Biochemical and Biophysical Research Communications **289,** 499–506 (2001)
doi:10.1006/bbrc.2001.5987, available online at http://www.idealibrary.com on IDEAL®

TCDD-Inducible Poly(ADP-ribose) Polymerase: A Novel Response to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Qiang Ma,*[,1] Kimberly T. Baldwin,* Anthony J. Renzelli,* Alison McDaniel,* and Liquin Dong†
**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, West Virginia 26505; and †Integra LifeSciences Corporation, San Diego, California 92121*

Received October 22, 2001

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) causes pleotropic effects in mammalian species through modulating gene expression. Here we analyzed TCDD-induced mRNA expression by using mRNA differential display and report the cloning of a novel TCDD-inducible poly(ADP-ribose) polymerase (TiPARP). TiPARP cDNA contains an open reading frame of 657 amino acid residues; the carboxyl half shares sequence similarity to the catalytic domain of PARP, a family of enzymes that catalyze poly ADP-ribosylation of proteins. Expression of the cDNA by *in vitro* transcription/translation reveals a protein of ~75 kDa. The expressed TiPARP exhibits PARP activity toward histone. TiPARP is highly homologous to RM1 which is induced during long-term potentiation, a memory formation process, and to TIL which is induced in T cells infiltrating progressing tumors. TiPARP mRNA is expressed in a broad range of mouse tissues. Together, these data demonstrate that TiPARP is a novel target of TCDD that may contribute to multiple responses to TCDD by modulating protein function through poly ADP-ribosylation. © 2001 Elsevier Science

Key Words: **TiPARP; PARP; TCDD; Ah receptor; RM1; TIL.**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a prototype for a class of structurally related halogenated aromatic hydrocarbons (HAHs). HAHs are mostly by-products of industrial processes and fuel combustion; moreover, HAHs are widespread in nature and are often resistant to biological degradation. As such, these chemicals are important environmental and industrial contaminants (1–3). Exposure to TCDD elicits a broad range of adaptive and toxic responses in animals, including induction of xenobiotic-metabolizing enzymes, tumor promotion, wasting syndrome, skin disorders, reproductive lesions, and liver toxicity (1–6). The possibility that TCDD causes cancer, diabetic conditions, and damage to reproductive functions in exposed human populations remains a public health concern (7, 8).

Molecular biochemical studies have revealed an AhR/DRE paradigm for the induction of *CYP1A1* by TCDD, a transcriptional response to TCDD that has served as a model for analyzing the mechanism of TCDD action (4). The aromatic hydrocarbon receptor (AhR) is a cytoplasmic, ligand-activated, basic helix–loop–helix (bHLH) PAS transcription factor (1, 2, 4, 9). Upon binding with TCDD, AhR is activated and translocated into the nucleus where it dimerizes with a partner protein called Arnt (Ah receptor nuclear translocator). The AhR/Arnt heterodimer binds to an enhancer sequence called the dioxin-responsive element (DRE) and mediates the transcription of *CYP1A1* (10). Recent studies have revealed two regulatory mechanisms that control the signal transduction of AhR. First, the unliganded cytoplasmic AhR is in a complex with hsp90 and AIP, an immunophillin type of chaperone molecule (11–13). The interaction among the proteins may determine the receptivity, protein stability, and other functional aspects of AhR. Second, agonist activated Ah receptor undergoes a rapid degradation through the ubiquitin-26S proteasome pathway (14, 15). The proteasome mediated turnover of AhR is controlled by a cycloheximide-sensitive factor (AhR degradation promoting factor, ADPF) (16). Thus, the AhR-mediated transcriptional responses to TCDD involve a regulated, multistep signal transduction/transcription process.

Genetic studies have implicated AhR in most of the responses to TCDD (17, 18). In addition, analyses using AhR null mice revealed a number of developmental and organ specific functional defects in the null mice (19–21). Studies in cultured cells suggest that AhR plays a role in cell growth and differentiation (22–24). Together, these findings suggest that AhR participates

[1] To whom correspondence and reprint requests should be addressed. Fax: (304) 285-5708. E-mail: qam1@cdc.gov.

