ACADEMIC PRESS

Archives of Biochemistry and Biophysics 404 (2002) 309–316

ABB

www.academicpress.com

Induction and superinduction of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible poly(ADP-ribose) polymerase: Role of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator transcription activation domains and a labile transcription repressor

Qiang Ma*

Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV 26505, USA

Received 7 May 2002, and in revised form 12 June 2002

Abstract

The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces a novel poly(ADP-ribose) polymerase (TiPARP). In this study, the signaling pathway of the induction was analyzed. Induction of TiPARP by TCDD occurs in both hepa1c1c7 cells and C57 mouse liver. Induction is concentration and time dependent. Genetic analyses reveal that induction is abolished in aromatic hydrocarbon receptor (AhR)- or aromatic hydrocarbon receptor nuclear translocator (Arnt)-defective variants but restored upon reconstitution of the variant cells with cDNAs expressing functional AhR or Arnt. Moreover, induction is largely reduced in cells expressing a deletion mutant of AhR or Arnt lacking the transcription activation (TA) domain, thus implicating the TA activities of both AhR and Arnt in the induction. Inhibition of protein synthesis by cycloheximide enhances the induction of TiPARP in the presence of an AhR agonist. The superinduction is transcriptional and does not require pretreatment with TCDD. Finally, inhibition of the 26S proteasomes by MG132 superinduces TiPARP. These findings establish that induction of TiPARP by TCDD is mediated through an AhR and Arnt transcription activation-dependent signal transduction that is repressed by a labile factor through the ubiquitin–26S proteasome-mediated protein degradation. Published by Elsevier Science (USA).

Keywords: TiPARP; TCDD; Ah receptor; Gene induction; Superinduction

The aryl hydrocarbon receptor (AhR),[1] a bHLH-PAS transcription factor, has been implicated in most of the biological responses to the environmental contaminant TCDD, a prototype of structurally related industrial/environmental halogenated aromatic hydrocarbons [1–4]. TCDD and certain HAHs elicit a wide range of adaptive responses, such as the induction of drug-metabolizing enzymes, and toxic effects, such as wasting, thymic atrophy, reproductive and developmental defects, tumor promotion, endocrine disorders, and neurocognitive lesions in animals [2,5–7]. The possibility that long-term exposure to TCDD and HAHs causes cancer and lesions in reproduction and neural development in humans is a particular concern.

The mechanism of action of AhR in mediating gene regulation to TCDD is best understood in the induction of *CYP1A1* through the AhR/DRE paradigm of gene transcription [3]. In liver cells, the cytoplasmic AhR is activated upon binding to TCDD and translocated into the nucleus where it dimerizes with a partner protein

* Fax: +1-304-285-5708.

E-mail address: qam1@cdc.gov (Q. Ma).

[1] *Abbreviations used:* AhR, aromatic hydrocarbon receptor; Arnt, Ah receptor nuclear translocator; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; CHX, cycloheximide; HAH, halogenated aromatic hydrocarbon; TA, transcription activation; DRE, dioxin responsive element; Me_2SO, dimethyl sulfoxide or DMSO; PCR, polymerase chain reaction; DIG, digoxygenin; PARP, poly(ADP-ribose) polymerase; TiPARP, TCDD-inducible poly(ADP-ribose) polymerase; EPO, erythropoietin.

0003-9861/02/$ - see front matter. Published by Elsevier Science (USA).
PII: S0003-9861(02)00339-9

Arnt; the AhR/Arnt dimer binds to an enhancer sequence (DRE) and activates the transcription of the gene [8]. The broad range of the responses to TCDD suggests multiple target genes. Indeed, recent cloning efforts using new screening strategies have uncovered several novel TCDD-regulated genes [9–16]. The mechanisms by which TCDD regulates the expression of the genes appear to be different for certain genes. For instance, induction of a major histocompatability gene MHC $Q1^b$ is mediated through AhR and Arnt [9], whereas suppression of gene expression of a cell cycle protein MAD2 by TCDD does not appear to require AhR, since the induction is observed in AhR null mice. The mechanism of this AhR-independent suppression of MAD2 is currently unclear [12]. Elucidating the signal transduction through which these new target genes of TCDD are regulated can reveal novel mechanistic aspects of AhR action in TCDD toxicity.

We have recently identified a novel TCDD-inducible gene, which is a new member of the growing family of poly(ADP-ribose) polymerase enzymes (TiPARP) [17]. PARPs use NAD^+ as a substrate to transfer ADP-ribose onto glutamic acid residues of a protein acceptor; repeated rounds of ADP-ribosylation leads to the formation of poly(ADP-ribose) chains on the protein, thereby altering the function of the target protein [18]. This poly-ADP-ribosylation of proteins by PARPs appears to serve as a general means of post-transcriptional regulation of protein functions, which include DNA repair [19], genomic stability [20], pancreatic islet β cell regeneration [21], telomere function [22], retroviral integration into host genome [23], and Golgi transport. TiPARP is highly homologous to RM1, which is induced during long-term potentiation, a memory formation process, in rat brain and to TIL, which is induced in T cells infiltrating progressing tumors [17]. Thus, TiPARP may participate in memory formation, T cell function, and tumor growth; these biological processes have been implicated in the toxicity of TCDD and related HAHs [7,24–26]. Induction of TiPARP represents a new transcriptional response to TCDD that may provide insights into AhR functions and the broad range of TCDD toxicity via modulating protein functions by poly-ADP-ribosylation.

