J. Biochem. **122**, 1075-1079 (1997)

Ah Receptor, a Novel Ligand-Activated Transcription Factor[1]

Kazuhiro Sogawa[2] and Yoshiaki Fujii-Kuriyama

Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-77

Received for publication, August 26, 1997

The aryl hydrocarbon receptor (AhR) is widely distributed in vertebrates and is known to be involved in metabolism of xenobiotics including man-made chemicals, most of which act as a ligand for the receptor, although no endogeneous ligand has yet been known. Upon binding a ligand, the receptor is activated to translocate to the nuclei, and during the nuclear translocation process, it is dissociated from the 90 kDa heat shock protein (Hsp90) to form a heterodimer with Arnt (*A*h *r*eceptor *n*uclear *t*ranslocator). The heterodimer complex binds a DNA response element termed xenobiotic responsive element (XRE) localized upstream of the target genes of many drug-metabolizing enzymes including cytochrome P4501A1 and glutathione S-transferase to activate their transcription. Recent cDNA cloning has revealed that the AhR, like Arnt, possesses characteristic structural motifs of basic helix-loop-helix and PAS domains responsible for DNA recognition, heterodimerization, and ligand binding, and functions as a novel receptor-type transcription factor.

Key words: bHLH domain, carcinogenesis, PAS domain, transcription factor, xenobiotics.

The Ah receptor (AhR) plays a key role in the metabolism of xenobiotics, including environmental pollutants such as polycyclic aromatic hydrocarbons (PAH). PAH such as 3-methylcholanthrene (3MC) and benzo[*a*]pyrene function as potent ligands for the AhR, and the liganded AhR complex (see below) activates a number of genes for phase I and phase II drug-metabolizing enzymes including cytochrome P-4501A1 (CYP1A1) and glutathione S-transferase Ya subunit, which detoxify PAH in most cases. Upon being biotransformed, however, a fraction of PAH acquires reactivity with DNA to act as an ultimate mutagen (*1-4*). Thus, the AhR is implicated in carcinogenesis, presumably by damaging protooncogenes or tumor-suppressor genes. Ligands for the Ah receptor also include halogenated dibenzo-*p*-dioxin, dibenzofurans, and biphenyls. These carcinogenic compounds, as represented by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are not or very poorly metabolized by CYP1A1 and other phase I enzymes, and therefore retained in the body of animals (*5-7*). Although these compounds show little genotoxicity in themselves, because of their metabolic stability, they exert adverse pleiotropic effects on organisms in addition to induction of the metabolizing enzymes. These effects include cancer promotion, teratogenicity, epithelial hyperplasia, thymic atrophy, a wasting syndrome, chloracne, and hepatotoxicity. It has been suggested that all of the pleiotropic biological effects of TCDD are mediated by the AhR (*5, 6*). Although evidence for involvement of the AhR in some of these effects of TCDD has been reported with AhR gene-disrupted mice (*8-10*), the molecular mechanisms for these diverse toxic effects generally remain mysterious. Now that the molecular entity of the AhR has been revealed, its functional mechanisms in these toxicological effects are being extensively pursued. This paper briefly summarizes the present information about the structure and function of the AhR with reference to transcription factors in the same gene family.

Genes controlled by AhR complex

The induction mechanism by the AhR complex has been most extensively studied on the CYP1A1 gene, because induction of the gene by TCDD or 3MC is so strong (more than 50-fold) that it can be easily detected by the conventional methods for gene expression. Other genes for drug metabolizing phase I and phase II enzymes known to be under the control of the AhR include genes for CYP1A2, CYP1B1, CYP2A8, glutathione S-transferase Ya subunit, aldehyde-3-dehydrogenase, NAD(P)H quinone oxidoreductase, and UDP-glucuronosyltransferase (*6*). Although a list of genes other than those for the drug-metabolizing enzymes whose expression is modulated by the AhR complex is not yet available, it has been reported that the expression of several genes with interesting functions was induced by TCDD (*11*). These genes include those for plasminogen activator inhibitor 2 (PAI-2) and interleukin 1β (IL-1β), which are important for cell growth and differentiation.

