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Ligand and Dimerization Dependent Transactivation Capability of Aromatic Hydrocarbon Receptor

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The aromatic hydrocarbon receptor (AhR) is a cytosolic protein that binds the environmental pollutant, dioxin. The liganded AhR translocates into the nucleus where it heterimerizes with a constitutive nuclear protein, AhR nuclear translocator (Arnt). The N-terminal regions of both AhR and Arnt contain basic helix-loop-helix (bHLH) and Per-AhR-Arnt-Sim (PAS) motifs that are required for DNA binding, dimerization, and ligand binding whereas the C-terminal regions of both AhR and Arnt contain transactivation domains. Here, results from the mammalian two-hybrid system indicate that Arnt can make a homodimer but AhR cannot. In the presence of dioxin, the interaction between AhR and Arnt is stronger than that of the Arnt homodimer, suggesting that Arnt prefers to make a heterodimer with the liganded AhR rather than a homodimer. Transfection analyses using the GAL4-driven reporter system suggest that AhR's N-terminal region represses its own transactivation domain, as well as exogenous transactivation domains such as Sp1 and VP16. Interestingly, the repressed transactivation domains of AhR are activated by ligand-dependent heterodimerization with Arnt. These observations suggest that heterodimerzation with Arnt is necessary not only for DNA binding but also for activation of the repressed transactivation capability of AhR.

Keywords: AhR, Arnt, Dioxin, Heterodimerization, Transactivation.

Introduction

The aromatic hydrocarbon receptor (AhR) is an intracellular protein that binds a variety of carcinogenic environmental pollutants, including polycyclic aromatic hydrocarbons such as bezo(α)pyrene, 3-methyl-cholanthrene, and halogenated aromatic hydrocarbons such as dioxin. Among these exogenous ligands, one of the dioxin isomers, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has the highest affinity to AhR. It is resistant to metabolic breakdown, resulting in an accumulation in the body which manifests sustained toxicity (Safe, 1986; Whitlock, 1993). The effects of TCDD include the induction of xenobiotic-metabolizing enzymes (Whitlock *et al.*, 1996), neoplasia, and reproductive toxicity that are associated with alterations in endocrine homeostasis, proliferation, and differentiation (Peterson *et al.*, 1993; Dohr and Abel, 1997; Ge and Elferink, 1998). Many of the TCDD effects are mediated by AhR. The reason why the environmental pollutant has its own receptor in mammalian cells is still a mystery. The following evidence strongly implies that the AhR must have evolved prior to the environmental pollutants (Poellinger *et al.*, 1992). AhR-deficient (AhR-/-) mice died shortly after birth and survivors showed reduced accumulation of lymphocytes in the spleen and lymph nodes. The livers of AhR-/- mice were reduced in size by 50% and showed bile duct fibrosis (Jain *et al.*, 1994; Fernandez-Salguero *et al.*, 1995). Therefore, the AhR plays an important role in the development of the liver and immune system and the putative endogenous ligand may activate AhR in certain developmental stages. TCDD may mimic the endogenous ligand to activate AhR (Poellinger *et al.*, 1992). Although the endogenous ligand for AhR is still unknown, the function of AhR is understood from analyses of the mechanism by which TCDD induces the transcription of CYP1A1 that encodes the microsomal enzyme cytochrome P4501A1 (Whitlock *et al.*, 1996). The unliganded AhR is located in the cytosol as a complex with heat shock protein 90 and perhaps two other proteins. After treatment of TCDD, liganded AhR is translocated into the nucleus and interacts with a nuclear protein AhR nuclear translocator (Arnt) to form a heterodimeric transcription factor

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(Hankinson, 1995). AhR/Arnt heterodimers specifically bind dioxin responsive elements (DRE; -TNGCGTG-) and function as enhancer-specific transcriptional activators. Upon its binding on the chromosomal enhancer, the AhR/Arnt complex increases the accessibility of the chromosomal promoter of the CYP1A1 gene from the distance to allow the binding of general transcription factors on the promoter (Kim and Hwang, 1995; Okino and Whitlock, 1995; Ko *et al.*, 1996). Both AhR and Arnt contain basic helix-loop-helix (bHLH) and Per-AhR-Arnt-Sim (PAS) motifs that are found in HIF-1α, Tango, Sim, AhR, and Arnt protein (Huang *et al.*, 1993). Based on the structure-function studies, the basic regions in the AhR/Arnt heterodimer are aligned in juxtaposition as a result of dimerization through HLH and PAS motifs. These basic regions specifically interact with the DRE site. Therefore, dimerization is a prerequisite for DNA binding (Kadesch, 1993; Lee *et al.*, 1997; Pongratz *et al.*, 1998). Several studies showed that the C-terminal regions of both AhR and Arnt contain transactivation domains (Jain *et al.*, 1994; Li *et al.*, 1994). This study presents a more detailed analysis of the transactivation capabilities of both proteins and how they are tightly regulated. The N-terminal region of AhR represses the transactivation capabilities of several transactivation domains. The transactivation function of AhR substantially changed during the ligand binding and dimerization processes. These findings give an insight into the general principle that the heterodimeric transactivators are regulated at multiple steps.

