Induction of *CYP1A1*. The AhR/DRE Paradigm: Transcription, Receptor Regulation, and Expanding Biological Roles

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Abstract: The *CYP1A1* gene encodes microsomal cytochrome P4501A1 that catalyzes the metabolism of many xenobiotics, including the oxygenation of polycyclic aromatic hydrocarbons (PAH). Induction of *CYP1A1* enhances the metabolism of PAHs, and therefore, represents an adaptive response to chemical exposure in mammalian cells. Mechanistic studies reveal an AhR/DRE paradigm for the induction, which involves activation of the aryl hydrocarbon receptor (AhR) by an agonist, dimerization of AhR with the Ah receptor nuclear translocator (Arnt), followed by binding of the AhR/Arnt heterodimer to the dioxin-responsive enhancer (DRE) and transcription of the gene. The AhR mediated transcription is tightly regulated through, at least, two mechanisms: (a) the cytoplasmic AhR interacts with hsp90 and an immunophilin chaperone AIP for proper folding and receptivity, and (b) the agonist-activated, nuclear AhR is degraded through the ubiquitin-26S proteasome mediated protein turnover, such that the transcription by AhR is controlled at a physiologically adequate level. In addition to *CYP1A1* induction, AhR mediates a broad range of biological responses to *CYP1A1* inducers, typified by the environmental contaminant dioxin, via modulating gene expression. Thus, mechanistic studies of *CYP1A1* induction have provided insights into P450 induction, PAH carcinogenesis, dioxin action, AhR function, and receptor-mediated mammalian gene expression.

INTRODUCTION

Cytochrome P450s are a superfamily of enzymes that play important roles in the metabolism of many drugs, carcinogens, steroid hormones, and environmental/occupational chemicals [1-5]. Cytochrome P4501A1, which is encoded by *CYP1A1*, is a major substrate-inducible form of microsomal P450s in mammalian species. P4501A1 oxygenates lipophilic substrates, including the carcinogenic polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP). Oxygenation by P4501A1 serves as an initial step in the metabolic conversion of the substrates to water-soluble metabolites for excretion from body; however, oxygenation of PAHs often generates electrophilic arene oxide, dioepoxide, and other reactive species that can bind to macromolecules and lead to tumor formation or toxicity [6-8]. A main feature of P4501A1 action is its high inducibility by substrates [9]. Furthermore, many of the inducers produce a wide range of biological effects in animals [10]. Over the past several decades, studies on *CYP1A1* induction have contributed significantly to our understanding of the mechanism of P450 induction, chemical carcinogenesis, and receptor mediated gene transcription and toxicity. In this review, I will summarize some recent advances in the understanding of the mechanism of *CYP1A1* induction, in particular the transcription through the AhR/DRE paradigm, regulation of AhR, and new insights into the mechanism of AhR action in mediating diverse biological effects of *CYP1A1* inducers via gene regulation.

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HISTORICAL ASPECTS OF *CYP1A1* INDUCTION

The discovery of cytochrome P4501A1 induction had its origin in the observation that PAHs induce their own metabolism [6]. Induction of cytochrome P450s reflects an adaptive change of cells to chemical exposure. Induction of the P450 isoforms that participate in the metabolism of xenobiotics, such as P4501A1, is usually advantageous in that it enhances the metabolism of lipophilic chemicals, a process that helps to detoxify and eliminate the chemicals from body. Since inducers are quite often substrates of the induced enzyme, the induction of the enzyme is generally limited in duration as the inducers are metabolized by the enzyme. This substrate/inducer interaction enables the cells to increase and maintain high levels of enzyme activity only as needed. Therefore, induction of P450s and other drug-metabolizing enzymes helps to maintain the metabolic homeostasis of cells as it confronts a potentially toxic chemical. However, induction can also be detrimental. For example, induction of P4501A1 leads to the formation of reactive species, such as arene oxides and dioepoxides during the metabolism of PAHs, resulting in cytotoxicity, mutagenicity, and carcinogenecity. In addition, since P450 enzymes often have broad substrate specificities, enzyme induction by one chemical may result in enhanced metabolism of other chemicals or drugs, thereby, altering the pharmacokinetic properties and the therapeutic or toxic effects of the others [11-13].

Mouse genetic studies contributed greatly to the understanding of the mechanism of *CYP1A1* induction. Induction of the gene exhibits a genetic polymorphism in inbred mouse strains [14,15]. Some strains, such as DBA/2, are resistant to induction by 3-methylcholanthrene (3-MC), a PAH inducer; whereas other strains, such as C57BL/6, are about 10-times more sensitive. The sensitive phenotype segregates as an autosomal dominant trait. The polymorphism defines a genetic locus that encodes a receptor protein designated as the aryl hydrocarbon receptor (AhR); the AhR binds an inducer and mediates the induction of P4501A1 [10,16]. In addition, studies in the polymorphic inbred mice implicate AhR in other biological effects of P4501A1 inducers, such as the toxicity of dioxin. Whether there is a polymorphism of AhR in the human population, and if so, whether the polymorphism affects the carcinogenic effect of PAHs or the toxicity of dioxin and related chemicals in humans, remain an active area in research as discussed later in this review [17].

The mouse hepatoma cell line, hepa1c1c7, constitutes a powerful cell system for studying the mechanism of induction of P4501A1. The induction in the cell is rapid and robust. Furthermore, variant hepatoma cells that are defective in the induction were obtained by using a single-step selection procedure, in which BaP induces P4501A1 in wild type, but not variant cells, leading to formation of toxic metabolites and cell death in wild type cells and selective growth of variant cells [18], or by using FACS (fluorescence activated cell sorter), in which variant cells were selected because they exhibit lower rates in the metabolism of BaP [19]. Genetic analyses of the variants using cell fusion revealed that the variant cells fell into several complementation groups; these findings imply that multiple gene products contribute to the induction mechanism. The class I variant exhibits diminished binding to inducers and is defective in AhR; whereas, the class II variant is defective in a protein termed "Ah receptor nuclear translocator" (Arnt), because the cells exhibit abnormal nuclear localization of ligand-activated AhR. In subsequent studies, Arnt was cloned by complementing Arnt-defective variants with genomic DNA from normal cells and AhR was cloned according to a cDNA fragment deduced from a partially purified AhR protein. Both AhR and Arnt belong to the bHLH (basic helix loop helix)/ PAS (Per, Arnt, and Sim) family of transcription factors, since they contain the corresponding modular structures at their amino halves. AhR and Arnt function as a heterodimer in the induction of *CYP1A1*. Reconstitution of class I or class II variant cells with AhR or Arnt cDNA coding sequences fully restored the induction of *CYP1A1* in the cells. These genetic studies using variant cells not only established AhR and Arnt as the principle mediators of *CYP1A1* induction, but also facilitated analysis of the transcriptional process by AhR/Arnt at a molecular level [20].

