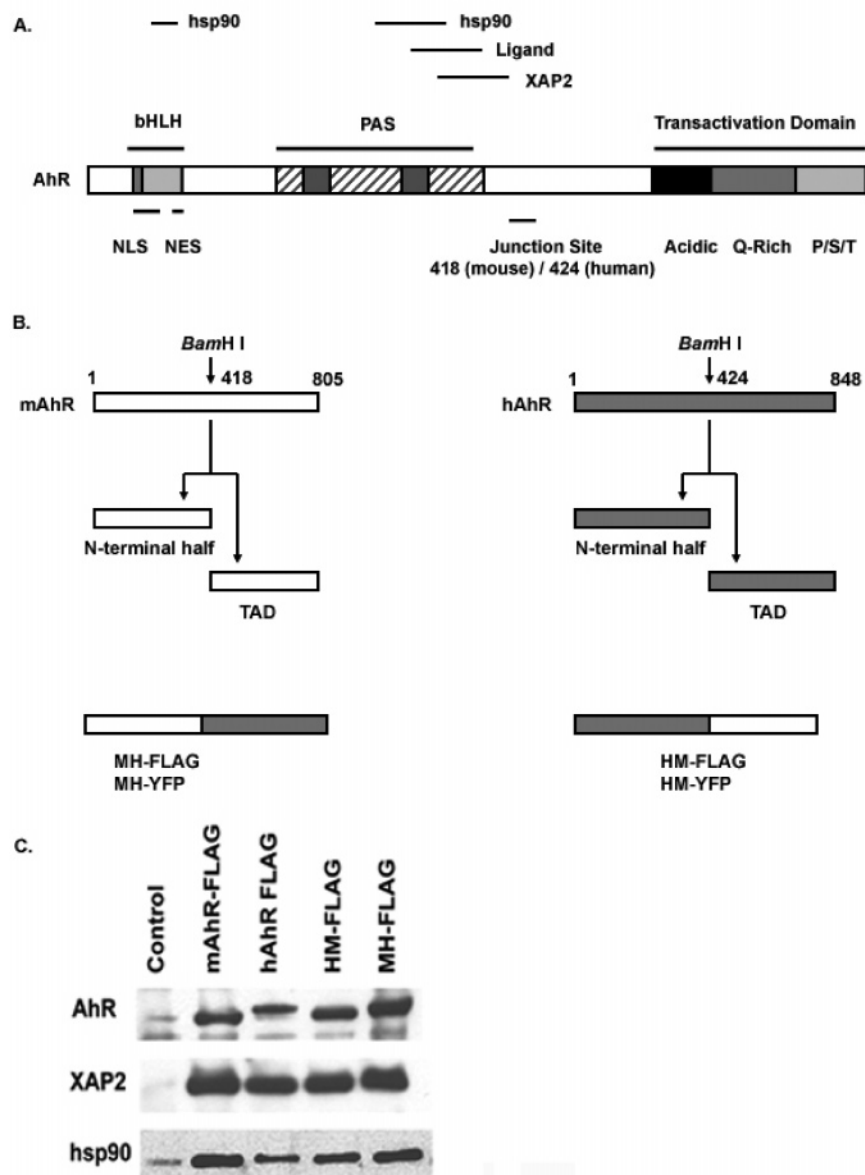


FIGURE 1: (A) Map depicting functional domains and the junction site used in the chimeric receptors. (B) Schematic showing how chimeric receptors were made. A unique and conserved BamHI site was used to digest the cDNAs of the receptors and switch the TADs and ligate them to the backbone of the nonparent receptor. (C) Immunoprecipitations of FLAG-tagged receptors to show formation of the AhR/XAP2/hsp90 tetrameric complex. COS-1 cells were transfected with pcDNA3/mAhR/FLAG, pCI/hAhR/FLAG, pEFV5/HM/FLAG or pcDNA3/MH/FLAG and pCI/XAP2. After 20 h, cells were lysed and complexes were immunoprecipitated from cytosol using Anti-FLAG M2 resin. Complexes were washed and separated by TSDS-PAGE. Western blot analysis was performed using antibodies against AhR, XAP2 and hsp90 and horseradish peroxidase (HRP)-conjugated secondary antibodies.

COS-1 cells (5, 8). The chimeras behave like the parent receptor whose TAD they contain; HM-YFP is nuclear, whereas MH-YFP is cytoplasmic (Figure 2A). Upon treatment of the sample with leptomycin B (a nuclear export inhibitor), all four receptors accumulate in the nucleus in the absence of cotransfected XAP2. When XAP2 is coexpressed, mAhR-YFP redistributes to the cytoplasm as previously reported, whereas there is no change in the localization of hAhR-YFP (Figure 2B), which was already cytoplasmic. The HM-YFP chimera remains nuclear, whereas the MH-YFP chimera remains cytoplasmic upon coexpression of XAP2. It is not clear why the HM receptor fails to redistribute to the cytoplasm upon coexpression of XAP2, but it is possible that switching the transactivation domains of the receptors affects this property, perhaps due to a change in the way the protein folds. In the presence of coexpressed

XAP2, mAhR-YFP remains cytoplasmic when treated with leptomycin B, whereas hAhR-YFP, HM-YFP, and MH-YFP accumulate in the nucleus in the presence of XAP2 with leptomycin B treatment (Figure 2B). These results suggest that XAP2 is capable of sequestering only mAhR-YFP and not hAhR-YFP or the chimeric receptors in the cytoplasm. Treatment with TCDD resulted in nuclear translocation of all four receptors with or without cotransfected XAP2 (Figure 2A,B and Table 1). These results suggest that the transactivation domains of mAhR and hAhR influence the receptors' localization in cells, perhaps through influencing the NLS and NES in the N-terminal half of the AhR.

The Transactivation Domain Regulates the Ability of XAP2 to Prevent Ligand-Independent Nuclear Import of the AhR. The results in Figure 2 suggest that the TAD determines localization and shuttling properties of the receptor. It has