Materials and Methods

Cell culture Wild-type (Hepa1c1c7), Arnt-defective mouse hepatoma cells were cultured as previously described (Miller *et al.*, 1983).

Subcloning of AhR and Arnt mutants A PCR-based method was used to obtain cDNA fragments of the AhR chimera and the deletion mutants of Arnt or AhR. The PCR reaction was performed in a volume of 100 μl containing 75 pmoles of forward and reverse primer, 30 nmoles of each dNTP, 10 ng of template pRC/CMVmAhR (Ma *et al.*, 1995), pBK-CMVArnt (Li *et al.*, 1994), pBS-Sp1-fl, pBS-Sp1-M37 (Kadonaga *et al.*, 1987; Gill *et al.*, 1994), or pNFT (Bryne *et al.*, 1989), and 3 units of Vent polymerase. The following PCR conditions were used: 94°C, 1 min; 55°C, 2min; 76°C, 3min for 30 cycles, and then a 76°C extension for 7min. The PCR products were digested by appropriate restriction endonucleases targeted to sites in the primers. A PCR-based site-directed mutagenesis approach was used to generate a point mutant of VP16 (VP16F442P) (Nelson *et al.*, 1989). The primers for point mutation were F442P forward, 5′-CTAGACGATCCCGATCTGGAC-3′ and F442P reverse, 5′-GTCCAGATCGGGATCGTCTAG-3′. The mutated nucleotides are underlined. The PCR segments of AhR or Arnt were subcloned into the *Bam*HI site of pG vector, the *Xho*I site of pN vector or AflIII, and the *Bcl*I sites of pMFG vector.

Retroviral expression of Arnt Three micrograms of pMFGArnt, pMFGArnt(1-743), and pMFGLacZ were transfected into the ecotropic packaging cell line, BOSC23, as previously described (Pear *et al.*, 1993). Forty-eight hours after transfection, 1 ml of the virus-containing medium was removed and mixed with 2 ml of hepatoma cell culture medium (α-minimal essential medium containing 10% fetal bovine serum) and polybrene (5 μg/ml). This virus cocktail was placed in 60-mm-diameter plates containing approximately 2×10^5 Arnt-defective cells; the plates were centrifuged at $1000 \times g$ for 90 min at 32°C as previously described (Kotani *et al.*, 1994). The infection efficiency was estimated from the percentage of blue cells in populations infected with virus containing lacZ cDNA and was always greater than 95%.

Transient transfection and CAT assay Expression plasmids (5 μg) were cotransfectd with 2.5 μg of the reporter plasmid pGCAT and β-galactosidase expression vector, pCHO110 (1 μg), into 2×10^6 Hepa1c1c7 cells or its variant cells in 60-mm plates, using the dimethyl sulfoxide (DMSO) method as described previously (Fisher *et al.*, 1990). The reporter plasmid pGCAT contains five copies of GAL4 binding sites followed by the E1b promoter and bacterial chloramphenicol acetyltransferase (CAT) cDNA (Fearon *et al.*, 1992). The transfected cells were treated with 1 nM TCDD (dissolved in DMSO) for 18 h prior to harvest. Forty eight hours after transfection, the cells were harvested in reporter lysis buffer according to the manufacturer's instruction (Promega, Madison, USA). Reaction was performed using 50 μl of cell extract for 1 h at 37°C. A thin layer chromatogaphy (TLC) assay was performed according to the manufacturer's instruction (Promega, Madison, USA). CAT activity was quantitated using a differential extraction/liquid scintillation assay. β-galactosidase activity and protein concentration were measured as previously described (Ko *et al.*, 1996).

Results and Discussion

Transactivation capability of AhR and Arnt To measure the transactivation capabilities of AhR or Arnt, AhR and Arnt were expressed as a chimera, and fused with the DNA binding domain of yeast protein GAL4 (1–147 aa). Transactivation capabilities were measured by using the GAL4 driven reporter system. The pG derivatives that encode GAL4-AhR or GAL4-Arnt fusion proteins were transfected into mouse hepatoma cells together with reporter plasmid pGCAT that contains 5 copies of the GAL4 binding site at the 5′ end of the E1b promoter and chloramphenicol acetyltransferase (CAT) gene. To ensure that measurements reflect the properties of the individual proteins, and not the AhR/Arnt heterodimer, AhR-defective and Arnt-defective hepatoma cells were used in the transfection experiments so that the cognate dimerization partner would not be present. The GAL4 fusion protein is able to bind GAL4 binding sites. If it has a transactivation domain, it increases the transcription of the reporter gene that is under the control of GAL4. The results presented in Fig. 1 reveal that DNA-bound Arnt

exhibits transactivation capability but AhR does not. In order to find the location of the transactivation domains, deletion mutants of both proteins were constructed as shown in Figs. 2. and 3. All C-terminal deletion mutants of Arnt lose their transactivation capabilities, whereas all N-terminal deletion mutants of Arnt keep their transactivation capability. The transactivation capability is located at the C-terminal, 33 amino acids of Arnt. The mutational analyses of AhR revealed that full-length AhR itself does not show the transactivation capability but its C-terminal fragments exhibit strong transactivation capability. At least three regions of AhR (aa 515–561, 649–740, 726–805) contained transactivation capabilities when they were tested as GAL4 fusion proteins without the N-terminal region of AhR. These observations imply that the N-terminal region of AhR represses the transactivation capability of AhR. It is uncertain whether these AhR's repressed transactivation capabilities are expressed in the AhR/Arnt complex that is a functional transactivator unit.

