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FUNCTIONAL ANALYSIS OF ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR INTERACTIONS WITH ARYL HYDROCARBON RECEPTOR IN THE YEAST TWO-HYBRID SYSTEM

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Abstract—The aryl hydrocarbon receptor (AHR) mediates dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin)-induced transcriptional activation of a battery of genes by interaction with a cofactor, called aryl hydrocarbon receptor nuclear translocator (ARNT) protein. Both AHR and ARNT belong to a family of proteins that includes the *Drosophila* circadian-rhythm protein and "single-minded" protein. These proteins share a domain called the PAS domain. In addition to the PAS domain, both AHR and ARNT contain basic helix-loop-helix (bHLH) and glutamine (Q)-rich domains. The roles of these domains in the receptor-mediated transcriptional activation are not understood completely. By using the yeast two-hybrid system with the N-terminal half of AHR as a probe, which contains the bHLH and PAS regions, to screen cDNA libraries prepared from human lymphocytes and C57BL mouse liver for clones encoding proteins capable of binding to these regions, we isolated a partial ARNT cDNA clone. These results demonstrated that the N-terminal half of AHR is capable of interacting with ARNT in yeast (probably through the bHLH motif). A fusion protein containing the GAL4 DNA binding domain (DB) linked to the full-length AHR was not capable of activating expression of a reporter gene containing the GAL4 DNA binding site, suggesting that ligand-free AHR alone has no transactivating properties in yeast. However, the C-terminal portion (amino acid residues 580–797) of the AHR, including the Q-rich domain, could confer transactivation of the reporter gene expression in the same system, suggesting that the N-terminal portion of the AHR contains transcription repression properties. In contrast, GAL4(DB)-ARNT fusion protein was able to activate expression of the same reporter gene. Deletion analysis of ARNT revealed that the C-terminal 75 amino acids, including the Q-rich domain, exhibited full transactivation function in yeast and mammalian cells. These results revealed different structural organizations for the transactivation properties between AHR and ARNT, although both contained transactivation domains at the C-termini.

Key words: dioxin; aryl hydrocarbon receptor; aryl hydrocarbon receptor nuclear translocator; yeast two-hybrid system; transcription activation; glutamine-rich domain

One of the important mechanisms of detoxifying environmental poisons in living organisms involves the activation of a group of metabolic enzymes called phase I and phase II enzymes [reviewed in Refs. 1–3]. Phase I enzymes, which include the cytochrome P450s, function by inserting one atom of atmospheric oxygen into substrates that are usually hydrophobic in nature. These oxygenated intermediates are then conjugated with a variety of endogenous moieties (e.g. glucuronide, glutathione, and sulfate) by phase II enzymes (e.g. UDP glucuronosyltransferases, glutathione transferases, and sulfotransferases), producing extremely hydrophilic products that are readily excreted. A subset of genes encoding these enzymes can be coordinately activated by dioxin and polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene.

Transcription activation of these xenobiotic metabolic enzymes by dioxins (most notably 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is mediated by an intracellular receptor called the AHR† [4, 5], which interacts with other cofactors, including ARNT [6, 7] and 90-kDa heat shock protein [8; also see review in Refs. 9–11]. The genes encoding AHR [4, 5] and ARNT [6] have been cloned, and their deduced amino acid sequences share distinct similarities. AHR and ARNT are each composed of three structural domains. The first domain, located in the N-terminal region of the molecule, consists of the bHLH domain found in many transcription factors (e.g. *MyoD*, E12, c-*myc*, and Max) [12–15]. The second domain is very similar to the *Drosophila* circadian rhythm gene *per* and the *Drosophila* single-minded protein *sim* and, therefore, is referred to as the PAS domain [4, 6, 16]. The third domain, located at the C-terminal end of the molecule, is glutamine (Q)-rich. The ligand-binding function apparently resides in the PAS region of AHR [17]. ARNT is a non-dioxin binding factor. *In vitro* studies suggested that ligand-bound AHR and ARNT heterodimerize through their bHLH domains to generate DNA-binding complexes [16]. The function of the Q-rich domain in AHR is not known, but *in vitro* analysis suggests that it may also modulate receptor/cofactor binding to the target genes [18].

The signal transduction mechanisms mediated by the AHR/ARNT nuclear receptors are not fully understood. A current model suggests that the AHR is associated with two copies of hsp90 in the cytosol, providing a

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† Abbreviations: AHR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; bHLH, basic helix-loop-helix; DB, DNA-binding domain; AD, activation domain; CAT, chloramphenicol acetyltransferase; and 3-AT, 3-amino-1,2,4-triazole.

conformation capable of ligand binding. Upon binding ligand, AHR is dissociated from hsp90 and translocated to the nucleus where it dimerizes with ARNT. The heterodimerized receptors recognize the xenobiotic responsive elements (XRE), which usually are located in the 5′-regions of the target genes [1, 9–11]. By interacting with general transcription factors, these receptors activate the transcription of the target genes.