Biochemical and molecular analyses of *CYP1A1* induction revealed an enhancer-dependent gene transcription. Several copies of sequences in the enhancer region of *CYP1A1* were identified as the DNA component specific for *CYP1A1* induction and were designated as DRE (dioxin responsive element) [21]. Thus, over the years, a combination of genetic, biochemical, and molecular approaches has been successfully utilized to delineate the pathway of *CYP1A1* induction; these studies established the AhR/DRE paradigm for transcriptional gene regulation by *CYP1A1* inducers, which involves a multi-step, receptor/transcription factor-mediated, transcriptional process [20,22].

Prototypical Inducers of *CYP1A1* include PAHs, typified by BaP [6], and halogenated aromatic hydrocarbons, typified by the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin, TCDD) [9,10]. Several notable differences between dioxin and BaP as *CYP1A1* inducers are observed. First, dioxin has a higher binding affinity for AhR than BaP; the high affinity of dioxin may reflect a better fit of dioxin in the ligand-binding pocket of AhR. Second, BaP is metabolized by P4501A1; consequently it has a short half life ($t_{1/2}$) and induces *CYP1A1* transiently. In contrast, dioxin is resistant to oxygenation by P4501A1 due to the symmetric distribution of chlorine atoms on its ring structure, and therefore is an exception to the inducer/substrate rule of P450 induction. The long half life of dioxin permits it to accumulate in cells and to cause prolonged effects. Lastly, dioxin produces a wide range of adaptive and toxic responses in animals in addition to induction of *CYP1A1*. Genetic studies implicate AhR in most of these responses to dioxin [23-26]. Thus, it is reasonable to speculate that dioxin's high receptor affinity and its capacity to produce sustained alterations in gene expression are the primary basis for its diverse and complex adverse health effects in comparison with other *CYP1A1* inducers, such as BaP. Although the molecular mechanism of dioxin action for most of its biological effects is not well understood, the AhR/DRE paradigm of *CYP1A1* induction has served as a model for mechanistic studies of gene regulation and complex toxic effects of dioxin.

In addition to man-made environmental chemicals, such as dioxin, certain indoles and other plant compounds are found to be relatively potent *CYP1A1* inducers and AhR agonists. Although the endogenous ligand for AhR is unknown, phycogenetic studies reveal that AhR is an ancient protein with both vertebrate and invertebrate homologs [27]. These findings imply that AhR has had important physiological functions and endogenous ligands for AhR exist during evolution. This view of AhR function is supported by findings from genetic studies using mouse or cell models that lack or are defective in AhR; these studies revealed that AhR is involved in the development of liver and the immune system, reproductive function, cell growth and differentiation, and vascular development in multiple organs [28-33]. These observations also suggest that AhR and the induction of *CYP1A1* are evolved together in response to naturally occurring chemicals; induction of *CYP1A1* by these chemicals help eliminating potentially harmful compounds of food ingestion [34,35]. This hypothesis of *CYP1A1* induction implys that dioxin and related chemicals, which are introduced into the environment only recently, mimics the action of an as-yet-unidentified, naturally occurring AhR ligand(s), which is from food consumption or produced endogenously, in their action of *CYP1A1* induction and other biological effects.

MECHANISM OF INDUCTION: THE AHR/DRE PARADIGM

DRE as the cis-Acting Component for *CYP1A1* Induction

As the result of adaptation to the ever-changing chemical environment, multiple mechanisms involving transcription and/or translation are evolved to induce the synthesis of P450 isoformes in response to foreign chemicals. In the case of *CYP1A1* induction, early studies revealed that the induction by PAHs in mouse hepatoma cells is fast and robust, and is due to an increase in the rate of *CYP1A1* mRNA synthesis [20]. These findings established that the induction is a primary response and is transcriptional.

Molecular analyses of the upstream sequences of the *CYP1A1* gene by Whitlock and associates led to the identification of the cis-acting elements that control the induction of the gene [For review, see ref. [20]]. Expression of chimerical genes (fusion of a reporter and the upstream sequence of *CYP1A1*) by transient expression identified a transcriptional enhancer, centered around 1000 and spanning about 600 base pairs, which conferred inducibility. Electrophoretic mobility shift analyses (EMSA) using nuclear extracts from hepa 1c1c7 cells revealed inducible protein-DNA interactions [21]. The interaction is AhR-, and Arnt-dependent, because it is not present in nuclear extracts from AhR-, or Arnt- defective variant cells. By using EMSA, a core sequence of 5' TNGCGTG 3' was identified. This core sequence is present in several copies in the enhancer region, and, together with several additional flanking base pairs, can confer induction in both reporter gene assay and EMSA. These sequences were designated as DRE for dioxin-responsive elements or XRE for xenobiotic-responsive elements. Further analyses by mutating the DREs identified a four-base pair motif, 5' CGTG 3', which resembles the "E-box" consensus sequence, 5' CANNTG 3'. Subsequent studies reveal that AhR and Arnt bind as a heterodimer to the DREs; Arnt binds to the 3' end and AhR to the 5' end [36-38].

The promoter element of the *CYP1A1* gene is located immediately upstream of the transcription start site. The promoter has binding sites for several general transcription factors, including the TATA-binding protein (TBP). The promoter is silent in the absence of the DRE enhancer and the protein-DNA interactions at the promoter are inducible. Thus, the function of the promoter is under the control of the enhancer of the *CYP1A1* gene [20].

