Identification of a Critical Amino Acid in the Aryl Hydrocarbon Receptor*

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Two aryl hydrocarbon receptors (rtAHR2α and rtAHR2β) have been identified in the rainbow trout (Oncohynchus mykiss). These receptors share 98% amino acid identity, yet their functional properties differ. Both rtAHR2α and rtAHR2β bind 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), dimerize with rainbow trout ARNTb (rtARNtb), and recognize dioxin response elements in vitro. However, in a transient transfection assay the two proteins show differential ability to enhance transcription, produce transactivation, and respond to TCDD. To identify the sequence differences that confer the functional differences between rtAHR2α and rtAHR2β, we constructed chimeric rtAHRs, in which segments of one receptor form was replaced with the corresponding part from the other isoform. This approach progressively narrowed the region being examined to a single residue, corresponding to position 111 in rtAHR2β. Altering this residue in rtAHR2β from the lysine to glutamate found in rtAHR2α produced an rtAHR2β with the properties of rtAHR2α. All other known AHRs resemble rtAHR2α and carry glutamate at this position, located at the N terminus of the PAS-A domain. We tested the effect of altering this glutamate in the human and zebrafish AHRs to lysine. This lysine substitution produced AHRs with transactivation properties that were similar to rtAHR2β. These results identify a critical residue in AHR proteins that has an important impact on transactivation, enhancer site recognition, and regulation by ligand.

The aryl hydrocarbon receptor (AHR) and its associated dimerization partner ARNT are members of the basic helix-loop-helix (bHLH) PAS family of proteins. These proteins transduce signals generated by environmental stresses into transcriptional responses. These stresses range from hypoxia to xenobiotic compounds (1, 2). The AHR is activated by a structurally broad range of ligands. Among these, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is one of the most potent and well-studied agonists (3). A broad spectrum of environmental contaminants, including TCDD, can produce toxic responses through activation of the AHR. Two hybrid and coexpression studies have revealed the presence of proteins that interact with AHR, including HSP90 and ARA9/AIP/XAP2 (4–7). These chaperone proteins stabilize and hold AHR in a conformation that is better able to bind ligand (8). TCDD binding causes the AHR protein to dissociate from cytosolic HSP90 and move into the nucleus where it forms a functional dimer with ARNT. This dimer then binds DNA to regulate the transcription of target genes. The AHR-ARNT dimer binds to specific enhancer elements that are often referred to as Dioxin Response Elements, or DREs. The best-characterized DREs lie upstream of genes encoding cytochrome P450s (CYP450s) (9, 10). Following nuclear localization and DNA binding, AHR exits the nucleus and is then degraded by the proteasome pathway (11–14).

The AHR protein is composed of several functional domains. The N terminus contains a domain rich in basic amino acids followed by a helix-loop-helix domain that is conserved among a variety of DNA binding proteins. The basic domain is required for DNA binding, whereas the helix-loop-helix domain is involved in dimer formation with ARNT. The N terminus also contains nuclear localization (NLS) and export (NES) domains (15, 16). C-terminal to the bHLH domain is a pair of PAS domains, PAS-A and PAS-B, that are conserved among a family of proteins. The PAS domains are named for several founding members of this protein family, Per, ARNT, and Sim (1). PAS domains act as regulated protein interaction surfaces and are involved in a wide variety of sensory/signaling processes in both eukaryotes and prokaryotes. These domains are involved in ligand binding to AHR, and the subsequent change in protein associations, subcellular location, and activity. The ligand-binding domain encompasses the PAS-B domain whereas HSP90 is thought to interact with the bHLH and PAS domains (17, 18). ARA9/AIP interacts with the PAS-B/ligand-binding domains (19, 20). Potential retinoblastoma protein binding sites have also been identified (21). The C-terminal domain is necessary for transcriptional activation and is the least conserved among AHR proteins (17).