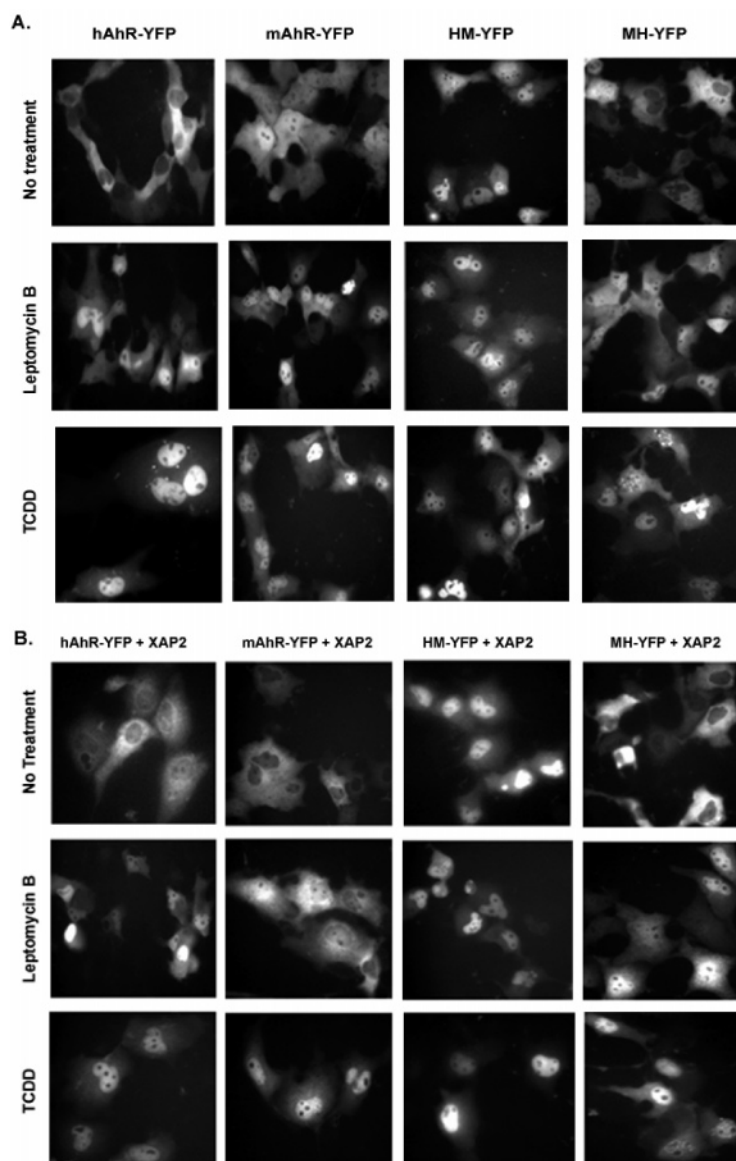


FIGURE 2: (A) Cellular localization of YFP-tagged receptors in COS-1 cells. Cells were transfected with pEFYFP/mAhR, pEFYFP/hAhR, pEFYFP/HM, or pEFYFP/MH and observed 20 h post-transfection. Cells were treated with 10 nM leptomycin B or 10 nM TCDD for 1 h. (B) Nucleocytoplasmic shuttling of YFP receptors in COS-1 cells. The same experiment as in A, but cells were cotransfected with pCI/XAP2.

Table 1: Overall Summary of the Results Presented in Figures 2–4

| Receptors | mAhR | hAhR | MH | HM |
|-----------------------|----------|--------------|--------|-----------------|
| no treatment | Nuc | Cyto | Cyto | Nuc |
| LMB | Nuc | Nuc | Nuc | Nuc |
| TCDD | Nuc | Nuc | Nuc | Nuc |
| XAP2 | Cyto | Cyto | Cyto | Nuc |
| XAP2 + LMB | Cyto | Nuc | Nuc | Nuc |
| XAP2 + TCDD | Nuc | Nuc | Nuc | Nuc |
| XAP2-NLS | Cyto | Nuc | Cyto | ND ^a |
| XAP2-NLS + LMB | Cyto | Nuc | Nuc | ND ^a |
| NLS-mutant receptors | mAhRK13A | hAhRΔ37–42 | MHK13A | |
| no treatment | Cyto | Cyto | Cyto | |
| XAP2-NLS | Cyto | Partical Nuc | Cyto | |
| XAP2-NLS + LMB | Cyto | Nuc | Cyto | |
| XAP2-NLS + TCDD | Cyto | Partical Nuc | Cyto | |
| XAP2-NLS + TCDD + LMB | Cyto | Nuc | Nuc | |

^a ND = not determined.

previously been demonstrated that mAhR complexes that translocate to the nucleus in both ligand-bound and ligand-

free states do not contain XAP2. In contrast, hAhR/hsp90 complexes that are nuclear contain XAP2 in both ligand-bound and ligand-free states. Thus, we wanted to determine whether XAP2 would be present in the chimeric receptor complex that translocates to the nucleus. If XAP2 is present in the MH complex that translocates to the nucleus either in the absence or in the presence of ligand, this would indicate that the TAD dictates whether XAP2 will be present in the complex that translocates to the nucleus, despite the XAP2 binding domain being present in the N-terminal half of the receptor. To this end, we utilized an XAP2-NLS construct that has an exogenous NLS fused to XAP2 that results in a predominantly nuclear localization of XAP2 (8). As demonstrated earlier (8), XAP2-NLS redistributes mAhR-YFP to the cytoplasm, whereas hAhR-YFP is dragged into the nucleus by XAP2-NLS (Figure 3A and Table 1). MH-YFP is cytoplasmic when XAP2-NLS is coexpressed, suggesting that it is not dragged into the nucleus by XAP2-NLS. However, this cytoplasmic localization may only reflect a higher rate of nuclear export, so, to address this question, cells were