It has been reported that the AhR can also act as an inhibitor to expression of the genes for estrogen receptor (ER) (*12*), tissue plasminogen activator (*13*), cathepsin D (*14*), and uterine peroxidase (*15*). Except for the ER gene, these genes are induced by estrogen and the induced expression is inhibited by the concomitant administration of TCDD. Inhibition of these gene expressions can be considered as antiestrogenic effects of TCDD. Several mechanisms of the inhibition were proposed. Firstly, the increased expression of CYP1A1 and CYP1A2 hydroxylates estrogens to less active forms. Secondly, the AhR

[1] This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Phone: +81-22-217-6591, Fax: +81-22-217-6594, E-mail: sogawa@mail.cc.tohoku.ac.jp

down-regulates the ER gene, resulting in a reduced level of nuclear ER. Recently a novel mechanism of the inhibition by the AhR has been proposed: the inhibition results from interference of the liganded AhR with the DNA binding of the activated ER. Reciprocally, it is also reported that estrogen-ER complex interferes with the transactivation potential exhibited by TCDD (*15*). Further studies are necessary to elucidate how liganded AhR interacts with the activated ER, leading to the mutual inhibition of their gene expression.

Structure of AhR and Arnt

The AhR cDNA was cloned in 1992, two decades after the discovery of the receptor, when problems associated with the receptor's low content in the cells and instability during purification processes were overcome (*16, 17*). The AhR had long been considered to be a member of the steroid/thyroid/retinoic acid receptor superfamily, because the molecular mechanism of its activation apparently resembles that of the glucocorticoid hormone receptor. However, structural analysis of the AhR cDNA revealed that the AhR exhibits no similarity to steroid hormone receptors, but has instead two characteristic structural domains, the basic helix-loop-helix (bHLH) and PAS domains in the N-terminal half of the molecule (*16, 17*) (Fig. 1). The PAS domain was designated as a common region found in *Drosophila* *P*er, human *A*rnt and *Drosophila* *S*im. Thus, the AhR represents a new class of a receptor-type transcription factor. To date, primary structures of the human, mouse, and rat AhR, consisting of 848 (*18-20*), 805 (*16, 17*), and 853 (*21*) amino acids, respectively, have been deduced from their cDNA sequences. The dimeric partner protein of the AhR, designated *A*h *r*eceptor *n*uclear *t*ranslocator (Arnt), also contains the two characteristic domains, HLH and PAS (*22, 23*) (Fig. 1). Amino acid identities of HLH and PAS domains between the AhR and Arnt are 25 and 18%, respectively. The AhR and Arnt form a heterodimer through interaction of the HLH and PAS domains between the two molecules to bind the DNA response element [xenobiotic responsive element (XRE) (*24*), or dioxin response element (DRE), CACGCNA/T] by the basic sequence just N-terminal to the HLH. Arnt is able to homodimerize with itself and heterodimerize with the AhR and other factors containing the HLH and PAS domains (*25*). The HLH domain is often found in transcription factors critical for cell growth and differentiation such as c-myc and Myo D, and functions as a dimerization interface. The PAS domain comprises approximately 250 to 300 amino acids containing two incomplete 50 amino acid repeats, and functions as a multifunctional domain interactive with ligands, Hsp90, and Arnt (*17, 26*). Recently, PAS-containing proteins have been discovered in increasing numbers and constitute a superfamily as shown in Fig. 2. It is interesting that two PAS proteins closely related to the AhR were found in *Caenorhabditis elegans*. Elucidation of the function of these proteins in nematoda may provide a clue to the innate functions and evolutionary process of the AhR. Transcriptional activation domains are localized in the C-terminal half of the AhR and Arnt (*27-29*) (Fig. 1). It has recently been found that the activation domain of Arnt interacts with the CBP or P300 at the CREB binding site (*30*).

