The Q-rich Subdomain of the Human Ah Receptor Transactivation Domain Is Required for Dioxin-mediated Transcriptional Activity*

Received for publication, May 25, 2001, and in revised form, September 6, 2001
Published, JBC Papers in Press, September 10, 2001, DOI 10.1074/jbc.M104798200

Mohan B. Kumar‡§, Preeti Ramadoss‡§, Rashmeet K. Reen‡, John P. Vanden Heuvel‡,
and Gary H. Perdew¶‡§

From the ‡Department of Veterinary Science and the Center for Molecular Toxicology and Carcinogenesis and the §Graduate Program in Biochemistry and Molecular Biology, Pennsylvania State University,
University Park, Pennsylvania 16802

The aryl hydrocarbon receptor (AhR), a basic helix-loop-helix/Per-Arnt-Sim transcription factor, mediates many of the toxic and biological effects of the environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin, which include the transcriptional activation of dioxin-responsive genes such as CYP1A1. Many aspects of this process are known; however, the mechanism of transcriptional activation and the proteins that are key to this process remain to be determined. The hAhR has a complex transactivation domain, composed of three potentially distinct subdomains. Deletional analysis of the hAhR transactivation domain indicates that removal of the P/S/T-rich subdomain enhances transcriptional activity, whereas the Q-rich subdomain is critical for hAhR transactivation potential, and the acidic subdomain by itself fails to activate a dioxin response element-driven reporter gene. Deletional analysis of the Q-rich subdomain identified a critical stretch of 23 amino acids between residues 666 and 688 of the hAhR, which are required for transactivation potential. Alanine scanning mutagenesis of this region identified a leucine residue (Leu-678), which is required for hAhR activity. Functional analysis of this point mutant revealed that it is capable of binding ligand, heterodimerization, and subsequent binding to dioxin response elements. Further, when hAhR/L678A and hAhR containing only the acidic subdomain were overexpressed they acted as dominant negative receptors and repressed wild-type hAhR activity. In addition, the hAhR/L678A failed to activate CYP1A1 gene transcription in transfected BP-8 cells and exhibited reduced binding to RIP140 in vitro. Thus, Leu-678 appears to be critical for efficient transactivation activity of the hAhR and appears to disrupt recruitment of co-regulators.

The aryl hydrocarbon receptor (AhR) binds to and mediates most, if not all, of the toxic responses to TCDD. These include body weight loss, thymic atrophy, immunotoxicity, hepatotoxicity, porphyria, chloracne and related dermal lesions, tissuespecific hypo- and hyperplastic responses, carcinogenesis, teratogenicity, and reproductive toxicity. In addition, AhR mediates various biochemical responses like the induction of phase I and II drug-metabolizing enzymes, such as CYP1A1, CYP1B1, CYP1A2, and GST-Ya (1, 2). The physiological role of the AhR is poorly understood; however, the generation of AhR null mice has yielded some clues. Although phenotypic differences in AhR null mice from different laboratories are evident, a common thread among the different mice is the lack of proper liver development including small liver size and fibrosis (3, 4). AhR null mice also exhibited immune system impairment and reproductive defects including female mortality, small litter size, and death of pups after weaning (4, 5). In addition, AhR null mice failed to develop subcutaneous tumors at the site of injection of benzo(a)pyrene, which were evident in the case of wild-type and AhR heterozygous mice, indicating that the AhR is necessary for the metabolic activation of some genotoxic aromatic hydrocarbon compounds (6).

The AhR in the unliganded state is found in the cytosol in a heterotetrameric complex, which comprises two Hsp90 molecules and XAP2 (7–12). Binding of ligand results in the translocation of the entire complex into the nucleus and presumably the shedding of Hsp90 and XAP2 occurs. After translocation to the nucleus the AhR heterodimerizes with AhR nucleolar translocator (ARNT), which resides in the nucleus (13–15). The AhR/ARNT heterodimer binds to DREs upstream of dioxin-responsive genes like CYP1A1 to up-regulate their transcription (16, 17). The binding of AhR/ARNT heterodimer to DREs upstream of CYP1A1 results in an AhR transactivation domain- and TCDD-dependent disruption of nucleosomal structure in the promoter-proximal regions following transcriptional activation of CYP1A1 (18–20). The details of assembly of a chromatin remodeling coactivator complex and the subsequent assembly of a pre-initiation complex are not completely understood. Certain basal transcription factors including TFIIB, TATA-binding protein, and TFIIF have been suggested to interact with the AhR in vitro (21, 22). Some corepressors and coactivators have been shown to interact with the AhR and/or ARNT and modulate the transactivation potential of the heterodimer. The coactivator ERAP140 and corepressor silencing mediator of retinoid and thyroid hormone receptor interact with the AhR and/or ARNT and alter AhR-mediated transcriptional activation of DRE-driven reporter genes (23). We have previously characterized the interaction of SRC-1 and RIP140 methylglycine; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-proanesulfonic acid; MOPS, 4-morpholinepropanesulfonic acid.
AhR Transactivation Requires Q-rich Subdomain

with the AhR TAD in vitro and in intact cells (24, 25). Both SRC-1 and RIPA140 enhance transcriptional activation of DRE-driven reporter genes in a TCDD- and AhR-dependent manner in various cell lines, although the pattern of response was dependent on the coactivator/co-regulator used. RIPA140 ectopic expression led to a biphasic response in reporter gene activity, whereas SRC-1 exogenous expression resulted in increased reporter gene activity, even at higher levels of transfected SRC-1. The mechanism of interaction was also distinct in that RIPA140 interacted with AhR in a TCDD- and LXXLL motif-independent manner, whereas SRC-1 interaction was TCDD- and LXXLL motif-dependent.