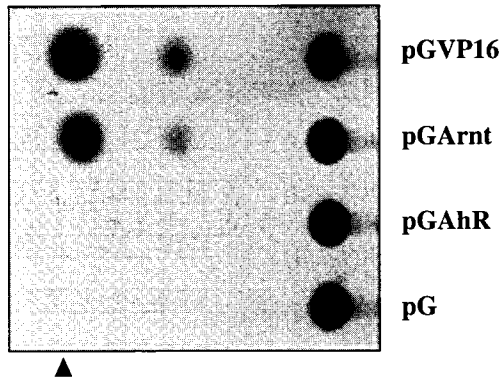
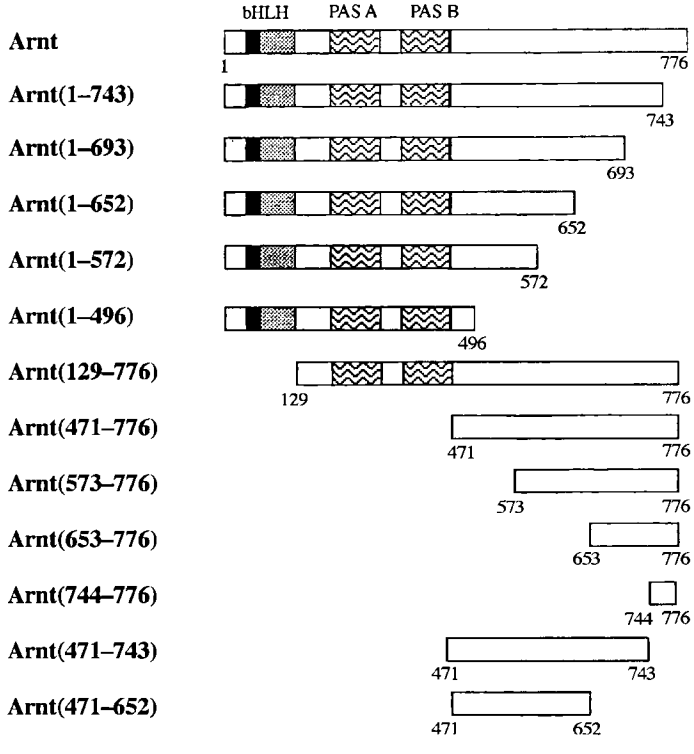
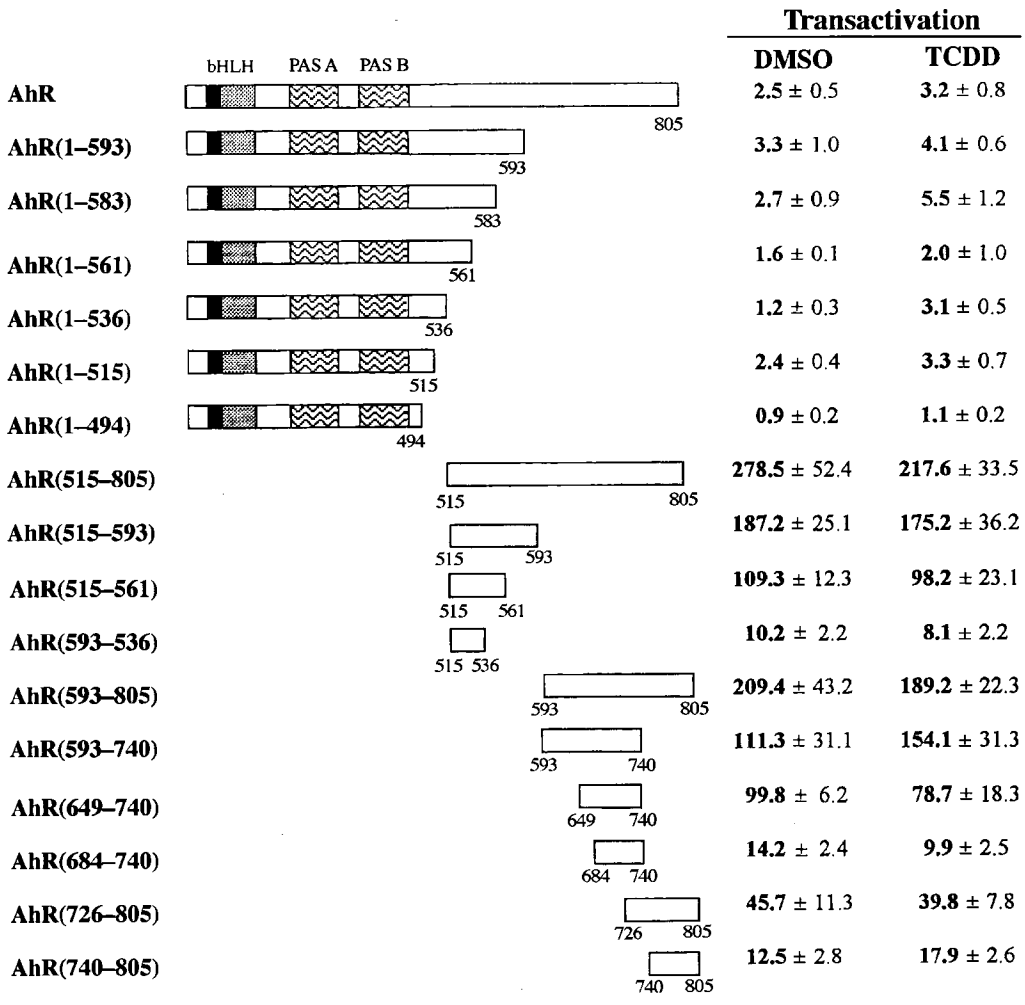