In this study, we used the yeast expression system to investigate how the AHR and ARNT interact and transactivate the target genes. It has been demonstrated that the basic molecular mechanisms involved in signal transduction by the nuclear receptors appear to be conserved between mammalian and yeast (*Saccharomyces cerevisiae*) cells. Expression of estrogen [19, 20], glucocorticoid [21, 22], and progesterone [23, 24] receptors in yeast cells can activate hormone-dependent transcription of genes linked to cognate responsive elements. The recently developed two-hybrid expression systems [25, 26] provide a powerful means of studying such protein–protein interactions, thereby allowing identification of important players in signal transduction pathways. Since AHR contains a bHLH motif that can dimerize with other bHLH partners, and since it has been reported that a number of transcription factors that play important roles in tissue-specific regulation of gene expression also contain such structural motifs, we first investigated whether AHR could dimerize with other transcription factors. Using the N-terminal half of AHR containing the bHLH and PAS to screen human T cell and mouse liver cDNA libraries, we isolated an ARNT cDNA clone. These results indicated that dimerization between AHR and ARNT occurred in yeast. Second, using fusion proteins consisting of GAL4(DB) and AHR or ARNT, we observed that the full-length AHR did not activate transcription, whereas ARNT did. However, further analysis demonstrated that the C-terminal portions of both AHR and ARNT contain transactivation properties. While this manuscript was being reviewed, two articles describing the identification of transactivation domains of the receptors were published [27, 28]. Our results are in agreement with those in these publications.

MATERIALS AND METHODS

Bacterial and yeast strains

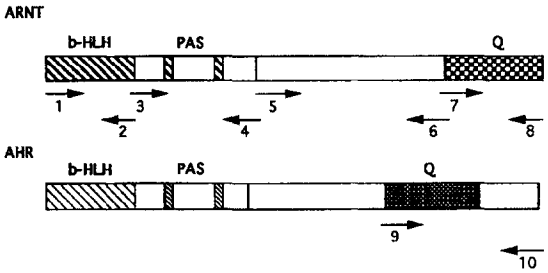
Escherichia coli strain DH10B was used for subcloning of cDNA inserts and for rescue of plasmids from yeast cells. For two-hybrid screenings and expression analysis, the yeast reporter strain (*S. cerevisiae*) Y153 (MATa gal4 gal80 his3 trypl-901 ade2-101 ura3-52 leu2-3, – 112 + URA::GAL → lacZ, LYS2::GAL → HIS3) [26] was used.

Plasmids

The yeast *E. coli* shuttle vector pAS1 [26], which contains GAL4(DB) (amino acid residues 1–147 [29]), was a gift from Dr. Stephen J. Elledge (Baylor College of Medicine, Houston, TX). pBM5/NEO-M1-1, which contains the complete coding sequence of human ARNT [6], was obtained from Dr. O. Hankinson (University of California, Los Angeles, CA). pcDNA AhR, which contains the full-length mouse AHR cDNA, was obtained from Dr. Christopher Bradfield (Northwestern University, Medical School, Chicago, IL). The pABgal vector containing GAL4(DB)(1–147) under the transcriptional control of the Rous sarcoma virus long terminal repeat [30] and plasmid p17-mertk CATΔH/N [31], which contains the CAT gene and thymidine kinase promoter linked to the GAL4 binding site were obtained from Drs. X. Leng and M-J. Tsai (Baylor College of Medicine).

The pAS1-AHR(1–419) fusion vector was constructed by first digesting pAS1 and pcDNA AhR with *Nde*I and *Hind*III, respectively. The sticky ends of the resulting fragments were filled with Klenow enzyme, digested with *Bam*HI, and ligated together. To prepare the pAS1-AHR vector, a *Bam*HI fragment containing the 3′-portion of the AHR gene was excised from pcDNA AhR and inserted into the *Bam*HI site of pAS1-AHR(1–419). To prepare pAS1-ARNT, the 2.6-kb full-length human ARNT cDNA was excised from pMB/Neo-M1-1 by *Bam*HI digestion and inserted into the *Bam*HI site of pAS1.