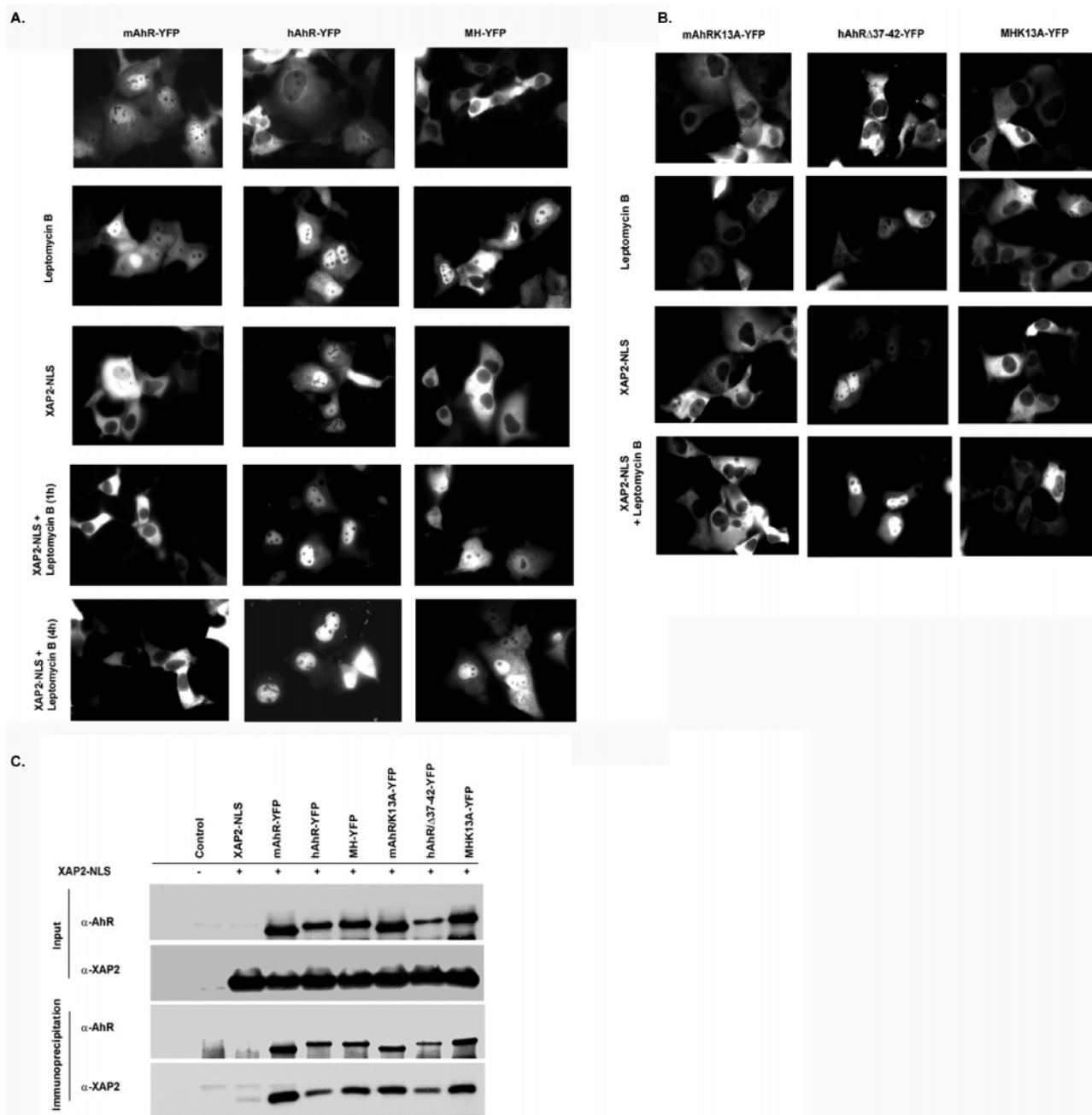


FIGURE 3: (A) Cellular localization of YFP-tagged receptors with coexpressed XAP2-NLS. Cells were transfected with pEFYFP/mAhR, pEFYFP/hAhR, pEFYFP/HM, or pEFYFP/MH with or without cotransfected pCI/XAP2-NLS and observed 20 h after transfection. Cells were treated with leptomycin B for 1 or 4 h and observed. (B) Cells were transfected with pEFYFP/mAhRK13A, pEFYFP/hAhRΔ37–42, or pEFYFP/MK13AH with or without cotransfected pCI/XAP2-NLS and observed 20 h after transfection. Cells were treated with leptomycin B for 1 or 4 h and observed. (C) COS-1 cells were transfected with 2 μ g of each YFP-tagged receptor construct and 2 μ g of pCI/XAP2-NLS. Cells were lysed, immunoprecipitations were performed using an anti-GFP antibody, and complexes were resolved by SDS–PAGE. Proteins were transferred to PVDF membrane and probed for AhR and XAP2.

treated with leptomycin B for 1 h. The mAhR–YFP remained cytoplasmic in the presence of XAP2-NLS and leptomycin B, whereas hAhR–YFP and MH–YFP were nuclear in the presence of XAP2-NLS and leptomycin B. This suggests that in the case of MH–YFP, XAP2-NLS does not sequester it to the cytoplasm. However, this does not determine whether XAP2 is present in the MH–YFP complex that translocates to the nucleus, since XAP2-NLS could be dissociated before MH enters the nucleus.

The Transactivation Domain Fails to Determine the Presence of XAP2 in the Unliganded Nuclear AhR/hsp90 Complex. To determine whether XAP2 is present in the

nuclear MH complex, we used an NLS-mutant of MH–YFP (MHK13A–YFP). This receptor cannot enter the nucleus unless XAP2-NLS drags it in as it does not have a functional NLS. mAhRK13A–YFP does not get dragged into the nucleus by XAP2-NLS, and this cytoplasmic localization is not due to enhanced nuclear export as treatment with leptomycin B does not result in nuclear accumulation of mAhRK13A–YFP (Figure 3B and Table 1). In contrast, hAhRΔ37–42–YFP is dragged into the nucleus by XAP2-NLS, and this effect is more evident when nuclear export is blocked using leptomycin B. This demonstrates that XAP2-NLS is present in the hAhR complex that shuttles to the

nucleus, but it is not present in the mAhR complex. XAP2-NLS failed to drag MHK13A-YFP into the nucleus, and the cytoplasmic localization was not due to enhanced nuclear export, as leptomycin B treatment did not result in nuclear accumulation. Therefore, while the wild-type chimera MH-YFP is not sequestered to the cytoplasm by XAP2 in the absence of ligand, it is not capable of entering the nucleus with XAP2 in the complex, suggesting that XAP2 is dissociated from the receptor complex before nuclear import. To ensure that the different YFP-tagged receptor proteins could bind XAP2-NLS, YFP-tagged receptor proteins and XAP2-NLS were coexpressed in COS-1 cells and receptor complexes were immunoprecipitated using an anti-GFP antibody. Immunoprecipitated complexes were resolved by SDS-PAGE, transferred to PVDF membrane, and probed for AhR and XAP2. XAP2-NLS coprecipitated with all receptor proteins tested (Figure 3C).

The Transactivation Domain Determines the Presence of XAP2 in the Ligand-Bound Nuclear AhR Complex. It has previously been shown that XAP2 may not be present in the mAhR complex that translocates to the nucleus in the presence of ligand but appears to be present in the liganded hAhR complex that translocates to the nucleus (8). In Figure 3B, we demonstrate that the TAD appears to influence the ability of XAP2 to sequester the receptor in the cytoplasm in the absence of ligand, but does not dictate the presence of XAP2 in the nuclear complex. We then wanted to investigate whether the TAD determines the presence of XAP2 in the nuclear complex in the presence of ligand. The NLS-mutants, mAhRK13A-YFP, hAhR Δ 37–42-YFP and MHK13A-YFP were expressed in the presence and absence of XAP2-NLS and treated with TCDD. As reported previously, hAhR, but not mAhR is dragged into the nucleus by XAP2-NLS in the presence of ligand (Figure 4 and ref 8). Treatment with leptomycin B for an hour after treatment with TCDD demonstrates that the cytoplasmic localization of mAhR is not due to an enhanced rate of nuclear export of the ligand-bound. MHK13A-YFP is cytoplasmic in the presence and absence of XAP2-NLS and remains cytoplasmic in the presence of TCDD. However, since it is possible that the ligand-bound complex was simply being exported out of the nucleus more rapidly than being imported, cells were treated with leptomycin B for an hour after TCDD treatment. MHK13A-YFP is predominantly nuclear in the presence of coexpressed XAP2-NLS with TCDD treatment for 1 h followed by leptomycin B treatment for 1 h, but it is cytoplasmic in the absence of coexpressed XAP2-NLS when treated with TCDD and Leptomycin B. Although this chimera does not accumulate in the nucleus as rapidly as hAhR Δ 37–42-YFP, it is not cytoplasmic like mAhRK13A-YFP. This demonstrates that the hAhR TAD that is present in the MH receptor appears to be able to determine whether XAP2 is present in the nuclear ligand-bound MH complex, since the only way this receptor could have entered the nucleus was through the nuclear import of XAP2-NLS. Table 1 is provided to facilitate the overall interpretation of the nucleocytoplasmic shuttling results (Figures 2–4).