In this study, cell genetics was used to examine the signaling pathway of TiPARP induction. The findings revealed that induction of TiPARP by TCDD is mediated through AhR/Arnt-dependent gene transcription and requires the TA domains of both AhR and Arnt. Furthermore, inhibition of protein synthesis by CHX or the 26S proteasomes by MG132 superinduces TiPARP in the presence of TCDD, suggesting a labile repressor that inhibits TiPARP transcription by controlling the ubiquitin–26S proteasome-mediated proteolysis.

Materials and methods

Materials

Restriction endonucleases and other general molecular biology reagents were purchased from New England Biolabs (Beverly, MA), Roche Molecular Biochemicals (Indianapolis, IN), and Promega (Madison, WI). TCDD was purchased from AccuStandard (New Haven, CT). CHX, puromycine, MG132, and other chemicals were purchased from Sigma Chemical (St. Louis, MO). Cell culture materials were from Life Technologies (Grand Island, NY).

Cell culture

The mouse hepa1c1c7 (Wt), AhR-defective variant (AhR-D), and Arnt-defective variant (Arnt-D) cells were from Dr. J.P. Whitlock, Jr. (Stanford University). The AhR-D cells expressing AhR or $AhR_{1–515}$ and the Arnt-D cells expressing Arnt or $Arnt_{1–652}$ were originally from the laboratory of Dr. J.P. Whitlock, Jr. These cell lines were generated by expressing cDNAs of corresponding recombinant proteins using the MFG retroviral expression system and were characterized previously [15,27]. The cells were grown as a monolayer in α-minimal essential medium, supplemented with 10% fetal bovine serum and 5% CO_2 at 37 °C, as described elsewhere [28].

Wild-type and AhR null skin kerotinocyte cells (Wt-K, AhR-D-K) were gifts from Dr. A. Poland (NIOSH, Morgantown, WV). Wild-type and AhR null mice [29] with C57BL/6 genetic background were crossed with the H-2Kb-tsA58 transgenic mice which express a temperature-sensitive mutant (tsA58) of the large T antigen of the SV 40 virus [30]. Keratinocytes were derived from the mice according to established procedures. The cells were cultured in KBM-2 keratinocyte culture medium with KGM-2 singlequots and low-calcium fetal bovine serum as suggested by Clonetics (BioWhitaker, Walkersville, MD) at 31 °C, 5% CO_2.

Treatment and preparation of total RNA

Cells were grown in 100-mm plates to near confluency in appropriate media and temperatures. The cells were treated with TCDD, CHX, or puromycine as described in figure legends; Me_2SO was used as the solvent control for TCDD. Total RNA was isolated from cells using the RNeasy Kit (Qiagen, Valencia, CA). RNA was stored at 80 °C for further use.

Female adult C57BL/6 mice (body weight of ~20 g) were treated with a single dose of TCDD (ip, 10 μg/kg body weight); control mice received the vehicle dioxane. Forty-eight hours later, the mice were sacrificed. The livers were removed and total RNA was prepared ac-

cording to Chomczynski and Sacchi [31]. Briefly, mouse liver was homogenized in a denaturing solution containing 4 M quanidinium thiocyanate. The homogenate was extracted sequentially with 2 M sodium acetate (pH 4), phenol, and chloroform/isoamyl alcohol. Total RNA was precipitated with isopropanol and glycogen was removed from the preparation by washing in 4 M lithium chloride. The RNA pellet was redissolved in denaturing solution, reprecipitated with isopropanol, and washed with 70% ethanol, followed by resuspension in diethyl pyrocarbonate-treated water. Animal treatment and care were performed according to NIOSH animal care and safety regulations.

RNA analysis

A cDNA fragment of TiPARP (designated DDF1) was subcloned into pCRII and used as a template to generate a riboprobe specific for TiPARP [17]. A 700-bp cDNA fragment encoding the 5′ untranslated region of the mouse *CYP1A1* mRNA was used to prepare a riboprobe for *CYP1A1*. A mouse actin cDNA fragment (~500 bp) was used to generate a riboprobe for actin. The riboprobes were synthesized in the presence of DIG-UTP using a DIG Labeling Kit (Roche Molecular Biochemicals). Total RNA (5 μg each lane) was fractionated on a 1% agarose–formaldehyde gel and transferred to a Nytran membrane by capillary action. After UV cross-linking, the membrane was hybridized with a DIG-labeled riboprobe at 68 °C overnight. Signals were visualized by chemiluminescence using a DIG RNA detection kit with CDP star as a substrate (Roche Molecular Biochemicals). Quantitation of the blotting result was performed by using the ImageQuaNT program (Molecular Dynamics, San Jose, CA). All data were corrected for loading variations by comparing the amount of actin of each sample analyzed.

Results

Induction of TiPARP by TCDD is mediated through AhR and Arnt

To analyze the mechanism of TiPARP regulation by TCDD, we characterized the induction of TiPARP mRNA. Northern analyses reveal that TiPARP is constitutively expressed at a low level and is induced to a large extent upon treatment with TCDD (Fig. 1) in mouse hepa1c1c7 cells, a highly responsive cell line to TCDD. To test whether TiPARP is induced in intact animals, C57BL/6 mice were treated with TCDD at 10 μg/kg body weight; TiPARP was found to be induced in the liver of the treated mice (Fig. 1). Induction of TiPARP is concentration dependent (Figs. 2A and B). Induction occurs at as low as 1 pM of TCDD and reaches a maximum at 1 nM with an EC_{50} value of 5 pM, which is similar to the induction of *CYP1A1* by TCDD [32] and thus implicates AhR in the induction. Induction of TiPARP is time dependent (Figs. 2C and D). The induction is observed as early as 30 min after TCDD treatment and reaches a maximal level at 2.5 h; the rapid induction suggests a primary response to TCDD.