To construct the chimeras containing various regions of ARNT and GAL4(DB), DNA sequences encoding amino acid residues 1–161, 160–474, 476–709, 704–778, 1–474, 476–778, and 1–709 were synthesized by polymerase chain reaction with appropriate oligonucleotide primers (as indicated in Fig. 1). These fragments were cloned into pCR™II (Invitrogen, San Diego, CA). The inserts were then released by *Sal*I/*Bam*HI digestion and recloned into pABgal. The resulting fusion vec-



oligonucleotides

1, 5- AC GTC GAC ATG GCG GCG ACT ACT GCC -3
1

2, 5- GA CTG CAG ATC AGT GAG GAA AGA CGG -3
161

3, 5- AC GTC GAC ACT GAT CAG GAA CTG AAA -3
160

4, 5- GA CTG CAG TGT AGG CCG TGG TTC TTG -3
474

5, 5- AC GTC GAC TCC AAC ACA ATC CAG AGG -3
476

6, 5- GA CTG CAG TCC AGT CTC AGG AGC AAA -3
709

7, 5- AC GTC GAC TTT GCT CCT GAG ACT GGA -3
704

8, 5- GA CTG CAG GAA TTC TTC ATT GTT GTA -3
788

9, 5- C AGG ATC CAG GAT TCC CTG AAC AAT TC -3
580

10, 5- C AGT CGA CTG AGA ACC TGG AAT TTC AGA -3
797

Fig. 1. Nucleotide sequences of oligonucleotides used as primers in the polymerase chain reactions described in this study. The upper panel shows the organizations of ARNT and AHR in which various domains are located; arrows point to the directions of nucleotide sequences (5′ to 3′). Numbers under the arrows refer to the oligonucleotide shown below. Codons defining the boundaries of ARNT or AHR in the polymerase chain reaction products are underscored and numbered.

tors were named pABgal-ARNT(1–161), pABgal-ARNT(160–474), pABgal-ARNT(476–709), pABgal-ARNT(704–778), pABgal-ARNT(1–474), pABgal-ARNT(476–778), and pABgal-ARNT(1–709). To generate the pAS1-ARNT series, *Sma*I-*Bam*HI fragments containing ARNT sequences were excised from the corresponding pABgal recombinants and re-inserted into the *Sma*I-*Bam*HI sites of pAS1. All the recombinant constructs were verified by DNA sequencing.

Yeast transformation

Y153 was used as the transformation recipient for GAL4(DB) fusion recombinants. Cells were grown in YPD medium or the appropriate selective minimal medium. The cells were transformed by the method described by Rose *et al.* [32].

cDNA library construction and library screening

A human T-lymphocyte cDNA library in λACT was provided by Dr. Stephen Elledge. A C57BL/6J mouse liver library was constructed in λACT by the method described by Durfee *et al.* [26]. Briefly, poly A^+ RNA was purified from the livers of C57BL/6J mice. Double-stranded cDNA was synthesized using a kit from GIBCO BRL (Gaithersburg, MD). About 1 μg of cDNA was ligated to the kinased adapters. The size of the ligated products was determined by 1% low-melting agarose gel electrophoresis. Fractions larger than 500 bp were used for library construction. One hundred nanograms of cDNA was then ligated to 2 μg of λACT plasmid DNA that had been digested with *Xho*I and filled-in with *Taq* polymerase as described by Durfee *et al.* [26]. The recombinant DNA was packaged with Gigapack Gold Packaging extract (Stratagene, La Jolla, CA) and converted into a plasmid library in BNN132 as described previously [26].

A single yeast colony transformed with pAS1-AHR(1–419) was grown in synthetic complete (SC) medium lacking tryptophan and transformed with the cDNA libraries. We obtained approximately 3.0×10^6 and 2.1×10^6 transformants from the human T-lymphocyte and mouse liver libraries, respectively, and then plated them on petri dishes containing SC medium without tryptophan, leucine, and histidine but with 25 mM 3-AT. The plasmids in positive yeast clones were rescued by transformation in *E. coli* by the method of Hoffman and Winston [33] and analyzed by DNA sequencing.

β-*Galactosidase assay*

Two assay methods were used: a biochemical assay and a colony filter assay. For the biochemical assay, 5 mL of yeast culture was grown in selective medium to $O.D._{600}$ 1.0 to 1.2 β-Galactosidase enzyme activity was determined by the method of Guarente [34]. For the colony filter assay, yeast colonies grown on the selective medium plates were transferred to a nitrocellulose filter. The filter was placed at −80° for 30 min, and incubated on Whatman No. 1 filter paper presoaked in a buffer containing 0.75 μg/mL 5-bromo-4-chloro-3-indolyl-β-D-galactoside. The colonies that turned blue within 8 hr of incubation were considered positive.

