A Novel Cytoplasmic Protein That Interacts with the Ah Receptor, Contains Tetrarcipeptide Repeat Motifs, and Augments the Transcriptional Response to 2,3,7,8-Tetrachlorodibenzo-p-dioxin*

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Qiang Ma and James P. Whitlock, Jr.‡
From the Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, California 94305-5322

To identify new proteins involved in dioxin-dependent signal transduction and transcriptional regulation, we used a yeast two-hybrid system to identify proteins that interact with the Ah receptor (AhR). We cloned a mouse cDNA, which encodes a novel 37-kDa protein that binds to AhR; we have designated the protein as Ah receptor-interacting protein (AIP). The amino acid sequence of mouse AIP exhibits homology with members of the FK506-binding protein family. AIP also contains three tetrarcipeptide repeat (TPR) motifs; the TPR sequence is present in proteins required for cell cycle control and RNA synthesis and in steroid receptor-binding immunophilins. Coimmunoprecipitation experiments in mouse hepatoma cells reveal that AIP is cytoplasmic and associates with unliganded Ah receptor and with hsp90; 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment disrupts the AhR-AIP-hsp90 interaction. Overexpression of AIP augments the response of the CYP1A1 gene to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Our data suggest that AIP influences ligand receptivity and/or nuclear targeting of AhR.

The aromatic hydrocarbon receptor (AhR) is a cytoplasmic, ligand-activated, basic helix-loop-helix (bHLH) transcription factor, which functions together with a second bHLH protein, the Ah receptor nuclear translocator (Arnt), in mediating the induction of CYP1A1 gene expression in response to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (1–3). CYP1A1 encodes the microsomal cytochrome P450 1A1 enzyme, which oxygenates polycyclic aromatic hydrocarbons, such as the carcinogen, benzo(a)pyrene (4). AhR and Arnt have similar modular structures. The bHLH domains are located toward the amino termini of the proteins; the HLH regions mediate dimerization between AhR and Arnt, whereas the basic regions are involved in DNA recognition by the AhR-Arnt heterodimer (5–8). Both AhR and Arnt contain two inverted repeats (designated as PAS regions), which share homology with Per and Sim, two Drosophila proteins involved in circadian rhythm and central nervous system development, respectively (9, 10). The PAS regions may contribute to AhR-Arnt dimerization, ligand/hsp90 binding, and suppression of AhR’s transactivation activity (7, 11, 12). The carboxyl regions of AhR and Arnt contain transactivation domains that contribute to transcriptional control by the AhR-Arnt heterodimer (12–15).

Induction of CYP1A1 gene transcription by TCDD is an interesting response for analyzing AhR function. Biochemical and genetic analyses of induction have revealed a multi-step pathway of AhR action (3, 16). In uninduced cells, the unliganded AhR is localized in the cytoplasm, complexed with hsp90. Upon ligand binding, AhR dissociates from hsp90 and enters the nucleus, where it interacts with Arnt. The AhR-Arnt heterodimer binds to a specific nucleotide sequence within an enhancer upstream of the CYP1A1 gene; this process is accompanied by alterations in chromatin structure, binding of general transcription factors to the promoter, and induction of transcription (16, and references therein).

Genetic and biochemical studies implicate AhR in numerous biological responses to TCDD, in addition to CYP1A1 induction (17–19). In experimental animals, TCDD induces a broad range of adaptive and toxic responses, including induction of other xenobiotic-metabolizing enzymes, tumor promotion, thymic involution, skin disorders, and alterations in endocrine homeostasis (17, 18). Recent studies imply that AhR is also required for normal liver development and immune function in mice (20, 21) and is involved in cell cycle control and differentiation in mouse and rat hepatoma cells (22, 23). Thus, AhR contributes to many complex cellular functions; therefore, we envisioned that AhR-mediated gene regulation may involve additional components that remain to be identified.

To test this hypothesis, we have used a yeast two-hybrid screening procedure to find new proteins that interact with AhR, and we have cloned a protein that we designate as AhR-interacting protein (AIP). Our findings reveal that AIP is a novel cytoplasmic protein, which binds to the unliganded AhR. Furthermore, overexpression of AIP in mouse hepatoma cells increases the response of the CYP1A1 gene to TCDD. Our results imply that AIP plays a positive role in AhR-mediated signaling, possibly by influencing AhR’s receptivity for ligand and/or AhR’s nuclear targeting.