Developing fish are especially sensitive to the toxic effects of TCDD (22). AHR and ARNT proteins have been identified in a variety of fish species and presumably mediate these effects. The ability of ligands to activate the AHR pathway is similar to their ability to cause TCDD-like toxicity (23–25). In contrast to mammals, most fish species appear to have at least two AHR...
genes. Generally, one AHR (AHR1) is more similar to the mamalian AHR and a second (AHR2) is fish-specific (26). Full-length AHRs have been cloned in tomodc and two each in rainbow trout, *Fundulus heteroclitus*, and zebrafish (27-30). In addition, partial AHR sequences have been cloned from several fish (26). ARNT isoforms have been cloned from *Fundulus (ARNT2)* rainbow trout (rtARNTa, b and d) and zebrafish (zfARNT2a, b, and c) (31-33). No ARNT1 has been identified in fish.

To date, salmonids are the group of fish species that are most sensitive to the effects of TCDD. Two AHR genes encoding rtaHR2 and rtaHR2β have been identified in rainbow trout. These two AHR isoforms are ~98% identical in primary sequence. Despite this similarity in structure, the two proteins have distinct properties. In general, rtaHR2α has stronger transactivation properties than rtaHR2β. This is somewhat surprising in light of the fact that these two proteins are identical in sequence in the C-terminal domain that is thought to mediate transcriptional activation. In addition, rtaHR2α and rtaHR2β have different enhancer sequence requirements. rtaHR2α appears to be active with a more limited set of enhancer sequences than rtaHR2β. To explore the structural nature of these differences, we constructed a set of chimeric proteins in which segments of rtaHR2β were exchanged with the cognate sequence from rtaHR2α. These experiments indicate that the functional differences between rtaHR2α and rtaHR2β are conferred by a single amino acid difference corresponding to position 111 in rtaHR2β.

**Experimental Procedures**

*Cell Culture—* COS-7 (monkey kidney epithelial) cells, obtained from ATCC (Manassas, VA), were maintained in 100% humidity in Dulbecco’s modified eagle’s medium supplemented with 10% fetal bovine serum in an atmosphere of 5%CO2 at 37°C. Cells were split and plated at a density of 6.0 × 10⁴ cells/well of a 24-well plate 1 day prior to transfections. Y1 cells (mouse adrenal cortex cells) generously provided by Dr. Collin Jefcoate (University of Wisconsin) were maintained in Ham’s F-10 media supplemented with 15% horse serum and 2.5% fetal bovine serum. Cells were plated at a density of 8.0 × 10⁴ cells/well of a 24-well plate one prior to transfections.

*Oligonucleotides—* Primers are displayed 5’ to 3’ in the following. Positions of the primers are relative to the initiation codon in rtaHR2β, hAHR, or zfAHR2. Mutated bases are in lowercase letters. Added restriction sites are shown in blue. 3’XbaI site (position 319), CGTGATGGAGGT; 5’Sal site (position 74), TGATGTCGAC.

**Key Difference between the Rainbow Trout AHRs**

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a density of $6 \times 10^4$ cells per well 1 day prior to transfection. Transient transfection was conducted using SuperFect (Qiagen, Chatsworth, CA). Each well was cotransfected with 400 ng of serum containing media, including wild-type or mutant AHR (450 ng), a species-specific ARNT (450 ng) expression vector, 100 ng of a luciferase reporter (pRL-luc or pGudluc1.1) and a β-galactosidase CMV reporter (50 ng) for estimation of transfection efficiency. Y1 cells were plated at 8 × 10^4 cells per well and transfected with the indicated AHR (250 ng/well), luciferase reporter (200 ng/well), and β-galactosidase CMV reporter (100 ng/well). Following a 2-h incubation at 37 °C, 600 μl of fresh serum-containing media was added to each well. After 20-h incubation, cells were exposed to MeSO_4 vehicle control or TCDD previously dissolved in Me_SO (0.1% media volume). Cells were harvested after a 20-h incubation. Media were aspirated, and each well was washed with 0.5 ml of phosphate-buffered saline. 100 μl of lysis buffer was added to each well (100 μM KPO_4, pH 7.8, 6 mM MgSO_4, 0.1% Triton X-100, 1 mM dithiothreitol, and 4 mM ATP trihydrate), 10-μl aliquots of cell lysate were transferred to a 96-well luminometer (Chantilly, VA). β-Galactosidase activity was determined for each well as follows. 15 μl of cell lysate was aliquoted to a 96-well plate. 200 μl of reaction buffer (0.1 M NaPO_4, 10 mM KCl, 1 mM MgCl_2, 0.385% β-mercaptoethanol) was added to each well followed by the addition of 40 μl of 0.1-nitrophenyl-β-D-galactopyranoside (4 mg/ml). The reaction was then incubated at 37 °C for 2–4 h. Plates were read at 405 nm using a Bio-Tek Instruments ELX808 plate reader (Winomski, VT). Results are expressed as luciferase activity normalized to the β-galactosidase activity in each sample. The fold induction was calculated by dividing the relative luciferase activity measured in the presence of TCDD by the activity measured in the corresponding vehicle-treated sample.

