Biochemical Pharmacology, Vol. 58, pp. 1649–1655, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00245-2

Antioxidant Response Element-Mediated 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Induction of Human NAD(P)H:Quinone Oxidoreductase 1 Gene Expression

*Venugopal Radjendirane and Anil K. Jaiswal**

DEPARTMENT OF PHARMACOLOGY, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX 77030, U.S.A.

ABSTRACT. Antioxidant response element (ARE) is required for high basal expression of the human NAD(P)H:quinone oxidoreductase 1 (*NQO1*) gene in tumor cells and its induction in response to β-naphthoflavone and phenolic antioxidants. In this study, we have demonstrated that ARE also is required for induction of human *NQO1* gene expression in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The various results suggest an alternate pathway for TCDD induction of human *NQO1* gene expression. This pathway is independent of xenobiotic response element (XRE) and aromatic hydrocarbon (Ah) receptor. It is presumed that TCDD-induced expression of CYP1A1 leads to increased oxidative stress, resulting in transcriptional activation and/or modification of ARE-binding factors and increased expression of the human *NQO1* gene. BIOCHEM PHARMACOL **58**;10:1649–1655, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. NAD(P)H:quinone oxidoreductase$_1$; TCDD; antioxidant response element; regulation of gene expression

ARE† has been found in the promoter regions of a battery of genes, including *NQO1* and *GST Ya* subunit genes [1, 2]. ARE is known to mediate high basal expression and coordinated induction of these genes in response to xenobiotics (e.g. β-NF) and antioxidants (e.g. BHA) [1, 2]. AREs from the various genes contain two AP1/AP1-like elements arranged as inverse or direct repeats at the interval of 3 or 8 bp followed by a 'GC' box [2]. Several nuclear factors including Jun, Fos, Fra, and Nrf bind to the human *NQO1* gene ARE and mediate signal transduction from β-NF and BHA [3–5].

TCDD is a potent inducer of cytochrome P450 1A1 (CYP1A1). It is known to play an important role in metabolic activation of polycyclic aromatic hydrocarbons, oxidative stress, and neoplasia [6]. The mechanism of TCDD induction of *CYP1A1* gene expression involves binding of aromatic hydrocarbon (Ah) receptor with TCDD, nuclear translocation of Ah receptor-TCDD complex, and interaction with XRE [6, 7]. Interestingly, TCDD also induces *NQO1* and *GST Ya* gene expression [8–10]. The promoters of the *NQO1* and *GST Ya* genes contain XRE/XRE-like elements [8, 10]. XRE and not ARE was shown to increase the expression of rat *NQO1* and *GST Ya* genes in response to TCDD [8]. Deletion mutagenesis of the human *NQO1* gene promoter identified a DNA segment between −780 and −365 that was required for TCDD induction of *NQO1* gene expression [10]. This DNA segment of the human *NQO1* gene promoter contains a single copy of each of the ARE and XRE-like elements.

In this report, we have presented evidence to demonstrate that TCDD induction of human *NQO1* gene expression is mediated by ARE located between nucleotide −471 and −447. We also have demonstrated that XRE-like element plays either an insignificant role or no role in TCDD induction of human *NQO1* gene expression. The possible mechanism of ARE-mediated TCDD induction of human *NQO1* gene expression is discussed.

MATERIALS AND METHODS

Plasmids

The construction of pNQO1CAT1.55 has been described [3]. Plasmid pNQO1CAT1.55 contains 1.55 kb of the 5′ flanking region and 110 bp of the first exon from the human *NQO1* gene. The plasmid pNQO1CAT1.55ΔARE was generated with selected primers and polymerase chain reaction (PCR). The human *NQO1* gene promoter was amplified between −1550 and −472 and between −446

* Corresponding author: Dr. Anil K. Jaiswal, Department of Pharmacology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Tel. (713) 798-7691; FAX (713) 798-3145; E-mail: ajaiswal@bcm.tmc.edu

† *Abbreviations*: ARE, antioxidant response element; NQO1, NAD(P)H:quinone oxidoreductase 1, also known as quinone reductase (QR), quinone:(acceptor) oxidoreductase (QAO), and DT diaphorase (EC 1.6.99.2); GSTs, glutathione *S*-transferases; XRE, xenobiotic response element; AP1 or TRE, TPA response element; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; β-NF, β-naphthoflavone; and BHA, 2 [3]-*tert*-butyl-4-hydroxyanisole.

