Molecular Biology of Ah Receptor-Mediated Gene Transcription and Implications for Risk Assessment

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds elicit a diverse spectrum of biochemical and toxic responses in laboratory animals and mammalian cells in culture [1-5]. Some of the toxic effects include chloracne and related dermal toxicity, reproductive and developmental toxicity, carcinogenesis, tissue-specific hypo- and hyperplastic responses, thymic atrophy and immune suppression, porphyria and hepatotoxicity, tumor promotion activity, acute lethality, and a wasting syndrome. The expression of these toxic responses are dependent on a number of factors including the age, sex, species and strain of the test animal, the target organ and cell type. TCDD also induces a number of genes and related enzyme activities and these include several drug-metabolizing enzymes and/or related genes including glutathione S-transferase, glucuronyl transferase, CYP1A1-, CYP1A2- and CYP1B1-dependent activities, and other enzymes including δ-aminolevulinic acid synthetase, epidermal transglutaminase, NAD(P)H quinone oxidoreductase (DT diaphorase), and aldehyde-3-dehydrogenase. TCDD and related compounds also decrease expression of several genes and/or their expressed proteins and these include uroporphyrinogen decarboxylase, phosphoenolpyruvate carboxy kinase, glucose-6-phosphatase, tryptophan-2,3-dioxygenase activities and mRNAs [6-8]; the binding activity of several cellular receptors (glucocorticoid, progesterone, estrogen and epidermal growth factor); c-fos, epidermal growth factor and transforming growth factor β mRNAs. These responses are also dependent on the target organ/cell type.

Characterization of the Ah Receptor Complex

The results of genetic and structure-activity studies which investigated the induction of CYP1A1 by both halogenated and polynuclear aromatic hydrocarbons suggested that the induction response may be mediated by a cellular aryl hydrocarbon (Ah) receptor [1]. Poland and coworkers [9] first identified the Ah receptor by demonstrating that radiolabeled TCDD specifically bound with high affinity to a hepatic protein from C57BL/6 mice and the Ah receptor has now been identified in multiple species, organs/tissues and cells, and has been extensively characterized using [^{3}H]TCDD and other radioligands [10]. The cytosolic *Ah receptor has been identified* as a low capacity, high affinity binding protein. Photoaffinity labeling studies indicate that the molecular weight of the Ah receptor is highly

variable between species (95- to 124-kDa) and the cytosolic Ah receptor forms a 270- to 310-kDa complex with two molecules of heat shock protein 90. The 9-10.5S cytosolic Ah receptor binds weakly to DNA; however, both temperature (i.e. 20° C) and salt transform the cytosolic Ah receptor into 170- to 220-kDa complex which sediments at 5.5-7.0S and to DNA. The transformed cytosolic and nuclear Ah receptor complexes are indistinguishable and have been characterized as a heterodimer containing the Ah receptor and aryl hydrocarbon nuclear translocator (Arnt) proteins [11]. The Ah receptor and Arnt genes have been cloned and the gene products have been identified as members of a family of proteins which include the Sim and Per proteins from *Drosophila* [12,13]. The results of several studies demonstrate that the nuclear Ah receptor complex is a ligand-activated transcription factor which is required for Ah receptor-mediated induction of gene expression [2,14].

Modulation of Gene Expression by TCDD

Induction of CYP1A1 Gene Expression. Induction of *CYP1A1* gene expression and related enzyme activities by TCDD and related Ah receptor agonists is one of the most common and sensitive indicators of exposure to these compounds [2]. Deletion analysis studies of 5'-upstream sequences in the *CYP1A1* gene promoter and their derived chimeric gene constructs showed that specific genomic sequences designated as dioxin or xenobiotic responsive elements (DREs/XREs) are required for ligand-induced gene transcription. These *cis*-acting enhancer sequences bind the Ah receptor-Arnt heterodimer and this interaction is required for transactivation of the *CYP1A1* gene. A core nucleotide sequence of 7 bases has been identified in most XREs/DREs and mutational analysis studies indicate that a 4-base sequence is required for binding of the nuclear heterodimer [2].