Western Blots—COS-7 cells at 70% confluency were transfected with 5 μg of either pBK-CMV empty vector, rtAHR2αFLAG, rtAHR2βFLAG, or rtAHR2βK111EFLAG as described above. Whole cell lysate was harvested 20 h later. Briefly, the cells were rinsed two times with phosphate-buffered saline (157 mM NaCl, 2.7 mM KCl, 8 mM Na_2PO_4, 1.5 mM Na_3EDTA, pH 7.4) containing EDTA and EGTA (1 mM each) and removed from the dish using a Teflon spatula, rinsed with 300 μl of extraction buffer (25 mM MOPS, pH 7.5, containing 1 mM EDTA, 5 mM EGTA, 0.02% Na_3 EDTA, 20 mM Na_2MoO_4, 0.1% Triton X-100, 1 mM dithiothreitol, 5 μg/ml leupeptin, 1 μg/ml aprotinin, and 5 μg/ml pepstatin A), and transferred to a 1.5-M centrifuge tube on ice. Cells were sonicated three times on ice and homogenized using a Dounce homogenizer. Debris was pelleted by centrifugation at 22,000 × g for 30 min. 20 μg of lysate was resolved by SDS-PAGE on an 8% gel and transferred to nitrocellulose. Blots were blocked with 5% dry milk in TBS-T (25 mM Tris (pH 7.6), 150 mM NaCl, 0.1% Tween 20) for 1 h and then washed three times with TBS-T. The FLAG epitope was then detected by incubation with anti-FLAG monoclonal antibody (Sigma Chemical Co., St. Louis, MO) diluted in TBS-T (2 μg/ml) containing 1% dry milk. The antibody was removed after 2 h, and blots were washed with TBS-T three times. Horseradish peroxidase-conjugated secondary antibody (Amersham Biosciences, Inc., Chicago, IL) diluted 1:4000 in TBS-T containing 5% dry milk was added for 1-h incubation. Blots were washed three times in TBS-T prior to chemiluminescence detection (Amersham Biosciences, Inc.).

RESULTS
The rtAHR2α and rtAHR2β proteins are almost 98% identical in primary sequence yet have distinctly different properties. We used transient transfection assays in COS-7 cells to measure transcription properties of these proteins, because these cells are devoid of endogenous AHR and express only a low level of reporter activity, which was not increased by the addition of TCDD (Fig. 1). However, when we used pGudluc1.1, a reporter driven by mammalian sequences derived from the zebrafish cyp1a1 gene (34), we observed a different response. In this case, both AHR proteins were able to produce a readily detected reporter signal that was induced by TCDD. Although rtAHR2α produced stronger transactivation with the pGudluc1.1 reporter than rtAHR2β, rtAHR2β was more responsive to TCDD, as indicated in the bottom right panel of Fig. 1. In this assay, rtAHR2α was induced ~10-fold by TCDD, whereas the activity of rtAHR2β was induced by more than 50-fold, owing to the very low activity in the absence of TCDD. This rtAHR2β basal activity is close to the limit of detection, making calculated values for-fold TCDD induction of rtAHR2β somewhat variable. However, both the low basal activity and the high-fold induction by TCDD were consistently observed with this receptor. These results demonstrate several different properties of these receptor molecules: First, rtAHR2α appears to have stronger transactivation properties than rtAHR2β, producing more luciferase expression with either reporter construct. Second, when assayed with the pGudluc1.1 reporter, rtAHR2β is more tightly regulated by TCDD, owing to the very low activity in the absence of TCDD. This rtAHR2β basal activity is close to the limit of detection, making calculated values for-fold TCDD induction of rtAHR2β somewhat variable. However, both the low basal activity and the high-fold induction by TCDD were consistently observed with this receptor. These results demonstrate several different properties of these receptors: First, rtAHR2α appears to have stronger transactivation properties than rtAHR2β, producing more luciferase expression with either reporter construct. Second, when assayed with the pGudluc1.1 reporter, rtAHR2β is more tightly regulated by TCDD, owing to the very low basal activity. Finally, the two AHR proteins appear to have different requirements for DNA target sequences.