ARNT has a 34-aa TAD, which is located COOH-terminal to a Q-rich segment of the protein (26). The AhR TAD, on the other hand, is more complex. The mouse AhR TAD has been studied extensively and can be divided into three subdomains: acidic-rich (aa 515–583), proline-rich (aa 643–740), and serine-rich (aa 726–848) (19). In the case of the mouse AhR, all three subdomains were potent transcriptional activators of the mouse CYP1A1 gene. The human AhR TAD also contains three subdomains: an acidic subdomain (aa 500–600), a Q-rich subdomain (aa 600–713), and a P/S/T subdomain (aa 713–848) (22). When tested in a yeast-based reporter gene assay, each of the three subdomains by themselves exhibit low levels of transactivation; however, any combinations of two of the subdomains can synergistically activate a reporter gene (23). In previous reports, we have characterized the binding of SRC-1 and RIPA140 to each of, or combinations of, the three subdomains of the hAhR (24, 25). The Q-rich subdomain appears to be required for in vitro recruitment of SRC-1 and RIPA140 to the TAD; however, an important role of the other subdomains cannot be completely ruled out.

In this study, we have characterized the transactivation potential of critical regions of the AhR TAD within the context of the AhR/ARNT heterodimer. We have found that the Q-rich TAD of the AhR was critical for transcriptional activation of dioxin-responsive genes. Detailed analysis of the Q-rich TAD indicated that the residues between aa 663 and 688 were necessary for transactivation, whereas the P/S/T-rich region appeared to be dispensable. Alanine scanning mutagenesis identified a critical leucine residue, Leu-678, which is required for efficient AhR transactivation potential. These findings underscore the critical role of the Q-rich region of the AhR in mediating transactivation activity.

MATERIALS AND METHODS

Plasmids and Site-directed Mutagenesis—The plasmid pEF-V5-hAhR was generated by subcloning a 2.6-kilobase pair KpnI-NoI fragment derived from pCI-hAhR into pEF-V5-HisC (Invitrogen, Carlsbad, CA). pEF-V5-hAhR was used for all subsequent experiments. Site-directed mutagenesis was performed using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) following manufacturer’s instructions. For generation of plasmids for expression of hAhR deletions, site-directed mutagenesis using the QuikChange kit was employed to introduce stop codons at appropriate sites.

Cell Culture and Transient Transfection Experiments—COS-1 and BP8 cells were obtained from American Type Culture Collection (Manassas, VA) and Mike Denison (University of California, Davis, CA), respectively, and were routinely cultured in α-modified Eagle’s medium containing 10% fetal bovine serum. BP8 cells are mutants of 5L rat V5-hAhR using the following protocol for LipofectAMINE Plus reagent. After a 15-min incubation of the mix, the entire volume was added to cells in each well in a six-well plate. The transfection was carried out for 3 h, at which point fetal bovine serum was added to the cells to a final volume of 10%. The transfection was continued for another 3 h, the transfection mixture was removed and washed twice with phosphate-buffered saline, and the cells were placed in serum-free medium containing 0.5 ng each of reporter plasmid, pGUDLUC containing 10% fetal bovine serum. BP8 cells are mutants of 5L rat V5-hAhR using the following protocol for LipofectAMINE Plus reagent. After a 15-min incubation of the mix, the entire volume was added to cells in each well in a six-well plate. The transfection was carried out for 3 h, at which point fetal bovine serum was added to the cells to a final volume of 10%. The transfection was continued for another 3 h, the transfection mixture was removed and washed twice with phosphate-buffered saline, and the cells were placed in serum-free medium containing 0.5 ng each of reporter plasmid, pGUDLUC containing 10% fetal bovine serum.

The plasmid pEF-V5-hAhR was used for all subsequent experiments. Site-directed mutagenesis was performed using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) following manufacturer’s instructions. For generation of plasmids for expression of hAhR deletions, site-directed mutagenesis using the QuikChange kit was employed to introduce stop codons at appropriate sites. For determining levels of expression of the different AhR mutants and deletions, 50–100 ng of total protein from cell extracts were electrophoresed on an 8% SDS-Tricine polyacrylamide gel. The separated proteins were transferred to a membrane and subjected to Western blotting using an anti-AhR mouse monoclonal antibody, RPT1 (1:100 dilution), and a goat anti-mouse secondary antibody peroxidase conjugate (1:100,000 dilution). Peroxidase activity was visualized using an ECL kit as described by the manufacturer (PerkinElmer Life Sciences).