Next, the involvement of AhR and Arnt in the induction of TiPARP was directly tested by using AhR- or Arnt-defective variant cells derived from hepa1c1c7; these variants offer the opportunity to analyze the induction of endogenous, chromosomal TiPARP gene in intact cells with exogenously introduced AhR or Arnt. Northern blots reveal that TiPARP is induced in wild-type, but not AhR-defective cells (AhR-D); reconstitution of the variants with functional AhR (AhR-D + AhR) fully restores the induction similarly to the induction of *CYP1A1* in the same cells (Fig. 3A). In another set of experiments, TiPARP induction by TCDD is abolished in Arnt-defective variants (Arnt-D),

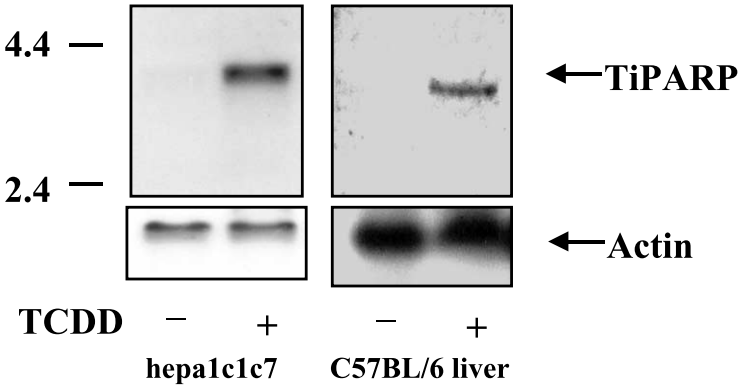

Fig. 1. Induction of TiPARP in hepa1c1c7 cells and mouse liver. Hepa1c1c7 cells were grown in 100-mm plates to near confluency and were treated with TCDD for 5 h at 1 nM. Total RNA was prepared and analyzed by Northern blotting for TiPARP and *CYP1A1*. The same RNA samples were blotted for actin to ensure equal loading. Adult female mice were treated with TCDD or vehicle; total RNA was prepared from liver and analyzed for TiPARP and actin expression by Northern blotting as described under Materials and methods.

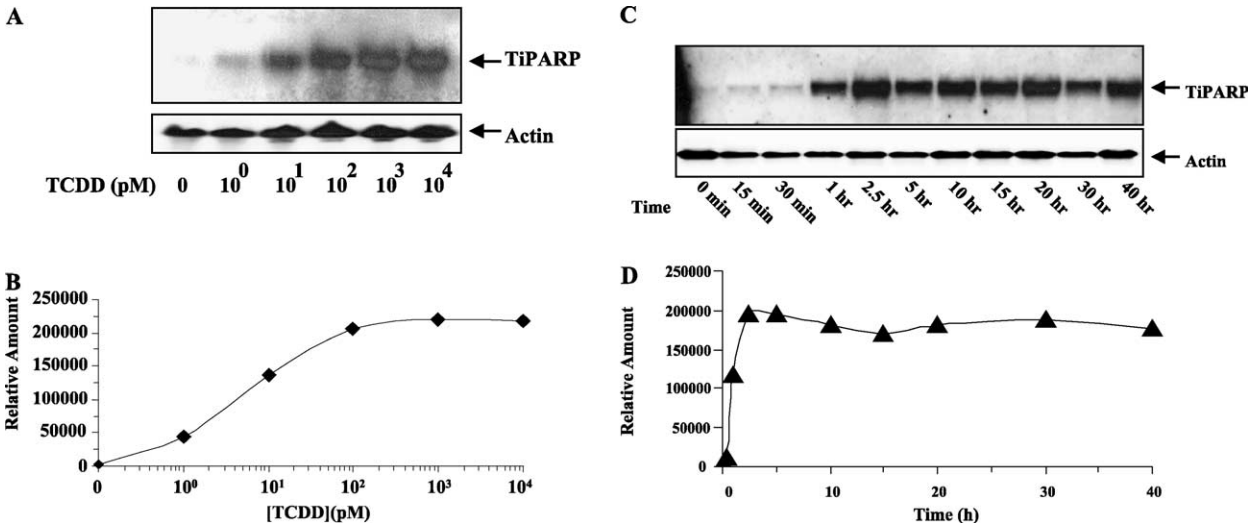

Fig. 2. Concentration and time dependence of TiPARP induction. Hepa1c1c7 cells were treated with TCDD for 5 h at increasing concentrations (A and B) or at 1 nM for different time periods (C and D). Total RNA was prepared and analyzed by Northern blotting for TiPARP and actin. The blots were analyzed by quantitating the bands using the ImageQuaNT program. All data were corrected for loading variations by comparing the amount of actin of each sample analyzed.

but is restored upon reconstitution of the cells with full length Arnt (Arnt-D + Arnt) (Fig. 3B). Thus, induction of TiPARP by TCDD is mediated through the AhR/Arnt-dependent signal transduction.