To identify the domain that confers these differences in activity, we constructed chimeric rtAHR2α proteins by exchanging similar domains between rtAHR2α and rtAHR2β and measured the activities of these chimeras with pRL-luc and pGudluc1.1. We then attempted to correlate the presence of a region in the α or β receptor isoforms with the different receptor characteristics. The N-terminal half of the AHR proteins. The first set of chimeric
proteins were made by taking advantage of conserved SphI and BglII restriction sites found in both rtAHR2/H9251 and rtAHR2/H9252. These were used to transfer domains from rtAHR2/H9251 to rtAHR2/H9252 and vice versa (Fig. 3, and see Fig. 2). In chimeras A through F, the ability to produce a robust transactivation signal with the prt1Aluc reporter, a characteristic of rtAHR2/H9251, was observed only in chimeras in which the N-terminal 250 amino acids were from rtAHR2/H9251. Chimeras carrying rtAHR2/H9252 sequence in this region were relatively inactive with the prt1Aluc reporter. Similarly, the tight regulation by TCDD observed in rtAHR2/H9252, characterized by very low basal expression and resulting high -fold induction by TCDD with the pGudluc1.1 reporter, correlated with the presence of the rtAHR2/H9252 sequence in this N-terminal SphI portion of the protein. In addition, the marked preference for pGudluc1.1, which characterizes rtAHR2/H9252, was also conferred by this part of the protein.

This N-terminal SphI fragment encoding the first 250 amino acids contains the majority of the differences between the rtAHR2/H9251 and rtAHR2/H9252 sequences. To narrow down the residues responsible for the functional differences between rtAHR2/H9251 and rtAHR2/H9252 and to possibly dissociate these functions, Fig. 2. Amino acid sequence alignment of rtAHR2α, rtAHR2β, and the human AHR. Sequence alignment of rtAHR2α (GenBank accession number AF065137), rtAHR2β (AF065138), and human AHR (L19872) was done using ClustalW1.8. Asterisks indicate perfect identity to rtAHR2α, and dashes indicate gaps in sequence alignment. The basic, HLH, and PAS domains are indicated by lines above the sequence. The ligand-binding domain is indicated by underlining (17, 18). The nuclear localization signal and nuclear import signal (16) are shaded.

rtAHR2α: M-LSN-TGVYAVKRR KEFPVTKSQAPDVY VKSMPSFKEIRELNG ELSDRTIPGLFPEMV ERSLDVKSLVRISVG YLKVFSFFKTMMKS
rtAHR2β: G-NS*:SANIT*R*E R***V*P**P**E I******T ******A*Q**O*E* I**K********L*E***R**A**A*D**S* 90

human AHR: N*SA*NI*T*E*R*E R***V*P**P**E I******T ******A*Q**O*E* I**K********L*E***R**A**A*D**S* 90

SphI

BglII

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tional differences, we made an additional set of chimeras, G and H. In these chimeras the regions between position 86 and the SphI site, containing the PAS-A domain, are swapped. The designation of the junction at position 86 is arbitrary, because this junction occurs in a region of sequence identity; the actual point of the junction can be considered anywhere between positions 84 and 93 on rtAHR2/H9251. The ability to produce a robust transactivation signal with the prt1Aluc reporter was observed in chimera H, which carried rtAHR2/H9251 sequence between positions 86 and 250, but not in the chimera containing rtAHR2/H9252 sequence in this region (Fig. 3). As observed with the previous set of chimeras, the tight regulation by TCDD observed with rtAHR2/H9252 and the pGudluc1.1 reporter was also conferred by this region of the protein. The chimera G, containing rtAHR2/H9252 sequence between positions 86 and 250, showed low activity with prt1Aluc and high -fold induction with pGudluc1.1. Thus, this portion of the protein confers both the rtAHR2/H9251- and rtAHR2/H9252-specific characteristics.