CAT assay

NIH/3T3 and ARNT-defective (Hepalclc-BP^rc1) cells (hereafter referred to as BpR) were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. These cells were cotransfected with 10 μg each of pABgal-ARNT constructs and the CAT reporter construct p17-mertk CATΔH/N DNA by the polybrene method [35]. In all cases, 10 μg of pH2B-βgal plasmid DNA (a gift of M. Perry, M.D. Anderson Cancer Center) was also included. pH2B-βgal contains a histone H2B promoter from *Xenopus laevis* to drive expression of *lacZ*. The levels of *lacZ* gene expression were measured 48 hr after transfection by a published procedure [34] and were used to normalize CAT activity, which was assayed and quantified using a differential extraction method according to the manufacturer's suggested protocol (Promega, Madison, WI).

RESULTS

Interactions of AHR and ARNT in yeasts

AHR and ARNT each contain a bHLH and a PAS motif [4, 6]. Since many transcription factors that play important roles in cell type-specific transcription have bHLH motifs, and since it has been suggested that the PAS motif may modulate protein–protein interactions via HLH dimerization [36], we first investigated whether the bHLH-PAS domains in AHR could interact with other cellular proteins. To this end, we used the yeast two-hybrid system to screen mammalian cDNA libraries. We constructed the recombinant pAS1-AHR(1–419) by ligating the DNA fragments encoding the GAL4(DB) (amino acids 1–147) and the N-terminal half (amino acids 1–419) of AHR (Fig. 2A). The AHR portion of the fusion protein contains the bHLH and PAS regions. This AHR sequence was used as a probe to screen cDNA libraries prepared from human T-lymphocytes and mouse livers in which cDNA was fused to the GAL4(AD) (amino acids 768–881). The yeast strain (Y153) used for these screenings contained the *HIS3* gene linked to *GAL1* UAS_G and the *E. coli lacZ* gene, whose expression was also under the transcriptional control of the *GAL1* promoter. Thus, expression of *HIS3* and *lacZ* depended upon functional GAL4 in the yeast transformants, and this was achieved by protein–protein interactions between the two hybrids.

Approximately 3.0×10^6 transformants from the human T-lymphocyte library were screened. We first selected for His^+ prototrophs and then for the expression of β-galactosidase activity to eliminate possible His^+ revertants in the first screen. Fifteen clones were positive for His^+ phenotype and were able to grow in the selective medium lacking tryptophan, leucine, and histidine in the presence of 25 mM 3-AT (an inhibitor to suppress residual *HIS3* expression in the absence of GAL4). Thirteen of these clones had β-galactosidase activity in the subsequent screening.

Plasmid DNA from these positive clones was rescued by bacterial transformation. All 13 clones contained inserts of about 2.6 kb. DNA sequencing at the junctions between the GAL4(DB) and the inserts revealed that all these clones were identical, i.e. they contained the ARNT sequence starting at nucleotide 77 from the translation starting site (Fig. 2B). These clones were designated pGAL4(AD)-ARNT(2.6). The encoded ARNT included the bHLH, PAS, and Q-rich domains. These results indicate that ARNT is capable of interacting with AHR in the yeast system. Moreover, it suggested that the bHLH and PAS regions of AHR were sufficient for het-