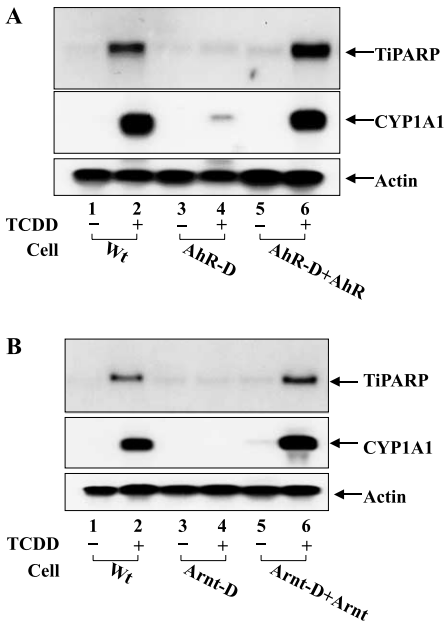

Fig. 3. Induction requires AhR and Arnt. (A) AhR dependence. Wt and AhR-D or AhR-D reconstituted with the full-length AhR coding sequence (AhR-D + AhR) were treated with TCDD (1 nM) or Me_2SO for 5 h. (B) Arnt dependence. Wt and Arnt-D or Arnt-D reconstituted with the full-length Arnt coding sequence (Arnt-D + Arnt) were treated with TCDD (1 nM) or Me_2SO for 5 h. Total RNA was prepared and analyzed by Northern blot for TiPARP, *CYP1A1*, and actin as described for Fig. 1.

Induction of TiPARP requires the TA domains of both AhR and Arnt

Both AhR and Arnt contain strong TA domains at carboxyl halves. The TA domain of AhR but not of Arnt is required for the induction of certain genes by TCDD, such as *CYP1A1* and ecto-ATPase [15,27]. We examined the role of the TA domains of AhR and Arnt in the induction of TiPARP. Fig. 4A shows that induction of TiPARP is largely diminished but detectable in AhR-D cells expressing a deletion mutant of AhR that lacks the AhR TA domain (AhR-D + $AhR_{1–515}$), whereas induction of *CYP1A1* in the cells is totally abolished as reported [27]. Induction of TiPARP is not observed in Arnt-D variant cells expressing a deletion mutant of Arnt that lacks the Arnt TA domain (Arnt-D + $Arnt_{1–652}$); in contrast, induction of *CYP1A1* in the same cells is comparable to Arnt-D reconstituted with full-length Arnt (Fig. 4B). These findings demonstrate that TCDD induction of TiPARP requires the TA domains of both AhR and Arnt and, therefore, is different from *CYP1A1* induction at a transcriptional level.

Cycloheximide superinduces TiPARP

Inhibition of protein synthesis by CHX enhances induction of *CYP1A1* by TCDD, a phenomenon termed superinduction. We tested whether induction of TiPARP by TCDD is regulated by CHX. As shown in Fig. 5A, TCDD (1 nM, 5 h) induces the TiPARP messenger RNA (lane 2), whereas cotreatment with TCDD and CHX enhances the induction by approximately sixfold (compare lane 4 with lane 2). The fold increase of

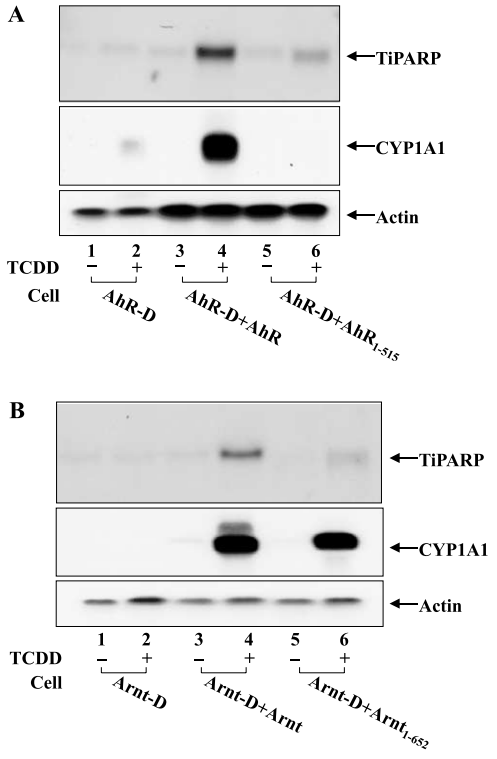

Fig. 4. Role of AhR and Arnt TA domains. (A) AhR-D cells and AhR-D cells reconstituted with AhR (AhR-D + AhR) or a deletion mutant (AhR-D + $AhR_{1–515}$) were treated with TCDD (1 nM) or Me_2SO for 5 h. (B) Arnt-D cells and Arnt-D cells reconstituted with Arnt (Arnt-D+Arnt) or a deletion mutant (Arnt-D + $Arnt_{1–652}$) were treated with TCDD (1 nM) or Me_2SO for 5 h. Total RNA was prepared and analyzed by Northern blot for TiPARP, *CYP1A1*, and actin.

induction and superinduction of TiPARP is comparable to those of *CYP1A1*. The superinduction is dose dependent; maximal superinduction occurs at a concentration of 10 μg/ml, a concentration at which over 95% of protein synthesis is inhibited in hepa1c1c7 cells (Fig. 5B), implying that superinduction requires substantial inhibition of protein synthesis in cells. Fig. 5C shows that superinduction is time dependent; both the induction and the superinduction are observable at 1 h after treatment and reach maximum levels at 2.5 h, suggesting a primary effect of CHX on TiPARP transcription. In addition, these studies revealed that CHX alone induces both TiPARP and *CYP1A1* in the absence of TCDD (Fig. 5A, compare lane 3 with lane 1). The induction by CHX is largely reduced in AhR-D variant cells; the small inducibility by CHX in the variants is likely due to the residue AhR activity (~10% of wild type) known to exist in the variant cells (Fig. 5D, top). Induction by CHX is restored in AhR-D cells reconstituted with AhR (AhR-D + AhR). In a separate experiment, CHX is shown to induce TiPARP in wild-type keratinocytes isolated from C57BL/6 mouse skin (Wt-K); however, the induction is totally lost in keratinocytes from skin tissue of AhR null mice (AhR-D-K) (Fig. 5D, bottom). These data demonstrate that induction of TiPARP by CHX in the absence of TCDD is AhR dependent.