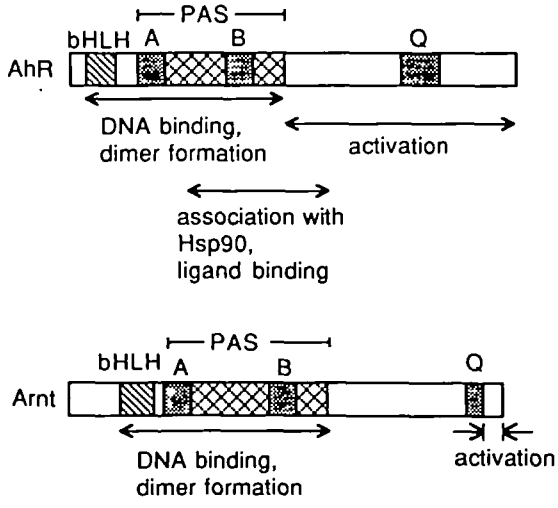

Fig. 1. Schematic representation of structure of the AhR and Arnt. bHLH, basic helix-loop-helix domain; PAS, PAS domain; Q, glutamine-rich region.

Gene structure, chromosomal localization, and expression

The mouse AhR gene is 37.5 kb long and separated into 11 exons (*31, 32*). It is a TATA-less gene, but has several GC boxes in the promoter region as is often observed with the house-keeping genes. Other *cis*-acting elements such as an AP-1 binding site, an E box, and a CRE are also found in

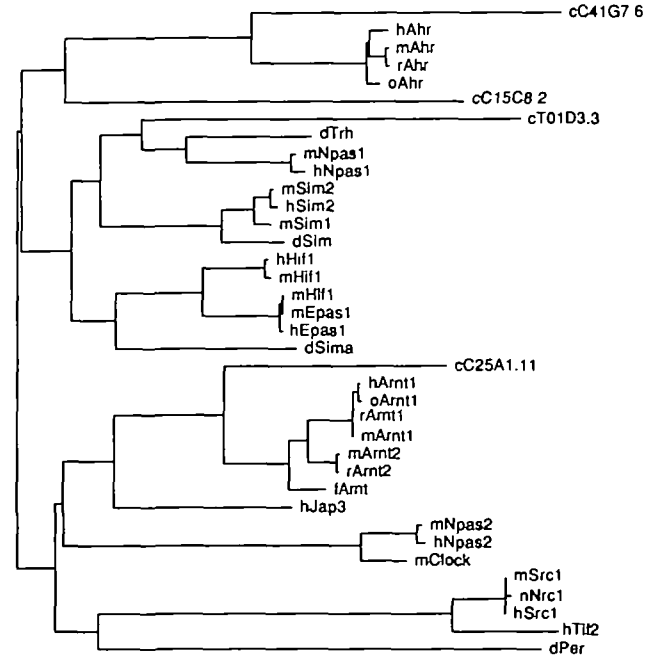

Fig. 2. A phylogenetic tree of the PAS protein superfamily. AhR, aryl hydrocarbon receptor; Trh, trachealess; Npas, neuronal PAS domain protein; Sim, single minded; Hif, hypoxia inducible factor; Hlf, Hif-like factor; Epas, endothelial PAS domain protein; Arnt, Ah receptor nuclear translocator; Src, steroid receptor coactivator; Nrc, nuclear receptor coactivator; Tif, transcriptional intermediary factor. h, human; m, mouse; r, rat; o, rabbit; f, traut; d, *D. melanogaster*; c, *C. elegans*.

the promoter region of the gene (*31, 32*). Deletion analysis of the upstream region of the AhR gene suggests that various combinations of these *cis*-acting elements regulate the transcription of the gene in cell type-specific manners (*33*).

Chromosomal localization of the human AhR gene was assigned to 7p21 (*34, 35*) and the mouse gene was localized on the syntenic region of chromosome 12 with the human chromosome (*31*). On the other hand, the human Arnt gene was mapped to chromosome 1q21 (*36*), a different chromosome from that of the AhR gene.

Expression of the AhR and Arnt genes was examined in various tissues of human (*37*), mouse (*16*), and rat (*21*) and found more or less similar patterns in these animals. The AhR mRNA is widely expressed with the highest level in lung, moderate levels in kidney and liver, and in low levels in spleen, heart, and skeletal muscle, while Arnt mRNA is relatively evenly expressed in these tissues. In general, expression of both genes appeared to be constitutive, except in special cases where the AhR mRNA was increased during monocytic differentiation of HL60 cells (*38*) and where Arnt mRNA was induced in Hep3B cells in response to hypoxic conditions (*39*).