0006-291X/01 $35.00
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in a range of cellular processes through regulating target gene expression and thereby, mediates the multiple effects of TCDD. One major challenge in explaining the mechanism of TCDD toxicity and AhR function is the lack of knowledge of AhR target genes. In this study, we utilized the mRNA differential display technique to identify new genes that are regulated by TCDD. We report here the cloning of a novel TCDD-inducible poly(ADP-ribose) polymerase (designated TiPARP). TiPARP contains a PARP catalytic domain and exhibits PARP activity toward histone. TiPARP shares over 90% sequence similarity with RM1 and TIL, suggesting that it plays a role in memory formation and T cell function. These studies provide new opportunities for analyzing the mechanism of TCDD toxicity and the transcriptional gene regulation by AhR.

MATERIALS AND METHODS

Materials and cell culture. General molecular biology reagents were purchased from New England Biolabs (Beverly, MA), Roche Molecular Biochemicals (Indianapolis, IN), Life Technologies, Inc. (Grand Island, NY), and Promega (Madison, WI). Radioactive compounds were from Amersham–Pharmacia Biotech (Piscataway, NJ). TCDD was purchased from AccuStandard (New Haven, CT). The mouse hepa1c1c7 (Wt) 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 (25).

Differential display. Differential display was performed using an mRNA differential display kit (GenHunter, Nashville, TN). Briefly, mouse hepa1c1c7 cells were exposed to 1 nM TCDD or Me_2SO for 10 h. Total RNA was isolated using an RNeasy kit (Qiagen, Valencia, CA). Three cDNA preparations were made by reverse transcribing the RNA with one of the three oligo (dT) primers (H-T_{11}M, where M is A, C, or G), which anneal to mRNA poly(A) tail. Polymerase chain reaction (PCR) was performed in the presence of [α-^{33}P]dATP, using one of the 80 arbitrary 5′ 13-mers and a corresponding 3′ oligo (dT) primer. Amplified cDNA fragments were resolved on a 6% denaturing polyacrylamide gel and visualized by autoradiography. Differentially expressed cDNA fragments (designated as differential display fragment or DDF) were eluted from the gel and reamplified using the same pair of PCR primers. Amplified cDNAs were subcloned into a TA cloning vector, pCRII (Invitrogen, San Diego, CA) for sequencing analysis.

cDNA cloning. Cloning of cDNAs was initially performed by screening a mouse liver cDNA library according to the D-loop technology developed by Pangene Corp. (Mountain View, CA). For cloning TiPARP, the sequence of DDF1 (~324 bp), which is induced by TCDD on a differential display analysis, was used to generate a pair of PCR primers: the sense primer, 5′-AGAGGCTATATGAGCAAAGTGAGG-3′, and the antisense primer, 5′-GTGGGTGATATATTTCAGTCCTGG-3′. DDF1 was amplified with the primers by PCR; the PCR fragment was used as a probe to screen a plasmid cDNA library made from C57BL/6 mouse liver mRNA. Complementary single stranded DNA probes were coated with the RecA protein to catalyze stable D-loop hybrids with homologous cDNA targets. These duplexes were selectively captured with magnetic beads. PCR was then performed to verify the presence of captured cDNA fragments with a pair of primers that anneal to sequences outside the capture domain. Two positive clones with inserts of 2.3 and 1.4 kb in length were selected and were designated as D-loop1 and D-loop2, respectively. The cDNA fragments were amplified by PCR with the above primers and subcloned into the pCRII vector for further analysis.

5′ rapid amplification of cDNA ends (5′ RACE). 5′ RACE was performed to obtain the full-length TiPARP cDNA according to the manufacturer's instructions (Clontech, Palo Alto, CA). Briefly, hepa1c1c7 cells were exposed to 1 nM TCDD or Me_2SO for 5 h. Total RNA was isolated. The cDNAs were prepared by reverse transcription of the RNA using a modified oligo(dT) primer, which contains two degenerate nucleotides at its 3′ end (5′-CDS primer), followed by addition of a SMART II primer, which has a stretch of guanine residues at its 3′ terminus. The cDNA was amplified using a gene specific primer and a universal primer mix (UPM long + UPM short) that recognizes the SMART II primer (UPM). Each amplified cDNA product was reamplified with nested primers to confirm a positive result. RACE8 was amplified with primers C52BGSP2 and the universal primer mix and was reamplified with nested primer C52QNGSP3 and NUPM. RACE32 was amplified with primers C5215GSP4 and the universal primer mix and was reamplified with nested primers C5215NGSP5 and NUPM. The RACE cDNA products were subcloned into pCRII for subsequent sequence analysis. The sequences of the primers used in the 5′ RACE are as follows: 5′-CDS, 5′-$(T)_{25}N_{-1}N$-3′ (N_{-1} = A, G, or C; N = A, C, G, or T); SMART II, 5′-AAGCAGTGGTAACAACGCAGAGTACGCGGG-3′; C52BGSP2, 5′-CAGAACCCTCACTTTGCTCATAGCCTC-3′; C52QNGSP3, 5′-CCAAACTTCTACCAGACCCAAGCAAG-3′; C5215GSP4, 5′-GGGTGGTGTAGACAGCCTTCGTAG-3′; C5215NGSP5, 5′-TCTGCCCAAAAGTCTTGTCCTCC-3′; UPM long, 5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTAACAACGCAGAGT-3′; UPM short, 5′-CTAATACGACTCACTATAGGGC-3′; NUPM, 5′-AAGCAGTGGTAACAACGCAGAGT-3′.