Superinduction requires a labile factor

Superinduction of TiPARP by CHX can result from inhibition of protein synthesis; alternatively, it can be due to CHX actions unrelated to protein synthesis. Puromycin, an analog of aminoacyl–tRNA, inhibits

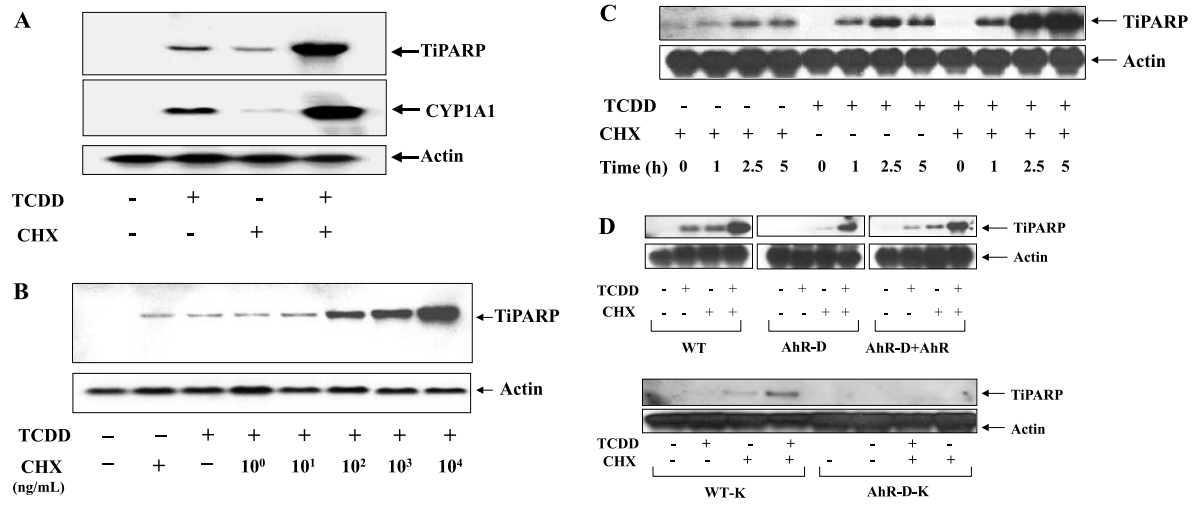

Fig. 5. Superinduction of TiPARP. (A) Superinduction by CHX. Cells were grown in 100-mm dishes to near confluency and were treated with Me_2SO, TCDD (1 nM), CHX (10 μg/ml), or TCDD+CHX for 5 h. (B) Concentration dependence. Cells were treated with TCDD (1 nM), CHX (10 μg/ml), or TCDD plus varying concentrations of CHX for 5 h. (C) Time dependence. Cells were treated with TCDD (1 nM), CHX (10 μg/ml), or both for indicated time periods. (D) AhR dependence of induction by CHX. Hepa1c1c7 and variants (Wt, AhR-D, AhR-D + AhR) at top and wild-type and AhR null keratinocytes (Wt-K, AhR-D-K) at bottom were treated as indicated for 5 h. Total RNA (5 μg/lane) was analyzed for TiPARP, *CYP1A1,* and actin by Northern blotting.

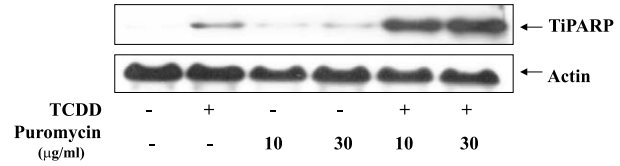

Fig. 6. Superinduction of TiPARP by puromycin. Hepatoma cells were treated with TCDD (1 nM), puromycin, or both for 5 h. Total RNA (5 μg/lane) was analyzed for TiPARP and actin.

protein synthesis with a similar potency to CHX. As shown in Fig. 6, cotreatment with puromycin and TCDD superinduces TiPARP at 10 and 30 μg/ml concentrations (compare lanes 5 or 6 with lane 2). Since puromycin and CHX inhibit protein synthesis through different mechanisms, the results suggest that inhibition of protein synthesis is sufficient for superinduction of TiPARP.

The sensitivity of TiPARP induction to inhibition of protein synthesis can be explained by the following mechanisms: TiPARP induction is regulated by a repressor, which is labile or inducible or both and therefore susceptible to inhibition of protein synthesis. To test the possibilities, cells were pretreated with TCDD for 10 h, followed by cotreatment with cycloheximide for 2.5 or 5 h. The data revealed that CHX superinduces TiPARP when cells were preexposed to TCDD (Fig. 7, compare lane 4 with 2 or lane 7 with 5); therefore, the repressor function requires constant protein synthesis and thus is mediated through a labile factor. Whether the repressor is also inducible by TCDD requires further studies.