Received 30 October 1998; accepted 26 April 1999.

and +110 with selected primers and PCR to delete the region between −471 and −447 containing the ARE. The nucleotide sequences of the various primers were as follows: Primers for PCR amplification of the −1550 to −472 region of human *NQO1* gene promoter:

Forward primer:
5′GGATCCGGGCTGCCTACCTAGCCC3′
Reverse primer: 5′ATTTGGAAGGCTGAGAGTC3′

Primers for PCR amplification of −446 to +110 region of human *NQO1* gene promoter:

Forward primer: 5′TGAGCCTAGGGCACCACA3′
Reverse primer:
5′GGATCCGGCTCTGGTGCAGTC CGG3′

The PCR-amplified DNA fragments were treated with Klenow and ligated to each other, followed by digestion with *Bam*HI. The 1.55kb of the *NQO1* gene promoter with an internal deletion of the ARE sequence was subcloned at the *Bam*HI site of pBLCAT3 to generate plasmid pNQO1CAT1.55ΔARE. This plasmid was sequenced to confirm the deletion of the ARE element from the *NQO1* gene promoter. The construction of pNQO1hARE-tk-CAT and pNQO1 mutant hARE-tk-CAT recombinant plasmids has been described [3, 11]. The ARE region between nucleotides −471 and −447 of the human *NQO1* gene was attached to the thymidine kinase (tk) basal promoter connected to the chloramphenicol acetyl transferase (CAT) gene by subcloning in the vector pBLCAT2. The resultant plasmid was designated as pNQO1hARE-tk-CAT. Plasmid pNQO1 mutant hARE-tk-CAT contains mutations in the ARE core sequence as described previously [11]. The two DNA strands of the XRE-like sequence between nucleotides −750 and −728 of the human *NQO1* gene promoter were synthesized, annealed, and subcloned in pBLCAT2 to generate plasmid pNQO1XRE-like-tk-CAT. The pSV2neo plasmid contained a neomycin resistance gene under the control of the SV40 promoter.

Transfection and Stable Expression of NQO1-CAT Plasmids

The recombinant plasmids pNQO1CAT1.55, pNQO1CAT 1.55ΔARE, pNQO1XRE-like-tk-CAT, pNQO1hARE-tk-CAT, and pNQO1 mutant hARE-tk-CAT were co-transfected with pSV2neo in separate experiments in mouse hepatoma (Hepa-1) cells by previously described procedures [3, 11]. The ratio of CAT plasmids with pSV2neo was 1:20. The transfected cells were selected in the presence of 400 μg/mL of G418. More than 100 selected colonies were pooled and propagated to study the effect of exposure to TCDD.

Transient Transfection and Expression of pNQO1hARE-tk-CAT in Hepa-1 (Wild-type), Hepa-1 C15 (AHR$^-$), and Hepa-1 C37 (CYP1A1$^-$) Cells

Hepa-1 C15 and Hepa-1 C37 are benzo[*a*]pyrene resistant mutant cell lines derived from Hepa-1 [12, 13]. Hepa-1 C15 is Ah receptor-deficient [12]. Hepa-1 C15 cells also lack TCDD induction of CYP1A1 because of the absence of an Ah receptor [12]. The Hepa-1 C37 cells contain functional Ah receptor but lack CYP1A1 activity because of mutations in the protein [13]. Ten micrograms of plasmid pNQO1hARE-tk-CAT was cotransfected with 5 μg of RSV-β-galactosidase in wild-type and mutant Hepa-1 cells by procedures also described previously [3, 11].

TCDD Treatment and CAT Activity

Hepa-1 cells permanently expressing the various NQO1-CAT plasmids were grown in monolayers and treated with different concentrations of TCDD for 24 hr. After the treatment period, the cells were scraped, homogenized, and analyzed for CAT activity by procedures described previously [3]. Similarly, the transiently transfected wild-type and mutant Hepa-1 cells were treated with TCDD for 24 hr and analyzed for β-galactosidase and CAT assays.

RESULTS AND DISCUSSION

The toxic and mutagenic effects of TCDD are mediated by Ah receptor and XRE-mediated induction of expression of cytochrome P450 genes including the *CYP1A1* gene [6, 7]. The mechanism of induction of *CYP1A1* gene expression by TCDD and Ah receptor is similar to that of members of the super family of steroid receptors [6, 7]. It involves binding of TCDD to the Ah receptor, resulting in dissociation of Hsp90 from the Ah receptor and formation of a TCDD-Ah receptor complex. The Ah receptor-TCDD complex translocates into the nucleus, binds to the XRE element of *CYP1A1* and other genes, and activates their transcription [7]. The complete mechanism of Ah receptor-mediated TCDD induction of the *CYP1A1* gene involves additional proteins including Ah receptor nuclear translocator (ARNT) and intermediary steps that are under investigation [7].