Activation mechanisms of AhR/Arnt complex

The AhR is stably associated with Hsp90 in the latent, ligand-free form in the cytosol (*40, 41*). The role of Hsp90 was considered to be to hold the AhR in a conformation having ligand-binding ability, and to mask its DNA binding activity. No evidence was reported that Arnt interacted with Hsp90. Treatment with a ligand such as TCDD initiates a sequence of molecular events leading to transcriptional activation of a group of genes by the AhR, including dissociation from Hsp90, nuclear translocation, and finally heterodimer formation with Arnt localized in the nucleus to bind XRE (*42, 43*). Figure 3 shows an illustration of these activation processes summarized from the work of several laboratories. It is still a matter of controversy whether dissociation of the AhR from Hsp90 occurs in the cytosol or in the nucleus.

It was investigated how the AhR-Arnt complex recognizes and binds its asymmetric XRE sequence of 5′-CACGCNA/T-3′. Arnt was found to form a homodimer to bind a symmetric E-box sequence of 5′-CACGTG-3′, and the basic sequence responsible for DNA recognition is very similar to those of E box-binding transcription factors such as USF and cMyc, while that of the AhR is more distantly related. It was therefore proposed that the CAC sequence of the XRE sequence is recognized by the basic sequence of Arnt, while the other half is bound by that of AhR (*25*). This mode of DNA recognition of the AhR/Arnt complex was confirmed by DNA cross-linking experiments (*44*). Concerning the inducible expression of the CYP1A1 gene, the AhR/Arnt complex which first bound the XRE sequence was found to facilitate the binding of Sp1 to the GC-box sequence localized upstream of the TATA box sequence through the interaction between the HLH/PAS region of the AhR/Arnt and the zinc finger domain of Sp1. The reciprocal facilitation of the DNA binding between AhR/Arnt complex and Sp1 presumably results in a synergistic enhancement of the inducible expression of the CYP1A1 gene. This sort of arrangement of the XRE and the GC box sequences is often found upstream of the genes for other drug-metabolizing enzymes which are induced by TCDD or 3MC (*45*).

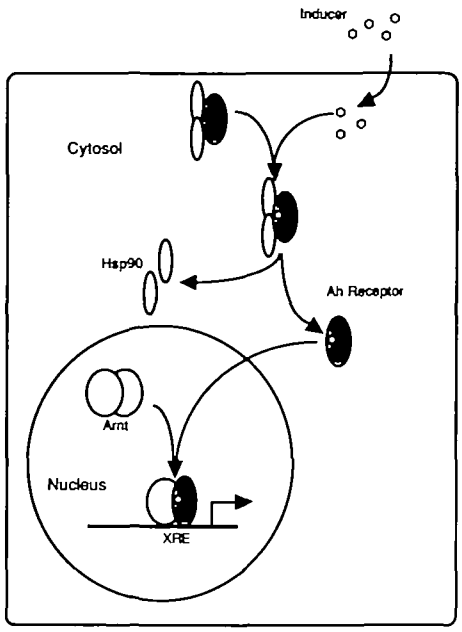

Fig. 3. **Model for the induction of xenobiotic responsive genes *via* the AhR.**

Several lines of evidence indicate that phosphorylation of the AhR/Arnt complex is important for its binding with the XRE sequence. *In vitro* treatment with protein phosphatase abolished the DNA-binding activity of the AhR/Arnt complex, and treatment of cultured cells with inhibitors of protein kinase C and tyrosine kinase inhibited the induction of CYP1A1 gene expression by TCDD or 3MC (*46, 47*). In addition, down-regulation of protein kinase C by prolonged exposure to phorbol esters probably results in a loss of the DNA-binding activity of AhR in human keratinocytes (*48*). It was also reported that long-term treatment of mice with a phorbol ester blocked the inducible expression of the CYP1A1 gene by TCDD (*49*). Although protein chemistry using metabolically ^{32}P-labeled AhR has localized two phosphorylation sites in the C-terminal half of the molecule (*50*), it remains to be seen whether these phosphorylation sites are related to the function of AhR and, if so, how.