Fig. 1. Transactivation capabilities of AhR and Arnt. Wild-type hepatoma cells were cotransfected with the indicated expression plasmid (5 μg) with the pGCAT reporter plasmid (2.5 μg). Transactivation capabilities were assayed by measuring the chloramphenicol acetyltransferase (CAT) activity from the pGCAT reporter.



	Transactivation	
	DMSO	TCDD
Arnt	**238.7** ± 88.5	**250.0** ± 24.6
Arnt(1–743)	**1.0** ± 0.3	**1.7** ± 0.6
Arnt(1–693)	**0.9** ± 0.3	**2.4** ± 0.7
Arnt(1–652)	**1.2** ± 0.7	**1.0** ± 0.2
Arnt(1–572)	**1.3** ± 0.6	**2.0** ± 0.6
Arnt(1–496)	**0.7** ± 0.3	**1.1** ± 0.4
Arnt(129–776)	**172.9** ± 8.3	**277.7** ± 40.5
Arnt(471–776)	**240.8** ± 55.4	**236.5** ± 55.6
Arnt(573–776)	**178.9** ± 67.2	**203.6** ± 27.7
Arnt(653–776)	**206.8** ± 19.9	**168.8** ± 35.9
Arnt(744–776)	**163.0** ± 64.5	**169.7** ± 41.9
Arnt(471–743)	**11.5** ± 4.4	**15.7** ± 3.6
Arnt(471–652)	**8.9** ± 2.5	**4.3** ± 1.3

Fig. 2. Transactivation capabilities of Arnt and its deletion mutants. The Arnt deletion mutants were generated as fusion protein that contains GAL4 DNA-binding domain and the indicated fragment of Arnt. AhR-defective mouse hepatoma cells were cotransfected with pG derivatives that encode the indicated GAL4-Arnt mutant and the pGCAT reporter plasmid. Transactivation capabilities were assayed by measuring chloramphenicol acetyltransferase (CAT) activity from the pGCAT reporter. Values are expressed as fold increases, relative to the pG control vector which contains only the GAL4 DNA-binding domain. The values represent the mean and standard deviation of three experiments. The numbers on the schematic diagrams indicate N- and C-terminal amino acids for the Arnt proteins. bHLH, basic helix-loop-helix domains; PAS A and PAS B, homologous domains among Per, Arnt, AhR, and Sim.



	Transactivation	
	DMSO	TCDD
AhR	2.5 ± 0.5	3.2 ± 0.8
AhR(1–593)	3.3 ± 1.0	4.1 ± 0.6
AhR(1–583)	2.7 ± 0.9	5.5 ± 1.2
AhR(1–561)	1.6 ± 0.1	2.0 ± 1.0
AhR(1–536)	1.2 ± 0.3	3.1 ± 0.5
AhR(1–515)	2.4 ± 0.4	3.3 ± 0.7
AhR(1–494)	0.9 ± 0.2	1.1 ± 0.2
AhR(515–805)	278.5 ± 52.4	217.6 ± 33.5
AhR(515–593)	187.2 ± 25.1	175.2 ± 36.2
AhR(515–561)	109.3 ± 12.3	98.2 ± 23.1
AhR(593–536)	10.2 ± 2.2	8.1 ± 2.2
AhR(593–805)	209.4 ± 43.2	189.2 ± 22.3
AhR(593–740)	111.3 ± 31.1	154.1 ± 31.3
AhR(649–740)	99.8 ± 6.2	78.7 ± 18.3
AhR(684–740)	14.2 ± 2.4	9.9 ± 2.5
AhR(726–805)	45.7 ± 11.3	39.8 ± 7.8
AhR(740–805)	12.5 ± 2.8	17.9 ± 2.6

Fig. 3. Transactivation capabilities of AhR and its deletion mutants. The AhR deletion mutants were generated as a fusion protein that contains the GAL4 DNA-binding domain and the indicated fragment of AhR. Arnt-defective mouse hepatoma cells were cotransfected with pG derivatives that encode the indicated GAL4-AhR mutant and the pGCAT reporter plasmid. Transactivation capabilities were assayed by measuring chloramphenicol acetyltransferase (CAT) activity from the pGCAT reporter. Values are expressed as fold increases, relative to the pG control vector which contains only the GAL4 DNA-binding domain. The values represent the mean and standard deviation of three experiments. The numbers on the schematic diagrams indicate N- and C-terminal amino acids for the AhR proteins. bHLH, basic helix-loop-helix domains; PAS A and PAS B, homologous domains among Per, Arnt, AhR, and Sim.