Rat *NQO1* and *GST Ya* genes that are induced by TCDD also contain XRE elements in their promoter regions [1, 8]. XRE was reported to mediate TCDD induction of rat *NQO1* gene expression [8]. Human *NQO1* gene transcription is also induced in response to TCDD [9, 10]. Deletion mutagenesis in the human *NQO1* gene promoter identified a region between −780 and −365 that was required for TCDD induction of human *NQO1* gene expression [10].

In the present report, we have performed experiments to identify the *cis*-element(s) responsible for transcriptional activation of human *NQO1* gene expression in response to TCDD. Nucleotide sequence analysis of the TCDD-response region between nucleotides −780 and −365 of the human *NQO1* gene promoter revealed the presence of a single copy of an XRE-like element between nucleotides −750 and −728 and a copy of the ARE element between −471 and −447 (Fig. 1, Ref. 10). Alignment of 1097 bp of the human *NQO1* gene promoter with similar bp of the rat

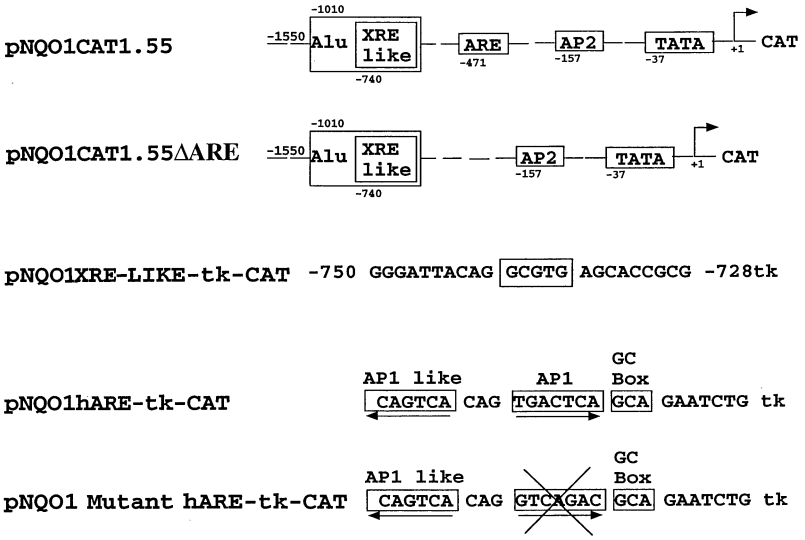

FIG. 1. List of the various human NQO1-CAT constructs. Plasmid pNQO1CAT1.55 contains 1.55 kb of the 5′ flanking region of the *NQO1* gene driving the CAT gene. Depicted in the figure is the 1.55 kb of the human *NQO1* gene promoter, showing the positions of various *cis*-elements identified by deletion mapping and nucleotide sequence analysis. Alu is a 300-bp repetitive and highly abundant sequence in the human genome. XRE is an element that binds to the Ah receptor-TCDD complex and increases *CYP1A1* gene transcription. ARE is an element that binds to nuclear factors Jun, Fos, Fra, and Nrf and increases expression of the *NQO1* gene in response to xenobiotics and antioxidants. AP2 is a cyclic AMP response element. ARE sequences were deleted from the 1.55-kb human *NQO1* gene promoter to generate plasmid pNQO1CAT1.55ΔARE. Plasmid pNQO1XRE-like-tk-CAT contains 24 bp of a human *NQO1* gene XRE-like element driving CAT gene expression. Similarly, plasmid pNQO1hARE-tk-CAT contains 26 bp of the human *NQO1* gene ARE driving CAT gene expression. The core of the ARE was mutated to generate plasmid pNQO1 mutant hARE-tk-CAT plasmid. Similar mutations in the ARE core sequence are known to reduce the expression and induction of target genes markedly [11].