Northern blot analysis. DDF1, D-loop1, and D-loop2, each subcloned into pCRII, were used as templates to generate riboprobes specific for TiPARP. 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. After UV cross-linking, the membrane was hybridized with a DIG-labeled riboprobe at 68°C for overnight. Signals were visualized by chemiluminescence using a DIG RNA detection kit with CDP star as a substrate (Roche Molecular Biochemicals).

In vitro expression of TiPARP. A coupled transcription/translation reticulocyte lysate system (Promega) was used to express the TiPARP protein in the presence of [^{35}S]methionine. The full length TiPARP cDNA coding region was amplified by PCR with two primers: (a) 5′-GTCGGAATTCCTCGAGATGGAAGTGGAAACCACTGAAC-3′ and (b) 5′-TGCCGTCGACTCAGGGCCCAATGGAAACAGTGTTACTGACTTC-3′. The PCR product was digested with *Xho*I and *Apa*I, and subcloned into the pcDNA3.1 vector (Invitrogen), which was modified by adding HA and poly histidine tags downstream of the *Apa*I site; the plasmid was designated as pTiPARPhahis/cDNA3.1. One microgram of the plasmid was mixed with rabbit reticulocyte lysate, T7 RNA polymerase, RNase inhibitor, unlabeled amino acid mixture without methionine plus [^{35}S]methionine, or a complete unlabeled amino acid mixture. The mixture was incubated at 30°C for 90 min. The radiolabeled TiPARP was resolved by SDS–polyacrylamide gel electrophoresis and visualized by fluorography; alternatively, the expressed TiPARP was analyzed by immunoblotting with a monoclonal antibody against the HA tag (Babco, Berkley, CA) and visualized by chemiluminescence.

PARP activity assay. The PARP activity assay was performed using a poly(ADP-ribose) polymerase assay kit (Trevigen, Gaithersburg, MD). Briefly, *in vitro* expressed TiPARP in reticulocyte lysate (10 μl) was added to a reaction mixture, which contains 10 μM NAD, 10 μg/ml histones, 2 μCi ^{32}P-NAD, and 1× PARP buffer in a total volume of 100 μl. The reaction was allowed to proceed for 10 min at room temperature and was stopped by mixing with 900 μl of ice cold

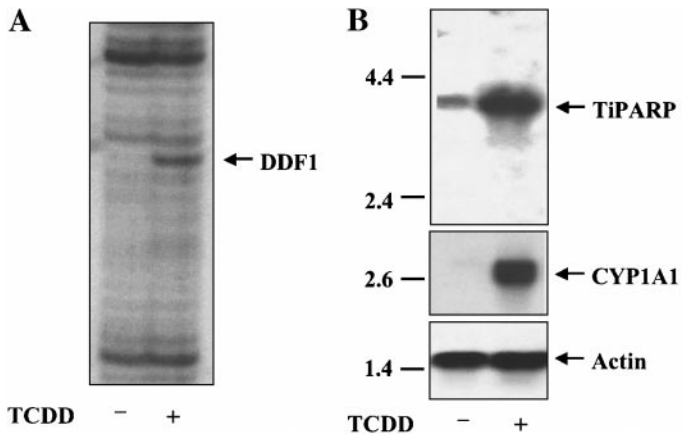

FIG. 1. Induction of TiPARP in hepa1c1c7 cells. (A) Identification of DDF1. Mouse hepa1c1c7 cells were treated with TCDD (1 nM) or Me_2SO for 10 h. Total RNA was analyzed by differential display. The arrow indicates the TCDD-inducible DDF1. (B) Induction of TiPARP. Mouse hepatoma cells were treated with TCDD (1 nM) or Me_2SO for 5 h. Total RNA (5 μg/lane) was analyzed by Northern blotting for DDF1 (top), *CYP1A1* (middle), or actin (bottom).