Gel Shift Assay—hAhR and hARNT were separately in vitro transcribed and translated using the TNT rabbit reticulocyte lysate system (Promega, Madison, WI). Translated hAhR and hARNT were mixed, and TCDD was added to a final concentration of 20 nM. The heterodimerization was allowed to occur at 30 °C for 15 min. Gel shift buffer (25 mM Hepes, pH 7.5, 10% glycerol, 1 mM KCl, 4 mM MgCl2, 2.5% CHAPS, 5 mM dithiothreitol, and 540 ng of poly[dI-dC] final concentration) was added to the in vitro translation mix along with 10 molar excess of competitor DNA as indicated in the figure. This was followed by the addition of 0.5 ng of 32P-labeled DRE oligonucleotide (provided by M. Denison, University of California, Davis, CA) and 15-min incubation at room temperature at which point 1× Hi-Density TBE Sample buffer (Invitrogen, Carlsbad, CA) was added. An aliquot of the final mix was run on a 6% non-denaturing polyacrylamide gel. The separated proteins were transferred to a membrane and subjected to Western blotting using an anti-AhR mouse monoclonal antibody, RPT1 (1:100 dilution), and a goat anti-mouse secondary antibody peroxidase conjugate (1:100,000 dilution). Peroxidase activity was visualized using an ECL kit as described by the manufacturer (PerkinElmer Life Sciences).

Cell Culture for QRT-PCR—Cells were routinely maintained in α-modified Eagle’s medium containing 10% fetal bovine serum (v/v). Cells were seeded in 100-mm dishes overnight and transfected with a total volume of 1 l of DNA/well, using LipofectAMINE Plus reagent (Life Technologies, Inc.) in six-well plates. The number of RNA molecules was standardized using a standard curve. Band intensities were measured using OptiQuant software (Packard Instruments Co.). The number of RNA molecules was determined as described previously (28)
AhR Transactivation Requires Q-rich Subdomain

In Vitro Binding Assay to Determine the Interaction of AhR with RIP140—GST and GST/RIP140(1–350) were expressed as described previously (25). For GST pull-down assays, GST and GST/RIP140(1–350) were pre-bound on 50 μl of glutathione-agarose for 60 min at 4 °C. The agarose pellet was washed three times in incubation buffer (25 mM MOPS, 2 mM EDTA, 0.02% sodium azide, 100 mM NaCl, 10% glycerol, 0.5% CHAPS, pH 7.4). In vitro translated [35S]methionine-labeled hAhR and 500 μl of incubation buffer containing 2 mg/ml bovine serum albumin was added to the agarose. This mixture was shaken at 4 °C for 2 h and washed four times with incubation buffer. SDS sample buffer was added to each agarose pellet and subjected to Tricine SDS-PAGE. The protein on the gel was transferred to membrane, and the radiolabeled protein was visualized by autoradiography and phosphorimaging.

RESULTS

Role of Individual Subdomains of the hAhR TAD—The human AhR transactivation domain has been characterized previously using a heterologous fusion protein system in yeast. The hAhR TAD was subdivided into three subdomains: the acidic subdomain (aa 500–600), Q-rich subdomain (aa 600–713), and P/S/T-rich subdomain (aa 713–848) (22). The individual subdomains possessed very little transcriptional activity by themselves when fused to a heterologous DNA binding domain and expressed in yeast. However, when combinations of two of the subdomains were examined the transcriptional activity was similar to the full-length AhR TAD, suggesting a possible effect of stabilized secondary structure or recruitment of additional factors by the various domain combinations (22). We have previously examined the role of the three subdomains in physical recruitment of SRC-1 and RIP140 in vitro using GST fusion protein pull-down assays. The Q-rich subdomain in both the cases appeared to be necessary for optimal coactivator interaction. However, the role of the other subdomains cannot be ruled out in recruiting other coactivators. In this report, the role of each of the subdomains and their contribution to the transactivation potential of the hAhR TAD in the context of the AhR/ARNT heterodimer were examined in mammalian cells.

Progressive deletions of the hAhR cDNA were made to generate hAhR mammalian expression vectors, which expressed either a hAhR protein with only the acidic subdomain, or a hAhR with the acidic and the Q-rich subdomains, or progressive deletions of the Q-rich region (Fig. 1). These AhR truncated mutants were tested in DRE-driven reporter assays in COS-1 and BP8 cells (Fig. 2, A and B). COS-1 cells contain relatively low levels of the AhR and thus provide a minimal background for testing the mutants. The BP8 cells are derived from 5L hepatoma cells, which do not express any detectable AhR, and hence are also suitable for testing the AhR mutants (27). The deletion of the P/S/T-rich subdomain significantly increased the transactivation potential of AhR, suggesting that the P/S/T-rich subdomain may repress the hAhR transactivation potential (Fig. 2, A and B). In contrast, the deletion of the P/S/T-rich and Q-rich subdomains together led to a significant loss of AhR transactivation potential, indicating that the acidic subdomain by itself had very little transcriptional activity. In fact, reporter gene activity dropped to 8% of levels obtained with liganded wild-type hAhR, suggesting a critical role for the Q-rich subdomain. Samples treated with carrier solvent showed minimal activity with all the hAhR plasmids tested. In the case of BP8 cells, the reporter activity in the absence of co-transfected hAhR was very low, indicating that the transcriptional activity observed is the result of transfected hAhR. Examination of the expression of various hAhR mutants using protein blot analysis indicated similar levels of expression of the AhR deletion proteins, and thus the level of expression was not a factor in the reporter gene activity obtained (Fig. 2C).