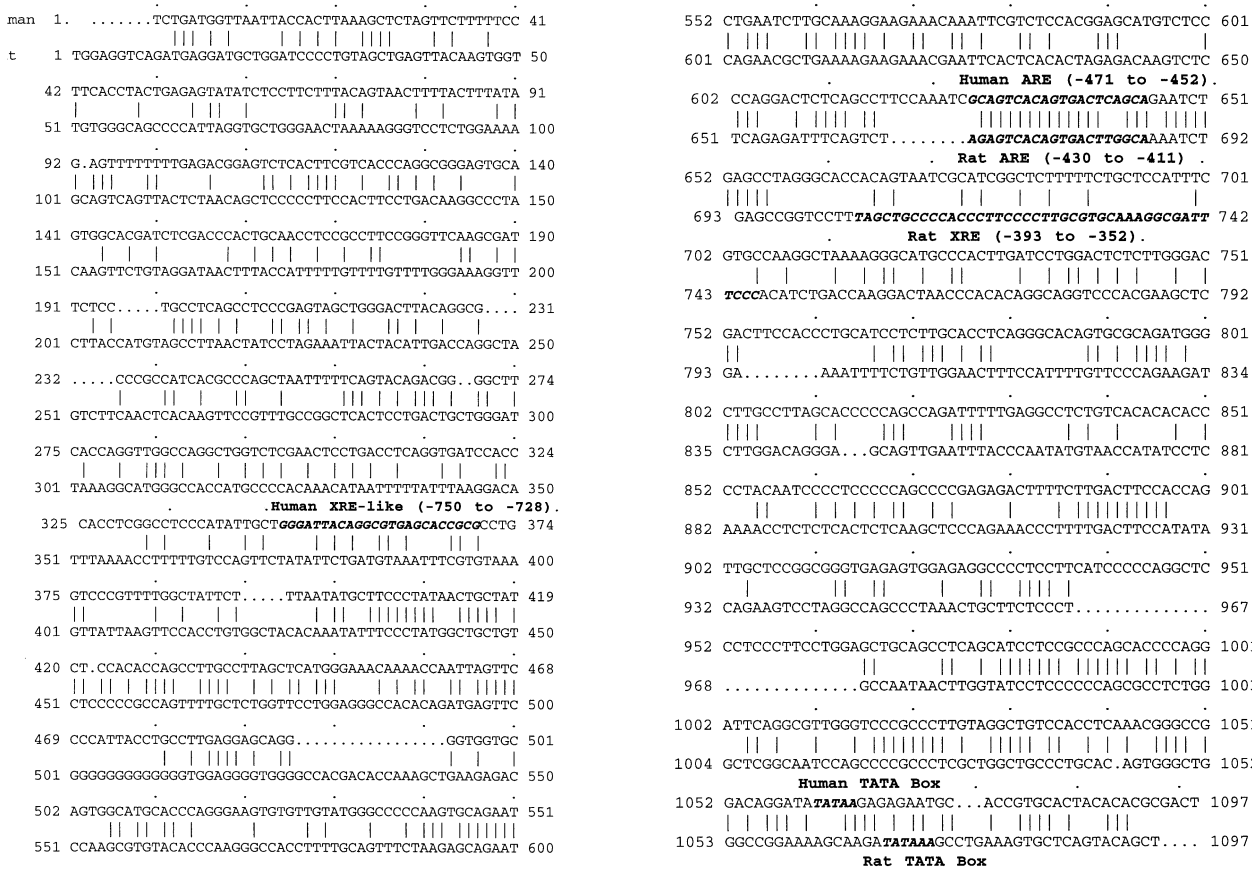

FIG. 2. Alignment of 1097 bp of the human *NQO1* gene promoter with 1097 bp of the rat *NQO1* gene promoter. ARE and XRE elements in both the genes are shown in bold and italic letters.