5'-T-GCGTG-3'	5'-CGTG-3'
3'-A-CGCAC-5'	3'-GCAC-5'

Figure 1 illustrates the model proposed for induction of Ah receptor-mediated responses by ligands which bind to the Ah receptor. There is evidence that this general mechanism may apply for Ah receptor-mediated induction of the biochemical and toxic responses elicited by TCDD and related compounds; however, results from several laboratories indicate the many other factors play a role in transactivation of Ah-responsive genes [2].

Inhibition of Gene Expression. TCDD and related compounds inhibit expression of several genes and influence multiple endocrine response pathways. For example, TCDD inhibits a diverse spectrum of estrogen (E2)-induced responses in the rodent uterus and mammary and in human breast cancer cell lines. The antiestrogenic activity of structurally-diverse Ah receptor agonists has been extensively investigated in MCF-7 cells and inhibition of the following E2-induced responses have been reported: postconfluent focus production, secretion of tissue plasminogen activator activity, procathepsin D (52-kDa protein), cathepsin D (34-kDa protein), a 160-kDa protein, PR binding sites, glucose to lactate metabolism, pS2 protein levels, and PR, cathepsin D, ER and pS2 gene expression [15-23]. Recent studies in this laboratory have focused on the mechanism of inhibition of E2-induced cathepsin D gene expression by TCDD and related compounds [20,24]. Results of

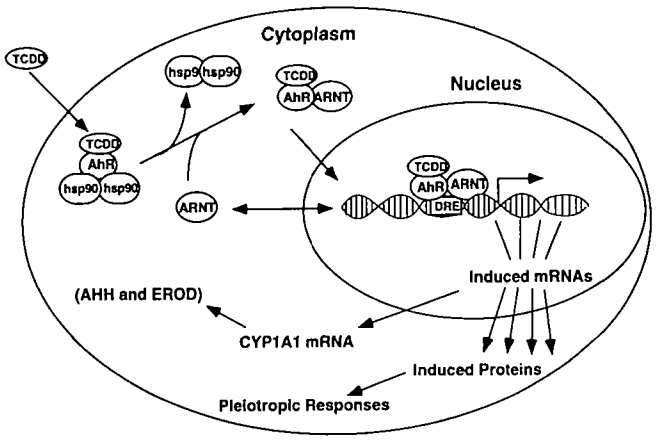

Figure 1. Molecular mechanism of induction of CYP1A1 gene expression by TCDD and related compounds.

initial studies suggested that an ER/Sp1 sequence (GGGCGG(n)$_{23}$ACGGG) in the 5'-flanking sequence of the cathepsin D gene is required for estrogen-responsiveness [20]. Subsequent analysis of the 5'-upstream DNA has shown that an imperfect DRE, GCGCGTG (-175/-181), is located between the ER and Sp1 genomic binding sites. *In vitro* gel electrophoretic mobility shift assays with the wild-type ER/Sp1 oligo showed that extracts from TCDD-treated cells do not form an ER/Sp1 complex; moreover, binding of the nuclear ER/Sp1 complex from MCF-7 cells to [^{32}P]ER/Sp1 oligo in a gel electrophoretic mobility shift assay is abolished by direct addition of transformed cytosolic Ah receptor complex from the same cells [24]. These results demonstrate a direct effect of the Ah receptor heterodimer on formation of the ER/Sp1 complex and this was confirmed in transient transfection assays which showed that TCDD inhibits E2-induced chloramphenicol acetyl transferase (CAT) activity and mRNA levels in MCF-7 cells transiently transfected with a construct containing a CAT reporter gene and the ER/Sp1 flanking sequence. Zacharewski and coworkers have also identified a DRE sequence near the transcription start site of the pS2 gene which is responsible for inhibition of E2-induced pS2 gene expression (personal communication). Ongoing studies in this laboratory suggest that other genes may also be inhibited via interactions of the Ah receptor with strategically-located DRE-like sequences. Thus, the nuclear Ah receptor complex serves to both induce and inhibit gene expression via similar pathways (Fig. 1) in which the nuclear Ah receptor complex binds to DRE/XRE sequences resulting in induction or inhibition of gene expression. The differences in these modulatory responses are regulated by several factors, including the DRE/XRE sequences (core and flanking) and their respective location in the 5'-promoter region of specific genes.

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