Deletions of the Q-rich subdomain were also tested to identify a critical stretch of amino acids, which are required for optimal transcriptional potential (Fig. 2, A and B). Finer deletion mutants, which resulted in AhR(1–688), AhR(1–663), and AhR(1–637), were made. Upon testing these mutants in DRE-driven reporter gene assays, it was observed that AhR(1–688) co-transfection resulted in activity similar to hAhR(1–713) in COS-1 and BP-8 cells (Fig. 2, A and B). In contrast, an additional deletion of 35 amino acids led to significant reduction in the levels of reporter gene activity to near background levels, suggesting that aa 663–688 contained critical residues required for optimal transcriptional activity of the hAhR. As expected, further deletion from the carboxyl terminus did not affect the AhR activity any further. Examination of protein blots of extracts from the transfected cells indicated similar
levels of expression (Fig. 2C). Overall, the data showed similar patterns in the BP8 cells, further reinforcing the data obtained using COS-1 cells.

**Alanine Scanning Mutagenesis of the Q-rich Subdomain**—Having identified a critical region required for hAhR transactivation, we turned to determining the possible role of individual amino acid residues by alanine scanning mutagenesis of the region between amino acids 663 and 688. Substitutions of individual residues with alanine did not result in the inactivation of the hAhR except in the case of Leu-678 (Fig. 3A). The replacement of Leu-678 by alanine led to the almost complete inactivation of the hAhR. All the other residues upon substitution with alanine resulted in near WT activity, although several residues adjoining Leu-678 displayed a slight reduction in activity. The expression levels of each of the mutants were examined and found to be similar, indicating that the low activity of the L678A mutant was not the result of low levels of expression (Fig. 3B). The ability of the WT and L678A mutant hAhR to enhance DRE-driven reporter activity was further assessed in triplicate, and the L678A mutant exhibited a very low level of transcriptional activity compared with WT hAhR (Fig. 4).

**Functional Analysis of L678A Mutant**—The L678A mutant was tested in gel shift assays for ligand binding, heterodimerization, and DRE binding activity. The L678A mutant heterodimerized and bound DRE similar to wild-type hAhR, indicating that the low level of activity seen in reporter gene assays was not the result of disruption of ligand binding, heterodimerization, or DRE binding.

**AhR Transactivation Requires Q-rich Subdomain**

![Figure 2: Effect of progressive truncations of the hAhR TAD on DRE-reporter gene activity in COS-1 and BP8 cells.](image-url)

COS-1 (panel A) or BP8 cells (panel B) were transfected in 6-well plates with a total of 1.6 μg of DNA composed of 50 ng of hAhR FL/hAhR, various deletion constructs or empty vector (indicated as control), 50 ng of DRE-driven luciferase gene plasmid, pGUDLUC 6.1, and the β-galactosidase internal control plasmid, pDJMβ-gal. The cells were harvested 10 h after treatment with TCDD or carrier solvent. Extracts were assayed for luciferase activity, which was subsequently normalized to β-galactosidase activity and expressed as Relative Luciferase Units (RLU). The expression levels of the different hAhR deletion mutants was assayed in duplicate by running 100 μg of total COS-1 cell extracts on an 8% polyacrylamide gel (panel C). The separated proteins were transferred to a polyvinylidene difluoride membrane and probed with an anti-AhR monoclonal antibody, RPT1, followed by ECL detection.
AhR Transactivation Requires Q-rich Subdomain

**Fig. 3.** Alanine scanning mutagenesis of the critical Q-rich subdomain region between amino acids 663 and 688. Summation of the results of alanine scanning mutagenesis of the Q-rich subdomain region between amino acid residues 663–688 (panel A). COS-1 cells were transfected in 6-well plates with a total of 1.6 µg of DNA which included 50 ng of hAhR FL/hAhR deletion plasmid or empty vector (indicated as control), 50 ng of DRE-driven luciferase gene plasmid, pGUDLUC 6.1, and the β-galactosidase internal control plasmid, pDJM-β-gal. The cells were harvested 10 h after treatment with TCDD or carrier solvent. Extracts were assayed for luciferase activity, which was subsequently normalized to β-galactosidase activity and expressed as Relative Luciferase Units (RLU). The numbers on the right indicate percent activity of each point mutant relative to wild-type AhR, which is set at 100%. The expression levels of the different hAhR point mutants was assayed by running 50 µg of total COS-1 cell extracts on a 8% polyacrylamide gel (panel B). The separated proteins were transferred to same polyvinylidene difluoride membrane and probed with an anti-AhR monoclonal antibody, RPT1 followed by ECL detection.