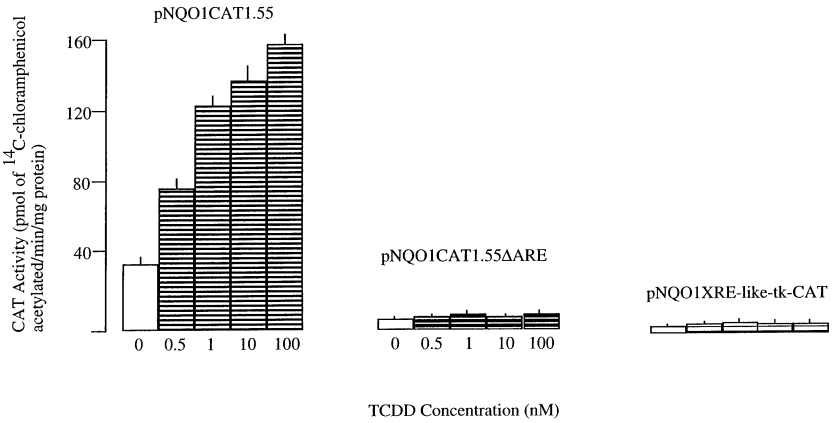

FIG. 3. Stable expression and TCDD induction of human NQO1-CAT plasmids in Hepa-1 cells. The plasmids pNQO1CAT1.55, pNQO1CAT1.55ΔARE, and pNQO1XRE-like-tk-CAT were co-transfected with pSV2neo in separate experiments in Hepa-1 cells. The transfected cells were selected in 400 μg of G418. The selected colonies for individual plasmids were pooled, propagated, and treated with various concentrations of TCDD for 24 hr. After the incubation period, the cells were harvested and analyzed for CAT activity. The data are means ± SEM of three independent treatments and assays.

NQO1 gene promoter revealed interesting observations on the conservation of location and nucleotide sequence of ARE and XRE elements between the two species (Fig. 2). The human *NQO1* gene ARE was highly conserved in the rat *NQO1* gene with respect to its location and nucleotide sequence (Fig. 2). Alignment of human and rat *NQO1* gene promoters also revealed that the location and nucleotide sequence of rat XRE were not conserved in the human *NQO1* gene (Fig. 2). The rat *NQO1* gene XRE that is known to mediate TCDD induction of rat *NQO1* gene expression was located between nucleotides −393 and −352 of the rat *NQO1* gene [8]. A comparison of homology between rat XRE and the corresponding region in human indicated less than 20% sequence similarity (Fig. 2). Analysis of human *NQO1* gene promoter for the presence of the XRE core sequence 'GCGTG' indicated the presence of a single copy of XRE at nucleotide position −740 (Fig. 2). However, this sequence was not conserved in the rat *NQO1* gene. The XRE-like sequence in the human *NQO1* gene was contained within an Alu repetitive sequence region between nucleotides −1010 and −710 (Fig. 1). It may be noteworthy that the human *NQO1* gene XRE-like sequence contained only the core XRE sequence. The flanking sequences that are important for TCDD induction of *CYP1A1* gene expression were not conserved in the human *NQO1* gene XRE-like element.

Several CAT constructs were generated in the present study and co-transfected with a plasmid containing a neomycin resistance gene in Hepa-1 cells to identify the *cis*-element(s) required for TCDD induction of human *NQO1* gene expression. Hepa-1 cells permanently expressing the various NQO1-CAT plasmids were selected in the presence of G418. All of the G418-resistant colonies for individual NQO1-CAT constructs were pooled and propagated for further studies on the TCDD induction of *NQO1* gene expression. The results are shown in Figs. 3 and 4. Exposure of Hepa-1-pNQO1CAT1.55 cells to 0.5 nM TCDD resulted in a 2-fold-increased expression of the CAT gene. An increase in the concentration of TCDD from 0.5 to 1 nM led to an additional fold increase in the expression of human *NQO1* gene promoter-regulated CAT gene expression (Fig. 3). Further increases in TCDD concentration increased the CAT gene expression only marginally (Fig. 3). Interestingly, in parallel experiments, internal deletion of ARE from 1.55 kb human *NQO1* gene promoter driving the CAT gene resulted in a marked decrease in basal expression and loss of TCDD induction of CAT gene expression. These results also indicated that ARE and not XRE-like element is required for TCDD induction of human *NQO1* gene expression. The absence of a significant role of the XRE-like element in TCDD-mediated induction of human *NQO1* gene expression was