AhR and Arnt Mediate the Induction of *CYP1A1*

By using a photoaffinity labeled ligand of AhR, Poland and associates labeled the AhR protein from the cytoplasmic fraction of mouse liver, and isolated the AhR protein to partial purity by using ion exchange chromatography and C4 reverse phase high performance liquid chromatography [39]. Sequence information obtained from a fragment of the AhR led to cloning of the AhR cDNA. Analyses of the amino acid sequence of mouse AhR cDNA revealed a structural organization that is now recognized as representative of bHLH/PAS transcriptional factors (Fig. 1) [20,22,40-42]. A bHLH domain is located near the N terminus. The basic region contributes to DNA binding and the HLH region to protein-protein dimerization. These

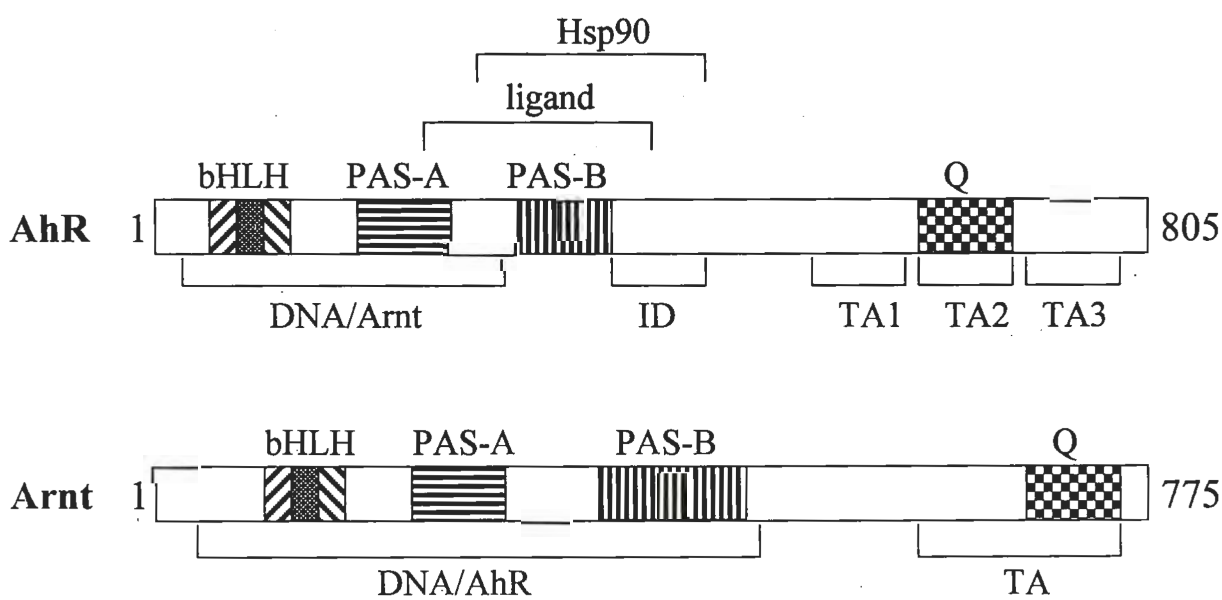

Fig. (1). Modular structures of AhR and Arnt. The basic (b), helix-loop-helix (HLH), Per-Arnt-Sim (PAS), and glutamine-rich (Q) domains are shown as shaded area; brackets indicate functional maps for binding to DNA and Arnt or AhR (DNA/Arnt or DNA/AhR), ligand of AhR (ligand), the Hsp90 protein (Hsp90), and for the inhibitory activity (ID) and the transcription activation function (TA). See the text for details and references.

regions also contain the nuclear localization and export signals. C terminal to the bHLH domain is the PAS region, which is an imperfect inverted repeat of about 300 amino acid residues. PAS was originally recognized because of the homology of the regions in Per (a Drosophila circadian protein), Arnt , and Sim (a Drosophila protein involved in CNS development). The PAS region in AhR contributes to dimerization with Arnt, ligand binding, and interaction with hsp90. The C-terminal region consists of three modular transcription activation (TA) domains, and an inhibitory domain (ID) that represses the activity of the TA domains [43]. These analyses of the modular structure of AhR have contributed significantly to the understanding of the mechanism of AhR action; it also served as a prototype for the analysis of other bHLH/PAS transcription factors. However, elucidating how the modular domains function at a molecular level remains a challenge in future studies.

By using cell genetic complementation, Hankinsen and associates cloned Arnt from a genomic DNA fragment that can complement the defect of class II variant cells, in which liganded AhR fails to accumulate in the nucleus [22,44]. The Arnt protein resides in the nucleus and heterodimerizes with ligand-activated AhR, thereby generating a DNA-binding transcription complex, which binds to the DREs of *CYP1A1*. Recent studies reveal that Arnt heterodimerizes with other bHLH/PAS proteins under different physiological or pathophysiological conditions; thus, Arnt appears to serve as a general partner in the function of a number of bHLH/PAS factors [45]. The modular structure of Arnt is similar to that of AhR (Fig. **1**). The N-terminal region has a bHLH domain that confers functions for DNA recognition, dimerization with AhR, and nuclear localization. The PAS region contributes to interaction with AhR. The C-terminal region contains a TA activity.

Transcription of *CYP1A1*

Since AhR and Arnt function as a heterodimer, the AhR/Arnt dimer serves as an interesting model for analyzing the mechanism of transcription by hetero-dimeric transcription factors. Protein-DNA cross-linking and mutational studies reveal that heterodimerization of AhR and Arnt orient the basic regions of AhR and Arnt such that the basic region of Arnt binds the 5' GTG 3' half, and the basic region of AhR to the other half of the core 5' to the GTG sequence [36,37]. The PAS regions of AhR and Arnt contribute to heterodimerization and DNA recognition. The PAS region of AhR is also involved in binding with hsp90 and ligands. EMSA experiments using DRE and *in vitro* expressed deletion mutants of AhR/Arnt demonstrated that the bHLH-PAS regions of AhR and Arnt are sufficient for ligand-induced binding to DRE sequences.