The Extended TAD of the hAhR Relative to the mAhR Does Not Contribute to Differences in Cellular Localization. The TAD of hAhR has 44 amino acids more than the mAhR at the C-terminal end, and we wanted to address the question of whether these extra amino acids are responsible for the

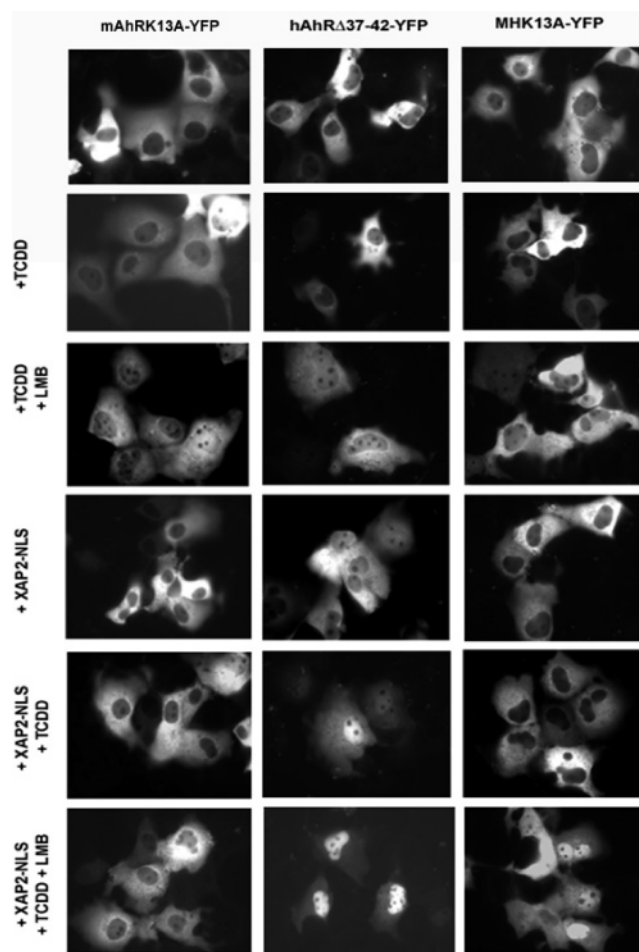


FIGURE 4: Cells were transfected with pEFYFP/mAhRK13A, pEFYFP/hAhR Δ 37–42, or pEFYFP/MK13AH with or without cotransfected pCI/XAP2-NLS and observed. Transfected cells were treated with TCDD for 1 h and observed, followed by LMB treatment for 1 h, and observed.

differences in localization and shuttling between the receptors. We generated a construct (pEFYFP/hAhR Δ 45), in which the last 45 amino acids were deleted from the hAhR-YFP while leaving the YFP moiety in frame. hAhR Δ 45-YFP was expressed in COS-1 cells with and without coexpressed XAP2, and its subcellular localization was observed. The hAhR Δ 45/YFP was cytoplasmic in COS-1 cells, and this localization did not change upon coexpression of XAP2. The hAhR Δ 45-YFP shuttles between the nucleus and cytoplasm in the presence and in the absence of XAP2 and accumulates in the nucleus upon treatment with ligand (Figure 5). Therefore, hAhR Δ 45/YFP shows the same cellular localization and shuttling properties as the full-length hAhR-YFP, both in the presence and absence of coexpressed XAP2. Thus, the differences in cellular localization between mAhR and hAhR are not due to the extra 45 amino acids of the TAD of the hAhR.

Difference in Ligand Affinity between mAhR and hAhR Is Not Due to the Difference in TAD Structure. It has been shown previously that mAhR and hAhR differ in their ligand affinity (32–34), and this difference is primarily due to a single amino acid variation in hAhR (34). It has been suggested previously that some amino acids in the C-terminal portion of the hAhR also contribute toward the difference in ligand affinity (33). To conclusively determine whether the C-terminal domain of the AhR influences ligand affinity,

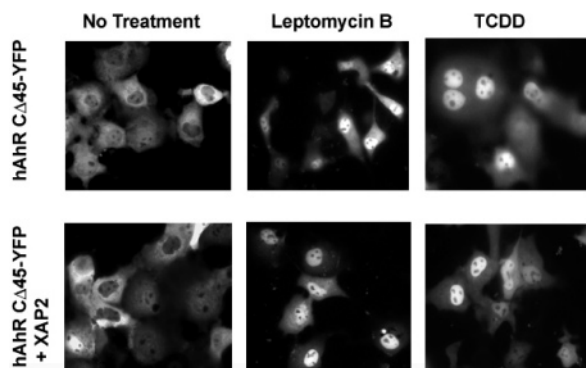


FIGURE 5: hAhRCΔ45-YFP shows the same localization and shuttling properties as hAhR-YFP. COS-1 cells were transfected with pEYFP/hAhRCΔ45 with or without pCI/XAP2 and observed. Cells were then treated with 10 nM TCDD or 10 nM leptomycin B for 1 h and observed.

we carried out ligand binding experiments using a photoaffinity ligand ($[^{125}\text{I}] \text{N}_3\text{Br}_2\text{DpD}$). Cytosol prepared from COS-1 cells transfected with pcDNA3-mAhR/FLAG, pCI-hAhR/FLAG, pcDNA3-MH/FLAG, or pEFV5-HM/FLAG was incubated with $[^{125}\text{I}] \text{N}_3\text{Br}_2\text{DpD}$ for 30 min, followed by incubation with a 200-fold molar excess of TCDBF for increasing amounts of time. Excess free ligand was removed using charcoal-dextran, and AhR–ligand complexes were photolyzed. The complexes were then resolved on 8% TSDBS–PAGE. The gels were dried and exposed to film, and radioactive bands corresponding to the AhR were cut from the gel and quantitated with a gamma counter (Figure 6). As previously reported, the photoaffinity ligand was displaced from hAhR-FLAG more readily by TCDBF than from mAHR-FLAG. The chimeras showed the same behavior as the parent receptors whose ligand binding domain they

contained, HM-FLAG showed a lower relative ligand affinity than MH-FLAG. These results suggest that the C-terminal domain of the AhR does not affect the relative ligand affinity of the receptor.

Transactivation Potential of mAHR and hAhR Are Similar in a Cell-Based Reporter Assay. To assess whether the transactivation potential of mAHR and hAhR are different and whether these differences are due to the poor homology in the transactivation domain, a reporter assay using a DRE-driven luciferase construct was performed in both COS-1 and BP8 cells. COS-1 cells and BP8 cells were transfected with pcDNA3/mAhR/FLAG, pCI/hAhR/FLAG, pEFV5/HM/FLAG or pcDNA3/MH/FLAG, pGUDLUC6.1 and pCMV- β gal using either LipofectAMINE-PLUS or GenePorter transfection reagents, respectively. After 20 h, cells were treated with 10 nM TCDD, an equal volume of DMSO, or no treatment, for 8 h. Cells were lysed in an appropriate buffer and analyzed for luciferase activity. mAHR-FLAG and hAhR-FLAG appear to have similar activity in both cell lines and do not show large differences in their ability to drive transcription of the reporter gene at a saturating dose of ligand (Figure 7). The chimeric receptors also show TCDD mediated DRE-driven luciferase activity comparable to wild-type receptors and are hence still functional receptors. The MH receptor shows an elevated constitutive activity in comparison to the HM receptor, which is more evident in BP8 cells. However, this difference is likely to be due to differences in expression of the two receptors and may not reflect a difference in activity. More importantly, there does not appear to be any difference between the mAHR and hAhR's transactivation potential at this ligand concentration. Therefore, there does not appear to be a difference in transactivation potential of hAhR and mAHR in a reporter