Arnt/AhR heterodimer and Arnt/Arnt homodimer In order to test the dimerization ability of AhR and Arnt, a mammalian two-hybrid system was used. Both proteins and its mutants were expressed as a chimeric protein, fused with either the DNA binding domain of yeast protein GAL4 (1–147 aa) or the transactivation domain of viral protein VP16 (414–490 aa). The dimerization capabilities were measured by using a GAL4-driven reporter system. As shown in Fig. 4, pG derivatives encode GAL4 (1–147 aa) and various bait fusion proteins and pN derivatives encode VP16 (414–490 aa) and target fusion protein. These two plasmid derivatives of pG and pN were cotransfected into the mouse hepatoma cell (hepa1c1c7) together with reporter plasmid pGCAT. If a pG derivative does not have intrinsic transactivation capability, but it can interact with a pN derivative, the interaction between two test proteins brings the transactivation domain of VP16 to the vicinity of the promoter and then the reporter gene can be transcribed.

The results in Fig. 4 indicate that AhR interacts with Arnt even in the absence of dioxin, but dioxin strengthens their interaction. Interaction between cytosolic AhR and nuclear protein Arnt requires the translocation of AhR and only liganded AhR is able to do so. Since the pG and pN vector contains nuclear localization signal, chimeric proteins can locate in the nuclear region without a ligand. The immunocytochemical study indicated that GAL4-linked chimeric AhR, like Arnt, is found in the nucleus in

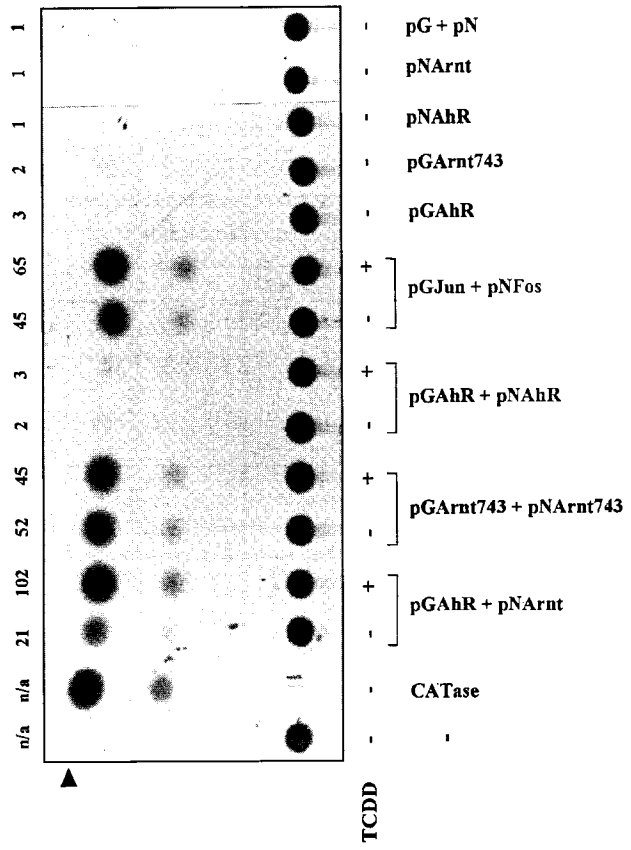


Fig. 4. Protein-protein interactions among AhR and Arnt. In order to measure the interaction between the two test proteins, a mammalian two-hybrid system was used. Wild-type hepatoma cells were cotransfected with the indicated plasmids with the pGCAT reporter plasmid. Interaction between two proteins was measured by CAT activity from the reporter plasmid. Untreated or TCDD-treated cell extracts were used for the CAT assay and visualized by thin layer chromatography (TLC). By using liquid scintillation counting, CAT activities were quantitated and presented as cpm/mg of protein at the top. An arrow head indicates acetylated chloramphenicol that was catalyzed by CAT activity. The other dots indicate unchanged chloramphenicol. The bottom line indicated '–', represents TLC of the negative control reaction from untransfected hepatoma cell extract. The second bottom line indicated 'CATase', represents TLC of positive control reaction with 10 units of CAT enzyme (Promega).

the absence of ligand (Jain *et al.*, 1994). Therefore, this result indicates that unliganded AhR has affinity for Arnt. However, it is not clear whether dioxin actually increases the affinity of AhR to Arnt or whether liganded GAL4-AhR can localize in the nucleus more efficiently than unliganded GAL4-AhR.