EXPERIMENTAL PROCEDURES

Materials—Vent polymerase was from New England BioLabs (Beverly, MA); Restriction endonucleases and other DNA-modifying enzymes were from Life Technologies, Inc. and Promega (Madison, WI). Radioactive compounds were purchased from Amersham Corp. Cell culture materials were from Life Technologies, Inc. Media for yeast culture were from Bio 101, Inc. (Vista, CA) and Difco. Reduced glutathione and glutathione-agarose were from Sigma. Expression plasmids
pGEX-4T3 and pET28a were from Pharmacia Biotech Inc. and Novagen (Madison, WI), respectively. TCDD was from the National Cancer Institute, Institute Chemical Carcinogen Reference Standard Repository.

Cell Culture—Wild-type hepali1c17, Ah receptor-defective (Ahr-D) cells, and ArnT-defective (ArnT-D) cells were grown as monolayers in alpha-MEM with 10% fetal bovine serum. The wild-type, AHR-D, and ArnT-D cells were maintained in DMSO-containing medium, in a 5% CO2 atmosphere, at 37 °C, as described previously (24).

Yeast Two-hybrid Screening—The full-length coding region of mouse AhR cDNA was generated by PCR (12) and was fused to the sequence encoding the Ga4 DNA-binding domain (DBD) in plasmid pG8T9 (Clontech) at the Sma1 site. The resulting plasmid expresses Ga4 DBD-Ahr fusion protein under the control of the HIS3, and was designated as pG8T9-Ahr. The plasmid was cotransformed into yeast strain HF7c together with a library in which cDNAs from HeLa cells were fused to the Ga4 transcriptional domain (AD) in pGaDGH (Clontech), using protocols suggested by Clontech. The interaction of the two hybrid proteins reconstitutes a functional Ga4 protein that activates transcription of two reporter genes, lacZ and HIS3, which were integrated into the genome of the yeast host and respond to the Ga4 transcription activator. Plasmids were isolated from His- and β-galactosidase-positive clones and were transformed into HB101, an Escherichia coli strain that is auxotrophic for leucine. Colonies containing the putative positive cDNAs were selected on minimal medium lacking leucine. To test the specificity of the interaction between AhR and ArnT, putative cDNA clones, plasmids were transformed into yeast strain SPY526 that carries a Ga4-responsive lacZ reporter gene (Clontech), together with the empty plasmid pG8T9, pG8T9-AhnTNT which encodes a fusion protein of Ga4 DBD, and the amino acids 1–470 of AhR (25) or pG8T9-AhR. Positive clones were identified by their β-galactosidase activity in filter assays. Using this approach, we isolated a HeLa cDNA fragment (~1 kb), which encodes a peptide that interacts with AhR.

Sequence Analysis and Cloning of Full-length cDNA—The nucleotide sequence of the HeLa 1-kb clone was determined by the dideoxynucleotide chain termination method. To obtain a full-length mouse cDNA, the 1-kb fragment was used to screen a mouse hepatoma (hepal1c17) cDNA library. A cDNA fragment (~1.35 kb) in the pBK/CMV vector (Stratagene) was isolated, as described previously (25), and was subcloned as pAIP/BKCMV. Sequence analyses were performed with GCG programs (Genetic Computer Group Sequence Analysis Software Package).

Protein Expression in E. coli and Preparation of Polyclonal Antibodies—The EcoRI-Xhol fragment of the 1-kb cDNA was excised from the two-hybrid positive clone and was inserted into a bacterial expression vector, pGEX-4T3 (Pharmacia-LKB) for expression and purification. The plasmid was transformed into E. coli strain BTH101, which carries the expression vector, and the recombinant E. coli was grown in 60-mm diameter plates and were treated with TCDD (1 nM). Dimethyl sulfoxide was the vehicle control. Sixteen hours later, the medium was removed and the plates were washed twice with phosphate-buffered saline. Cells were scraped into 400 μl of buffer A (see above) and were sonicated for 5 s. Aliquots of the whole cell lysate were mixed with 600 μl of buffer A and were incubated with anti-T7.tag antibody (Novagen) for 1 h at 4 °C with shaking. In other experiments, aliquots of the whole cell lysate were incubated with anti-AhR serum at 4 °C for 1 h, followed by incubation with protein A-agarose beads (Bio-Rad) for an additional 1 h with shaking. The beads were pelleted by brief centrifugation and were washed five times with ice-cold 25 mM HEPES, pH 7.5, 1.5 mM EDTA, 1 mM dithiotreitol, 10% glycerol, 400 mM NaCl, and 0.5% Triton X-100. The beads were boiled for 5 min in 40 μl of SDS sample buffer. Equal aliquots were fractionated on three separate SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and probed with different antibodies, as indicated under “Results.”