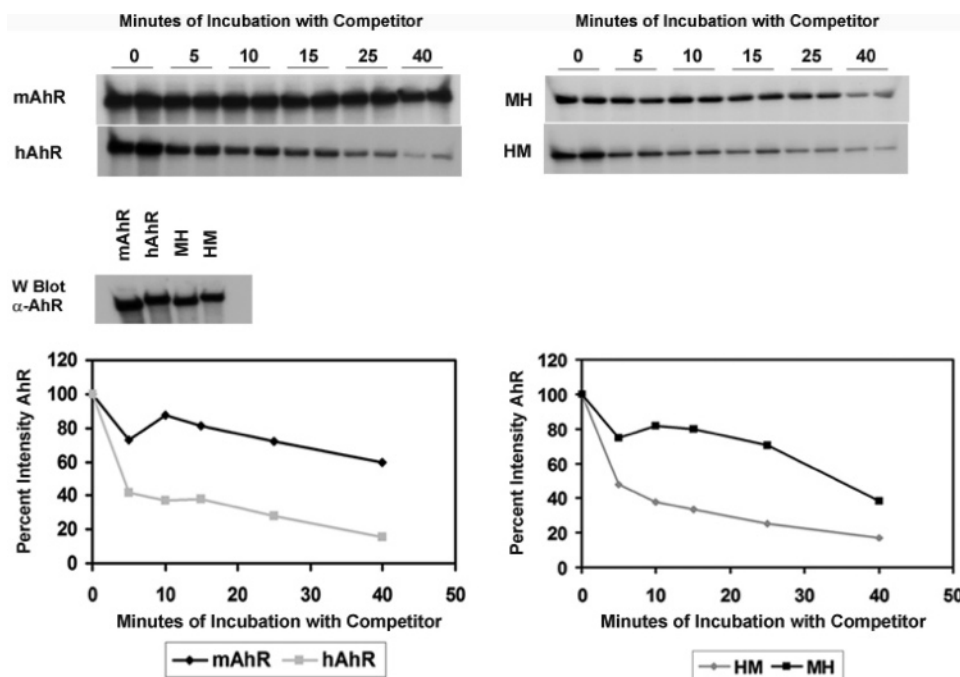


FIGURE 6: Relative ligand affinity of chimeric receptors. Cytosol from COS-1 cells transfected with pcDNA3/mAhR/FLAG, pCI/hAhR/FLAG, pEFV5/HM/FLAG, or pcDNA3/MH/FLAG was incubated with photoaffinity ligand ($[^{125}\text{I}] \text{N}_3\text{Br}_2\text{DpD}$) for 30 min, followed by the addition of 200-fold molar excess of TCDBF for the indicated time. Samples were then incubated on ice and charcoal-dextran was added. Samples were centrifuged and then photolyzed, and radioligand–receptor complexes were resolved by TSDBS–PAGE. Gels were dried, exposed to film, and bands were cut out and gamma-counted. A graphical representation of the data is also shown. Western blot analysis revealed that these receptors were expressed at comparable levels in the cells.

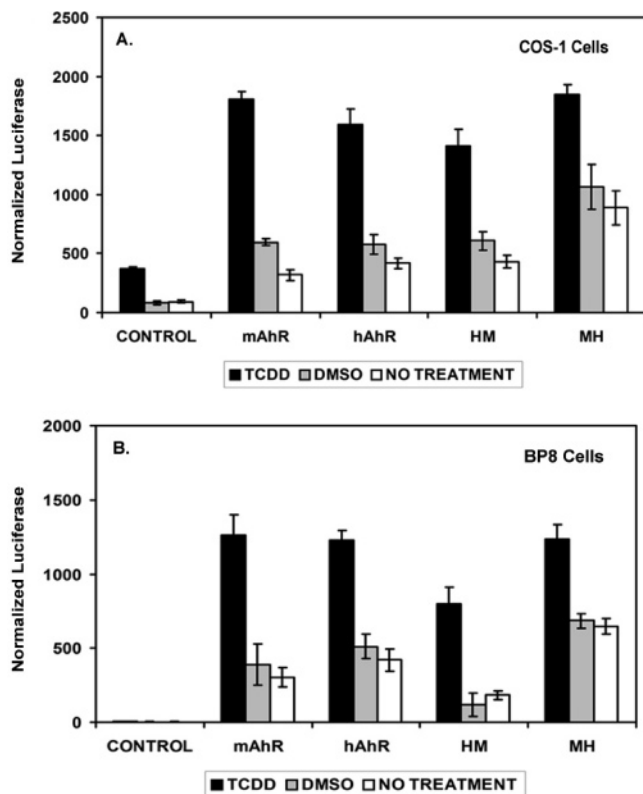


FIGURE 7: Luciferase reporter assay measuring transcriptional activity of receptors. COS-1 or BP8 cells were transfected with pcDNA3/mAhR/FLAG, pCI/hAhR/FLAG, pEFV5/HM/FLAG or pcDNA3/MH/FLAG, pGudLuc 6.1 and pCMV- β gal. At 20 h post-transfection, cells were treated with 10 nM TCDD, DMSO, or no treatment for 8 h. Cells were lysed and luciferase activity was measured and normalized to β -gal activity.

based system, but whether this is true in the case of endogenous genes in tissues remains to be determined.

DISCUSSION

Differences between the mouse and the human AhRs is of considerable importance because mice are used in toxicological studies to evaluate risk upon exposure to AhR ligands. The aim of this study was to determine whether the transactivation domains of the two receptors, which show more divergence than the N-terminal half, are responsible for the observed differences between the two receptors. Some of the properties of the two receptors that have been compared earlier include cellular localization, nucleocytoplasmic shuttling, effect of XAP2 on localization and shuttling, and differences in ligand binding potential (8, 34). The domains responsible for these properties, such as the nuclear localization and export signals (NLS and NES), the XAP2 binding region and ligand binding domain, are all found in the conserved N-terminal half of the receptor. However, we hypothesized that the transactivation domain in the C-terminal half of the receptor may play a role in determining the differences between these receptors by influencing the properties found in the N-terminal half in the folded state. To determine whether this was the case, chimeric receptors which have the N-terminal half of the receptor from one species (mouse or human) and the C-terminal half of the receptor from the other species were generated.

In COS-1 cells, mAhR-YFP is predominantly nuclear, whereas hAhR-YFP is cytoplasmic (5, 8; Figure 2A) and both receptors shuttle between the nucleus and the cytoplasm. XAP2 is capable of sequestering the mAhR-YFP but not hAhR-YFP in the cytoplasm and preventing its shuttling. The chimeras distribute to the nucleus or cytoplasm depending on the transactivation domain they contain, rather than the NLS or NES in the N-terminal half (Figure 2A), suggesting that in COS-1 cells, the TAD influences the cellular localization of the receptor. MH-YFP is unaffected by the coexpression of XAP2, in that it remains cytoplasmic, similar to hAhR-YFP (Figure 2B). Interestingly, however, while mAhR-YFP is redistributed to the cytoplasm by coexpression of XAP2, HM-YFP remains nuclear in the presence of XAP2. The reason for this lack of an effect by XAP2 is not clear, since the chimera does form a complex with XAP2 (Figure 1). It is possible that the chimera behaves differently because of altered folding of the receptor upon switching domains. Alternatively, the quality of interaction between the HM chimera and the XAP2 may mirror hAhR's interaction with XAP2 and HM-YFP may therefore remain unaffected by XAP2 coexpression. The chimeras show ligand-dependent translocation into the nucleus and nuclear accumulation upon blocking nuclear export similar to mAhR-YFP and hAhR-YFP, and XAP2 is capable of sequestering only mAhR to the cytoplasm in the absence of ligand.