The C terminal half of AhR contains three modular TA domains, i.e. an acidic domain (TA1), a glutamine (Q)-rich domain (TA2), and a serine and threonine-rich domain (TA3); the TA domains exhibit synergistic activities with each other. The C terminus of Arnt contains a single glutamine (Q)-rich TA domain. The transcription activation activities of AhR and Arnt TA domains exhibit different characteristics. The TA activity of Arnt is constitutive, whereas that of AhR is regulated through a modular inhibitory domain (ID), adjacent to the amino end of the TA domains of AhR. Genetic reconstitution experiments involving expression of AhR/Arnt mutants in AhR or Arnt-defective variant cells reveal that the TA function of AhR is both required and sufficient for the induction of *CYP1A1 in vivo*; the TA of Arnt, however, is dispensable [43,46,47].

Analyses of *CYP1A1* transcription using *in vivo* DNA foot printing established that both the enhancer and promoter regions of *CYP1A1* are in a nucleosomal configuration, and therefore, are not accessible to transcription factors in the absence of an AhR agonist [48]. Activation of AhR by an agonist leads to the disruption of the chromatins in the enhancer and promoter. Furthermore, these analyses revealed that binding of AhR/Arnt dimer to the enhancer is associated with loss of the nucleosomal structure in the enhancer; this process does not require the TA domains of AhR or Arnt. However, protein binding at the promoter region is dependent upon the presence of functional TA domains of AhR. Therefore, the TA domains of AhR at the enhancer region mediate the chromatin disruption, protein binding, and transcription of the gene at the promoter region, a phenomenon referred to as enhancer-promoter communication [20].

The mechanism by which the TA domains of AhR mediate *CYP1A1* transcription is not clear. By analogy with the findings on the transcription by steroid hormone receptors such as estrogen receptor, it is conceivable that the TA domains of AhR recruit coactivators and other factors, which in turn initiate the assembly of general transcription factors and the polymerase II complex (Pol II) at the promoter region, followed by transcription by Pol II. The protein factors recruited by AhR TA domains may contain a histone acetyltransferase (HAT) activity, which catalyzes acetylation of histones, and thereby facilitates the opening of chromatin structures at *CYP1A1* promoter. Cloning these cofactors and analyzing the mechanism of their action in the transcription of endogenous, chromosomal *CYP1A1* remain a major challenge in future [49,50].

REGULATION OF AHR

Recent studies have identified several mechanisms by which the AhR/DRE mediated *CYP1A1* transcription is regulated. These findings reveal new insights into the mechanism of controlling receptor-mediated gene transcription in mammalian cells.

The Cytoplasmic AhR-Hsp90-AIP Complex

The unliganded AhR resides in the cytoplasm in a complex with Hsp90 (Heat shock protein 90) [51] and AIP (Ah receptor-interacting protein), an immunophilin type of chaperone protein [52-54]. Hsp90 is involved in several ligand-activated receptor signaling pathways, including glucocorticoid receptor and estrogen receptor. Genetic analyses in Hsp90-defective yeast strains revealed that Hsp90 is required for AhR function in DRE-mediated reporter gene induction [55]. Hsp90 may function as a chaperone to maintain AhR in a configuration that facilitates ligand binding and responsiveness.

AIP is a 38 kDa protein that exhibits homology with FK506-binding proteins and contains three tetratricopeptide repearts (TPR) [52-54]. AIP was first cloned by the yeast two-hybrid screening procedure using mouse AhR as a bait [52]. Immunoprecipitation study confirmed that AIP is a component of the cytoplasmic AhR/Hsp90 complex; the TPR motifs may mediate interactions of AIP with other components of the complex. Overexpression of AIP enhances the function of AhR by two-fold. AIP may act as a chaperone that influences the receptivity and nuclear targeting of AhR. In addition, biochemical analyses suggest that AIP is involved in determining the stability and the protein level of AhR in mouse tissues, thereby contributing to diverse tissue-specific toxicities of AhR ligands [56,57]. Understanding the molecular basis for the interaction among AhR, Hsp90, and AIP, and the functional role of such interactions in AhR action will provide new insights into the mechanism of AhR regulation and ligand-activated receptor signal transduction in general.

Superinduction and Degradation of Nuclear AhR Through the Ubiquitin-26S Proteasome Pathway

Cycloheximide enhances the induction of *CYP1A1* gene expression by dioxin by an order of magnitude, a phenomenon termed "superinduction" [58]. Since cycloheximide is known to inhibit protein synthesis, two conclusions can be made from the observation: (a) induction of *CYP1A1* does not require protein synthesis; thus the factors mediating *CYP1A1* induction (i.e. AhR/Arnt) preexist in cells. (b) An additional regulatory mechanism exists that negatively controls the transcription of *CYP1A1* by AhR/Arnt. Analyses of *CYP1A1* transcription during the superinduction established that the superinduction requires functional DREs and involves an increase in the rate of transcription of the gene [58,59]. These findings suggest that a cyclohexmide-sensitive, labile regulatory factor inhibits the AhR/DRE-mediated transcription.

Analyses of the AhR protein by immuno blotting and pulse-chase labeling revealed that, in addition to activating AhR, TCDD induces a rapid reduction in the level of the AhR protein in cells, which is attributed to an increase in the turnover of AhR (TCDD reduces the $t_{1/2}$ of AhR from 28 h to 3 h) [60,61]. Mechanistic studies established that the TCDD-induced AhR turnover is mediated through the 26S proteasome, involves ubiquitination of AhR, and requires the transcription activation domain of AhR [61]. Furthermore, cycloheximide is found to totally block TCDD induced AhR turnover. Blocking of the AhR degradation by cycloheximide is time and dose-dependent and is accompanied by an increase in the amount of the nuclear AhR protein and the DNA-binding activity of the AhR/Arnt complex. These analyses implicate a cycloheximide-sensitive, labile factor (designated as AhR degradation promoting factor, or ADPF) in controlling the removal of agonist-activated AhR in nucleus [62]. Inhibition of the synthesis of ADPF by cycloheximide releases the negative control, resulting in an increase in the stability of nuclear AhR, thereby superinducing *CYP1A1*. Several lines of observations support this conclusion: (a) inhibition of agonist-induced AhR degradation by using the 26S proteasome inhibitors superinduces *CYP1A1*; (b) puromycin, which inhibits protein synthesis through a different mechanism from that of cycloheximide, also blocks AhR degradation and superinduces *CYP1A1*, implying that inhibition of protein synthesis is sufficient for both phenotypes; and (c) cyclohexmide superinduces several other dioxin-inducible genes, including *CYP1B1*, Aldh3a1 (an aldehyde dehydrogenase), and ectoATPase (a plasma membrane ATPase) (Ma, *et al*, unpublished observations). ADPF may regulate the degradation of nuclear AhR by promoting ubiquitination of AhR similarly to the degradation of p53 (a tumor suppressor protein) by Mdm2, an oncogene product with an ubiquitin-ligase activity for p53 [63,64]. Cloning of ADPF will reveal new aspects of regulation of AhR and other transcription factors in nucleus through the 26S-proteasome mediated protein degradation.