20% trichloroacetic acid (TCA), followed by a centrifugation at 12,000*g* for 10 min. The precipitate was washed with 1 ml of ice cold 10% TCA, dissolved in 2 ml of liquid scintillation cocktail, and counted for ^{32}P in a liquid scintillation counter.

RESULTS

Identification of a Novel TCDD-Inducible Gene

To identify new target genes of TCDD, we utilized the mRNA differential display to analyze the mRNA expression patterns in mouse hepatoma cells, a highly responsive cell line to AhR ligands. The cells were treated with TCDD (1 nM, 10 h) or Me_2SO; the total RNA was analyzed by differential display. A cDNA fragment of 324 bp was shown to be induced by TCDD and was designated as differential display fragment 1 (DDF1) (Fig. 1A). A sequence homology search of nucleotide databases using the Net Blast program showed no significant similarity of DDF1 to known gene sequences. Northern blot analysis of total RNA prepared from mouse hepa1c1c7 cells using DDF1 as a probe revealed a single mRNA band (Fig. 1B). TCDD at 1 nM (5 h) dramatically induces the expression of the mRNA similarly to the induction of *CYP1A1* by TCDD, except that the basal expression of DDF1 is higher than that of *CYP1A1* (Fig. 1B). Thus, DDF1 represents a novel TCDD-inducible gene in mouse hepatoma cells.

Cloning of Full-Length cDNA

To clone the full length cDNA, we screened a mouse liver cDNA library with DDF1 as a probe. Two positive clones were isolated and were shown to contain cDNA fragments of 2310 and 1402 bp, respectively (designated as D-loop1 and D-loop2). Northern blot analysis with probes for D-loop1 and D-loop2 reveals that both recognize the same TCDD-inducible mRNA band as DDF1 (data not shown). The nucleotide sequence of D-loop2 overlaps with the 3′ half of D-loop1; D-loop1 contains a partial open reading frame, which lacks an initiation methionine.

To clone the 5′ end, we performed 5′ RACE, a PCR-based procedure that rapidly amplifies 5′ ends of cDNAs. The first round of 5′ RACE yielded a 1725-bp fragment (designated as RACE8). The nucleotide sequence of RACE8 overlaps with the 5′ end of DDF1, D-loop1, and D-loop2, and contains a partial open reading frame. A second round of RACE yielded a 1063-bp fragment (designated as RACE32). Northern blot analysis confirmed that probes from RACE8 and RACE32 hybridize to the same mRNA band as the DDF1 probe (data not shown). The nucleotide sequence of RACE32 overlaps with RACE8. The contig sequence generated from DDF1, D-loop1, RACE8, and RACE32 is 3851 bp in length, which is close to the size of the mRNA band revealed by Northern blotting. Figure 2 shows the nucleotide and predicted amino acid sequences with a putative Kozac sequence for translation (26) and a poly(A) tail signaling sequence. The sequence contains an open reading frame of 657 amino acid residues with a start codon 5′ to and in frame with the open reading frame. The predicted peptide has a molecular mass of 75,879 Daltons and an isoelectric point of 6.63.

Next, we expressed the coding sequence using *in vitro* transcription/translation. The expressed protein is analyzed by SDS–polyacrylamide gel electrophoresis and visualized by fluorography for ^{35}S-labeled proteins, or by immunoblotting with a monoclonal antibody specific for the HA tag, which is at the carboxyl terminus of the expressed protein. Both assays detected a protein band of ~80 kDa, a value identical to the size predicted from the open reading frame (~75 kDa) plus the tag residues at the carboxyl terminus of the protein (Figs. 3A and 3B).

Full-Length DDF1 Encodes a PARP

Homology search of nucleotide databases reveals that DDF1 represents a novel gene. Inspecting the amino acid sequence of the gene revealed a partial homology with the catalytic domain of PARP enzymes (43% similarity over 87 amino acid residues) (Figs. 2 and 4) (27). PARPs are a family of enzymes that catalyze the formation of poly(ADP-ribose) onto a protein target (28). PARPs have been shown to be involved in multiple cellular functions, such as DNA damage-induced DNA repair (28, 29). These findings suggest that DDF1 is related to PARP and may function as a protein modulator by catalyzing the formation of poly(ADP-ribose) on protein targets. To test if DDF1 encodes a PARP activity, the *in vitro* expressed DDF1 was assayed for PARP activity using histone as a sub-