Superinduction is transcriptional

Superinduction of TiPARP mRNA can be due to an increase in the rate of transcription or in the stability of the mRNA. The effect of CHX on the half-life ($t_{1/2}$) of the TiPARP mRNA was analyzed. Hepatoma cells were induced with TCDD for 2.5 h, followed by treatment with CHX, actinomycin D, or both for indicated time periods in fresh medium. TCDD induces TiPARP (Fig. 8, compare lane 2 with 1). CHX superinduces TiPARP in cells pretreated with TCDD (compare lanes 3 through 7 with lane 2). In the presence of actinomycin D, an

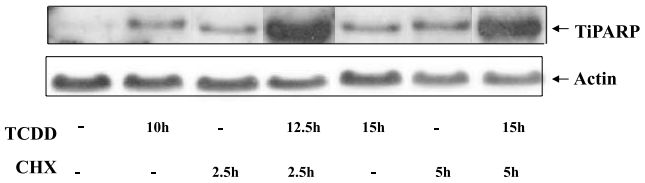

Fig. 7. Effect of TCDD pretreatment on superinduction of TiPARP. Hepa1c1c7 cells were induced with TCDD for 10 h and then cotreated with CHX for 2.5 or 5 h. Total RNA (5 μg/lane) was analyzed for TiPARP and actin.

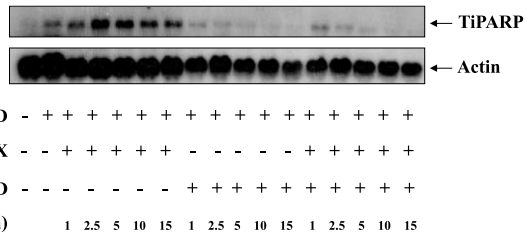

Fig. 8. TiPARP mRNA stability. Hepatoma cells were induced with TCDD for 2.5 h. Cells were then washed with fresh medium three times and recultured in fresh medium with CHX (10 μg/ml), actinomycin D (2 μg/ml), or both for indicated time periods. Total RNA (5 μg/lane) was analyzed for TiPARP and actin expression.

inhibitor of RNA synthesis, the induced TiPARP mRNA undergoes degradation with a $t_{1/2}$ of about 1 h; cotreatment with actinomycin D and CHX did not change the half-life of the TiPARP mRNA ($t_{1/2} = 1$ h) (Fig. 8 and data not shown). These findings demonstrate that CHX does not affect the stability of TiPARP mRNA, suggesting that superinduction of TiPARP mRNA is due to an increase in the transcription of TiPARP mRNA.

Inhibition of the 26S proteasomes superinduces TiPARP

Since inhibition of TCDD-induced degradation of AhR by inhibitors of the ubiquitin–26S proteasomes superinduces *CYP1A1*, the effect of proteasome inhibitors on TiPARP induction was examined. As shown in Fig. 9, cotreatment with TCDD and MG132 (an inhibitor of the 26S proteasomes) superinduces TiPARP (compare lane 4 with lane 2); lactacystin, also a proteasome inhibitor, superinduces TiPARP similarly to MG132 (data not shown). The findings suggest that repression of TiPARP induction is at least in part through the ubiquitin–26S-proteasome-mediated proteolysis controlled by a labile factor.

Discussion

It is generally accepted that AhR mediates diverse functions by controlling the expression of a spectrum of

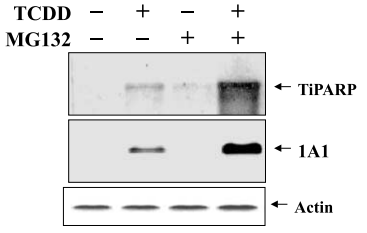

Fig. 9. Superinduction of TiPARP by MG132. Cells were cultured in 100 mm dishes and were treated with TCDD (1 nM), MG132 (25 μM), or both for 5 h. Total RNA (5 μg/lane) was analyzed for TiPARP, *CYP1A1*, and actin expression.

target genes, which include but are not limited to a previously characterized AhR battery of genes such as CYPs 1A1, 1A2, and 1B1, GST A1, NQOR, and aldh3a1 [3]. Indeed, a number of novel TCDD-regulated genes have been identified recently, including a major histocompatibility complex gene MHC $Q1^b$ [9], Epo [10], low-molecular-weight prekininogen [11], a cell cycle check point protein MAD2 [12], cytochrome P450 2S1 [13], Cu/Zn superoxide dismutase [14], Ecto-ATPase [15], *N*-myristoyltransferase 2 [16], and TiPARP [17]. The broad range of functions that these genes may associate with suggests that regulation of the expression of these genes by TCDD contributes to the diversity and tissue specificity of TCDD toxicity. In this study, we analyzed the signaling pathway of TiPARP induction by TCDD. We first examined the roles of AhR and Arnt in the induction by using cell genetics with AhR- or Arnt-defective variant cells. The results show that TiPARP induction requires both AhR and Arnt, because the induction is diminished to a large extent in AhR- or Arnt-defective cells, but is restored in the defective cells reconstituted with functional AhR or Arnt. Whether a DRE(s) is present in the enhancer region of the TiPARP gene and mediates the induction is currently unclear; analysis of the enhancer sequence of the gene will address the question.

Transcription by heteromeric dimers of transcription factors serves as a general theme of gene regulation [33]. This paradigm of gene transcription has several advantages. For example, it allows efficient control of transcription by regulating the activation of one subunit, such as the activation of AhR by TCDD. It also allows sharing of a common subunit among several signaling pathways, such as sharing Arnt by AhR and HIF1α, a transcription factor mediating gene transcription to hypoxic signals [34]. The molecular mechanism of transcription by heterodimeric transcription factors is, however, largely unclear. Analyses of *CYP1A1* transcription in intact cells by AhR/Arnt heteromer reveal that the TA of AhR is repressed by an inhibitory domain of AhR, but is activated and required for the induction of *CYP1A1* in the presence of Arnt and an agonist. On the contrary, the TA of Arnt is constitutively active, but is dispensable for the induction of endogenous *CYP1A1* in intact cells [27,35,36]. These findings suggest a mechanism by which the TA activities of the AhR/Arnt heteromer are regulated during the transcription of *CYP1A1*. In this study, we found that both of the TA domains of AhR and Arnt are required for the induction of TiPARP, because the induction of the gene is largely diminished in a reconstitution experiment with a TA domain deletion mutant of either AhR or Arnt. This is in contrast to the induction of *CYP1A1* by TCDD. Therefore, we envision that the mechanism of transcription of TiPARP by AhR/Arnt is different from that of *CYP1A1*. Differences in the sequences and associated protein factors of the promotor and enhancer of TiPARP and CYP1A1 may influence the regulation of the TA activities of AhR and Arnt. Alternatively, variations in the chromatin configurations of the two genes contribute to the differential activities of AhR and Arnt TA domains in the induction of the two genes.