Since AhR and Arnt have bHLH and PAS motifs, it is possible that both proteins can make a homodimer. To test this hypothesis, pGArnt743 was used to test its homodimeric interaction since full-length Arnt has its own transactivation capability. The results presented in Fig. 4 indicate that Arnt743 can make a homodimer regardless of dioxin. *In vitro* analysis using immunoprecipitation experiments also showed that Arnt could make a homodimer and that it has an affinity for the E-box DNA motif (Antonsson *et al.*, 1995; Sogawa *et al.*, 1995; Lee *et al.* 1997). These transfection analyses support that Arnt can make a homodimer *in vivo*. However, it is still unknown whether the Arnt homodimer binds an endogenous gene and functions as a real transactivator. In contrast, AhR fails to make a homodimer even in the presence of ligand. It suggests that each HLH-PAS domain may have its own specificity for a partner protein's HLH-PAS domain. Quantitative analyses of the CAT activity reveal that the interaction between liganded AhR and Arnt is stronger than that between Arnt and Arnt. Therefore, it can be hypothesized that Arnt exists as an homodimer in the absence of dioxin, but in the presence of dioxin, heterodimerization between liganded AhR and Arnt prevails over homodimerization of Arnt.

Ligand and dimerization dependent transactivation capability of AhR Several studies have suggested that deletion of AhR's C-terminal region reduces AhR/Arnt-dependent transcription of dioxin-induced genes *in vivo* (Whitelaw *et al.* 1994). This observation implies that the repressed transactivation capability of AhR is functional *in vivo*, even though as a monomer its activity is repressed when it is linked to the N-terminal region. Therefore, the following questions are raised: (1) How is repressed transactivational capability of AhR activated? (2) Does AhR's N-terminal region generally repress any other transactivation domain or is it specific for the AhR's transactivation domain? (3) What is the role of Arnt for the activation of AhR's transactivation domain? In order to approach these questions, the chimeric AhR mutants were generated and these mutants were linked to the GAL4 DNA binding domain as shown in Fig. 5. The AhR mutants commonly contain an N-terminal region AhR (1–494 aa) that is linked at several transactivation domains: AhR (495–805 aa), VP16 (414–454 aa), VP16F442P. Transactivation capability was lost by substitution of phenylalanine to proline at amino acid 442 of VP16, Sp1 (263–542 aa), Sp1-M37 that lose its transactivation capability by change of 10 amino acids (455–464 aa) of the transactivation domain of Sp1 (263–542 aa) (Gill *et al.*, 1994). The fragments of AhR (495–805 aa), Sp1 (263–542 aa), VP16 show strong transactivation capabilities when they are tested as GAL4 fusion proteins without the N-terminal region of AhR in the GAL4-driven reporter system (Cress *et al.*, 1990; Gill *et al*, 1994; Ma *et al.*, 1995). The transactivation capabilities of these AhR chimeras were measured in three types of hepatoma cells in which Arnt is expressed differently; viz., Arnt-defective cell; Arnt-defective cells reconstituted with full-length Arnt; Arnt-defective cells reconstituted with C-terminal

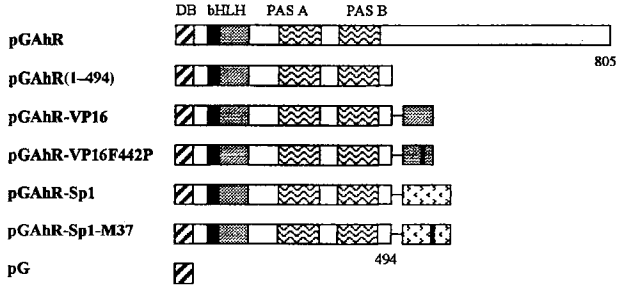


Fig. 5. Schematic structures of AhR chimeric proteins. The N-terminal region of AhR (1–494 aa) was linked to various transactivation domains such as AhR (494-805 aa), Sp1 (263–541 aa) and Sp1 mutant (Sp1–M37), VP16 (414-454 aa) and VP16 mutant (VP16F442P) as shown. N-terminals of all mutant AhR were linked to the DNA binding domain of the yeast GAL4 protein.

deleted Arnt (1–743). Arnt-defective hepatoma cells were reconstituted with full-length or mutant Arnt by using retroviral gene transfer. These reconstituted cells express Arnt or its mutant more than wild-type hepatoma cells and Arnt can interact with the HLH-PAS domain of the GAL4-AhR fusion protein (Ko *et al.*, 1996). Therefore, in Arnt-expressing cells, the transactivational capability of each AhR mutant represents the AhR/Arnt heterodimer and not AhR by itself. The results in Fig. 6 indicate the following: (1) The N-terminal region of AhR blocks not only AhR's own transactivation capabilities but also exogenous ones such as Sp1 or VP16 in the absence of ligand and Arnt; (2) In Arnt-expressing cells (Arnt-defective cells reconstituted with full-length Arnt), dioxin increases the transactivational capabilities of pGAhR and all AhR chimeric mutants (AhR-VP16, AhR-VP16F442P, AhR-Sp1, and AhR-Sp1-M37) regardless of whether the transactivation domains are mutated or not; (3) In mutant Arnt expressing cells [Arnt-defective cells reconstituted with mutant Arnt (1–743)], AhR and AhR-VP16 chimeric proteins exhibit dioxin-dependent transactivation capabilities, but AhR-VP16F442P and AhR-Sp1 chimeric proteins can not.