Immunoblot, Northern, and Slot Blot Analyses—For immunoblotting, total cell lysates, cytosolic and nuclear fractions, or coprecipitated pellets were prepared, fractionated on SDS-polyacrylamide gels, and transferred to nitrocellulose membranes and probed with different antibodies, as indicated under “Results.”

For RNA analysis, total RNA was isolated from hepatoma cells using the Qiagen total RNA purification system. Ten μg of RNA were fractionated on a 1% agarose gel, transferred to a Nitro membrane using the Rapid-hyb buffer (Amersham Life Sciences, Inc.). After autoradiography, the blots were stripped and were reprobed with a radiolabeled probe specific for mouse β-actin, as a control. For slot blots, 5 μg of total RNA were blotted to a Nitro membrane using a Minifold II slot-blotter (Schleicher & Schuell). The blots were probed with the CYP1A1 and the actin probes as described above.

RESULTS

Cloning and Sequence Analysis of AhR-interacting Protein—To find new proteins that interact with AhR, we used a yeast two-hybrid system, which identifies proteins that interact with a particular bait protein (28). For bait, full-length AhR...
Fig. 1. Primary structure of mouse AIP. A, the nucleotide and predicted amino acid sequences of mouse AIP. A region with sequence similarity to FKBP12 is shown in italics. Three tetratricopeptide repeat (TPR) regions are underlined. B, alignment of TPR sequences. The amino acid sequences are given in one-letter code.
TPR-containing Ah Receptor-interacting Protein

Figure 2. Expression of AIP in mouse hepatoma cells. A, Northern blot of AIP mRNA. Total RNA (10 μg) from wild-type hepa1c1c7 cells was analyzed. Upper panel, AIP; lower panel, β-actin. B, immunoblot of AIP protein. Aliquots of total cell lysate (20 μg) from wild-type hepa1c1c7, AhR-D, and Arnt-D were analyzed as described under “Experimental Procedures.”

cDNA was fused to cDNA encoding the Gal4 DNA-binding domain. The Gal4-AhR fusion protein is incapable of transcriptional activation by itself, possibly due to the existence of an inhibitory domain(s) in AhR (12).

Using Gal4-AhR as bait, we screened a yeast two-hybrid library derived from HeLa cell cDNAs. After screening 6 million colonies, we obtained several positive clones that grew on selective media lacking Leu, Trp, and His and were positive for β-galactosidase. Four of the clones interacted with AhR following transformation into yeast strains containing various bait constructs (data not shown). Sequence analyses of the clones implied that they were probably derived from the same mRNA species. Northern blot analysis of total RNA from HeLa cells, using the largest cDNA fragment (~1 kb) as a probe, revealed a mRNA band of ~1.35 kb (data not shown).

To obtain full-length cDNA, we used the HeLa 1-kb fragment as a hybridization probe to screen a mouse hepatoma (hepa1c1c7) cDNA library. Ten overlapping clones were isolated and analyzed. The largest clone contains a 1367-base pair insert (Fig. 1A). Two lines of evidence support the conclusion that this 1.37-kb cDNA clone represents the full-length mouse homologue of the HeLa 1-kb clone. (i) The 1.37-kb fragment exhibits high homology with the HeLa cDNA sequence (~87% identity). (ii) Northern blot analysis of mouse hepatoma mRNA using the 1.37-kb fragment as a probe identified a single band of ~1.37 kb (Fig. 2A), which agrees with the size of the mRNA transcript recognized by the HeLa 1-kb fragment. We designated the mouse 1.37-kb clone as “AhR-interacting protein” (AIP).