The domain from residues 86 to 250 found to be responsible for the differential transactivation properties of the two rtAHR2s is differentiated by 8 residues. Seven of these differences lie near the N terminus of the PAS-A domain (Fig. 2). To identify specific residues that confer the differential transactivation properties, we made a chimera in which the residues in rtAHR2β sequence between positions 86 and 250, showed low activity with prt1Aluc and high -fold induction with pGudluc1.1. This, thus portion of the protein confers both the rtAHR2α- and rtAHR2β-specific characteristics.

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The glutamate at position 110 in rtAHR2α corresponding to lysine 111 in rtAHR2β is conserved among the known AHRs that bind TCDD. To be certain that the lysine identified at residue 111 in rtAHR2β was not an allelic variant or cloning artifact, RNA was isolated from four separate hatchery strains:

FIG. 3. TCDD responsiveness of rtAHR2 chimeras in a transient transfection assay. COS-7 cells were transiently transfected with expression vectors for the indicated rtAHR2 chimeras as described for Fig. 1. Maps of the AHR open reading frames indicate the positions of the chimera junctions. Dark bars indicate rtAHR2α sequence, and the light bars represent rtAHR2β sequence. Data are expressed in the upper panels as β-galactosidase normalized relative light units: light bars, TCDD-exposed; dark bars, vehicle control. Results are expressed as -fold induction by TCDD in the lower panels. The results are expressed as the means of three independent replicates ± S.E.
Arlee, Eagle Lake, McConoughy, and Shasta (n = 3 for each strain). Amplified cDNAs for rtAHR2α and rtAHR2β were generated. In addition, clones were obtained from cDNA prepared from a rainbow trout gonadal cell line (RTG-2). All samples generated. In addition, clones were obtained from cDNA prepared

FIG. 4. TCDD responsiveness of rtAHR2 chimeras created by point mutations in rtAHR2β. COS-7 cells were transiently transfected with expression vectors for the indicated rtAHR2 chimeras as described for Fig. 1. The change in amino acid altered in rtAHR2β to that found in rtAHR2α is labeled as the rtAHR2β residue followed by the position number and corresponding altered amino acid. A, data are expressed in the upper panels as β-galactosidase normalized relative light units. Light bars, TCDD-exposed; dark bars, vehicle control. B, data are expressed as -fold induction by TCDD. The results in both A and B are expressed as the means of three independent replicates ± S.E. C, Western blot of the transfected FLAG epitope-tagged rtAHR2α, rtAHR2β, and the rtAHR2β K111E substitution chimera proteins.

We also determined whether the presence of lysine at position 111 in other AHRs, an rtAHR2β mutant was constructed in which the lysine at position 111 was replaced by alanine (rtAHR2βK111A). This produced a receptor with characteristics that were somewhat intermediate between rtAHR2α and rtAHR2β (Fig. 5). This protein was a stronger transactivator than rtAHR2β, but weaker than rtAHR2α. Similarly, when assayed with the pGudluc1.1 reporter, the rtAHR2βK111A mutant had a higher -fold induction by TCDD than the K111E mutant, but lower -fold induction than the normal rtAHR2β. This suggests that the glutamate in rtAHR2α and the corresponding lysine in rtAHR2β both play important roles in determining the characteristics of their receptors.

We also determined whether the presence of this lysine at the corresponding position in other AHR proteins would produce characteristics of rtAHR2β in these AHRs. When the zebrafish AHR2 was mutated from glutamate to lysine at position 114 (corresponding to position 111 in rtAHR2β) the mutated zfAHR2 displayed the characteristics of rtAHR2β. These included decreased transactivation, a requirement for prtGudluc1.1 as a reporter, and a low basal level of activity with a corresponding increase in -fold responsiveness to TCDD (Fig. 5).