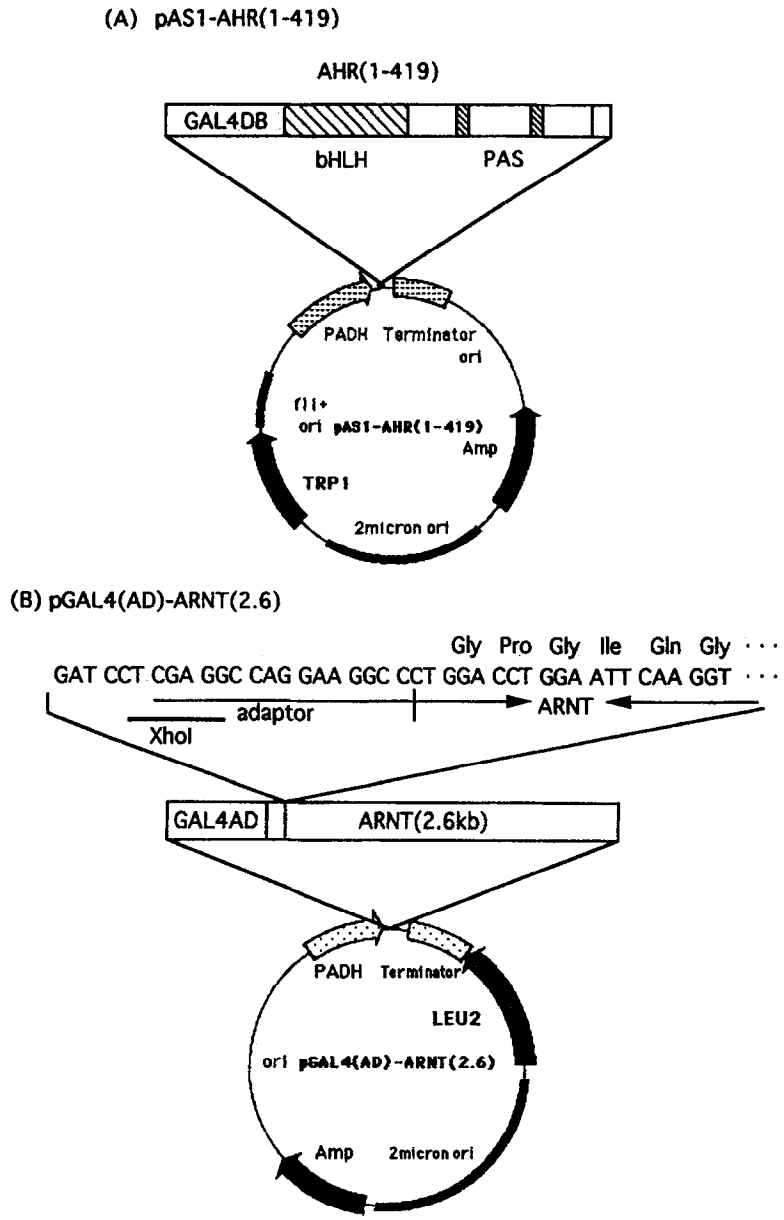


Fig. 2. Structures of pAS1-AHR(1–419) and pGAL4(AD)-ARNT(2.6). pAS1-AHR(1–419) (panel A) contains the bHLH and PAS regions of AHR fused to the DNA-binding domain of GAL4 and the TRP1 selectable marker. pGAL4(AD)-ARNT(2.6) (panel B) was isolated by the two-hybrid screening from the human cDNA library using pAS1-AHR(1–419) as a probe. The amino acid sequence of the cDNA insert was deduced by DNA sequencing with a primer upstream of the junction of GAL4(AD). The nucleotide sequence and deduced open reading frame for a portion of the sequence are shown.

erodimerization with ARNT, probably via HLH–HLH interactions. This result also suggests that yeast may be a powerful system to elucidate the signal transduction mediated by AHR [8].

Identification of transactivation properties of AHR and ARNT

Recent studies have identified several domains important for the function of the AHR and ARNT molecules. However, the transactivation domains in these molecules have not been determined. The yeast GAL4 system has been used successfully to identify such domains in many transcription factors [37]. To investigate the transcription activation properties of the AHR and ARNT, we first constructed two plasmids in which GAL4(DB) was linked to the full-length AHR (pAS1-AHR, Fig. 3b) and ARNT (pAS1-ARNT, Fig. 3c). These plasmids were used to transform Y153, and the transformants were selected by growth in medium lacking tryptophan. The positive transformants were tested for β-galactosidase activity, first by the filter-lift assay and subsequently by the enzyme activity assay in cell extracts. In the filter-lift assay, pAS1-AHR transformants did not exhibit *lacZ* expression after 8 hr of staining, whereas pAS1-ARNT