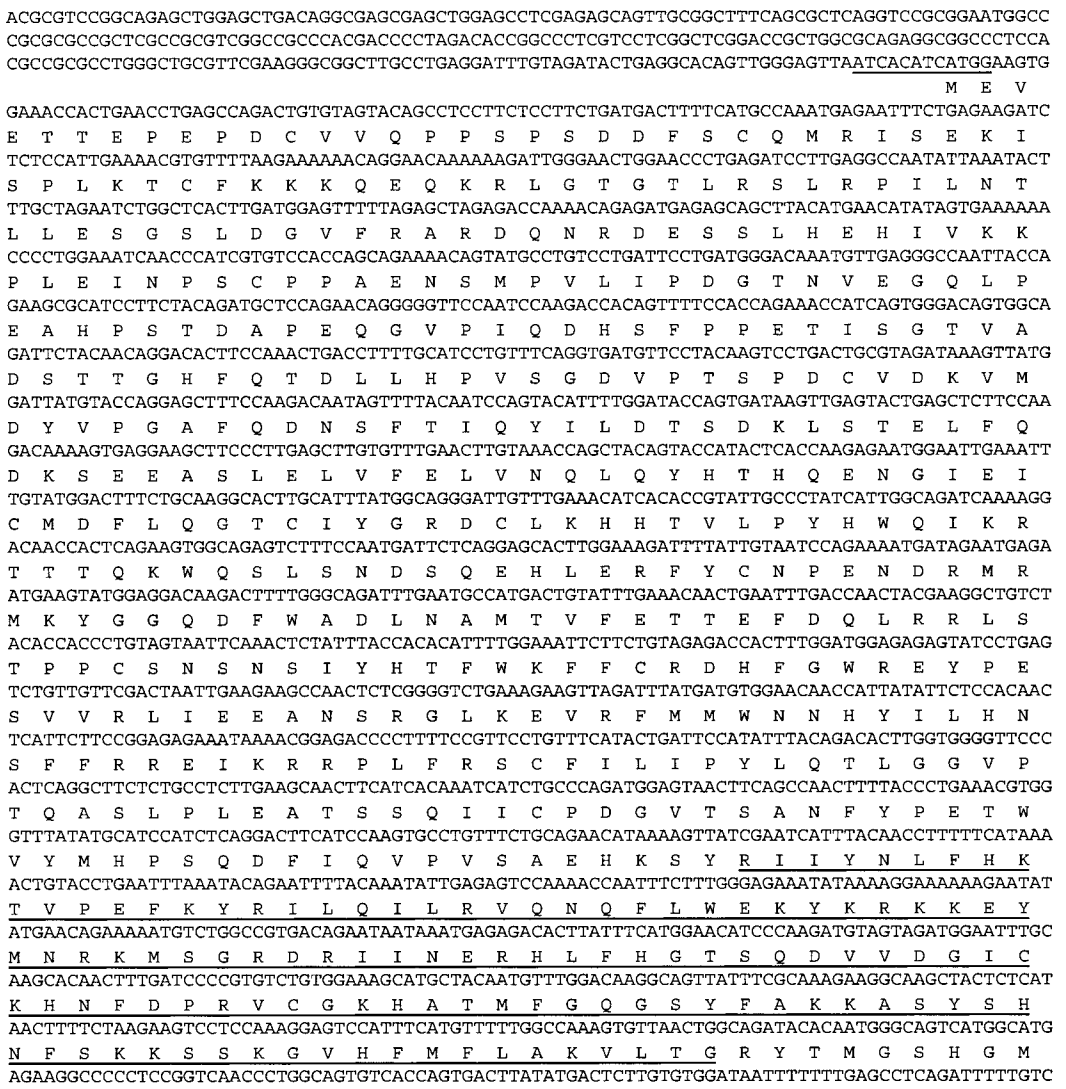

FIG. 2. Nucleotide and predicted amino acid sequences of TiPARP. Numbers in regular face indicate positions of nucleotide residues and numbers in bold face indicate positions of predicted amino acid residues. A putative Kozac sequence, the PARP domain, and a poly(A) signal sequence are indicated.