Since Arnt (1–743) does not contain transactivation capability (Fig. 2.), dioxin induced reporter gene expression by pGAhR and pGAhR-VP16 in Arnt (1–743) expressing cells is mediated through transactivation domains of AhR or VP16. These results imply that dioxin-dependent Arnt-binding activates transactivation domains of AhR that are otherwise repressed by the N-terminal region of AhR. Therefore, ligand-dependent heterodimerization with Arnt may cause allosteric changes in AhR to activate its transactivation domains to then interact with coactivator or general transacription factors.

These results also indicate that the N-terminal region of AhR represses the transactivation domain of not only its

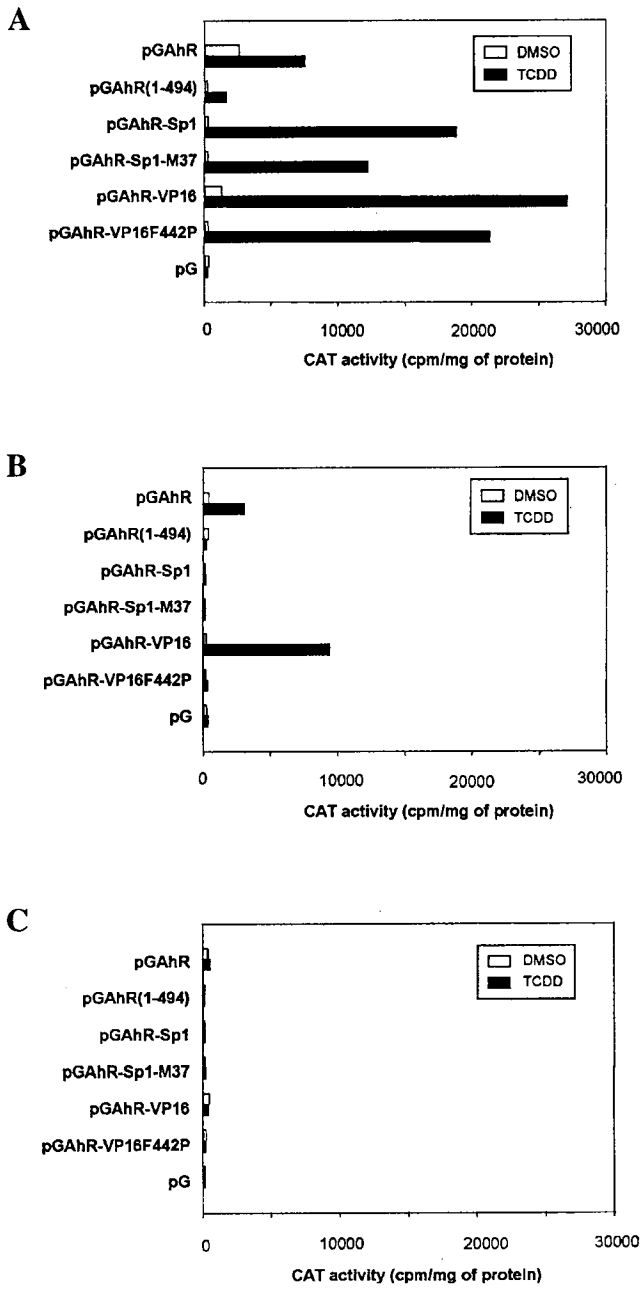


Fig. 6. Transactivation capabilities of AhR and AhR chimera proteins in Arnt-defective cells, Arnt, or Arnt (1–743) mutants expressing cells. The indicated AhR and AhR chimeras were subcloned into a pG vector to generate fusion proteins of GAL4-AhR or GAL4-AhR chimeras. These proteins were expressed in three different cells respectively; A. Arnt-defective cells reconstituted with Arnt; B. Arnt-defective cells reconstituted with Arnt (1–743) mutants; C. Arnt-defective cells. Arnt-defective cells were reconstituted with Arnt or its mutant by using the retroviral gene transfer system. These reconstituted cells express Arnt (A) or Arnt (1–743) (B), respectively. The transactivation capability of each chimera or AhR was assayed by measuring CAT activity from reporter plasmid pGCAT. Values are expressed as fold increases, relative to the pG control vector which contains only the GAL4 DNA-binding domain.

own but also the heterogeneous transactivation domains, such as Sp1 and VP16 (Ma *et al.*, 1995). This observation excludes the possibility that this inhibitory effect of the N-terminal region of AhR is mediated by specific intramolecular interactions between the N-terminal and C-terminal regions of AhR (Huang *et al.*, 1995). Another possibility is that the N-terminal region of AhR interacts with an inhibitory protein that prevents the transactivation domain from interacting with the coactivator or general transcription factors. Once liganded AhR translocates into the nucleus, Arnt may replace the inhibitory protein and activate the transactivation domains of AhR. This activation process discriminates between two different types of transactivation domains; Sp1 and VP16. The transactivation capabilities of AhR and VP16 are activated in heterodimerization with Arnt but Sp1 is not. All transactivation domains of AhR, VP16, and Sp1 are able to transcribe from the Elb promoter of the reporter plasmid when they are linked directly to the GAL4 DNA binding domain without the N-terminal region of AhR (Cress and