The agonist-activated AhR is ubiquitinated, followed by a rapid degradation of AhR through the 26S proteasome-mediated protein turnover [37]. The agonist-induced degradation can be blocked by inhibition of protein synthesis or the 26S proteasome-mediated proteolysis. Thus, a labile repressor (designated AhR degradation promoting factor, ADPF) negatively controls the transcription of *CYP1A1* by regulating the ubiquitin–26S proteasome-mediated turnover of nuclear AhR; inhibition of the synthesis of ADPF by inhibiting protein synthesis or inhibition of the 26S proteasome pathway downstream of ADPF removes the repression by ADPF, resulting in a superinduction [38]. Since ADPF is a *trans*-acting factor, it can be predicted from this model that other target genes of AhR are also negatively regulated by ADPF. In this study, we found that induction of TiPARP by TCDD is enhanced by CHX or puromycin. The superinduction of TiPARP is similar to that of *CYP1A1* in magnitude and time course. The superinduction occurs at a transcriptional level and does not require pretreatment with TCDD, but involves blocking the synthesis of a labile repressor or the 26S proteasome activities. Thus, the findings support the model in which the transcription of AhR target genes is regulated at a second level after agonist activation of the receptor through labile ADPF, which controls the degradation of nuclear AhR via the ubiquitin–26S proteasome-mediated receptor degradation. Whether a single factor or more than one labile proteins is responsible for the ADPF activity remains to be established.

The observation that cycloheximide or puromycin alone (Figs. 5 and 6) can induce AhR target genes through AhR (i.e., CYP1A1 and TiPARP) in hepatoma cells can be explained by the following mechanism: AhR is activated in hepatoma cells at a low level in the absence of TCDD. This low level of AhR activity is subject to regulation by ADPF; blocking the synthesis of ADPF by CHX enhances the activity of AhR and leads to the induction of the genes. These findings are consistent with the observations that AhR is required in a number of cellular or developmental processes in the absence of TCDD [39–44]. Thus, the induction and superinduction of AhR target genes by CHX can be useful in analyzing the signal transduction of AhR in the absence of an exogenous agonist, which is poorly understood at present.

TCDD causes multiple toxic responses in a tissue-, developmental stage-, and species-dependent manner.

Previous studies revealed that TiPARP mRNA is constitutively expressed in a wide range of tissues in mouse, including heart, brain, lung, liver, kidney, testis, and, to a lesser extent, spleen and skeletal muscle [17]. In this report, we demonstrate that TiPARP is inducible in mouse liver. Together, these findings implicate TiPARP in a biological process(s) that is constitutively present and is induced by foreign chemicals in multiple animal tissues. In particular, the role of TiPARP in memory formation, T cell function, and tumor progression and their relation to TCDD toxicity should be examined in future studies.

Acknowledgments

The author thanks Dr. J.P. Whitlock, Jr. for providing AhR-D and Arnt-D variants of hepa1c1c7 and their reconstituted cells, Dr. A. Poland for the wild-type and AhR null keratinocyte cells (Wt-K, AhR-D-K), and Ms. H. Michael for secretarial assistance.