Polymorphism of AhR

Interstrain polymorphism of AhR is known in mice. The polymorphic forms of AhR have different ligand-binding activities and consequently show different biological responses such as teratogenic and cancer susceptibility to TCDD and 3MC. The C57BL/6 strain is a representative strain with high inducibility in the CYP1A1 gene expression in response to inducers such as TCDD and 3MC, while the DBA/2 strain has with low inducibility of the gene expression. The AhR alleles in the C57BL/6 and DBA/2 mice are designated AhR$^{b\text{-}1}$ and AhR^{d}, respectively. cDNA cloning revealed that the AhR$^{b\text{-}1}$ allele encodes a polypeptide of 805 amino acids, while the gene product of the AhR^{d} allele consists of 848 amino acids (*51-53*). The molecular weights deduced from their cDNA sequences are in good agreement with those estimated by gel electrophoresis of the endogeneous AhR affinity-labeled with ^{125}I-TCDD (*52*).

Expression of these polymorphic forms of AhR in a cell-free system or in cultured cells by DNA transfection allowed us to estimate their ligand-binding affinities (*52, 53*). The K_d value for TCDD of the AhR^{b-1} allele is about 6 times lower than that of the AhR^d. There are ten nucleotide differences between the AhR^{b-1} and AhR^d alleles. While five of them are silent, the others cause amino acid alterations, with one of them on the termination codon resulting in a C-terminal extention of 43 amino acids in the AhR^d protein. Reciprocal changes of amino acid sequences between the two AhRs and introduction of specific mutations defined the amino acid changes responsible for affecting the ligand binding activity of the AhR. The A381V replacement together with the C-terminal extension are able to explain the change from the high-affinity form to the low-affinity form of AhR. It has been reported that two other high affinity forms of AhR, designated AhR^{b-2} and AhR^{b-3}, have apparent M_r of 104,000 and 105,000, respectively, which are close to that of the low-affinity form (*52*).

Because of the close association of polymorphic forms of AhR and cancer susceptibility in mice, human AhR polymorphism has been extensively investigated, especially in cancer patients. Thus far, however, no evidence has been obtained for the presence of a high affinity form of human AhR, although some polymorphic forms have been reported (*54-56*).

We thank Dr. O. Gotoh (Saitama Cancer Institute) for generously providing us with the figure of the phylogenetic tree of PAS proteins.