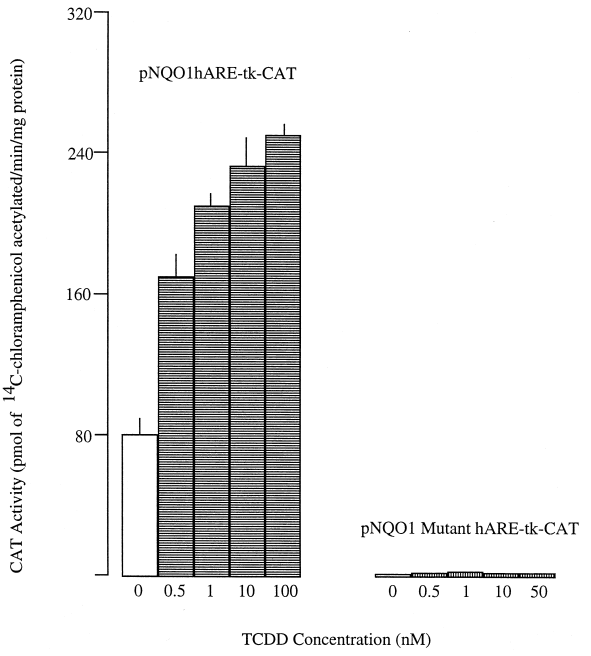

FIG. 4. Stable expression and TCDD induction of human pNQO1hARE-tk-CAT and pNQO1 mutant hARE-tk-CAT plasmids in Hepa-1 cells. The plasmids pNQO1hARE-tk-CAT and pNQO1 mutant hARE-tk-CAT were co-transfected with pSV2neo in separate experiments in Hepa-1 cells. The transfected cells were selected in 400 μg of G418. The selected colonies for individual plasmids were pooled, propagated, and treated with various concentrations of TCDD for 24 hr. After the incubation period, the cells were harvested and analyzed for CAT activity. The data are means ± SEM of three independent treatments and assays.

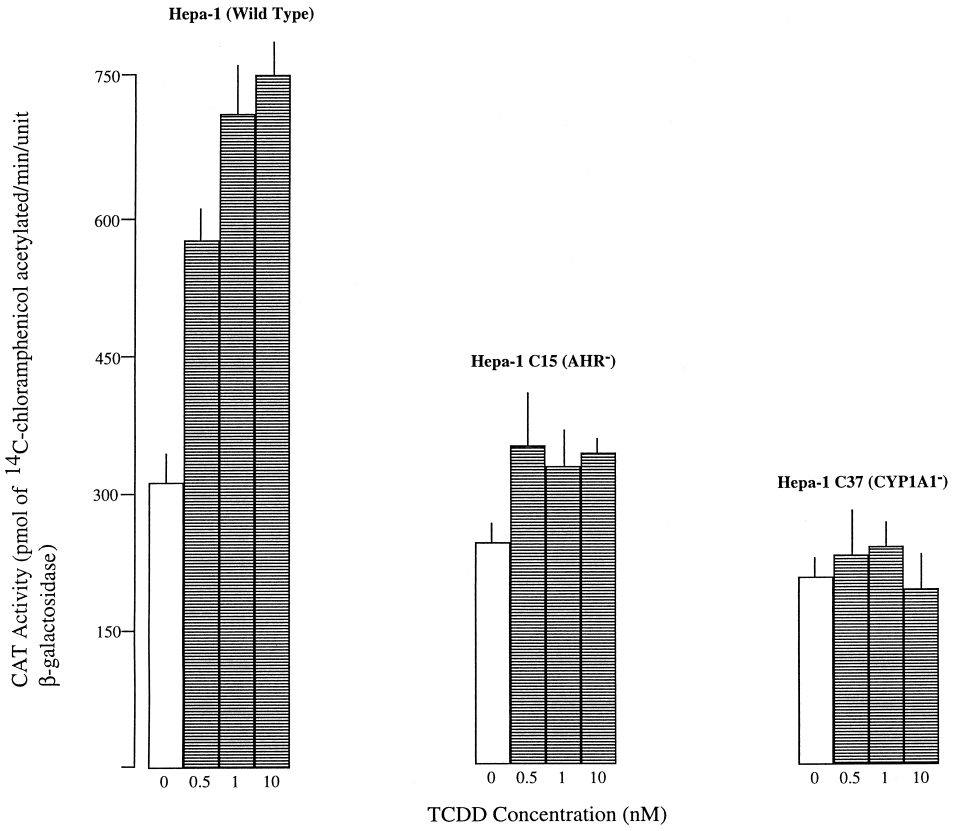

FIG. 5. Transient expression and TCDD induction of pNQO1hARE-tk-CAT in wild-type and mutant Hepa-1 cells. Ten micrograms of pNQO1hARE-tk-CAT plasmid was cotransfected with 5 μg of RSV-β-galactosidase plasmid in wild-type Hepa-1, Hepa-1 C15 (AHR$^-$), and Hepa-1 C37 (CYP1A1$^-$) cells. Twenty-four hours after the transfection, the cells were treated with either DMSO or TCDD. Twenty-four hours after the treatment, the cells were harvested, homogenized, and analyzed for β-galactosidase and CAT activities. The data are means ± SEM of three independent experiments.

confirmed by Hepa-1 cells permanently expressing plasmid pNQO1XRE-like-tk-CAT. The pNQO1XRE-like-tk-CAT plasmid contained a human *NQO1* gene XRE-like element attached to the tk basal promoter connected to the CAT gene. Hepa-1 cells permanently transfected with plasmid pNQO1XRE-like-tk-CAT failed to show an increase in XRE-like element-mediated CAT gene expression upon treatment with TCDD (Fig. 3).