Other Mechanisms of AhR Regulation

Induction of *CYP1A1* requires the TA domains of AhR; the TA activity of AhR is inhibited by an inhibitory domain (ID) of AhR located N-terminal to the TA domains between residues 340 and 421 [43]. The inhibitory domain is rich in serine and threonine and functions as a modular structure, for it suppresses both AhR and Arnt TA activities in a heterologous context. Releasing the inhibition follows activation of AhR by dioxin and requires the presence of Arnt; these findings implicate a mechanism for the regulation of the TA activities of the AhR/Arnt heterodimer. Inhibition of the AhR TA activity by the inhibitory domain may involve interaction of the domain with a trans-, inhibitory protein, since the inhibitory activity of the modular domain varies in different cell types. Identification and cloning of the inhibitory protein factor will provide new insights into transcriptional regulation by heterodimeric transcription factors.

Inhibitors of protein kinase C block the induction of *CYP1A1*, implying that protein phosphorylation/dephosphorylation plays a role in the function of AhR and/or Arnt [65]. Regulation of AhR through phosphorylation/dephosphorylation of AhR or its partner(s) may contribute to cross talking of the AhR/DRE pathway with other signal transduction pathways. However, direct evidence of phosphorylation of AhR or Arnt is lacking at the present stage.

Screening of a mouse genomic library using AhR cDNA as a probe led to cloning of a novel bHLH protein that shares high homology with the bHLH and PAS-A domains of AhR. By using a combination of *in vitro* coprecipitation and luciferase reporter assays, the AhR-like protein was found to repress AhR function by competing with AhR for dimerizing with Arnt. Furthermore, the gene of the protein was inducible by 3-methylcholanthrene (3MC) through the AhR/DRE pathway. The protein was designated as AhR repressor or AhRR [66]. Thus, AhR and AhRR may form a regulatory circuit in AhR signal transduction. However, whether the competition between AhR and AhRR for Arnt and the induction of AhRR by AhR ligands occur in intact cells and animal tissues remain to be established.

EXPENDING ROLES OF THE AHR/DRE PARADIGM

One prominent feature of AhR action is that it mediates a broad range of diverse biological responses to its ligands, typified by dioxin, ranging from induction of drug-metabolizing enzymes to complex phenotypes such as tumor formation, birth defect, wasting, and thymic involution. Since AhR is a transcription factor, it is assumed that AhR modulates the expression of a spectrum of target genes in response to dioxin, which in turn cause the biological phenotypes. This hypothesis raises the questions of whether and how AhR regulates different target genes through the AhR/DRE paradigm. During the past few years, certain progress has been made with respect to the mechanism of AhR action in regulating the expression of various target genes that may contribute to the diversity and specificity of the responses to AhR ligands.

Induction of Other Phase I and Phase II Drug-Metabolizing Enzymes.

In addition to inducing *CYP1A1*, AhR agonists, such as BaP and dioxin, induce several other phase I and phase II drug-metabolizing enzymes that include *CYP1A2* (cytochrome P4501A2) [67], *CYP1B1* (cytochrome P4501B1) [68,69], NQOR [NAD(P)H:quinone oxidoreductase, DT-diaphorase][70-72], GSTYa (glutathione S-transferase Ya subunit) [73], Aldh3a1 (an aldehyde dehydrogenase) [74], and UGT1A6 (a UDP glucuronosyltransferase) [75]. Induction of these enzymes results in enhanced metabolism of the inducers and closely related chemicals, such that the products of these enzymatic reactions are more hydrophilic, and therefore can be excreted from the body through urine or bile ducts. Thus, induction of the enzymes constitutes an important mechanism of defense against foreign chemicals. Analyses of the mechanism by which dioxin induces the enzymes reveal that the induction requires functional AhR and Arnt and involves DRE or DRE like sequences found in the enhancer regions of the genes; therefore, induction of these phase I and phase II enzymes is mediated through the AhR/DRE pathway.

Several differences are noted in the expression and induction of the genes. First, several of the genes, including *CYP1A2,* GstYa, NQOR, and UGT1A6, are constitutively expressed in certain tissues such as liver. For instance, *CYP1A2* is a major constitutive form of the P4501A family in the livers of human and other mammalian species, whereas *CYP1A1* is nearly undetectable, but is highly inducible in the liver. The liver specific, constitutive expression of *CYP1A2* may be mediated through several liver-specific transcription factors. Indeed, a DNA responsive element for HNF-1α (hepatocyte nuclear factor 1α) was identified in the enhancer of *CYP1A2* and disruption of the HNF-1α gene in mice down-regulated the expression of *CYP1A2* [76-78]. Second, the enhancer regions of the dioixn-inducible genes contain varying copy numbers of DREs. For example, the human *CYP1A2* [67], rat NQOR [70], and rat Gstya [73] contain a single copy of DRE in their enhancer regions, whereas the enhancers of *CYP1A1* and Aldh3a1 contain multiple copies of DREs [21,74,79]. The difference in DRE copy numbers in the enhancers contributes to the variation in the inducibility of the genes through the AhR/DRE pathway. Lastly, induction of certain genes exhibits species, tissue, and cell type specificity. For example, human *CYP1B1* is induced by dioxin in MCF-7, a human breast adenocarcinoma cell line, but not in HepG2, a human hepatoma cell line [68]; the cell type specific repression of *CYP1B1* induction may involve a cell type specific inhibitor(s) or a higher order chromatin structure of the *CYP1B1* regulatory region in HepG2 cells. Elucidating the interaction of the AhR/DRE pathway with other regulatory mechanisms that contribute to the regulation of dioxin-inducible drug-metabolizing enzymes will reveal new aspects of the mechanism of gene regulation by AhR.