A similar mutation was made in the human AHR (hAHR) in which glutamate 118 corresponding to K111 in rtAHR2β was mutated to lysine (hAHRK118E) (Fig. 6). This also produced a decrease in transactivation and an increase in TCDD responsiveness characteristic of rtAHR2β. These results underscore the importance of this residue, which is consistently conserved among all known AHRs, other than rtAHR2β.

DISCUSSION

Our results indicate that the lysine at position 111 in rtAHR2β and the corresponding glutamate at position 110 in rtAHR2α play important roles in the functions of the proteins that they reside in. The residue at this position affects transactivation, the degree of activation by ligand, and enhancer specificity. In rtAHR2α the glutamate confers increased transactivation function at either of the two reporters used in our experiments. Substitution of glutamate for the lysine at position 111 on rtAHR2β produced a mutant rtAHR2β with increased transcriptional activity, similar to that of rtAHR2α. Although the rtAHR2β K111E mutant gained these rtAHR2α-like properties, it lost other properties, i.e. high -fold induction by TCDD with pGudluc1.1 and enhancer specificity, that are characteristic of rtAHR2β. These changes in receptor properties are apparently entirely the result of the lysine at position 111. The residues that differ at this position between rtAHR2α and rtAHR2β (glutamate in rtAHR2α and lysine in rtAHR2β) are substantially different in charge and could therefore be
expected to produce different conformations in the respective proteins.

With the exception of rtAHR2β, all other known AHRs have a glutamate at the residue corresponding to rtAHR2β’s lysine 111. This complete conservation in sequence implies a conserved function among these AHR proteins. Because rtAHR2β does not share this conserved residue, our results suggest that rtAHR2β diverges in function in some way from these other AHRs.

Our results complement a body of work that has identified distinct functional domains in the AHR protein. A number of laboratories have identified residues that are required for transactivation, ligand binding, protein-protein interactions, and DNA binding. In addition to identifying the C terminus as a transactivation domain (17, 37-39) specific subdomains within the C terminus that are necessary for transactivation have been identified (40, 41). Mutation of residue 678 in hAHR blocks transactivation without affecting DNA binding (41). The domain spanning residues 230-421 containing the PAS-B domain has been shown to bind ligand, and residue 381 in hAHR is important for ligand binding (35). Alterations at positions 78 and 216 in the mouse AHR affect DNA binding (42, 43). Substitutions in the basic domain alter DNA binding activity (44). Differences in sensitivity to TCDD toxicity among rodent species have been attributed to differences in the C-terminal sequences of the respective AHRs (45, 46). Our results are significant, because they identify a part of the AHR protein that appears to play a role in the ligand-regulated interactions between these previously identified domains. This suggests that the residue at this position in AHR is in communication with domains responsible for these different processes.

Position 111 in rtAHR2β corresponds to the N-terminal border of the PAS-A domain that is strongly conserved in AHR proteins (Fig. 2). This position lies between the bHLH and PAS domains. Thus, it is close to regions involved in DNA binding, dimerization with ARNT and other proteins, as well as nuclear import and export signals. In addition, ligand binding in the PAS-B region must in some way transmit conformational differences.
changes to the PAS-A and helix-loop-helix domains. The residue at position 111 may be in a position to affect these processes. Clearly, a difference in residue polarity at such a central location could alter the function of the rTAHR2s.

The crystal structure for the PAS domain of photoactive yellow protein (PYP) from Halorhodospira halophila has been determined (47). This structure has been proposed as a model for PAS domains in eukaryotic proteins such as ARNT and AHR (48). Fig. 7 shows the structure of the PYP PAS domain with the position corresponding to the location of the rTAHR2β K111 highlighted in yellow on the polypeptide backbone (in PYP, this residue is methionine). Although lysine in position 111 in rTAHR2β is unique among AHRs, this region is not strongly conserved among all PAS proteins, and lysine is found at this position in some bacterial PAS family proteins. The degree of conservation between PAS domains from different proteins is indicated by color as well, with warmer colors indicating higher conservation (Fig. 7). It can be seen that the residue in question lies at the juncture between the conserved PAS-A domain and the remainder of the N terminus of the protein. In PYP, this segment is referred to as the N-terminal cap, a part of the structure that helps enclose the hydrophobic portion of the PAS core (48). PAS domains are thought to function as protein interaction domains that can be regulated more than the process of translocation to the nucleus and dimerization with ARNT, processes that by themselves should be DNA-independent and unaffected by the enhancers.