To determine whether the TAD dictates the presence of XAP2 in the nuclear Ah receptor complex, mAhR-YFP, hAhR-YFP, and MH-YFP were cotransfected with XAP2-NLS. mAhR-YFP is redistributed to the cytoplasm, whereas hAhR-YFP is dragged into the nucleus by XAP2-NLS. Interestingly, the chimeric receptor, MH-YFP, remained cytoplasmic in the presence of XAP2-NLS, but upon treatment with LMB accumulated in the nucleus. This demonstrates that MH-YFP was not sequestered to the cytoplasm like mAhR but was shuttling in the presence of XAP2-NLS. NLS mutant receptors were used to determine whether XAP2-NLS was present in the complex that shuttles to the nucleus in the absence of ligand. mAhRK13A-YFP is cytoplasmic in the absence and presence of XAP2-NLS and this is not due to enhanced nuclear export (Figure 3B). The hAhR Δ 37–42-YFP is cytoplasmic in the absence of XAP2-NLS, partially nuclear in the presence of XAP2-NLS, and completely nuclear after treatment with Leptomycin B, but only in the presence of XAP2-NLS demonstrating that XAP2 is present in the hAhR complex that enters the nucleus. MHK13A-YFP is cytoplasmic in the absence and presence of XAP2-NLS, and this is not due to enhanced nuclear export. Therefore, XAP2-NLS is not present in the MH-YFP complex that enters the nucleus. This demonstrates that while the hAhR TAD prevents MH-YFP from being sequestered to the cytoplasm by XAP2, it does not mediate the presence of XAP2 in the nuclear hAhR/hsp90 complex, since XAP2 appears to be dissociated before nuclear entry in the case of MH-YFP. It is significant to note that XAP2 is present in nuclear hAhR/hsp90 complexes but not in mAhR/hsp90 complexes. It is possible that XAP2 may play a role in the nucleus through interactions with other proteins and nuclear entry via the hAhR may mediate these interactions. In the case of the mAhR, XAP2 does not enter the nucleus, and logically, it would not play a role in the nucleus, unless there was some other means of nuclear entry. This is important

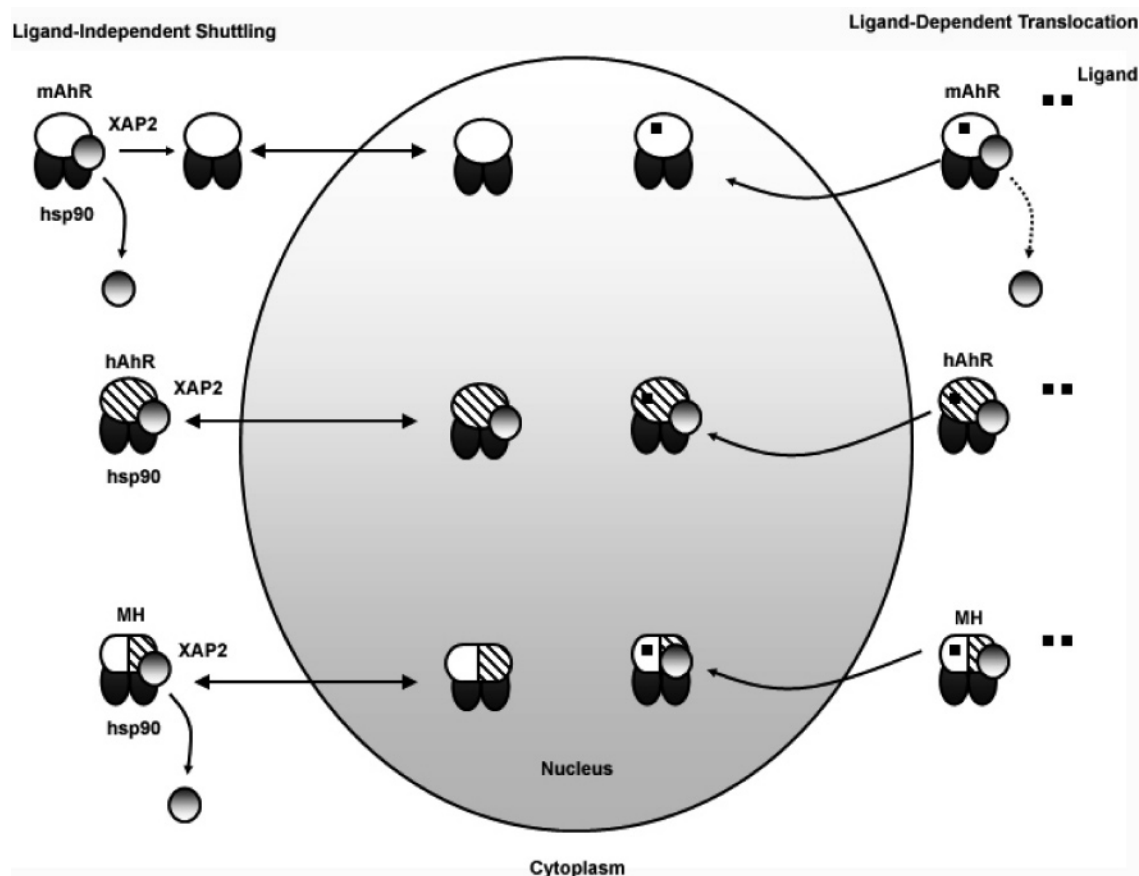


FIGURE 8: Model summarizing the presence of XAP2 in ligand-free and ligand-bound nuclear AhR/hsp90 complexes. XAP2 is present in nuclear hAhR/hsp90 complexes in both ligand free and ligand-bound states but not in the analogous mAhR complexes. The hAhR TAD determines the presence of XAP2 in ligand-bound but not ligand-free nuclear MH/hsp90 complexes.

considering that XAP2 appears to have a predominantly cytoplasmic localization (39).

This study also demonstrated the ability of the TAD to determine the presence of XAP2 in the nuclear ligand bound AhR/hsp90 complex by using NLS mutants of mAhR-YFP, hAhR-YFP, and MH-YFP. In the presence of XAP2-NLS, TCDD and LMB, mAhR13A-YFP remains cytoplasmic, whereas MHK13A-YFP starts to accumulate in the nucleus, demonstrating that XAP2-NLS can drag the chimera into the nucleus in the presence of ligand, albeit not as efficiently as it drags hAhR-YFP. In the case of the MH complex, the nuclear accumulation effect may not be as pronounced as in the case of the hAhR because the chimera still bears the XAP2 binding domain of the mAhR, and this may negatively influence translocation of the MH receptor into the nucleus in the presence of ligand. Also, as we have reported previously (8), hAhR Δ 37–42-YFP does show a small degree of nuclear import without XAP2-NLS, but there is a much greater amount of nuclear translocation in the presence of XAP2-NLS. Figure 8 provides a model summarizing nucleocytoplasmic shuttling properties and ligand-dependent nuclear import of mAhR, hAhR, and MH as determined by the results in Figures 2–4. It has been shown previously that the AhR contains two putative NES sequences (3, 16), and these sequences are used differently by the ligand-free versus the ligand-bound receptor complex (16). Since both the ligand-free and the ligand-bound mAhR complexes do not enter the nucleus with XAP2 in the complex, it is unlikely that nuclear export plays a role in the differences between mAhR and hAhR.