Regulation of Ecto-ATPase and MHC Q1^b by Dioxin

Through differential display, a technique that analyzes the mRNA expression patterns, the cytoplasmic membrane protein ecto-ATPase, which may regulate the homeostasis of ATP in and outside cells, was found to be induced, and a class I major histocompatibility molecule MHC Q1^b, which may be involved in antigen presentation, repressed in response to dioxin in mouse hepatoma cells [80,81]. Genetic analyses using AhR or Arnt-defective variant cells established that the regulation of both genes by dioxin is dependent upon the presence of functional AhR and Arnt. Thus, ecto-ATPase and MHC Q1^b represent novel target genes of AhR; whether the regulation of the genes by dioxin is mediated through a DRE-dependent mechanism, and what are the functional implications of such regulation in dioxin action remain to be established.

Induction of EPO by Dioxin and Hypoxia

The hypoxia-inducible EPO gene encodes erythropoietin, which stimulates the growth of red blood cells in response to low oxygen tension. Induction of EPO by hypoxia is mediated through the hypoxia inducible factor 1α (HIF1α), a bHLHPAS transcription factor. HIF1α dimerizes with Arnt and binds to the hypoxia responsive element (HRE) located in the enhancer of EPO. A recent study revealed that the EPO enhancer contains 5 copies of DRE-like sequences that can mediate induction by dioxin in a reporter assay [82]. EPO is weakly induced by dioxin in cell culture. Moreover, hypoxia enhances the induction of EPO by dioxin. The additive effect of dioxin and hypoxia points out the cross-interaction between the AhR/DRE and the HIF1α/HRE signaling pathways and raises the possibility that AhR plays a role in the physiology of hypoxia.

Repression of AhR/DRE Function in Fibroblast

Induction of dioxin-inducible genes, such as *CYP1A1* and *CYP1B1*, is repressed in fibroblast cells. EMSA and UV cross-linking studies identified a heterodimer complex in the nuclear extract of fibroblast cells, which binds to DRE constitutively, and therefore, is proposed to be responsible for inhibiting AhR/DRE function in fibroblast cells [83]. The complex contains Arnt and an as-yet-unidentified protein factor. Since trichostatin A, a potent inhibitor of histone deacetylase (HDAC), derepresses the induction in fibroblast cells, it was postulated that the fibroblast-specific, Arnt-interacting factor recruits HDAC, which in turn inhibits transcription of dioxin target genes in fibroblast cells.

Cross Talk Between AhR and NF-κB

Immunoprecipitation studies demonstrate that AhR interacts with NF-κB in a ligand-dependent manner in hepatoma cells; interaction of AhR and NF-κB forms a heterodimer that suppresses gene transcription through the DRE or the NF-κB binding elements in reporter assays [84]. In mammary cells, AhR and the RelA NF-κB subunit cooperate to bind to NF-κB binding elements and induce c-myc gene expression [85]. These findings may provide new opportunities to study the mechanism by which AhR mediates the immunotoxicity and tumor formation by dioxin.

Hormonal Action of Dioxin

Animals exposed to dioxin and related HAHs exhibit certain endocrine dysfunction or perturbation [86]. For example, exposure of male rats to TCDD during the perinatal period leads to demasculination/feminization of sexual behavior during adulthood [87]; whereas in female rats, prenatal/perinatal exposure is associated with malformations of the external genitalia, reduced ovarian weight, delayed puberty, disruption of the estrous cycle, and reduced fertility [88]. The endocrine actions of dioxin may involve both pharmacokinetic and pharmacodynamic mechanisms. In the former case, induction of drug metabolizing enzymes may lower the concentrations of certain components of the endocrine function. For instance, dioxin induces UDP - glucuronosyltransferase (UGT) that catalyzes the conjugation of thyroxine (T4) with glucuronic acid [89], leading to enhanced excretion of T4 and a compensatory increase in thyroid-stimulating hormone (TSH), which in turn can contribute to the induction of a thyroid tumor by dioxin. Alternatively, dioxin may alter the expression of hormone-dependent genes. For example, dioxin inhibits the induction of cathepsin D and pS2 gene expression by estradiol [90,91]. Inhibition of the gene expression is mediated through DRE sequences in the enhancer regions of the genes. Thus, the AhR/DRE pathway can antagonize estrogene-responsive gene expression at a transcription level. Dioxin may also reduce the concentration of the receptor for a hormone, thereby decreasing the responsiveness of the tissues to the hormone. For example, exposure of mice to dioxin causes a reduction in the tissue levels of estrogen receptor, which may be mediated through an AhR-dependent transcriptional process [92,93].

AhR Action in the Absence of Exogenous Ligands

Although the endogenous ligand(s) for AhR has not yet been identified, studies on the AhR null mice show that AhR is involved in immune and liver development and reproductive function [24,29]. A recent study revealed a role of AhR in vascular formation during development, for the AhR null mice exhibit massive portosystemic shunting, immature sinusoidal architecture in the liver, persistent hyaloid arteries in the eyes, and aberrations in kidney vascular patterns [33]. Identifying the molecular mechanism by which AhR regulates vascular development represents a challenge in the understanding of AhR biology in future. Analyses of AhR function in AhR-defective cells implicate AhR in the growth and differentiation of the cells [30,31,94]. The mechanism by which AhR modulates cell growth may involve cross talking with the retinoblastoma protein (pRB) pathway and induction of p27Kip1, a cyclin-dependent kinase inhibitor [32,95]. Arnt is expressed in many tissues and can dimerize with a number of bHLH/PAS proteins besides AhR, suggesting that Arnt functions in a broad range of physiological responses [45]. The Arnt null condition is lethal to embryos; abnormal vascular development in the yolk sac has been observed in Arnt null mouse embryo [96]. The availability of AhR or Arnt-null mice, combined with the advance in the molecular understanding of AhR action, will facilitate elucidating the physiological functions and mechanism of action of AhR and Arnt. In addition, recent studies have identified several isoforms of AhR and Arnt in mice, whose expression appears to be more restricted to certain tissues; the functions of the isoforms of AhR and Arnt remain to be identified.