The role of ARE-mediated TCDD induction of *NQO1* gene expression was confirmed further by Hepa-1 cells permanently transformed with hARE-tk-CAT plasmid (Fig. 4). The treatment of Hepa-1 cells permanently expressing human *NQO1* gene ARE-mediated CAT gene expression with TCDD showed a concentration-dependent increase in CAT gene expression. The kinetics of induction was similar to that observed with the 1.55 kb *NQO1* gene promoter. The treatment of cells with 0.5 nM TCDD led to a slightly more than 2-fold increase in ARE-mediated CAT gene expression. Raising the TCDD concentration from 0.5 to 1 nM increased the induction of CAT gene expression to approximately 3-fold. Further increases in TCDD concentration resulted in only marginal increases in the ARE-mediated CAT gene expression. Mutations in the core sequence of the ARE resulted in the loss of basal expression and TCDD induction of *NQO1* gene expression (Fig. 4).

The transient transfection of wild-type Hepa-1 cells with plasmid pNQO1hARE-tk-CAT expressed CAT activity, which was also induced by 2-fold in response to 0.5 nM TCDD (Fig. 5). Raising the TCDD concentration further increased hARE-mediated CAT gene expression in wild-type Hepa-1 cells. However, in similar experiments, mutant Hepa-1 cells demonstrated marked decreases in TCDD induction of hARE-mediated CAT gene expression, as compared with wild-type Hepa-1 cells (Fig. 5). Hepa-1 C15 (AHR$^-$) cells are deficient in Ah receptor and, thus, in TCDD induction of CYP1A1 [12]. The results with Hepa-1 C15 (AHR$^-$) cells indicated involvement of either Ah receptor and/or CYP1A1 in TCDD induction of hARE-mediated CAT gene expression. However, the experiments with Hepa-1 C37 cells, which contain Ah receptor but lack CYP1A1, clearly indicated that it was the absence of CYP1A1 activity and not Ah receptor that led to marked decreases in TCDD induction of hARE-mediated CAT gene expression. CYP1A1 activity is required to generate TCDD-induced oxidative stress, which may signal the activation of *NQO1* gene expression.

The transfection results clearly indicated an ARE-mediated mechanism for TCDD induction of human *NQO1* gene expression. The *NQO1* gene ARE is highly conserved between human and rat genes [2]. The only difference

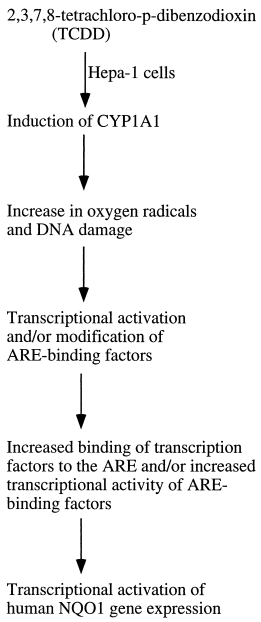

FIG. 6. Hypothetical model showing the mechanism of ARE-mediated TCDD induction of *NQO1* gene expression.

between the human and rat *NQO1* gene AREs is the presence of a perfect AP1 binding site in the human ARE [2]. The rat *NQO1* gene ARE contained both imperfect (AP1-like) elements. In contrast to the human *NQO1* gene, the TCDD-induced rat *NQO1* gene expression has been reported to be mediated by XRE and not by ARE [8]. The obvious discrepancy in results with human and rat *NQO1* gene AREs may be attributed to species differences or differences in the presence/absence of a perfect AP1 element in the ARE. These results showing ARE-mediated TCDD induction of human *NQO1* gene expression were significant because they demonstrated a pathway for TCDD induction of gene expression independent of XRE and Ah receptor. The results also raised questions regarding the molecular mechanism of ARE-mediated TCDD induction of *NQO1* gene expression. A hypothetical model for TCDD induction of ARE-mediated gene expression based on the current information is proposed in Fig. 6. It is an established fact that TCDD treatment of Hepa-1 cells leads to increased expression of CYP1A1 [6, 7, 14]. The studies have also demonstrated that the TCDD-mediated increased expression of CYP1A1 in Hepa-1 cells was associated with increased oxidative damage of DNA as evidenced by increased excretion of 8-oxoguanosine, a biomarker of oxidative DNA damage and the major repair product of 8-oxo-2′-deoxyguanosine residues in DNA [14]. The TCDD-induced oxidative damage to Hepa-1 cells may lead to transcriptional activation and/or modification of ARE-binding factors, resulting in increased binding to the ARE and/or increased transcriptional activity of ARE-binding factors. These changes lead to transcriptional activation of *NQO1* gene expression. Several nuclear factors have been identified that bind to the human *NQO1* gene ARE [2–5]. These include Jun (c-Jun, Jun-B, Jun-D), c-Fos, Fra (Fra1 and Fra2), and Nrf (Nrf1 and Nrf2). Among these nuclear factors, Nrf1 and Nrf2 positively and c-Fos and Fra1 and Fra2 negatively regulate ARE-mediated expression and β-NF induction of *NQO1* gene expression [5]. It is not clear at this time whether TCDD recruits a similar mechanism and factors as β-NF for induction of *NQO1* gene expression or activates a distinct mechanism. In either case, it will be interesting to identify the ARE-binding nuclear factors that are transcriptionally activated and/or modified by TCDD to activate *NQO1* gene expression. It may be noteworthy that TCDD is known to increase the expression of c-*jun* and c-*fos* proto-oncogenes [15]. However, the role of c-Jun and c-Fos in ARE-mediated TCDD induction of human *NQO1* gene expression remains to be determined.