REFERENCES

1. Fujii-Kuriyama, Y. and Gotoh, O. (1995) Molecular biology of cytochrome P-450: Evolution, structure and regulation in *Molecular Aspects of Oxidative Drug Metabolizing Enzymes*, NATO ASI Series, Vol. H90 (Arinc, E., Schenkman, J.B., and Hodgson, H., eds.) pp. 65-85, Springer-Verlag, Berlin/Heidelberg
2. Sogawa, K. and Fujii-Kuriyama, Y. (1993) Regulation of cytochrome P450 expression in *Handbook of Experimental Pharmacology*, Vol. 105 Cytochrome P450 (Schenkman, J.B. and Greim, H., eds.) pp. 493-501, Springer-Verlag, Berlin/Heidelberg
3. Hankinson, O. (1995) The aryl hydrocarbon receptor complex. *Annu. Rev. Pharmacol. Toxicol.* **35**, 307-340
4. Whitlock, J.P., Jr. (1986) The regulation of cytochrome P-450 gene expression. *Annu. Rev. Pharmacol. Toxicol.* **26**, 333-369
5. Rose, J.Q., Ramsey, J.C., Wentzler, T.H., Hummel, R.A., and Gehring, P.J. (1976) The fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin following single and repeated oral doses to the rat. *Toxicol. Appl. Pharmacol.* **36**, 209-226
6. Poland, A. and Knutson, J.C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**, 517-554
7. Bock, K.W. (1993) Aryl hydrocarbon or dioxin receptor: Biologic and toxic responses. *Rev. Physiol. Biochem. Pharmacol.* **125**, 1-42
8. Fernandes-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., Lee, S.S.T., Kimura, S., Nebert, D.W., Rudikoff, S., Ward, J.M., and Gonzalez, F.J. (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* **268**, 722-726
9. Schmidt, J.V., Su, G.H.-T., Reddy, J.K., Simon, M.C., and Bradfield, C.A. (1996) Characterization of a murine Ahr null allele: Involvement of the Ah receptor in hepatic growth and development. *Proc. Natl. Acad. Sci. USA* **93**, 6731-6736
10. Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T.N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M., and Fujii-Kuriyama, Y. (1997) Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells*, in press
11. Sutter, T.R. and Greenlee, W.F. (1991) Targets for dioxin: genes for plasminogen activator inhibitor-2 and interleukin-1. *Science* **254**, 415-418
12. Lin, F.H., Stohs, S.J., Birnbaum, L.S., Clark, G., Lucier, G.W., and Goldstein, J.A. (1991) The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptor in congenic strains of Ah responsive and Ah nonresponsive C57BL/6J mice. *Toxicol. Appl. Pharmacol.* **108**, 129-139
13. Gierthy, J.F., Lincoln, D.W., Gillespie, M.B., Seeger, J.I., Martines, H.L., Dickerman, H.W., and Kumar, S.A. (1987) Suppression of estrogen-regulated extracellular plasminogen activator activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Cancer Res.* **47**, 6198-6203
14. Biegel, L. and Safe, S.J. (1990) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on cell growth and secretion of the estrogen-induced 34-, 52- and 160-kDa proteins in human breast cancer cells. *Steroid Biochem. Mol. Biol.* **37**, 725-732
15. Kharat, I. and Saatcioglu, F. (1996) Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are mediated by direct transcriptional interference with the liganded estrogen receptor: cross-talk between aryl hydrocarbon- and estrogen-mediated signaling. *J. Biol. Chem.* **271**, 10533-10537
16. Ema, M., Sogawa, K., Watanabe, N., Chujoh, Y., Matsushita, N., Gotoh, O., Funae, Y., and Fujii-Kuriyama, Y. (1992) cDNA cloning and structure of mouse putative Ah receptor. *Biochem. Biophys. Res. Commun.* **184**, 246-253
17. Burbach, K.M., Poland, A., and Bradfield, C.A. (1992) Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proc. Natl. Acad. Sci. USA* **89**, 8185-8189
18. Dolwick, C.M., Schmidt, J.V., Carver, L.A., Swanson, H.I., and Bradfield, C.A. (1993) Cloning and expression of a human Ah receptor cDNA. *Mol. Pharm.* **44**, 911-917
19. Eguchi, H., Hayashi, S., Watanabe, J., Gotoh, O., and Kawajiri, K. (1994) Molecular cloning of the human AH receptor gene promoter. *Biochem. Biophys. Res. Commun.* **203**, 615-622
20. Ema, M., Matsushita, N., Sogawa, K., Ariyama, T., Inazawa, J., Nemoto, T., Ota, M., Oshimura, M., and Fujii-Kuriyama, Y. (1994) Human aryl hydrocarbon receptor: Functional expression and chromosomal assignment to 7p21. *J. Biochem.* **116**, 845-851
21. Carver, L.A., Hogenesch, J.B., and Bradfield, C. (1994) Tissue specific expression of the rat Ah-receptor and ARNT mRNAs. *Nucleic Acids Res.* **22**, 3038-3044
22. Hoffman, E.C., Reyes, H., Chu, F.F., Sander, F., Conley, L.H., Brooks, B.A., and Hankinson, O. (1991) Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* **252**, 954-958
23. Nambu, J.R., Lewis, J.O., Wharton, Jr. K.A., and Crews, S.T. (1991) The *Drosophila* single-minded gene encodes a helix-loop-helix protein that acts as a master regulator of CNS midline development. *Cell* **67**, 1157-1167
24. Fujisawa-Sehara, A., Sogawa, K., Yamane, M., and Fujii-Kuriyama, Y. (1987) Characterization of xenobiotic responsive elements upstream from the drug-metabolizing cytochrome P-450c gene: a similarity to glucocorticoid regulatory elements. *Nucleic Acids Res.* **15**, 4179-4191
25. Sogawa, K., Nakano, R., Kobayashi, A., Kikuchi, Y., Ohe, N., Matsushita, N., and Fujii-Kuriyama, Y. (1995) Possible function of Ah receptor nuclear translocator (Arnt) homodimer in transcriptional regulation. *Proc. Natl. Acad. Sci. USA* **92**, 1936-1940
26. Antonsson, C., Whitelaw, M.L., McGuire, J., Gustafsson, J.-A., and Poellinger, L. (1995) Distinct roles of the molecular chaperone hsp90 in modulating dioxin receptor function via the basic helix-loop-helix and PAS domains. *Mol. Cell. Biol.* **15**, 756-765
27. Dong, L.H. and Whitlock, J.P., Jr. (1994) Transcriptional activation function of the mouse Ah receptor nuclear translocator. *J. Biol. Chem.* **269**, 28098-28105
28. Whitelaw, M.L., Gustafsson, J.A., and Poellinger, L. (1994) Identification of transactivation and repression functions of the