References

[1] J.P. Whitlock Jr., Annu. Rev. Pharmacol. Toxicol. 39 (1999) 103–125.
[2] F.J. Gonzalez, P. Fernandez-Salguero, Drug Metab. Dispos. 26 (1998) 1194–1198.
[3] Q. Ma, Curr. Drug Metab. 2 (2001) 149–164.
[4] H.I. Swanson, C.A. Bradfield, Pharmacogenetics 3 (1993) 213–230.
[5] J. Mimura, K. Yamashita, K. Nakamura, M. Morita, T.N. Takagi, K. Nakao, M. Ema, K. Sogawa, M. Yasuda, M. Katsuki, Y. Fujii-Kuriyama, Genes Cells 2 (1997) 645–654.
[6] A. Poland, D. Palen, E. Glover, Nature 300 (1982) 271–273.
[7] W.D. Niemi, J. Audi, B. Bush, D.O. Carpenter, Exp. Neurol. 151 (1998) 26–34.
[8] M.S. Denison, J.M. Fisher, J.P. Whitlock Jr., Proc. Natl. Acad. Sci. USA 85 (1988) 2528–2532.
[9] L. Dong, Q. Ma, J.P. Whitlock Jr., J. Biol. Chem. 272 (1997) 29614–29619.
[10] W.K. Chan, G. Yao, Y.-Z. Gu, C.A. Bradfield, J. Biol. Chem. 274 (1999) 12115–12123.
[11] M. Nukaya, Y. Takahashi, F.J. Gonzalez, T. Kamataki, Biochem. Biophys. Res. Commun. 287 (2001) 301–304.
[12] K. Oikawa, T. Ohbayashi, J. Mimura, R. Iwata, A. Kameta, K. Evine, K. Iwaya, Y. Fujii-Kuriyama, M. Kuroda, K. Mukai, Cancer Res. 61 (2001) 5707–5709.
[13] S.P. Rivera, S.T. Saarikoski, O. Hankinson, Mol. Pharmacol. 61 (2002) 255–259.
[14] J.S. Cho, M.S. Chang, H.M. Rho, Mol. Genet. Genom. 266 (2001) 133–141.
[15] L. Gao, L. Dong, J.P. Whitlock Jr., J. Biol. Chem. 273 (1998) 15358–15365.
[16] S.K. Kolluri, C. Balduf, M. Hofmann, M. Gottlicher, Cancer Res. 61 (2001) 8534–8539.
[17] Q. Ma, K.T. Baldwin, A.J. Renzelli, A. McDaniel, L. Dong, Biochem. Biophys. Res. Commun. 289 (2001) 499–506.
[18] S. Smith, Trends Biochem. Sci. 26 (2001) 174–179.
[19] S. Shall, G.d. Murcia, Mutat. Res. 460 (2000) 1–15.
[20] M.E. Smulson, C.M. Simbulan-Rosnethal, A.H. Boulares, A. Yakovelv, B. Stoica, S. Iyer, R. Luo, B. Haddad, Z.Q. Wang, T. Pang, M. Jung, A. Dritschilo, D.S. Rosenthal, Adv. Enzyme Regul. 40 (2000) 183–215.
[21] T. Akiyama, S. Takasawa, K. Nata, S. Kobayashi, M. Abe, N.J. Shervani, T. Ikeda, K. Nakagawa, M. Unno, S. Matsuno, H. Okamoto, Proc. Natl. Acad. Sci. USA 98 (2001) 48–53.
[22] S. Smith, I. Giriat, A. Schmitt, T. de Lange, Science 282 (1998) 1484–1487.
[23] C.H. Ha, K. Juluri, Y. Zhou, S. Leung, M. Hermankova, S.H. Snyder, Proc. Natl. Acad. Sci. USA 98 (2001) 3364–3368.
[24] W.J. Rogan, B.C. Gladen, K.L. Hung, S.-L. Koong, L.Y. Shih, J.S. Taylor, Y.-C. Wu, D. Yang, N.B. Ragan, C.-C. Hsu, Science 241 (1988) 334–336.
[25] R.W. Miller, Environ. Health Perspect. 60 (1985) 375–396.
[26] M.A. Fingerhut, W.E. Halerpin, D.A. Marlow, L.A. Piacitelli, P.A. Honchar, M.H. Sweeney, A.L. Greife, P.A. Dill, K. Steenland, A.J. Suruda, N. Engl. J. Med. 324 (1991) 212–218.
[27] H.P. Ko, S.T. Okino, Q. Ma, J.P. Whitlock Jr., Mol. Cell Biol. 16 (1996) 430–436.
[28] A.G. Miller, D.L. Israel, J.P. Whitlock Jr., J. Biol. Chem. 258 (1983) 3523–3527.
[29] J.V. Schmidt, G.H. Su, J.K. Reddy, M.C. Simon, C.A. Bradfield, Proc. Natl. Acad. Sci. USA 93 (1996) 6731–6736.
[30] P.S. Jat, M.D. Noble, P. Ataliotis, Y. Tanaka, N. Yannoutsos, L. Larsen, D. Kioussis, Proc. Natl. Acad. Sci. USA 88 (1991) 5096–5100.
[31] P. Chomczynski, N. Sacchi, Anal. Biochem. 162 (1987) 156–159.
[32] P.B.C. Jones, A.G. Miller, D.I. Israel, D.R. Galeazzi, J. Whitlock, P. James, J. Biol. Chem. 259 (1984) 12357–12363.
[33] J.D. Klemm, S.L. Schreiber, G.R. Crabtree, Annu. Rev. Immunol. 16 (1998) 569–592.
[34] G.L. Semenza, Curr. Opin. Genet. Dev. 8 (1998) 588–594.
[35] Q. Ma, L. Dong, J.P. Whitlock Jr., J. Biol. Chem. 270 (1995) 12697–12703.
[36] H.P. Ko, S.T. Okino, Q. Ma, J.P. Whitlock Jr., Mol. Cell Biol. 17 (1997) 3497–3507.
[37] Q. Ma, K.T. Baldwin, J. Biol. Chem. 275 (2000) 8432–8438.
[38] Q. Ma, A.J. Renzelli, K.T. Baldwin, J.M. Antonini, J. Biol. Chem. 275 (2000) 12676–12683.
[39] P.M. Fernandez-Salguero, J.M. Ward, J.P. Sundberg, F.J. Gonzalez, Vet. Pathol. 34 (1997) 605–614.
[40] G.P. Lahvis, S.L. Lindell, R.S. Thomas, R.S. McCuskey, C. Murphy, E. Glover, M. Bentz, J. Southard, C.A. Bradfield, Proc. Natl. Acad. Sci. USA 2000 (2000) 10442–10447.
[41] T.M. Lin, K. Ko, R.W. Moore, D.L. Buchannan, P.S. Cooke, R.E. Peterson, J. Toxicol. Environ. Health A 64 (2001) 327–342.
[42] Q. Ma, J.P. Whitlock Jr., Mol. Cell. Biol. 16 (1996) 2144–2150.
[43] S. Kolluri, C. Weiss, A. Koff, M. Gottlicher, Genes Dev. 13 (1999) 1742–1753.
[44] N.-L. Ge, C. Elferink, J. Biol. Chem. 273 (1998) 22708–22713.