Analyses of the nucleotide sequence of mouse AIP cDNA indicate that it contains an open reading frame with a putative Kozak sequence (29) for translation initiation upstream of the first ATG codon of the reading frame. A 22-nucleotide poly(A) tail is located at the end of the 3′-untranslated region of the cDNA. The predicted amino acid sequence of mouse AIP (Fig. 1A) reveals 330 amino acid residues, a molecular mass of 37,602 daltons, and a pI of 5.96. Immuno blot analysis of wild-type, AhR-defective, and Arnt-defective cells using polyclonal antibodies against AIP revealed a single band of ~37 kDa in wild-type, Arnt-defective, and AhR-defective hepatoma cells (Fig. 2B), in good agreement with the protein’s predicted molecular weight.

Inspection of AIP’s amino acid sequence revealed a region (between residues 12 and 160) that exhibits sequence homology with human FKBP12 (30), with 52% similarity and 32% identity (Fig. 1A). AIP also exhibits homology with human FKBP59 (also known as p59 and hsp56, Ref. 31) (50% similarity and 27% identity) and mouse p51 (32) (50% similarity and 28% identity). These findings indicate that AIP is structurally related to the FKBP family of proteins, which function as molecular chaperones in steroid receptor signaling, heat shock responses, and drug-induced immunosuppression (33, 34). Analyses of the AIP sequence also reveal three regions that exhibit homology with the tetratricopeptide repeat (TPR), a motif found in several proteins that mediate a variety of functions, including the targeting of steroid receptors to the cell nucleus (35, 36). This finding suggests that AIP might participate in the movement of AhR from cytoplasm to nucleus.

Characterization of the AIP-AhR Interaction—To examine the functional nature of the AIP-AhR interaction and to determine whether AIP can also interact with Arnt, we used a Gal4-responsive, yeast two-hybrid lacZ reporter system in which testing proteins were fused to the DNA binding (DBD) or activation (AD) domains of Gal4. Fig. 3A shows a representative result of such studies. These results reveal that neither AIP nor AhR, by itself, activates the lacZ reporter gene; however, the two proteins interact to produce an increase in lacZ gene expression comparable with that produced by the AhR-Arnt interaction. Since Arnt protein contains a constitutive transactivation activity toward its carboxyl terminus (25), we used a Gal4-ArntNT fusion in which Gal4 DBD was fused with the amino-terminal region of Arnt to test the interaction of AIP with Arnt. Our results indicate that AIP does not interact productively with Arnt’s amino-terminal region, which contains its bHLH and PAS domains. Together, these observations imply that AIP exhibits selectivity toward AhR (compared with Arnt) and that the AIP-AhR interaction is capable of producing a biological response.

Immunoprecipitation experiments using T7-tagged AIP reveal that AhR, but not Arnt, coprecipitates with AIP (Fig. 3B). We obtained similar results using GST-tagged AIP (data not shown). These findings are consistent with our observations in yeast; they imply that AIP preferentially interacts with AhR and not with Arnt, even though AhR and Arnt have similar modular organizations. The basis for the preferential interaction remains to be determined.

To study the AIP-AhR interaction under more physiological conditions, and to determine the effect of TCDD upon the interaction, we performed immunoprecipitation experiments using wild-type, AhR-defective, and Arnt-defective cells, in which we expressed a AIP-T7.tag fusion protein (AIP-T7) that permits immunochemical studies with a monoclonal antibody against the T7.tag sequence at the amino terminus of the fusion protein. Fig. 4A shows that AIP-T7 was overexpressed in wild-type, Arnt-D, and AhR-D hepatoma cells as compared with endogenous native AIP, and the expression levels were comparable in these cell lines. Immunoprecipitation studies (Fig. 4B) reveal that, in extracts from uninduced wild-type cells, AIP coprecipitates with AhR and hsp90. Because unliganded AhR and hsp90 are cytoplasmic proteins (Fig. 5, Ref. 37),...
Our findings imply that AIP, hsp90, and AhR exist as a complex in the cytoplasm of uninduced cells. In contrast, we find that little hsp90 and virtually no AhR coprecipitates with AIP in TCDD-induced wild-type cells. We obtained similar results using uninduced and TCDD-induced wild-type cells that express AIP in normal amounts; these findings verify that AIP interacts with AhR under physiological conditions (Fig. 4C). Our observations imply that TCDD binding by AhR is associated with disruption of the AhR-AIP-hsp90 complex. Immunoprecipitation experiments using extracts from Arnt-defective cells reveal that AIP, hsp90, and AhR coprecipitate even in extracts from TCDD-treated cells (Fig. 4B). These findings indicate that liganded AhR can maintain an interaction with AIP and hsp90 and that the presence of Arnt is necessary for disruption of the three-protein complex. Studies in AhR-defective cells reveal that AIP coprecipitates with hsp90 even in the absence of detectable AhR (Fig. 4B). These findings imply that AIP interacts with hsp90, as well as with AhR.