dioxin receptor and its basic helix-loop-helix/PAS partner factor Arnt: inducible versus constitutive modes of regulation. *Mol. Cell. Biol.* **14**, 8343-8355
29. Sogawa, K., Iwabuchi, K., Abe, H., and Fujii-Kuriyama, Y. (1995) Transcriptional activation domains of the Ah receptor and Ah receptor nuclear translocator. *J. Cancer Res. Clin. Oncol.* **121**, 612-620
30. Kobayashi, A., Numayama-Tsuruta, K., Sogawa, K., and Fujii-Kuriyama, Y. (1997) CBP/P300 functions as a transcriptional coactivator of Ah receptor nuclear translocator (Arnt) *J. Biochem.* **122**, 703-710
31. Schmidt, J.V., Carver, L.A., and Bradfield, C.A. (1993) Molecular characterization of the murine Ahr gene. Organization, promoter analysis, and chromosomal assignment. *J. Biol. Chem.* **268**, 22203-22209
32. Mimura, J., Ema, M., Sogawa, K., Ikawa, S., and Fujii-Kuriyama, Y. (1994) A complete structure of the mouse Ah receptor gene. *Pharmacogenetics* **4**, 349-354
33. FitzGerald, C.T., Fernandez-Salguero, P., Gonzalez, F.J., Nebert, D.W., and Puga, A. (1996) Differential regulation of mouse Ah receptor gene expression in cell lines of different tissue origins. *Arch. Biochem. Biophys.* **333**, 170-178
34. Le Beau, M.M., Carver, L.A., Espinosa, R., 3rd., Schmidt, J.V., and Bradfield, C.A. (1994) Chromosomal localization of the human AHR locus encoding the structural gene for the Ah receptor to 7p21→p15. *Cytogenet. Cell Genet.* **66**, 172-176
35. Ema, M., Matsushita, N., Sogawa, K., Ariyama, T., Inazawa, J., Nemoto, T., Ota, M., Oshimura, M., and Fujii-Kuriyama, Y. (1994) Human arylhydrocarbon receptor: functional expression and chromosomal assignment to 7p21. *J. Biochem.* **116**, 845-851
36. Johnson, B., Brook, B.A., Heinzman, C., Diep, A., Monadas, T., Sparks, R., Reyes, H., Hoffman, E., Gratti, R.A., Xin, Y.-R., Lusis, A.J., and Hamkinson, O. (1993) The Ah receptor nuclear translocator gene (ARNT) is locatedon q21 of human chromosome 1 and mouse chromosome 3 near Cf-3. *Genomics* **17**, 592-598
37. Hayashi, S., Watanabe, J., Nakachi, K., Eguchi, H., Gotoh, O., and Kawajiri, K. (1994) Interindividual difference in expression of human Ah receptor and related P450 genes. *Carcinogenesis* **15**, 801-806
38. Hayashi, S., Okabe-Kado, J., Honma, Y., and Kawajiri, K. (1995) Expression of Ah receptor (TCDD receptor) during human monocytic differentiation. *Carcinogenesis* **16**, 1403-1409
39. Wang, G.L., Jiang, B.-H., Rue, E.A., and Semenza, G.L. (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tenion. *Proc. Natl. Acad. Sci. USA* **92**, 5510-5514
40. Perdew, G.H. (1988) Association of the Ah receptor with the 90 kD heat shock protein. *J. Biol. Chem.* **263**, 13802-13805
41. Wilhelmsson, A., Cuthill, S., Denis, M., Wikstrom, A.-C., Gustafsson, J.-A., and Poellinger, L. (1990) The specific DNA binding activity of the dioxin receptor is modulated by the 90 kd heat shock protein. *EMBO J.* **9**, 69-76
42. Pollenz, R.S., Sattler, C.A., and Poland, A. (1993) The aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator protein show distinct subcellular localizations in HePa 1c1c7 cells by immunofluorescence microscopy. *Mol. Pharmacol.* **45**, 428-438