As noted previously by Whitelaw et al. (37) transcriptional activation and TCDD responsiveness are substantially influenced by the enhancer elements used in the experiments. rTAHR2α is more responsive to TCDD than rTAHR2β with pRT1αuc, but this is reversed with gPuduc1.1. Although rTAHR2α appears to be a stronger transactivator than rTAHR2β, we cannot rule out the possibility that there might be promoter sequences with which rTAHR2β is the stronger transactivator. Thus, using the in vitro activities of these proteins alone to draw conclusions about function is limited by our current uncertainty about the gene targets for these receptors. Identifying these targets will be an important step forward in understanding AHR function in fish.

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REFERENCES

1. Ga, Y. Z., Hugeneesch, J. B., and Bradfield, C. A. (2000) Annu. Rev. Pharmacol. Toxicol. 40, 519–561
2. Rowlands, J. C., and Gustafsson, J. A. (1997) Crit. Rev. Toxicol. 27, 109–134
3. Denison, M. S., and Heath-Pagliuso, S. (1998) Bull. Environ. Contam. Toxicol. 60, 557–566
4. Carver, L. A., Jackiw, V., and Bradfield, C. A. (1994) J. Biol. Chem. 269, 30109–30112
5. Carver, L. A., and Bradfield, C. A. (1997) J. Biol. Chem. 272, 11452–11456
6. Chen, H. S., and Perdew, G. H. (1994) J. Biol. Chem. 269, 27554–27558
7. Ma, Q., and Whitlock, J. P., Jr. (1997) J. Biol. Chem. 272, 8878–8884
8. Meyer, B. K., Petrusis, J. K., and Perdew, G. H. (2000) Cell Stress Chaperones 5, 243–254
9. Schmidt, J. V., and Bradfield, C. A. (1996) Annu. Rev. Cell Dev. Biol. 12, 58–89
10. Whitlock, J. P., Jr (1999) Annu. Rev. Pharmacol. Toxicol. 39, 103–125
11. Ma, Q., Renzelli, A. J., Baldwin, K. T., and Antonini, J. M. (2000) J. Biol. Chem. 275, 12676–12683
12. Ma, Q., and Baldwin, K. T. (2000) J. Biol. Chem. 275, 8432–8438
13. Pollenz, R. S. (1996) Mol. Pharmacol. 49, 391–398
14. Pollenz, R. S., and Barbour, E. R. (2000) Mol. Cell. Biol. 20, 6905–6914
15. Berg, P., and Pongrizer, I. (2001) J. Biol. Chem. 276, 43231–43238
16. Ikuta, T., Eguchi, H., Tachibana, T., Yoneda, Y., and Kawajiri, K. (1998) Biochim. Biophys. Acta 1385, 293–301
17. Fukunaga, B. N., Probst, M. R., Reiss-Porszasz, S., and Hankinson, O. (1995) J. Biol. Chem. 270, 29270–29278
18. Coumarine, P., Puellinger, L., Gustafsson, J. A., and Whitelaw, M. L. (1995) J. Biol. Chem. 270, 25291–25300
19. Bell, D. R., and Poland, A. (2000) J. Biol. Chem. 275, 36407–36414
20. Meyer, B. K., and Perdew, G. H. (1999) Biochemistry 38, 8907–8917
21. Puga, A., Barnes, S. J., Dalton, T. P., Chang, C., Knudsen, E. S., and Maier, M. A. (2000) J. Biol. Chem. 275, 2943–2950
22. Elmen, G. E., Sphar, R. L., Holcombe, G. W., and Johnson, R. D. (1998) Environ. Toxicol. Chem. 17, 472–483
23. Walker, M. K., and Peterson, R. E. (1991) Aquat. Toxicol. 21, 219–238
24. Zabel, R. W., Cook, P. M., and Peterson, R. E. (1995) Aquat. Toxicol. 31, 315–328
25. Zabel, E. W., Pollenz, R., and Peterson, R. E. (1996) Environ. Toxicol. Chem. 15, 2120–2138
26. Hahn, M. E., Karchner, S. I., Shapira, M. A., and Perera, S. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13743–13748
27. Abnet, C. C., Tanguay, R. L., Hahn, M. E., Heideman, W., and Peterson, R. E. (1999) J. Biol. Chem. 274, 15159–15166
28. Karchner, S. I., Powell, W. H., and Hahn, M. E. (1999) J. Biol. Chem. 274, 33814–33824
29. Roy, N. K., and Virgin, I. (1997) Arch. Biochem. Biophys. 344, 373–386
30. Tanguay, R. L., Abnet, C. C., Heideman, W., and Peterson, R. E. (1999) Biochim. Biophys. Acta 1444, 35–48
31. Pollenz, R. S., Sullivan, H. R., Holmes, J., Necela, B., and Peterson, R. E. (1996) J. Biol. Chem. 271, 30886–30896
32. Powell, W. H., Karchner, S. I., Bright, R., and Hahn, M. E. (1999) Arch. Biochem. Biophys. 361, 156–163
33. Tanguay, R. L., Andreasen, E., Heideman, W., and Peterson, R. E. (2000) Biochim. Biophys. Acta 1494, 117–128