**Fig. 4.** DRE-driven reporter gene activity of the L678A mutant compared with the WT hAhR. COS-1 cells were transfected in 6-well plates with a total of 1.6 µg of DNA which included 50 ng of hAhR FL/hAhR deletion plasmid or empty vector (indicated as control), 50 ng of DRE-driven luciferase gene plasmid, pGUDLUC 6.1, and the β-galactosidase internal control plasmid, pDJM-β-gal. The cells were harvested 10 h after treatment with TCDD or carrier solvent. Extracts were assayed for luciferase activity, which was subsequently normalized to β-galactosidase activity and expressed as Relative Luciferase Units (RLU). The numbers on the right indicate %WT hAhR activity of each of the point mutants.

ization, or DNA binding (Fig. 5). We also tested the hAhR L678A mutant for its ability to function as a dominant negative in an AhR-driven reporter gene assay with the hypothesis that the increasing expression of L678A mutant would function as a dominant negative in the presence of the wild-type hAhR by competing for the available ARNT pool and subsequent binding to DREs. Indeed, increasing amounts of hAhR/L678A mutant expression down-regulated WT hAhR activity in reporter gene
15 min incubation at 30°C. Mixed and 20 nM TCDD or carrier solvent was added and incubated for 15 min incubation at 30°C. Followed by the addition of [P-32]DRE, whereas competitor DRE was added to control incubations, and each sample was incubated at room temperature for 15 additional min. An aliquot of each sample was applied to a 6% nondenaturing gel and run for 1 h at 100 V. The gel was dried and exposed to film.

**Fig. 5. Functional analysis of the hAhR L678A mutant using gel shift assays.** The L678A mutant or the WT hAhR, and hARNT cDNAs were in vitro transcribed/translated using TNT rabbit Reticulocyte Lysate kit. Aliquots of hAhR and hARNT translations were mixed and 20 nM TCDD or carrier solvent was added and incubated for 15 min incubation at 30°C. Followed by the addition of [P-32]DRE, whereas competitor DRE was added to control incubations, and each sample was incubated at room temperature for 15 additional min. An aliquot of each sample was applied to a 6% nondenaturing gel and run for 1 h at 100 V. The gel was dried and exposed to film.

The L678A mutant or the WT hAhR, and hARNT cDNAs were in vitro transcribed/translated using TNT rabbit Reticulocyte Lysate kit. Aliquots of hAhR and hARNT translations were mixed and 20 nM TCDD or carrier solvent was added and incubated for 15 min incubation at 30°C. Followed by the addition of [P-32]DRE, whereas competitor DRE was added to control incubations, and each sample was incubated at room temperature for 15 additional min. An aliquot of each sample was applied to a 6% nondenaturing gel and run for 1 h at 100 V. The gel was dried and exposed to film.

**Discussion**

The AhR binds to xenobiotic compounds like polycyclic aromatic hydrocarbons and halogenated aromatic hydrocarbons, which are environmental contaminants; the most toxic member of this class of chemicals is TCDD. AhR mediates many, if not all, of the adaptive and biological responses to the exposure of TCDD, which includes carcinogenesis in rodents, and the induction of cytochrome P450 genes like CYP1A1. A great deal is known about the activation of CYP1A1 gene by the mouse AhR. The mAhR via its TAD up-regulates the transcription of CYP1A1 by a process that involves chromatin remodeling of the CYP1A1 gene. The different subdomains of mAhR appear to be equally potent individually in activating transcription of mCYP1A1. The role of hAhR and its complex TAD has not been studied to the same degree. The three subdomains of the hAhR were originally characterized by Rowlands et al. (22) and seem to be structured differently from the mouse AhR. The mouse and human AhRs show considerable divergence in amino acid sequence of the TAD, although a number of amino acid sequences are highly conserved among a number of species. In the case of the hAhR, the individual subdomains were incapable of sufficiently driving a yeast-based reporter gene when fused to a heterologous DNA binding domain. In addition, different combinations of the subdomains were capable of synergistically increasing transcriptional activation. However, the role of subdomains of the hAhR TAD has not been studied in the context of the full-length protein, or in a mammalian system.