We determined AIP's subcellular location by immunoblotting (Western) analyses of cytosolic and nuclear extracts from wild-type cells. Our findings (Fig. 5) reveal that AIP is present in the cytosol, but not in the nucleus, in both uninduced and TCDD-induced cells. In contrast, AhR is cytosolic in uninduced cells but is nuclear in TCDD-induced cells, as expected. These findings imply that AIP is cytoplasmic. Its location in the cytoplasm imposes a constraint upon the possible mechanisms by which AIP could influence AhR-mediated signaling.

**Fig. 3. Characterization of the AIP-AhR interaction.** A, interaction between AIP and AhR in yeast. The indicated fusion proteins were coexpressed in yeast (strain SFL5326) as described under "Experimental Procedures." β-Galactosidase activity was detected in a colorimetric filter assay. Three independent yeast clones were included for each test (labeled as A, B, and C). DBD, fusion proteins containing Gal4 DNA-binding domain; AD, fusion proteins containing Gal4 activation domain. The Gal4 AD-AIP and Gal4 AD-Arnt fusions were constructed by subcloning AIP and Arnt into pGADGH and pGAD424, respectively. Other Gal4 fusion constructs were described under "Experimental Procedures." B, interaction between AIP and AhR in vitro. AhR and Arnt proteins were translated in a reticulocyte lysate system in the presence of β3H)methionine and were coprecipitated using the AIP-T7 fusion protein and an anti-T7-agarose conjugate. The bound proteins were fractionated by SDS-polyacrylamide gel electrophoresis and were visualized by fluorography.

**Fig. 4. Interaction between AIP and AhR in situ.** A, expression of AIP-T7 fusion protein. Wild-type, Arnt-D, and AhR-D hepatoma cells were infected with viruses that express AIP-T7 fusion protein. The AIP-T7 (upper band) and native AIP (lower band) proteins were analyzed by immunoblotting of cell lysates with antibodies against AIP, as described under "Experimental Procedures." B, immunoprecipitation of AIP, AhR, and hsp90 from cells that express AIP-T7 fusion protein. Cell lysates from dimethyl sulfoxide- or TCDD-treated cells were precipitated with antibody against the T7.tag. Precipitates were fractionated by SDS-polyacrylamide gel electrophoresis, transferred to membranes, and probed with antibodies against T7.tag (upper panel), AhR (middle panel), or hsp90 (lower panel). The positions of molecular weight markers are indicated. Cytosolic proteins from wild-type cells were analyzed (lane 9) to show the positions of AIP-T7, AhR, and hsp90. C, interaction between native AIP and AhR in situ. Lysates from wild-type hepa1c1c7 cells were adsorbed to antibodies for AIP, and the precipitates were analyzed by immunoblotting using antibodies against AhR as described above.

**Effect of AIP Overexpression on AhR-mediated CYP1A1 Induction by TCDD**—To analyze the functional importance of AIP, we asked whether overexpression of AIP influenced the response of the CYP1A1 gene to TCDD. Our findings reveal that induction of CYP1A1 mRNA is increased 2–3-fold in wild-type cells that overexpress AIP (Fig. 6). Thus, AIP has a positive influence on AhR-mediated signaling. Dose-response studies (Fig. 6A) reveal that AIP has no detectable effect on the sensitivity of the CYP1A1 induction mechanism to TCDD.
These findings imply that AIP does not alter the affinity of AhR for TCDD. Instead, AIP increases the extent of CYP1A1 induction at each time point studied (Fig. 6B). Therefore, our data imply that AIP increases the amount of AhR that is transcriptionally active.

**DISCUSSION**

We have cloned and characterized a novel 37-kDa protein that interacts with AhR in the cytoplasm of the cell. We have designated the protein as AhR-interacting protein (AIP). Our studies suggest that AIP may act in concert with hsp90 and may contribute molecular chaperone and/or nuclear targeting activities to AhR-mediated signaling. In doing so, AIP produces a positive effect on TCDD-inducible CYP1A1 gene expression.