Fig. 7. Location of rTAHR2β residue K111 on a model of PAS domain structure. The amino acid sequence for the PAS-A domain from rTAHR2β was aligned by RPS-BLAST to the consensus PAS domain sequence in the NCBI database. The structure model was produced by the Cn-3D 3.0 structure viewer (www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml). Red indicates strongly conserved PAS-domain sequences. The position of the K111 residue is indicated in yellow.
Key Difference between the Rainbow Trout AHRs

34. Garrison, P. M., Tullis, K., Aarts, J. M., Brouwer, A., Giesy, J. P., and Denison, M. S. (1996) *Fundam. Appl. Toxicol.* 30, 194–203
35. Ema, M., Ohe, N., Suzuki, M., Mimura, J., Sogawa, K., Ikawa, S., and Fuji-Kuryama, Y. (1994) *J. Biol. Chem.* 269, 27337–27343
36. Abnet, C. C., Tanguay, R. L., Heideman, W., and Peterson, R. E. (1999) *Toxicol. Appl. Pharmacol.* 159, 41–51
37. Whitelaw, M. L., Gustafsson, J. A., and Poellinger, L. (1994) *Mol. Cell. Biol.* 14, 8343–8355
38. Jain, S., Dolwick, K. M., Schmidt, J. V., and Bradfield, C. A. (1994) *J. Biol. Chem.* 269, 31518–31524
39. Ma, Q., Dong, L., and Whitlock, J. P., Jr. (1995) *J. Biol. Chem.* 270, 12697–12703
40. Jones, L. C., and Whitlock, J. P., Jr. (2001) *J. Biol. Chem.* 276, 25037–25042
41. Kumar, M. B., Ramadoss, P., Reen, R. K., Vanden Heuvel, J. P., and Perdew, G. H. (2001) *J. Biol. Chem.* 276, 42302–42310
42. Levine, S. L., Petrusis, J. R., Duhl, A., and Perdew, G. H. (2000) *Mol. Pharmacol.* 58, 1517–1524
43. Sun, W., Zhang, J., and Hankinson, O. (1997) *J. Biol. Chem.* 272, 31845–31854
44. Bacsi, S. G., and Hankinson, O. (1996) *J. Biol. Chem.* 271, 8843–8850
45. Korkalainen, M., Tuomisto, J., and Pohjanvirta, R. (2000) *Biochem. Biophys. Res. Commun.* 273, 272–281
46. Korkalainen, M., Tuomisto, J., and Pohjanvirta, R. (2001) *Biochem. Biophys. Res. Commun.* 285, 1121–1129
47. Borgstahl, G. E., Williams, D. R., and Getzoff, E. D. (1995) *Biochemistry* 34, 6278–6287
48. Pellequer, J. L., Wager-Smith, K. A., Kay, S. A., and Getzoff, E. D. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 5884–5890