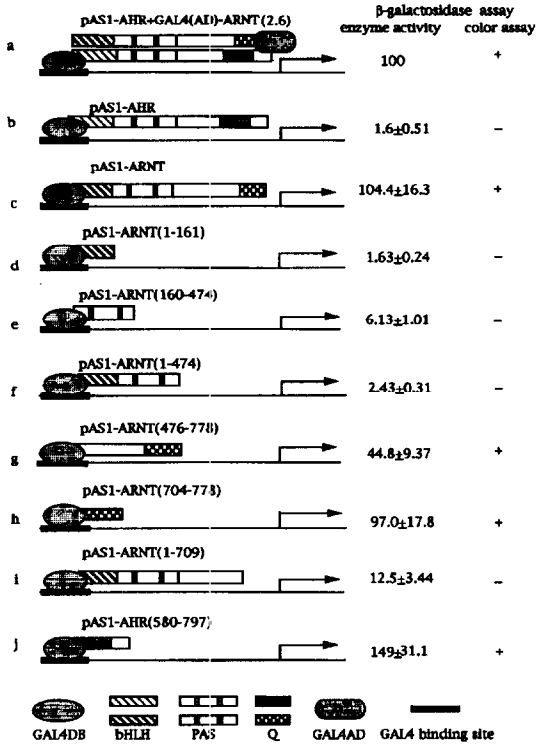


Fig. 3. Analysis of AHR and ARNT by deletion mutation in yeast expression system. Full-length AHR (recombinants a and b), and ARNT (c) and various deletion mutations of ARNT (d–i) and AHR (j) were fused to GAL4(DB). The amino acid residues in the mutants are indicated in parentheses, and the structural domains are schematically labeled. These recombinant DNAs were used to transform yeast Y153, which carries a *lacZ* reporter gene under the control of the *GAL1* promoter. The positive transformants were tested for β-galactosidase activity by both filter and biochemical assays. In the filter assay, blue color developing within 8 hr of staining was considered positive. In the biochemical assay, the results were expressed as a percentage of expression of construct a, which contains two hybrids [pAS1-AHR + pGAL4(AD)-ARNT(2.6)]. The means ± SD of six determinations are shown.

transformants showed positive signals within 10 min of staining. When pGAL4(AD)-ARNT(2.6) was subsequently transfected into the pAS1-AHR transformant (Fig. 3a), *lacZ* expression was detected by filter staining within 10 min. These results suggested that the full-length ARNT exhibited a transactivation property, whereas AHR did not [27, 28]. Enzymatic activity assays of cell extracts prepared from the corresponding transformants were consistent with the filter-lift assay (Fig. 3).

To further dissect the transactivation domain in ARNT, we constructed several fusion recombinants in which various domains of ARNT were linked to GAL4(DB) (Fig. 3, c–i). These fusion plasmids were transformed into Y153, and the expression of β-galactosidase activities was determined. Two transformants, pAS1-ARNT(704–778) (Fig. 3h) and pAS1-ARNT (476–778) (Fig. 3g) produced β-galactosidase staining within 10 and 30 min, respectively, in filter-lift assays. Enzymatic activity assays of cell extracts prepared from these transformants were also consistent with these findings. These two transformants contain the Q-rich regions. Transformants containing only the bHLH (Fig. 3d), the PAS (Fig. 3e), or the bHLH plus PAS domains (Fig. 3, f and i) failed to express β-galactosidase signals after 8 hr of staining. Enzymatic activity assays revealed only marginal β-galactosidase activity in these clones. None of these transformants contained the Q-rich region. Our data indicated that the 75 amino acid residues in the Q-rich domain of ARNT (Fig. 3h) are sufficient to fully support transcriptional activity in the yeast assay system.

Since AHR also contains a Q-rich domain in the C-terminal region, it would be of importance to determine whether this region exhibits transactivation properties. To this end, we constructed a fusion recombinant in which the C-terminal amino acid residues (580–797) were linked to the GAL4(DB). We observed that the C-terminal region was capable of conferring expression of the reporter gene (Fig. 3j). These results suggest that the C-termini of both AHR and ARNT contain transactivation domains. The fact that the C-terminal region but not the full-length AHR exhibited transactivation function suggests that its N-terminal region contains a repressor region for the transactivation function. Our interpretation is consistent with the recent finding by Whitelaw *et al.* [28], showing that the PAS domain contains a repressor region that conditionally represses both dimerization with ARNT and transactivation.

To determine whether these results could be reproduced in mammalian cells, we constructed recombinant DNAs containing various domains of ARNT linked to the GAL4(DB) vector. These recombinant DNAs were cotransfected into mammalian cells with a reporter plasmid DNA that contains the GAL4 DNA binding site to the CAT gene and the thymidine kinase promoter. Cotransfection of the plasmid harboring GAL4(DB) with the CAT reporter gene into both NIH/3T3 cells and the ARNT-defective mouse hepatoma cell line BpR yielded very low CAT activity (Fig. 4a). However, cotransfection with a plasmid expressing the full-length GAL4(DB) ARNT fusion protein produced high levels of CAT expression (Fig. 4b). Likewise, fusion proteins expressing the Q-rich domain (amino acids 704–778, Fig. 4f; and amino acids 476–778, Fig. 4h) exhibited CAT activities comparable to that of the full-length ARNT construct (Fig. 4b). Fusion proteins lacking the Q-rich residues had significantly lower CAT activities in the cotransfection assay in both NIH/3T3 and BpR cell lines (Fig. 4c, g). These results are consistent with the notion that the Q-rich region of ARNT contains the transactivation properties in mammalian cells.