The hAhR does not appear to have as high an affinity for ligand as does the mAhR, and one study shows that this difference may be due to the difference in a single amino acid change in the ligand binding domain (valine 381 in hAhR is an alanine in mouse) in combination with a portion of the TAD (33). Another study showed that this difference is predominantly due to the single amino acid change in the ligand binding domain (34). Differences in the way these experiments were carried out in the two studies (density gradient analysis versus photaffinity labeling of the receptor in cells) may actually be the cause of the observed differences. In addition, the mAhR^d allele which has a lower affinity for ligand than the mAhR^b allele also has a valine at amino acid 375 (28). In this study, we utilized the chimeras to determine whether the TAD influences ligand binding in *in vitro* photoaffinity labeling experiments. We determined that the chimera behaves like the receptor whose ligand binding domain they contain, i.e., MH-FLAG has a higher relative ligand affinity than HM-FLAG (Figure 6). Therefore, the difference between mAhR and hAhR in terms of relative ligand affinity appears to be primarily due to the single amino acid change in the ligand binding region and not due to the differences between their TADs.

In COS-1 and BP8 cell lines, mAhR-FLAG and hAhR-FLAG showed a comparable ability to drive transcription of a DRE-driven luciferase reporter gene (Figure 7). It is possible that there may be differences in the ability of these receptors to drive transcription of their endogenous target genes, both from a qualitative and quantitative perspective, but this remains to be tested. However, generating an

appropriate system to address this question has not yet been possible and determining the ability of the two receptors to drive transcription in different cell lines (mouse versus human cells) is not a valid way to address the question, since other differences between the cell lines may contribute to observed differences in transactivation potential. We have observed that in cell lines with low levels of AhR or in AhR-null cells obtained from knockout models, the constitutive activity of exogenously introduced receptor is very high, and generating stable cell lines does not alleviate this problem (unpublished results). It is therefore difficult to assess whether the two receptors can show differences in transactivation of their target genes in such a system. It is possible that in the absence of functional AhR, there is an accumulation of an endogenous ligand that activates receptor when it is transfected into the cells. Generation of an appropriate system, where the mAHR and hAhR can be expressed at comparable levels and show inducible transactivation properties, will enable us to address the question of whether these receptors can transactivate their target genes comparably, both at low and high levels of ligand. Different ligand specificities can also be evaluated in such a system. If there are differences between mAHR's and hAhR's ability to drive transcription, using a high-affinity form of the hAhR that binds ligand as efficiently as mAHR or the MH chimera could determine whether the differences in the TAD are responsible for this variation.

Overall, this report demonstrates that some of the differences between the mouse and human AhR are due to the degeneracy in the TADs of the two receptors. Although the domains influencing properties such as cellular localization and shuttling, ligand binding, and XAP2 binding are present in the well-conserved N-terminal half, the TAD appears to be able to influence some of these properties. Significant differences in the behavior of mouse versus human receptors, demonstrated in this report as well as earlier reports, may eventually warrant the use of a more appropriate model for toxicological studies, such as the generation of a "humanized" AhR mouse. This model would not be able to control for differences in absorption, metabolism or excretion of compounds between species, but differences at the receptor level between species could be assessed. A humanized mouse model has been generated previously (40), where hAhR cDNA was knocked in to the AhR locus in AhR^{b/b} mice, thus disrupting the mAHR gene and allowing for the expression of hAhR under the control of the native mAHR promoter. The hAhR-knock in model showed a weaker induction of CYP1A1 upon exposure to TCDD and 3-Methylcholanthrene than AhR^{b/b} mice. The difference between mAHR's and hAhR's ability to induce CYP1A1 in this system lends support to the possibility that they do not transactivate endogenous genes to the same extent. However, whether this occurs due to a lower relative ligand affinity of hAhR or due to differences in the TAD remains to be determined. Interestingly, while TCDD caused hydronephrosis in the AhR^{b/b} mice and to a lesser extent in the hAhR knock-in mice, another teratological effect of TCDD, namely cleft palate was absent only from the hAhR knock-in mice. However, one of the potential problems with this model is that the actual level of hAhR protein expressed in the hAhR-knock-in mouse was not shown, thus it is not clear whether hAhR was expressed comparably to the wild-type mAHR. Therefore, a humanized mouse model with hAhR protein

levels comparable to mAHR protein levels may need to be generated. Overall, the distinct differences in response to TCDD found in this study between the wild-type mouse and the humanized mouse would suggest that the humanized mouse may serve as a better model for toxicological studies.

ACKNOWLEDGMENT

We thank Dr. Mike Denison for the construct pGudLuc 6.1 and Dr. Steve Safe for TCDD.

REFERENCES

- Huff, J. E., Moore, J. A., Saracci, R., and Tomatis, L. (1980) Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans, *Environ. Health Perspect.* 36, 221–240.
- Petrulis, J. R., and Perdew, G. H. (2002) The role of chaperone proteins in the aryl hydrocarbon receptor core complex, *Chem. Biol. Interact.* 141, 25–40.
- Ikuta, T., Eguchi, H., Tachibana, T., Yoneda, Y., and Kawajiri, K. (1998) Nuclear localization and export signals of the human aryl hydrocarbon receptor, *J. Biol. Chem.* 273, 2895–2904.
- Ikuta, T., Tachibana, T., Watanabe, J., Yoshida, M., Yoneda, Y., and Kawajiri, K. (2000) Nucleocytoplasmic shuttling of the aryl hydrocarbon receptor, *J. Biochem. (Tokyo)* 127, 503–509.
- Petrulis, J. R., Hord, N. G., and Perdew, G. H. (2000) Subcellular localization of the aryl hydrocarbon receptor is modulated by the immunophilin homolog hepatitis B virus X-associated protein 2, *J. Biol. Chem.* 275, 37448–37453.
- Pollenz, R. S., and Barbour, E. R. (2000) Analysis of the complex relationship between nuclear export and aryl hydrocarbon receptor-mediated gene regulation, *Mol. Cell Biol.* 20, 6095–6104.
- Petrulis, J. R., Kusnadi, A., Ramadoss, P., Hollingshead, B., and Perdew, G. H. (2003) The hsp90 Co-chaperone XAP2 Alters Importin beta Recognition of the Bipartite Nuclear Localization Signal of the Ah Receptor and Represses Transcriptional Activity, *J. Biol. Chem.* 278, 2677–2685.
- Ramadoss, P., Petrulis, J. R., Hollingshead, B. D., Kusnadi, A., and Perdew, G. H. (2004) Divergent Roles of Hepatitis B Virus X-Associated Protein 2 (XAP2) in Human versus Mouse Ah Receptor Complexes, *Biochemistry* 43, 700–709.
- Reyes, H., Reisz-Porszasz, S., and Hankinson, O. (1992) Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor, *Science* 256, 1193–1195.
- Mimura, J., and Fujii-Kuriyama, Y. (2003) Functional role of AhR in the expression of toxic effects by TCDD, *Biochim. Biophys. Acta* 1619, 263–268.
- Kolluri, S. K., Weiss, C., Koff, A., and Gottlicher, M. (1999) p27-(Kip1) induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells, *Genes Dev.* 13, 1742–1753.
- Kolluri, S. K., Balduf, C., Hofmann, M., and Gottlicher, M. (2001) Novel target genes of the Ah (dioxin) receptor: transcriptional induction of N-myristoyltransferase 2, *Cancer Res.* 61, 8534–8539.
- Matikainen, T., Perez, G. I., Jurisicova, A., Pru, J. K., Schlezinger, J. J., Ryu, H. Y., Laine, J., Sakai, T., Korsmeyer, S. J., Casper, R. F., Sherr, D. H., and Tilly, J. L. (2001) Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals, *Nat. Genet.* 28, 355–360.
- Burbach, K. M., Poland, A., and Bradfield, C. A. (1992) Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor, *Proc. Natl. Acad. Sci. U.S.A.* 89, 8185–8189.
- Fukunaga, B. N., Probst, M. R., Reisz-Porszasz, S., and Hankinson, O. (1995) Identification of functional domains of the aryl hydrocarbon receptor, *J. Biol. Chem.* 270, 29270–29278.
- Berg, P., and Pongratz, I. (2001) Differential usage of nuclear export sequences regulates intracellular localization of the dioxin (aryl hydrocarbon) receptor, *J. Biol. Chem.* 276, 43231–43238.
- Ma, Q., Dong, L., and Whitlock, J. P., Jr. (1995) Transcriptional activation by the mouse Ah receptor. Interplay between multiple stimulatory and inhibitory functions, *J. Biol. Chem.* 270, 12697–12703.