This work was supported by NIH Grant GM 47466. We would like to thank Dr. Oliver Hankinson, University of California at Los Angeles, for providing us with the Hepa-1 mutant cell lines.

References

1. Rushmore TH and Pickett CB, Glutathione-*S*-transferases, structure, regulation, and therapeutic implications. *J Biol Chem* **268**: 11475–11478, 1993.
2. Jaiswal AK, Antioxidant response element. *Biochem Pharmacol* **48**: 439–444, 1994.
3. Li Y and Jaiswal AK, Regulation of human NAD(P)H: quinone oxidoreductase gene. Role of AP1 binding site contained within human antioxidant response element. *J Biol Chem* **267**: 15097–15104, 1992.
4. Jaiswal AK, Jun and Fos regulation of NAD(P)H: quinone oxidoreductase gene expression. *Pharmacogenetics* **4**: 1–10, 1994.
5. Venugopal R and Jaiswal AK, Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase$_1$ gene. *Proc Natl Acad Sci USA* **93**: 14960–14965, 1996.
6. Whitlock JP, Mechanistic aspects of dioxin action. *Chem Res Toxicol* **6**: 754–763, 1993.
7. Sogawa K and Fujiikuriyama Y, Ah receptor, a novel ligand-activated transcription factor. *J Biochem (Tokyo)* **122**: 1075–1079, 1997.
8. Favreau LV and Pickett CB, Transcriptional regulation of the rat NAD(P)H:quinone reductase gene. Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J Biol Chem* **266**: 4556–4561, 1991.
9. Jaiswal AK, McBride OW, Adensik M and Nebert DW, Human dioxin-inducible cytosolic NAD(P)H:menadione oxidoreductase. cDNA sequence and localization of gene to chromosome 16. *J Biol Chem* **263**: 13572–13578, 1988.
10. Jaiswal AK, Human NAD(P)H:quinone oxidoreductase (NQO_1) gene structure and induction by dioxin. *Biochemistry* **30**: 10647–10653, 1991.
11. Xie T, Belinsky M, Xu Y and Jaiswal AK, ARE- and TRE-mediated regulation of gene expression: Response to xenobiotics and antioxidants. *J Biol Chem* **270**: 6894–6900, 1995.
12. Zhang J, Watson AJ, Probst MR, Minehart E and Hankinson O, Basis for the loss of aryl hydrocarbon receptor gene expression in clones of a mouse hepatoma cell line. *Mol Pharmacol* **50**: 1454–1462, 1996.
13. Sun W, Zhang J and Hankinson O, A mutation in the aryl

hydrocarbon receptor (AHR) in a cultured mammalian cell line identified a novel region of AHR that affects DNA binding. *J Biol Chem* **272**: 31845–31854, 1997.
14. Park J-YK, Shigenaga MK and Ames BN, Induction of cytochrome P4501A1 by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or indolo(3,2-*b*)carbazole is associated with oxidative DNA damage. *Proc Natl Acad Sci USA* **93**: 2322–2327, 1996.
15. Puga A, Nebert DW and Carrier F, Dioxin induces expression of c-*fos* and c-*jun* proto-oncogenes and a large increase in transcription factor AP1. *DNA Cell Biol* **11**: 269–281, 1992.