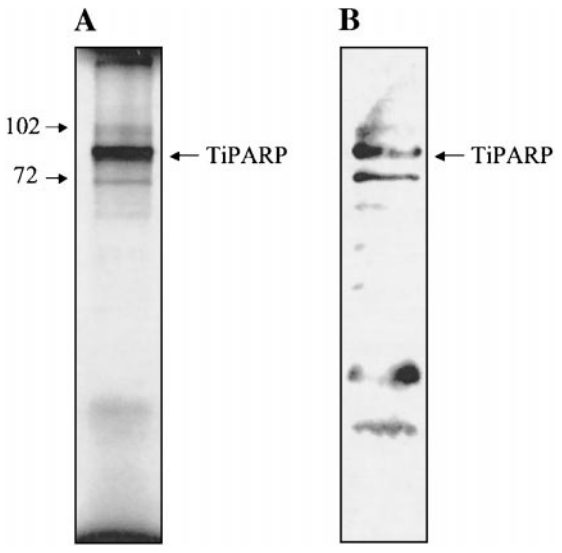

FIG. 3. *In vitro* expression. The full length coding sequence of TiPARP was subcloned into a pcDNA3.1-based expression vector. *In vitro* transcription/translation was performed using a coupled reticulocyte lysate system, (A) in the presence of [^{35}S]methionine and the [^{35}S] labeled proteins were resolved by SDS–PAGE and visualized by fluorography or (B) in the absence of [^{35}S]methionine and the protein products were immunoblotted with an anti-HA monoclonal antibody.

strate. Figure 5 shows that the reticulocyte lysate control expresses little PARP activity. Expression of DDF1 increases the PARP activity to fourfold higher. Thus, DDF1 represents a novel member of the PARP family that is induced by TCDD. Hence, we designate the gene as TCDD-inducible poly(ADP-ribose) polymerase (TiPARP).

Sequence analyses also identified two partial cDNA sequences (RM1 and TIL) in the GenBank database that are highly homologous to the 3′ end and the 5′ end sequences of TiPARP (>90%), respectively. RM1 is expressed in rat hippocampus and is induced during the long-lasting long term potentiation (LTP), a process involved in memory formation (30). TIL is expressed in T cells isolated from a progressing murine mammary carcinoma, but not in T cells from a regressing tumor (31). The high homology of TiPARP with RM1 and TIL suggests that it serves as a homolog of RM1 and TIL, and plays a role in memory formation and tumor progression. Further study is needed to address these issues.

Expression of TiPARP in Mouse Tissues

To examine the expression of TiPARP in mouse tissues, we performed multiple tissue Northern blot analysis. As shown in Fig. 6, TiPARP is expressed in mouse heart, brain, lung, liver, kidney, and testis and, to a lesser extent, spleen and skeletal muscle. The broad tissue expression suggests that TiPARP is involved in multiple tissue functions and may contribute to multiple responses to TCDD.

DISCUSSION

We have identified a novel gene inducible by TCDD, which is a new member of the PARP family proteins and is designated TCDD-inducible PARP or TiPARP. PARPs are a growing family of enzymes that 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 (28).

The best understood example of PARPs is PARP1, a nuclear protein that is induced and activated by strand-break DNA damage and participates in the early events of DNA repair in response to alkylation or ionizing irradiation. PARP1 is highly conserved through evolution. Loss of the PARP1 activity in PARP1 null mice leads to enhanced susceptibility to DNA damaging agents. Recent studies have revealed two new aspects of the PARP family. First, analyses of the PARP1 null mice demonstrated novel functions of PARP1 (29). For instance, PARP1 coordinates with p53, the Ku proteins, and cell cycle regulators to mediate apoptosis, cell cycle arrest, and DNA repair in response to DNA damage, and with Nf-κB to regulate cytokine expression in inflammatory responses. PARP1 is required for maintenance of genomic stability in cells (32) and for efficient integration of retroviral DNA, such as HIV-1, into host genome (33). In addition, PARP1 plays a critical role in determining the sensitivity of mice to chemical-induced diabetic conditions by regulating the NAD^+ homeostasis and the transcription of the *Reg* gene, which controls the regeneration of pancreatic islet β cells (34). Second, recent studies have identified a number of novel PARPs, including PARP2, PARP3, VPARP (vault PARP), and tankyrase (28). The new PARPs each have a PARP domain that shares sequence similarity to the catalytic domain of PARP1, and exhibit PARP activities toward certain protein substrates. However, the new PARPs show large differences from PARP1 in structure, cellular localization, and function. For instance, tankyrase was identified as a component of the human telomeric complex, which is essential for chromosome maintenance and stability. Tankyrase regulates telomere function by interacting with TRF1, a negative regulator of telomere length (35). Tankyrase is also found in the peripheral membrane of the Golgi apparatus and may be involved in the metabolism of GLUT4 vesicles by interacting with the insulin-responsive amino peptides (or IRAP) of GLUT4 vesicles (36). Thus, poly ADP-ribosylation of proteins by PARPs appear to serve as a general means of posttranscriptional regulation of protein function and is involved in a diverse spectrum of cellular functions.