The role of each of the subdomains in contributing to the transactivation potential of the hAhR/ARNT heterodimer was assessed. Results presented here indicate that the P/S/T subdomain may repress other domains and thus its deletion leads to an increase in overall transactivation potential of the AhR. It is possible that the P/S/T-rich subdomain is involved in recruiting certain co-regulators, which moderate the transcriptional response. On the other hand, the P/S/T-rich subdomain may pose a conformational constraint on the Q-rich subdomain, which could lead to greater transactivation potential. Interestingly, rainbow trout express two splice variants of ARNT: rt ARNTa, which has a 104-aa carboxyl-terminal domain rich in proline, serine, and threonine residues; and rt ARNTb, which expresses a protein with a 190-aa COOH-terminal domain rich in glutamine and asparagine residues. The rt ARNTb was found to induce endogenous CYP1A1 mRNA 20-fold higher than ARNTa (29). Studies on the mutant form MHC class II transactivator, which lacks the acidic TAD but retains the P/S/T-rich subdomain, was found to behave as a dominant negative.
protein, suggesting a repressor function for the P/S/T-rich subdomain (30). Our observations would appear to be the first evidence for a potential repressive role for the P/S/T-rich subdomain in the hAhR TAD. This is in contrast to earlier indications that the P/S/T-rich subdomain in combination with the Q-rich subdomain was able to activate reporter gene activity similar to the full-length TAD fused to a heterologous DNA binding domain (22). However, these studies were carried out with yeast-based reporter genes and hAhR TAD subdomains fused to a heterologous DNA binding domain. In the present study, deletions in the full-length hAhR proteins were generated and the roles of the subdomains were examined in a mammalian reporter assay system. The mouse AhR TAD subdomains have been investigated in great detail, and when examined as Gal4 DNA binding domain fusion proteins, the Q-rich subdomain was found to harbor most of the mouse AhR's transactivation potential. The deletion of the carboxyl-terminal subdomain rich in proline and serine residues did not appear to impair mAhR activity (31). On the other hand, the proline/serine-rich subdomain of the mAhR examined within the context of mAhR/ARNT heterodimer appears to be able to transactivate CYP1A1 similar to that of WT mAhR (19). Thus, the distinction between Q-rich and P/S/T-rich subdomain transactivation potential of the mAhR was minimal, in contrast to studies with the mAhR TAD subdomain Gal4 DNA binding domain fusion protein studies (31).

**Fig. 6.** The hAhR L678A mutant exhibits a dominant negative effect on wt hAhR. BP8 cells were transfected in 6-well plates with a total of 500 ng of DNA composed of 10 ng of wt hAhR plasmid, empty vector (indicated as control), or increasing amounts up to 390 ng of either the hAhR L678A, hAhR D668A, or the hAhR(-1-599) mutants, 50 ng of DRE-driven luciferase gene plasmid, pGUD-LUC 6.1, and the β-galactosidase internal control plasmid, pDJM-β-gal. The cells were harvested 10 h after treatment with TCDD or carrier solvent. Extracts were assayed for luciferase activity, which was subsequently normalized to β-galactosidase activity and expressed as Relative Luciferase Units (RLU).

**Fig. 7.** The hAhR L678A mutant fails to enhance CYP1A1 mRNA levels. BP8 cells were transfected with hAhR constructs in 100 mm dishes as described in materials and methods. The cells were harvested 2 h after treatment with TCDD or carrier solvent and RNA was isolated. QRT-PCR was utilized to determine the levels of CYP1A1 mRNA in each transfection. The three graphs summarize the data from three separate transfection experiments and the agarose gel pictures show a representative example of an internal standard curve and QRT-PCR of the individual samples from a single experiment.

**Fig. 8.** Reduced binding of hAhR L678A to RIP140. hAhR and hAhR L678A were in vitro translated in the presence of [35S]methionine and incubated with immobilized GST RIP 140-(1–350) or GST in the presence of TCDD. After a 2-h incubation, samples were washed, subjected to SDS-PAGE, followed by transfer to membrane, and autoradiography. The results shown are representative of three separate experiments.
Deletion of the Q-rich subdomain from the hAhR TAD led to complete inactivation of the hAhR, which suggests that the Q-rich subdomain is critical for the hAhR TAD and the acidic subdomain by itself is incapable of transactivating dioxin-responsive genes. The Q-rich subdomain has been shown to be necessary for optimal mAhR transactivation potential, although perhaps not required (26, 31). We have shown previously that SRC-1 and RIP140 both interact in vitro mainly through the Q-rich subdomain of the hAhR TAD (24, 25). In addition, the retinoblastoma tumor suppressor protein has been shown to interact with the Q-rich subdomain via amino acid residues 589–774 (32). Collectively, this suggests that, indeed, the Q-rich subdomain of the hAhR TAD is critical for functionality. An alignment of the Q-rich subdomains from various species indicates that a significant number of residues are highly conserved across species (Fig. 8), further pointing to the importance of the Q-rich subdomain. Interestingly, hamster AhR TAD has been shown to contain a longer Q-rich subdomain with 49 glutamine residues, compared with 27 and 25 glutamine residues in the mouse and human AhR TADs, respectively (33). Hamsters are known to be extremely resistant to TCDD lethality, but curiously, CYP1A1 is highly inducible in hamsters, which may suggest that, although the glutamine enriched TAD is still functional, other AhR-mediated events that lead to TCDD lethality are somehow impaired. Whether the aberrant Q-rich subdomain may alter other activities of the AhR requires further investigation. Several transcription factors containing glutamine-rich TADs have been identified, and the glutamine-rich TAD has been shown to be indispensable for transcriptional potential. These include Sp1 (34, 35), the GAGA factor (36–38), TF(II)130 (35), and NF-Y (39) among others. In fact, the GAGA factor glutamine-rich TAD is necessary for DNA distortion (37) and amyloid fiber formation in vitro, although it is dispensable for chromatin remodeling (36). The glutamine-rich transcription factors are generally considered to be promoter-proximal factors, which synergistically enhance transcriptional activity of cognate genes from a promoter proximal region (40). This may be a result of direct interaction with TATA-binding protein (41) and/or with hTAF(II)130 (35). However, glutamine-rich transcription factors have also been shown to stimulate transcription when bound distal and proximal to the promoter region (42). Other glutamine-rich transcription factors, including the hAhR may behave in a similar fashion. Another transcription factor PU.1, which plays a major role in hematopoiesis and the generation of mature macrophages actually has a TAD with several subdomains similar to those in the hAhR. The PU.1 TAD includes multiple NH2-terminal acidic and glutamine-rich subdomains, along with a PEST domain. The glutamine-rich TAD and a portion of the PEST domain were found to be required for myelopoiesis; however, the deletion of the three acidic subdomains apparently did not affect PU.1 activity (43).