Mouse AIP exhibits amino acid sequence homology with FKBP12, a prototype of the FKBP family (30), and FKBP59, an immunophilin that interacts with unliganded steroid receptors (31). The FKBP family is implicated in several biological processes, such as protein folding, steroid receptor signaling and nuclear targeting, heat shock responses, and drug-induced immunosuppression (33, 34). Studies of steroid receptor-binding immunophilins reveal that FKBP59 is a component of the multi-protein complex containing unliganded steroid receptor as well as hsp90, hsp70, and an acidic protein, p23 (34, 38). FKBP59, together with other proteins in the complex, may act as a molecular chaperone that maintains the steroid receptor in a ligand-responsive configuration; the peptidylprolyl cis-trans-isomerase activity of FKBP59 may be important in this regard (39). Like the glucocorticoid receptor, AhR is a ligand-activated transcription factor that resides in the cell cytoplasm complexed with hsp90. By analogy with glucocorticoid receptor, the cytoplasmic AhR complex may also contain an immunophilin-like component. Previous immunoochemical studies reveal that FKBP59 and CyP-40, two steroid receptor-binding immunophilins, are not associated with AhR (40). Our findings suggest that AIP might contribute an FKBP-like function(s) to AhR-mediated signaling.

The AIP protein contains three regions that exhibit homology with the tetratricopeptide repeat (TPR) motif, a degenerate conserved sequence of 34 amino acid residues (35). The TPR motif was first noted in CDC23, CDC16, and NUC2, three cell cycle genes required for completion of mitosis in yeast, and in SSN6 and SKI2, two genes required for mRNA synthesis in yeast (41). Subsequent studies have revealed that TPR exists in multiple copies in a diverse group of proteins that function in mitosis, transcription, RNA splicing, protein import, serine/threonine phosphorylation, and neurogenesis (35). Structural analyses of the TPR motif suggest that it may serve as an interface for protein-protein interactions (35, 42). Mutational analyses of some TPR domains indicate that the motif is essential for function (42, 43). It is notable that the two steroid receptor-binding immunophilins FKBP59 and CyP-40 each contain three TPR motifs, as does AIP (44, 45). Biochemical studies reveal that the TPR regions of FKBP59 are required for binding to hsp90 (46). Others have proposed that FKBP59 or CyP-40 interacts with TPR acceptor(s) in the glucocorticoid receptor-hsp90 complex and thereby directs the multi-protein complex to appropriate cellular targets (36). By analogy, we hypothesize that AIP contributes to the nuclear targeting of AhR through interactions between its TPR motifs and other proteins. Identification of the proteins that interact with AIP's TPR motifs may shed light on the molecular basis for the nuclear targeting and transport of liganded AhR.

AhR does not coprecipitate with AIP in TCDD-induced wild-type hepatoma cells (Fig. 4B). This observation could indicate that AIP dissociates from AhR following ligand binding. However, in Arnt-defective cells, AIP, AhR, and hsp90 coprecipitate in the presence of TCDD (Fig. 4B); this finding implies that AIP (as well as hsp90) remains associated with liganded AhR. Therefore, we infer that AIP has an effect on AhR-mediated signaling that occurs subsequent to ligand binding, and we hypothesize that AIP helps target the liganded AhR to the nucleus. Our findings also indicate that Arnt is required for the disruption of the AhR-AIP-hsp90 complex. Given the nuclear location of Arnt (37), we envision that Arnt binds liganded AhR upon its arrival in the nucleus, thereby stabilizing its nuclear localization.

Overexpression of AIP in mouse hepatoma cells enhances the response of the CYP1A1 gene to TCDD. These findings impute functional importance to the AIP-AhR interaction in AhR-mediated signaling. There are several possible mechanisms by which AIP may increase CYP1A1 induction. First, AIP might facilitate transport of the liganded AhR to the nucleus; thus, overexpression of AIP would enhance the nuclear accumulation

![Diagram](image-url)
of liganded AhR. Second, AIP might act as a molecular chaperone that maintains AhR in a ligand-responsive configuration; thus, overexpression of AIP would increase the amount of functional AhR in the cytoplasm. Third, AIP might interfere with AhR degradation; thus, overexpression of AIP might increase the overall cellular content of AhR. Each scenario would likely produce a positive effect on the induction of CYP1A1 gene expression in response to TCDD. Testing these hypotheses will provide new insights into the mechanism by which AhR transduces the signals of TCDD and other ligands.

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