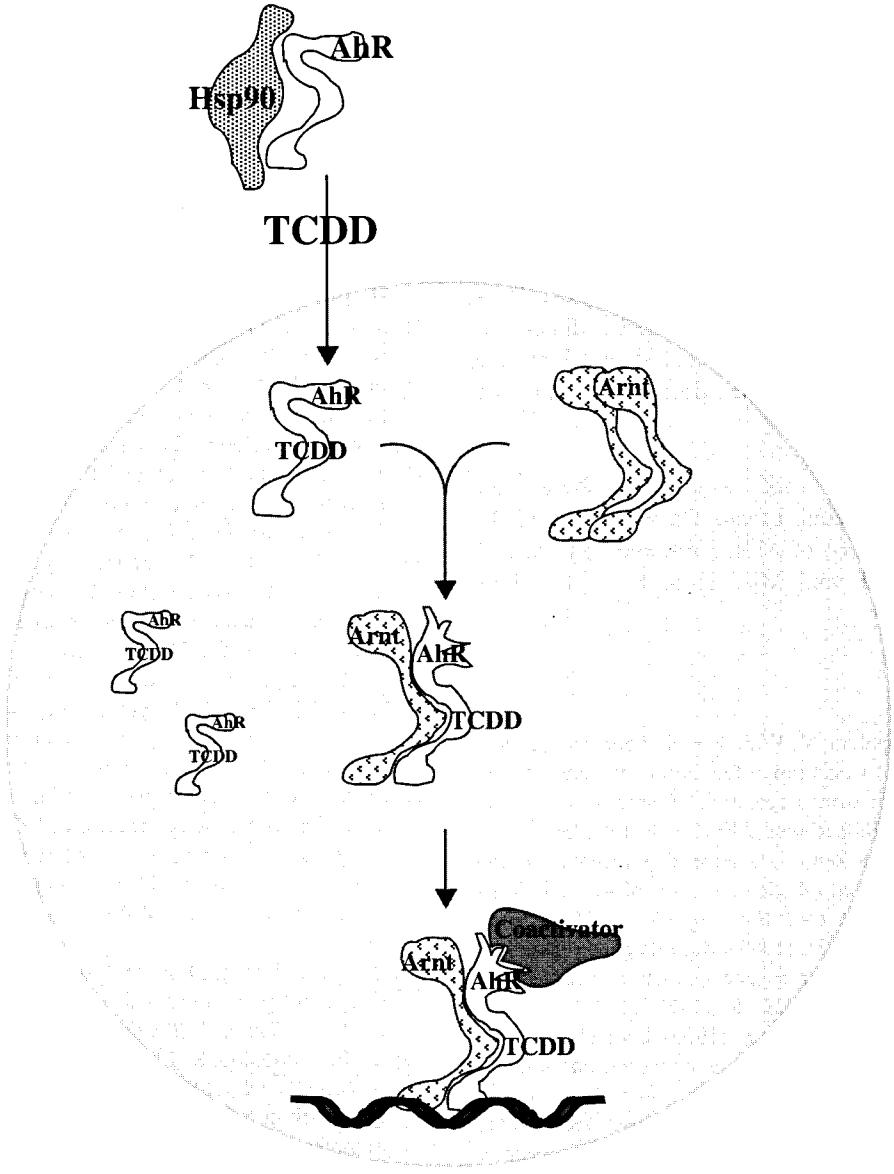


Fig. 7. Ligand and dimerization dependent transactivation capability of AhR. In the presence of TCDD, liganded AhR dissociates from heat shock protein 90 and translocates into the nucleus where it interacts with Arnt. Arnt may exist as a homodimer in the absence of dioxin. Once liganded AhR translocates into the nucleus, it heterodimerizes with Arnt resulting in the conformational change of AhR, thereby activating the repressed transactivation domains of AhR. Two basic regions of AhR and Arnt contact with regulatory elements of genes and the transactivation domains of AhR/Arnt interact with coactivators which facilitate the recruitment of the general transcriptional machinery.

Triezenberg, 1990; Gill *et al.*, 1994). Therefore, the functional difference between Sp1 and VP16 is not due to the promoter specificity (Emami *et al.*, 1995; Gerber *et al.*, 1995; Blau *et al.*, 1996; Lee, 1997).

It is possible that dimerization with Arnt facilitates the recruitment of coactivators or targets general transcription factors for the AhR or VP16, whereas those for Sp1 are not favorable to interact with Sp1 in the context of the AhR/Arnt heterodimer. Therefore, it is to be investigated whether dimerization between AhR and Arnt increases the ability of AhR/Arnt to interact with its target coactivator (Rowlands *et al.*, 1996). In hepatoma cells, the expression levels of AhR exceeds those of Arnt, raising the possibility that upon the exposure of dioxin, there may be two pools of AhR in the nucleus: viz., liganded AhR/Arnt heterodimer and liganded AhR monomer, that may either interact with target genes or be functionally inert. The transactivation domains of inert, liganded AhR monomers are repressed by its own N-terminal region, whereas the transactivation domains of functional AhR/Arnt are activated so that they interact with both the DNA element and other coactivators to induce target genes. It is beneficial to cells that only the functional heterodimer that is able to bind a cognate DNA element should have the privilege to interact with the limited number of coactivators.

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