The acidic subdomain of the hAhR appears to be insufficient to elicit significant transcriptional activity in the absence of the Q-rich subdomain. In addition, the acidic domain of the hAhR was unable to enhance CYP1A1 activity in BP8 cells (Fig. 7). This is in contrast to findings obtained examining the mAhR TAD, where the acidic subdomain was able to transactivate the mouse CYP1A1 gene (19). This may be indicative of the functional differences between the hAhR and the mAhR TADs. The acidic subdomain, if it behaves like that of VP16 and others, should up-regulate genes from promoter-proximal regions and thus should function within the context of the reporter construct used in this report.

The presence of multiple subdomains in the AhR raises several questions about the role of each subdomain in mediating transactivation potential. In addition, the lack of a positive role...
AhR Transactivation Requires Q-rich Subdomain

for the acidic and the P/S/T-rich subdomains under these experimental conditions is curious. It is possible that the different subdomains may be differentially used depending on the promoter, enhancer, cell line, species, temporal-specific context. One intriguing possibility is the role of AhR in regulating a myriad of genes, which include dioxin-responsive genes such as the cytochrome P450 genes, but also modulating other unrelated receptors, which are involved in cross-talk with the AhR. The estradiol-induced cathepsin D gene, for example, contains a DRE, which is adjacent to a Sp1 site in the promoter-proximal region and may be involved in modulating transcription of the gene (44). The gene for Epo also harbors DREs in the promoter proximal region, which mediate up-regulation by TCDD (45). In addition, dioxin-responsive genes harbor several DREs, which are located distally and proximally to the promoter region. The flexibility provided by multiple activation subdomains, which are all highly conserved. Alanine scanning mutagenesis indicated that, except for one leucine residue (Leu-678), none of the other residues is required for optimal recruiting/assembly of transcription activators that are recruited by the Q-rich subdomain that are necessary for efficient transactivation potential.

Acknowledgments—We thank Chris Bradfield for the human Ah receptor cDNA, as well as Mike Denison and Martin Göttlicher for BP8 cells.