The exact function of TiPARP is currently unclear; however, it is conceivable that induction of TiPARP

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TiPARP        475 RIIYN..LFH .K.T.VPEF.K YR.ILQILRVQ NQFLWEKYKR. .KKEYM.NRKMS
PARP1
   human      816 KYVkN..T.H .ATT.HNAY.D LE.VIDIFKIE REGECQRYKP. FKQ..LHNRRL.
   rat        816 KYVkN..T.H .ATT.HNAY.D LE.VIDIFKIE REGESQRYKP. FRQLH..NRRL.
   mouse      815 KYVkN..T.H .ATT.HNAY.D LE.VIDIFKIE REGESQRYKP. FRQLH..NRRL.
   bovine     818 KYVkN..T.H .ATT.HNAY.D LE.VVDIFKIE REGESQRYKP. FKQLH..NRRL.
   chick      815   VkN..T.H .AAT.HNAY.D LK.VVEIFRIE REGESQRYKP. FKQLH..NRQL.
   frog       799  YVkN..T.H .ADT.HNAY.D LE.VLEIFKID REGEYQRYKP. FKQLH..NRQL.
   fruit fly  800   VkN..T.H .AST.HKSY.D LK.IVDVFKVS RQGEARRFKP. FKKLH..NRKL.
TANKYRASE    1130 RE.H.RDGG. NAG.GI..FNR Y.NVIRIQKVV NKKLRERF.CH RQKE.VS..E..


TiPARP        520 GR.DRIINERH LFHGTSQDVV. DG..I.CKHN.FD .P.R.VCGK.HAT MFGQGSYFAK
PARP1
   human      860 ..........  LWHG.SRTTNF AG..ILSQ.GLRI APPE.APVTGY.. MFGKGIYFAD
   rat        860 ..........  LWHG.SRTTNF AG..ILSQ.GLRI APPE.APVTGY.. MFGKGIYFAD
   mouse      859 ..........  LWHG.SRTTNF AG..ILSQ.GLRI APPE.APVTGY.. MFGKGIYFAD
   bovine     862 ..........  LWHG.SRTTNF AG..ILSQ.GLRI APPE.APVTGY.. MFGKGIYFAD
   chick      857 ..........  LWHG.SRTTNF AG..ILSQ.GLRI APPE.APVTGY.. MFGKGIYFAD
   frog       844 ..........  LWHG.SRTTNF AG..ILSQ.GLRI APPE.APVTGY.. MFGKGIYFAD
   fruit fly  842 ..........  LWHG.SRLTNF VG..ILS.HGLRI APPE.APPTGY.. MFGKGIYFAD
TANKYRASE    1172 .ENHNHHNERM LFHG.S..P.F I.NAII..HKGFD E..RHAYIGGY.. MFGAGIYFAE


TiPARP        568 KA.SYSHN.F.. .SK....KSSKGV.HFM FLAKV.LTG
PARP1
   human      900 MV.SKSAN.YCH TSQ....GDPIGL...I LLGEVAL.G
   rat        900 MV.SKSAN.YCH TSQ....GDPIGL...I LLGEVAL.G
   mouse      899 MV.SKSAN.YCH TSQ....GDPIGL...I MLGEVAL.G
   bovine     902 MV.SKSAN.YCH TSQ....GDPIGL...I LLGEAAL.G
   chick      997 MV.SKSAN.YCH TSQ....ADPIGL...I LLGEVAL.G
   frog       894 MV.SKSAN.YCH A....MPGSPIGL...I LLGEVAL.G
   fruit fly  882 MV.SKSAN.YCC TSQ....QNSTGL...M LLSEVAL.G
TANKYRASE    1217 N.SSKS.NQYVY G..IGGGTGCP..THK. D..R.S.C.
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FIG. 4. Sequence comparison of TiPARP with the catalytic domain of PARPs. The sequence of TiPARP between amino acid residues 475 and 594 is aligned with the catalytic domains of PARP1 from human (Accession No. P09874), rat (Accession No. P27008), mouse (Accession No. P11103), bovine (Accession No. P18493), chick (Accession No. P26446), frog (Accession No. P31669), and fruit fly (Accession No. P35875), and the human tankyrase (Accession No. AAC79842). Numbers indicate the positions of amino acid residues. Letters in bold face represent sequence identity and underlined letters represent sequence similarity of TiPARP to PARPs.