Cross Talk with Other bHLH/PAS Proteins

Recent studies have revealed a family of transcription factors that possess bHLH/PAS modules. The bHLH/PAS proteins appear to participate in a broad range of biological processes, such as circadian rhythm by Per [97], and the response to hypoxia by HIF1α [45,98]. Both *in vitro* and *in vivo* studies have indicated that the bHLH/PAS proteins may share partners under different physiological or pathophysiological conditions. For example, HIF1α is induced in response to low oxygen, and dimerizes with Arnt; the HIF1α/Arnt dimer binds to an enhancer containing the same core tetranucleotide motif 5' CGTG 3' that AhR/Arnt recognizes. The HIF1α/Arnt controls the induction of a number of glycolytic enzymes, erythropoitin, and genes important in vascular growth in response to hypoxia. Cross talk of the AhR/DRE pathway with different bHLH/PAS systems has been demonstrated *in vitro*; its existence and biological significance in intact animals remain to be studied. Mechanistic understanding of the signal transduction of the AhR/DRE pathway will provide insights into the mechanism of action and functions of other bHLH/PAS systems.

POLYMORPHYSM IN *CYP1A1* GENE EXPRESSION

P4501A1 catalyzes the bioactivation of many toxic chemicals, in particular, the metabolic conversion of PAHs, which are mostly non-reactive (or precarcinogenes), to reactive species (or ultimate carcinogens), such as arene oxides and dioepoxides; the reactive products can cause mutations in DNA and/or damages in protein functions, leading to cancer and other adverse effects [6]. Humans are exposed to PAHs and related chemicals through tobacco smoking and automobile exhaust. Variations in the activity of P4501A1 in human populations could contribute to the differences in the susceptibility of individuals to lung cancer by PAHs. Thus, the potential association between a polymorphysm in P4501A1 and susceptibility to lung cancer by PAHs represents a gene-environment interaction in the etiology of cancer. Understanding the molecular basis of the variations in P4501A1 in humans can facilitate identifying individuals or populations that exhibit high susceptibilities to cancer and other toxic effects by PAHs, thereby contributing to the prevention of the disease.

The polymorphysm in P4501A1 activity could involve variations in either cytochrome P4501A1 enzyme function or *CYP1A1* gene expression. A phenotypic polymorphysm affecting P4501A1 activity in humans was first reported by Kellermann *et al* [99]. The data revealed that the induction of *CYP1A1* by 3-MC in mitogen-activated lymphocytes exhibit a trimodal distribution with approximatedly 20% of individuals having a "high inducibility" phenotype. Subsequent studies revealed a correlation of the "high inducibility" of *CYP1A1* with lung cancer [100, 101], though contradictory results were also reported in literature [102].

The polymorphism within the *CYP1A1* gene includes four genetic alleles. Allele CYP1A1*2A exhibits a polymorphysm in the 3'-flanking region that can be detected by Msp1 digestion [103,104]. Allele CYP1A1*2B can also be detected by Msp1, but has a second polymorphysm in the coding region of exon 7, in which Ile462 is replaced by Val [105]. Allele CYP1A1*3 is a polymorphysm in the 3'-noncoding region and is high in frequency in African-Americans [106]. The allele CYP1A1*4 has a polymorphysm in residue 461 (Thr to Val) [107]. The association of the alleles with lung cancer or "high inducibility" varies among the alleles. For example, CYP1A1*2A has been associated with increased lung cancer susceptibility in Japanese populations [108]. The relevance of CYP1A1*3 to lung cancer is currently controversial [106,109,110]; whereas, CYP1A1*4 does not appear to be a susceptibility factor for lung cancer [107]. A recent study described three new alleles of *CYP1A1* involving base substitutions (C4151T, G-469A, and C-459T); however, these new alleles do not appear to be associated with high inducibility of *CYP1A1* in the study group [111].

Since AhR is the major protein factor that controls the induction of *CYP1A1*, a polymorphysm in the AhR function could change the inducibility of *CYP1A1*, thereby affecting the susceptibility to *CYP1A1* substrates. Screening of the exons of AhR by single-strand conformational polymorphysm (SSCP) identified polymorphisms G1721A (Arg554Lys) and G1768A (Val570Ili); the alleles are present more frequently in African-Americans than in Caucasians [111]. Individuals with at least one copy of the G1721A allele showed a significantly higher level of induced *CYP1A1* activity compared with individuals negative for the polymorphysm. However, allele G1721A does not appear to be associated with lung cancer in Japanese populations [112]. A recent study confirmed the presence of the two alleles and identified a third allele Pro517Ser [113]. It is noteworthy that all three alleles (Arg554Lys, Val570Ili, and Pro517Ser) are in the region identified as the acidic transcription activation domain (TA1) of AhR [43] (Fig. **1**). Analysis of the role of the residues in the transcription activation function of AhR may provide new insights into the functional relevance of the polymorphism in AhR function and cancer susceptibility in human populations. In addition to AhR, other proteins of the AhR/DRE pathway, such as Arnt, AIP, and ADPF, may also exhibit polymorphisms in humans that contribute to the variations in *CYP1A1* inducibility and risk to lung cancer.

Polymorphysms in other genes that affect the metabolism of PAHs may also influence the incidence of smoking-induced lung cancer. For example, the phase II enzyme GSTM1 contributes to the detoxification of dioepoxide of PAHs. Individuals with a particular *CYP1A1* polymorphysm and a null allele at GSTM1 locus may be at a substantially greater risk than individuals with either polymorphism alone [114,115]. This gene-gene interaction is a clear example of the complexity of factors that determine genetic susceptibility to cancer or other diseases caused by environmental chemicals.