REFERENCES
1. Safe, S. H. (1986) Annu. Rev. Pharmacol. Toxicol. 26, 371–379
2. Poland, A., and Knutson, J. C. (1982) Annu. Rev. Pharmacol. Toxicol. 22, 523–554
3. Fernandez-Salgueiro, P., Pneau, T., Hilbert, D. M., McPhail, T., Lee, S. S., Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M., and Gonzalez, F. J. (1995) Science 268, 722–728
4. Schmidt, J. V., Su, G. H., Reddy, J. K., Simon, M. C., and Bradfield, C. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6731–6736
5. Abbott, B. D., Schmid, J. E., Pitt, J. A., Buckley, A. R., Wood, C. R., Held, G. A., and Dible, E. J. (1999) Toxicol. Appl. Pharmacol. 155, 62–79
6. Shimizu, Y., Nakatsuru, Y., Ichinose, M., Takahashi, Y., Kume, H., Mimura, J., Fujii-Kuriyama, Y., and Ishikawa, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 779–782
7. Meyer, B. K., and Perdew, G. H. (1999) Biochemistry 38, 8907–8917
8. Ma, Q., and Whitlock, J. P., Jr. (1997) J. Biol. Chem. 272, 8878–8884
9. Carver, I., LaPres, J. J., Jain, S., Dunham, E. E., and Bradfield, C. A. (1998) J. Biol. Chem. 273, 35555–35567
10. Carver, L. A., and Bradfield, C. A. (1997) J. Biol. Chem. 272, 11452–11456
11. LaPres, J. J., Glover, E., Dunham, E. E., Bunger, M. K., and Bradfield, C. A. (2000) J. Biol. Chem. 275, 6153–6159
12. Meyer, B. K., Pray-Grant, M. G., Vanden Heuvel, J. P., and Perdew, G. H. (1998) Mol. Cell. Biol. 18, 978–988
13. Henry, E. C., and Gasiewica, T. A. (1993) Biochem. J. 294, 95–101
14. Hord, N. G., and Perdew, G. H. (1994) Mol. Pharmacol. 46, 618–626
15. Reyes, H., Reisz-Porszasz, S., and Hankinson, O. (1992) Science 256, 1185–1195
16. Kresse, L., Reichert, J., and Schwarz, M. (1998) Eur. J. Biochem. 258, 803–812
17. Tukey, R. H., and Nebert, D. W. (1984) Biochemistry 23, 6003–6008
18. Ko, H. P., Okino, S. T., Ma, Q., and Whitlock, J. P., Jr. (1996) Mol. Cell. Biol. 16, 430–436
19. Ko, H. P., Okino, S. T., Ma, Q., and Whitlock, J. P., Jr. (1997) Mol. Cell. Biol. 17, 3497–3507
20. Okino, S. T., and Whitlock, J. P., Jr. (1995) Mol. Cell. Biol. 15, 3714–3721
21. Simonson, H. I., and Yang, J. H. (1998) Mol. Pharmacol. 54, 671–677
22. Rowlands, J. C., McEwan, I. J., and Gustafson, J. A. (1996) Mol. Pharmacol. 50, 538–548
23. Nguyen, T. A., Hoivik, D., Lee, J. E., and Safe, S. (1999) Arch. Biochem. Biophys. 367, 250–257
24. Kumar, M. B., and Perdew, G. H. (1999) Gene Expr. 8, 273–286
25. Kumar, M. B., Turpey, R. W., and Perdew, G. H. (1999) J. Biol. Chem. 274, 21255–21264
26. Sogawa, K., Iwabuchi, K., Abe, H., and Fujii-Kuriyama, Y. (1995) J. Cancer Res. Clin. Oncol. 121, 612–620
27. Kolluri, S. K., Weiss, C., Koff, A., and Gottlicher, M. (1999) Genes Dev. 13, 1742–1753
28. Vanden Heuvel, J. P. (ed) (1998) PCR Protocols in Molecular Toxicology, pp. 69–77, CRC Press, New York
29. Polenz, R. S., Sullivan, H. E., Holmes, J., Necele, B., and Petersen, R. E. (1990) J. Biol. Chem. 271, 30886–30896
30. Zhou, H., Su, H., Zhang, X., Douhan, J., III, and Glimcher, L. H. (1997) ImmunoL 158, 4741–4749
31. Jain, S., Dolwick, K. M., Schmidt, J. V., and Bradfield, C. A. (1994) J. Biol. Chem. 269, 31518–31524
32. Go, S. H., and Elferink, C. J. (1998) J. Biol. Chem. 273, 22708–22713
33. Korkalainen, M., Tuomisto, J., and Pohjanvirta, R. (2000) Biochem. Biophys. Res. Commun. 273, 272–281
34. Rojo-Niersbach, E., Furukawa, T., and Tanese, N. (1999) J. Biol. Chem. 274, 33778–33784
35. Saluja, D., Vassallo, M. F., and Tanese, N. (1998) Mol. Cell. Biol. 18, 5734–5743
36. Agianian, B., Leonard, K., Bonte, E., Van der Zandt, H., Becker, P. B., and Agard, D. A. (1999) Arch. Biochem. Biophys. 367, 620–626
37. Serra, E., Zemzoumi, K., di Silvio, A., Mantovani, R., Lardans, V., and Dissous, C. (1998) Nucleic Acids Res. 26, 3860–3865
38. Stuhr, K., Greigour, O., and Schaffner, W. (1992) EMBO J. 11, 4961–4968
39. Emili, A., Greenblatt, J., and Inglee, C. J. (1994) Mol. Cell. Biol. 14, 1582–1593
40. Courey, A. J., Holtzman, D. A., Jackson, S. P., and Tjian, R. (1989) Cell 59, 827–836
41. Fisher, R. C., Olson, M. C., Pongubala, J. M., Perkell, J. M., Achinson, M. L., Scott, E. W., and Simon, M. C. (1998) Mol. Cell. Biol. 18, 4437–4457
42. Wang, F., Hovick, D., Polenz, R., and Safe, S. (1998) Nucleic Acids Res. 26, 3044–3052
43. Chan, W. K., Yao, G., Gu, Y. Z., and Bradfield, C. A. (1999) J. Biol. Chem. 274, 12115–12120
44. Dong, L., Ma, Q., and Whitlock, J. P., Jr. (1997) J. Biol. Chem. 272, 29614–29619
45. Shiau, A. K., Barstad, D., Loria, P. M., Cheng, L., Kushner, P. J., Agard, D. A., and Greene, G. L. (1996) Cell 85, 927–937
The Q-rich Subdomain of the Human AhReceptor Transactivation Domain Is Required for Dioxin-mediated Transcriptional Activity
Mohan B. Kumar, Preeti Ramadoss, Rashmeet K. Reen, John P. Vanden Heuvel and Gary H. Perdew

J. Biol. Chem. 2001, 276:42302-42310.
doi: 10.1074/jbc.M104798200 originally published online September 10, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M104798200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 46 references, 29 of which can be accessed free at
http://www.jbc.org/content/276/45/42302.full.html#ref-list-1