serves as an adaptive response to chemical exposure (TCDD, for instance) and thereby mediates certain effect(s) of the chemicals. Sequence homology search identified two cDNA sequences in the GenBank database that share over 90% homology with TiPARP. RM1, a partial sequence of a rat gene, is homologous to the 3′ end of TiPARP. RM1 mRNA is expressed in hippocampus and other parts of the brain; RM1 is induced during the induction of long-lasting long term potentiation (LTP). Thus, RM1 is proposed to play a role in memory formation in rats (30). It has been shown that exposure to polychlorinated biphenols (PCB), a class of chlorinated AhR ligands, selectively reduces long term potentiation in the CA1 region of rat hippocampus (37). Behavioral and neurological studies in animals have also provided evidence that PCBs impair nervous system functions, including decrements in learning and memory (38). Furthermore, epidemiological studies indicated that prenatal exposure to PCBs is associated with deficits in intelligence and behavioral development in childhood (39–41). Therefore, it is possible that TiPARP is a mouse homolog of the rat RM1 and plays a role in LTP; induction of TiPARP by AhR ligands interferes with LTP formation, leading to impairment of memory function. TIL is a partial cDNA sequence homologous to the 5′ end of TiPARP. TIL is induced in T cells from progressing tumors, but not in T cells from regressing tumors; whether TIL promotes or inhibits tumor growth is cur-

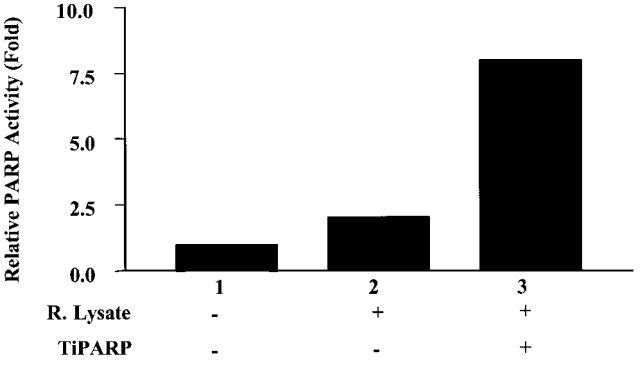

FIG. 5. PARP activity of TiPARP. The PARP activity of TiPARP was measured by using *in vitro* expressed TiPARP and a poly(ADP-ribose) polymerase assay kit.

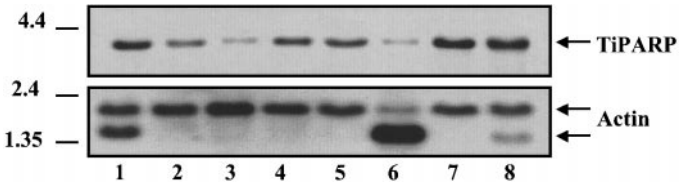

FIG. 6. Expression of TiPARP in mouse tissues. A mouse multiple tissue Northern blot was probed with a riboprobe for TiPARP or actin according to the manufacturer's instructions (Clontech). The tissues represented are as follows: 1, heart; 2, brain; 3, spleen; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; and 8, testis.

rently unclear (31). It is known that TCDD is a potent tumor promoter in animals (3) and exposure to TCDD is associated with certain forms of cancer in human beings (7). These findings raise the possibility that TiPARP is involved in T cell function and induction of TiPARP by TCDD contributes to the tumor promotion by TCDD. Generation of TiPARP null mice by directed gene knockout will provide opportunities to test the role(s) of TiPARP in memory formation, T cell function, and TCDD-induced responses.

TCDD causes multiple toxic responses in a tissue, developmental stage, and species-dependent manner. TiPARP mRNA is constitutively expressed in a wide range of tissues in mouse, that includes heart, brain, lung, liver, kidney, testis, and to a lesser extent, spleen and skeletal muscle. These findings implicate TiPARP in a biological process(s) that is constitutively present and is regulated by environmental chemicals in multiple animal tissues. Studying the induction of TiPARP by TCDD and the functional consequences of the induction in animal tissues and during development will provide new opportunities to elucidate the biological function of AhR and the toxicity of TCDD and related environmental/industrial chemicals.

ACKNOWLEDGMENTS

We thank Drs. A. Little, Jr. and J. Matheson for internal review of the manuscript and Ms. H. Michael for secretarial assistance.

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