43. Hord, N.G. and Perdew, G.H. (1994) Physicochemical and immunochemical analysis of the aryl hydrocarbon receptor nuclear translocator: Characterization of two monoclonal antibodies to the aryl hydrocarbon receptor nuclear translocator. *Mol. Pharmacol.* **46**, 618-626
44. Bacsi, S.G., Reisz-Porszasz, S., and Hankinson, O. (1995) Orientation of the heterodimeric aryl hydrocarbon (dioxin) receptor complex on its asymmetric DNA recognition sequence. *Mol. Pharmacol.* **47**, 432-438
45. Kobayashi, A., Sogawa, K., and Fujii-Kuriyama, Y. (1996) Cooperative interaction between AhR/Arnt and Sp1 for the drug-inducible expression of CYP1A1 gene. *J. Biol. Chem.* **271**, 12310-12316
46. Carrier, F., Owens, R.A., Nebert, D.W., and Puga, A. (1992) Dioxin-dependent activation of murine cyp1a-1 gene transcription requires protein kinase C-dependent phosphorylation. *Mol. Cell. Biol.* **12**, 1856-1863
47. Berghard, A., Gradin, K., Pongratz, I., Whitelaw, M.L., and Poellinger, L. (1993) Cross-coupling of signal transduction pathway: the dioxin receptor mediates induction of cytochrome P-450IA1 expression via a protein kinase C-dependent mechanism. *Mol. Cell. Biol.* **13**, 677-689
48. Gradin, K., Whitelaw, M.L., Toftgard, R., Poellinger, L., and Berghard, A. (1994) A tyrosine kinase-dependent pathway regulates ligand-dependent activation of the dioxin receptor in human keratinocytes. *J. Biol. Chem.* **269**, 23800-23807
49. Okino, S.T., Pendurthi, U.R., and Tukey, R.H. (1992) Phorbol esters inhibit the dioxin receptor-mediated transcriptional activation of the mouse Cyp1a-1 and Cyp1a-2 genes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* **267**, 6991-6998
50. Mahon, M.J. and Gasiewicz, T.A. (1995) Ah receptor phosphorylation: Location of phosphorylation sites to the C-terminal half of the protein. *Arch. Biochem. Biophys.* **318**, 166-174
51. Chang, C., Smith, D.R., Prasad, V.S., Sidman, C.L., Nebert, D.W., and Puga, A. (1993) Ten nucleotide differences, five of which cause amino acid changes, are associated with the Ah receptor locus polymorphism of C57BL/6 and DBA/2 mice. *Pharmacogenetics* **3**, 312-321
52. Poland, A., Palen, D., and Glover, E. (1994) Analysis of the four alleles of the murine aryl hydrocarbon receptor. *Mol. Pharmacol.* **46**, 915-921
53. Ema, M., Ohe, N., Suzuki, M., Mimura, J., Sogawa, K., Ikawa, S., and Fujii-Kuriyama, Y. (1994) Dioxin binding activities of polymorphic forms of mouse and human aryl hydrocarbon receptors. *J. Biol. Chem.* **269**, 27337-27343
54. Kawajiri, K., Watanabe, J., Eguchi, H., Nakachi, K., Kiyohara, C., and Hayashi, S. (1995) Polymorphisms of human Ah receptor gene are not involved in lung cancer. *Pharmacogenetics* **5**, 151-158
55. Jones, J.E., Huckaby, C.S., Stafford, M.D., and Linnoila, R.I. (1994) An MspI RFLP of the human AHR gene. *Human Mol. Genet.* **3**, 2083
56. Perdew, G.H. and Hollenback, C.E. (1995) Evidence for two functionally distinct forms of the human Ah receptor. *J. Biochem. Toxicol.* **10**, 95-102