DISCUSSION

The initial goal of this study was to investigate cellular proteins that interact with AHR. Identification of proteins that cross-talk with AHR would provide important insights into the signal transduction mechanism mediated by the *Ah* locus in mammalian cells. For this purpose, we used the yeast genetic approach. We first used recombinant bHLH-PAS as a probe in cDNA library screening in the two-hybrid system. This system has been used successfully to clone a number of important genes recently. Our screening of a human lymphocyte library resulted in the isolation of a cDNA encoding ARNT, demonstrating that ARNT can indeed form heterodimer with AHR in the yeast. No other HLH-containing cellular proteins were identified. We also

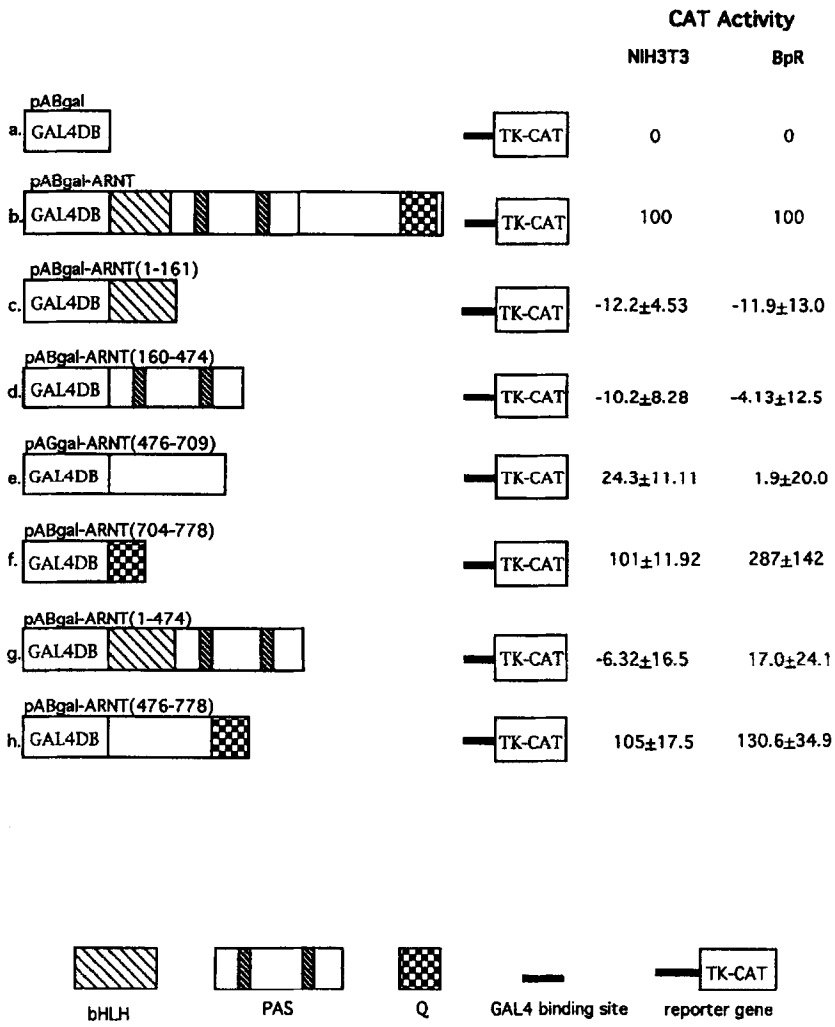


Fig. 4. Analysis of ARNT by deletion mutation in mammalian cells. Full-length ARNT (b) and various deletion mutations of ARNT were fused to GAL4(DB). The amino acid residues included in the mutants are indicated in parentheses, and the corresponding structural domains are schematically shown. The recombinant DNAs were cotransfected with a CAT reporter gene whose expression is controlled by thymidine kinase promoter and upstream GAL4 DNA binding site. NIH/3T3 and ARNT-defective BpR cells were used for transfection. The results are expressed as a percentage of expression of the full-length construct (b). The means ± SD of six determinations are shown.

screened 2.1×10^6 transformants from the mouse C57BL6 library but failed to identify new binding partners. These results suggest that either other potential bHLH-PAS binding proteins were not present in the library or that only a subset of HLH-containing proteins (e.g. ARNT) can form stabilized heterodimers with the HLH-PAS domains of AHR in yeast. Consistent with the latter possibility, we found that the muscle cell-specific transcription regulators MyoD and Id (a gift of Dr. Eric Olson, M.D. Anderson Cancer Center), which also contain bHLH sequences [38], were unable to interact with the HLH domain of AHR (unpublished results).

In analogy to some nuclear receptors, such as glucocorticoid receptor, progesterone receptor, and mineralocorticoid receptors [reviewed in Refs. 9 and 39], AHR is known to associate with hsp90 [8, 40]. This heat-shock protein is bound to the PAS domain of AHR in the absence of ligand, and is dissociated in the liganded AHR. The failure to detect hsp90 in the two-hybrid library screening may be due to the same reasons as mentioned above.