18. Poland, A., and Glover, E. (1987) Variation in the molecular mass of the Ah receptor among vertebrate species and strains of rats, *Biochem. Biophys. Res. Commun.* **146**, 1439–1449.
19. Dolwick, K. M., Schmidt, J. V., Carver, L. A., Swanson, H. I., and Bradfield, C. A. (1993) Cloning and expression of a human Ah receptor cDNA, *Mol. Pharmacol.* **44**, 911–917.
20. Korkalainen, M., Tuomisto, J., and Pohjanvirta, R. (2000) Restructured transactivation domain in hamster AH receptor, *Biochem. Biophys. Res. Commun.* **273**, 272–281.
21. Korkalainen, M., Tuomisto, J., and Pohjanvirta, R. (2001) The AH receptor of the most dioxin-sensitive species, guinea pig, is highly homologous to the human AH receptor, *Biochem. Biophys. Res. Commun.* **285**, 1121–1129.
22. Schwetz, B. A., Norris, J. M., Sparschu, G. L., Rowe, U. K., Gehring, P. J., Emerson, J. L., and Gerbig, C. G. (1973) Toxicology of chlorinated dibenzo-p-dioxins, *Environ. Health Perspect.* **5**, 87–99.
23. McConnell, E. E., Moore, J. A., Haseman, J. K., and Harris, M. W. (1978) The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs, *Toxicol. Appl. Pharmacol.* **44**, 335–356.
24. Henck, J. M., New, M. A., Kociba, R. J., and Rao, K. S. (1981) 2,3,7,8-tetrachlorodibenzo-p-dioxin: acute oral toxicity in hamsters, *Toxicol. Appl. Pharmacol.* **59**, 405–407.
25. Hakansson, H., Johansson, L., Manzoor, E., and Ahlborg, U. G. (1994) Effect of 2,3,7,8-tetrachlorodibenzoy-p-dioxin on the hepatic 7-ethoxyresorufin O-deethylase activity in four rodent species, *Eur. J. Pharmacol.* **270**, 279–284.
26. Chapman, D. E., and Schiller, C. M. (1985) Dose-related effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57BL/6J and DBA/2J mice, *Toxicol. Appl. Pharmacol.* **78**, 147–157.
27. Poland, A., and Glover, E. (1990) Characterization and strain distribution pattern of the murine Ah receptor specified by the Ah^d and Ah^{b-3} alleles, *Mol. Pharmacol.* **38**, 306–312.
28. Poland, A., Palen, D., and Glover, E. (1994) Analysis of the four alleles of the murine aryl hydrocarbon receptor, *Mol Pharmacol* **46**, 915–921.
29. Pohjanvirta, R., Unkila, M., Tuomisto, J. T., Vuolteenaho, O., Leppaluoto, J., and Tuomisto, J. (1993) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on plasma and tissue beta-endorphin-like immunoreactivity in the most TCDD-susceptible and the most TCDD-resistant rat strain, *Life Sci.* **53**, 1479–1487.
30. Kumar, M. B., Ramadoss, P., Reen, R. K., Vanden Heuvel, J. P., and Perdew, G. H. (2001) The Q-rich subdomain of the human Ah receptor transactivation domain is required for dioxin-mediated transcriptional activity, *J. Biol. Chem.* **276**, 42302–42310.
31. Pohjanvirta, R., Wong, J. M., Li, W., Harper, P. A., Tuomisto, J., and Okey, A. B. (1998) Point mutation in intron sequence causes altered carboxyl-terminal structure in the aryl hydrocarbon receptor of the most 2,3,7,8-tetrachlorodibenzo-p-dioxin-resistant rat strain, *Mol. Pharmacol.* **54**, 86–93.
32. Harper, P. A., Golas, C. L., and Okey, A. B. (1988) Characterization of the Ah receptor and aryl hydrocarbon hydroxylase induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin and benz[a]-anthracene in the human A431 squamous cell carcinoma line, *Cancer Res.* **48**, 2388–2395.
33. Ema, M., Ohe, N., Suzuki, M., Mimura, J., Sogawa, K., Ikawa, S., and Fujii-Kuriyama, Y. (1994) Dioxin binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors, *J. Biol. Chem.* **269**, 27337–27343.
34. Ramadoss, P., and Perdew, G. H. (2004) Use of 2-azido-3-[125I]-iodo-7,8-dibromodibenzo-p-dioxin as a probe to determine the relative ligand affinity of human versus mouse aryl hydrocarbon receptor in cultured cells, *Mol. Pharmacol.* **66**, 129–136.
35. Meyer, B. K., Pray-Grant, M. G., Vanden Heuvel, J. P., and Perdew, G. H. (1998) Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity, *Mol. Cell Biol.* **18**, 978–988.
36. Makarova, O., Kamberov, E., and Margolis, B. (2000) Generation of deletion and point mutations with one primer in a single cloning step, *Biotechniques* **29**, 970–972.
37. Perdew, G. H., Abbott, B., and Stanker, L. H. (1995) Production and characterization of monoclonal antibodies directed against the Ah receptor, *Hybridoma* **14**, 279–283.
38. Poland, A., Glover, E., Ebetino, F. H., and Kende, A. S. (1986) Photoaffinity labeling of the Ah receptor, *J. Biol. Chem.* **261**, 6352–6365.
39. Kuzhandaivelu, N., Cong, Y. S., Inouye, C., Yang, W. M., and Seto, E. (1996) XAP2, a novel hepatitis B virus X-associated protein that inhibits X transactivation, *Nucleic Acids Res.* **24**, 4741–4750.
40. Moriguchi, T., Motohashi, H., Hosoya, T., Nakajima, O., Takahashi, S., Ohsako, S., Aoki, Y., Nishimura, N., Tohyama, C., Fujii-Kuriyama, Y., and Yamamoto, M. (2003) Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 5652–5657.

BI050948B