A MODEL FOR AHR/DRE TRANSCRIPTION AND REGULATION

The identification of DRE as the cis-acting DNA component and the AhR and Arnt as the principle protein components for *CYP1A1* induction, the characterization of the interactions between the DNA and protein components, and the cloning of other protein factors contributing to AhR signaling, provide a model for the transcription and regulation through the AhR/DRE pathway (Fig. **2**). TCDD or other inducers binds to AhR, which resides in the cytoplasm in a complex with Hsp90 and AIP. Activation of the AhR by a ligand triggers the dissociation of AhR from Hsp90 and AIP, followed by translocation of the AhR into the nucleus, dimerization with Arnt, binding of the AhR/Arnt dimer to DREs, activation of the TA domains of AhR, and transcription of *CYP1A1*. The transcription involves the disruption of the chromatin structures associated with the gene. ADPF, a labile factor, promotes ubiquitination of agonist-activated AhR in nucleus, which leads to rapid degradation of the AhR protein through the 26S proteasome pathway, thereby negatively controls the transcriptional activity of nuclear AhR. In addition, other regulatory mechanisms involving protein-protein, protein-DNA interactions cross-react with the AhR/DRE pathway, thereby contributing to the species, tissue, and cell type-specific regulation of AhR target genes. This model is useful for generating testable hypothesis for future mechanistic studies on the induction of *CYP1A1*, the other biological responses to AhR ligands, such as dioxin, and transcriptional gene regulation by other bHLH-PAS transcription factors.

CONCLUSION

The induction of cytochrome P4501A1 by xenobiotics represents an interesting model for analyzing the mechanism by which a small, lipophilic chemical activates the transcription of a specific mammalian gene, which is also an example of an adaptive cellular response to chemical

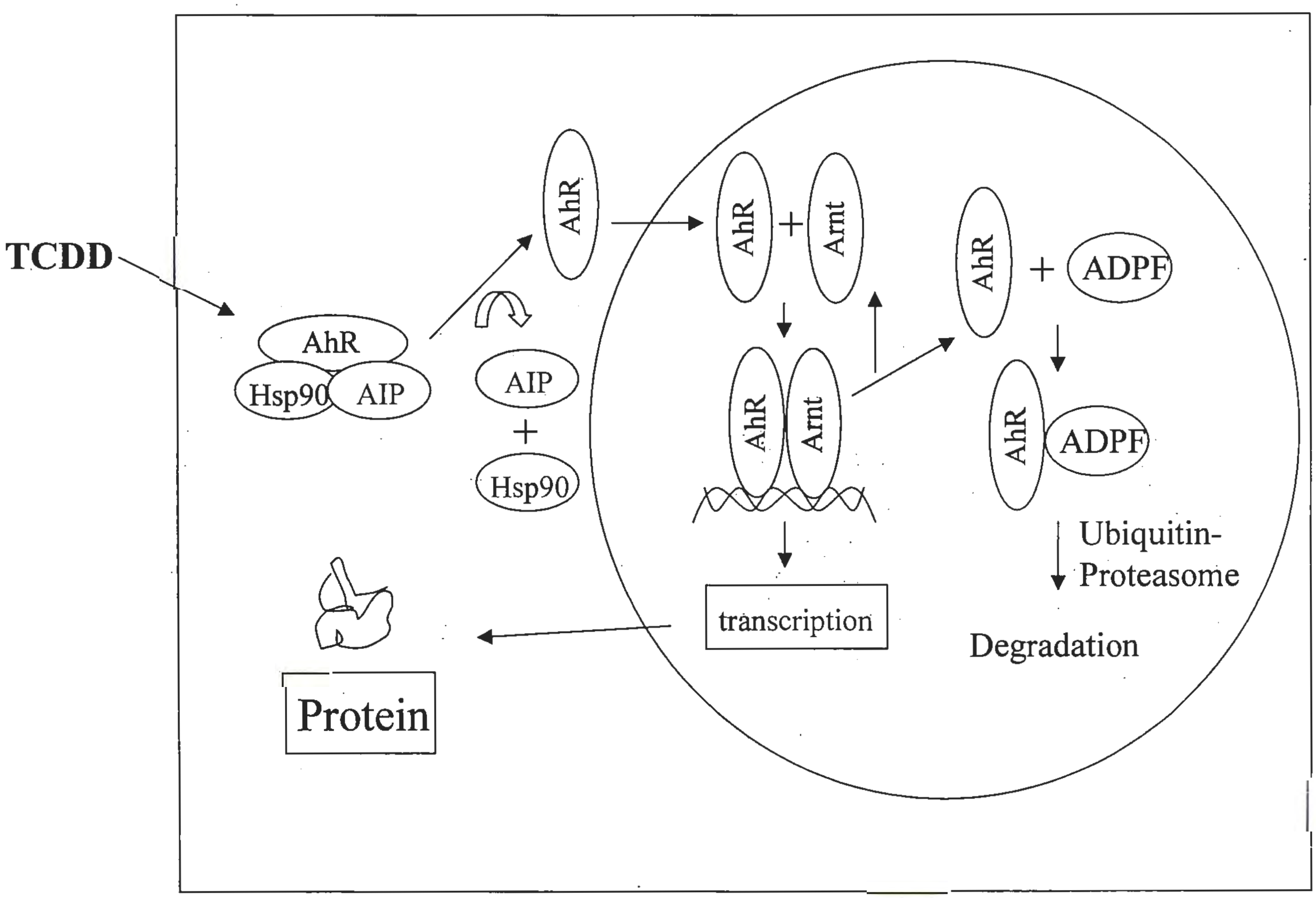

Fig. (2). A model for the induction of cytochrome P4501A1. For more detailed discussion, see the text.

exposure. The AhR/DRE paradigm derived from analyses of the induction involves a novel set of transcription factors characterized by the presence of bHLH/PAS modular structures. Studies of the domain structure, the interactions of the domains with DNA and other protein factors, and cloning of regulatory proteins that control the activities of cytoplasmic or nuclear AhR have provided valuable information into the mechanism of action of AhR/Arnt and other bHLH/PAS transcription factors, and into mammalian transcription in general. These studies also lead to a better understanding of the mechanism by which dioxin elicits toxic responses, which is of public health interest.

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