We then turned our attention to the investigation of transactivation properties of the AHR/ARNT. Our results showed that full-length AHR *per se* failed to transactivate the reporter gene, whereas ARNT did. In addition, the C-termini of both AHR and ARNT exhibited transactivation activities. Further analyses resulted in the identification of a 75-amino acid region in the Q-rich domain of ARNT that displayed transactivation in both yeast and mammalian expression systems. These results are consistent with the results recently published by several groups [27, 28, 41]. Our results, together with those published in these studies, suggest that ARNT has a pivotal role in the AHR receptor-mediated transactivation pathway, as proposed in a model schematically shown in Fig. 5. According to this model, ARNT first acts as a binding partner to dimerize with AHR. The receptor complex uses the dimerized bHLH to bind the

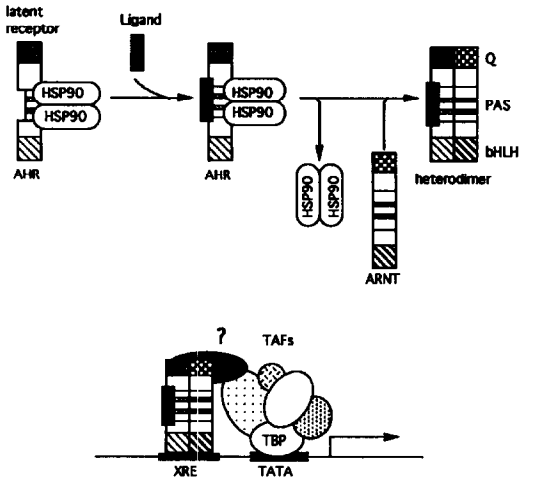


Fig. 5. Schematic diagram of a model of AHR/ARNT-mediated transcriptional activation of genes encoding xenobiotic-metabolizing enzymes (modified from Poellinger *et al.* [9]). The latent form of AHR is associated with hsp90. Ligand binding leads to dissociation of hsp90 from AHR. AHR then forms a heterodimer with ARNT via the HLH motifs. The dimerized receptor complex then interacts with xenobiotic responsive element (XRE) of the target genes by the dimerized bHLH, and activates transcription, probably by direct contact between the Q-rich domains of AHR and ARNT with transcriptional activation factors (TAFs) in the preinitiation complex.

XRE in the 5′-regions of the target genes. The C-termini of AHR/ARNT then transactivate expression of target genes through their Q-rich regions.

The mechanism by which the Q-rich domains of ARNT and AHR activates transcription is presently unknown. The Q-rich domain was initially found in the human transcription factor Sp1 gene and was shown to be necessary for a high level of transcriptional activation [42]. The Q-rich region was also found in other sequence-specific transcriptional factors, including the *Drosophila zeste* gene [43], the homeobox-containing *cut* genes [44], and cyclic AMP response element-binding (CREB) protein [45]. The mechanism by which AHR/ARNT activates transcription may be similar to that of Sp1. It has been demonstrated that Q-rich activation domains of Sp1 can interact selectively with transcriptional activation factor $TAF_{II}110$, a 110-kDa component of TFIID [46, 47]. Furthermore, analysis of a mutant Sp1 activation domain revealed a correlation between loss of transcriptional activation and failure to bind to $TAF_{II}110$ [48]. Therefore, the Q-rich domains in AHR/ARNT might work, in analogy to Sp1, by recruiting TFIID to the DNA template by binding specifically to $TAF_{II}110$, thus enhancing the stability of the transcriptional complex at the promoters of the responsive genes. This hypothesis places $TAF_{II}110$ downstream of the ARNT transactivation pathway. Consistent with this idea is the finding that a 110-kDa protein copurifies with AHR and ARNT during DNA recognition site chromatography [49]. It is noteworthy that although $TAF_{II}110$ can bind several different Q-rich activation domains, including Sp1 regions A and B [46] and CREB protein [45], a number of Q-rich domains in transcriptional factors fail to bind to $TAF_{II}110$ [46]. Therefore, the involvement of $TAF_{II}110$ in AHR receptor-mediated transcriptional activation remains to be demonstrated. Alternatively, it is possible that these nuclear receptors may interact with other coactivators in the transcriptional machinery in the transactivation process. Further studies are required to examine these possibilities and should reveal how the dimerized AHR/ARNT interacts with basic transcriptional machinery in the process of transactivating expression of xenobiotic-metabolizing enzymes.

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