Human NAD(P)H:Quinone Oxidoreductase (NQO_1) Gene Structure and Induction by Dioxin[†,‡]

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ABSTRACT: The human NAD(P)H:quinone oxidoreductase (NQO_1) gene, 1850 base pairs (bp) of the 5′ flanking region, and 67 bp of the 3′ flanking region have been sequenced. The human NQO_1 gene is approximately 20 kb in length and has six exons interrupted by five introns. The start site of transcription was determined by primer extension analysis. The first exon is 118 bp in length and codes for two amino acids including the initiating methionine and one G for the first codon of the second exon. The sixth exon is the largest among the exons and is 1833 bp in length. The sequence analysis of the sixth exon revealed the presence of four potential polyadenylation signal sequences (AATAAA) and a single copy of human Alu repetitive sequence. The second intron is the smallest of all the introns (116 bp). Nuclear run-on experiments performed using nuclei isolated from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) treated and untreated human hepatoblastoma (Hep-G2) cells demonstrated that TCDD treatment increases the rate of transcription of endogenous NQO_1 gene by 3-fold. The sequence analysis of the 5′ flanking region of NQO_1 gene showed the presence of a TATA box in the −37 to −32 bp region, one CCAAT box at nucleotide −649, an AP1 binding site at position −462, an AP2 site at nucleotide position −156, and one copy of the nucleotide sequence GCGTG (at position −740) similar to that found in the xenobiotic/dioxin response elements (XREs and DREs) present in the cytochrome P-450$\underline{1}$A1 (CYP$\underline{1}$A1) gene and known for binding to the Ah receptor–inducer complex and increasing the expression of CYP$\underline{1}$A1 gene in response to polycyclic aromatic hydrocarbons (PAHs) and TCDD. The sequence analysis also showed the presence of one copy of an antioxidant response element (ARE) between residues −467 and −447 of the human NQO_1 gene. The ARE sequence has recently been identified in the regulatory region of ya subunit of glutathione *S*-transferase gene and rat NAD(P)H:quinone reductase gene and shown to mediate the basal expression and its induction in response to planar aromatic compounds and phenolic antioxidants. It is noteworthy that XRE in human NQO_1 gene is located 5′ to the ARE compared to its 3′ location in the rat quinone reductase gene. The nucleotide sequence of the ARE characterized in the rat quinone reductase gene is highly conserved in the human NQO_1 gene. Interestingly, the consensus sequence for binding to AP1 protein (**TGACTCA**) is contained within the ARE sequence (TCACAG**TGACTCA**GCAGAATC) of the human NQO_1 gene. The 1850 bp of 5′ flanking region of the NQO_1 gene containing one copy each of the XRE and the ARE, when attached to the CAT gene and transfected into human hepatoblastoma (Hep-G2) and mouse hepatoma (Hepa-1) cells, increased the expression of CAT gene upon treatment with TCDD. By deletion mutagenesis and transfection studies, we have identified a segment of DNA in the upstream region of the human NQO_1 gene (between positions −780 and −365) required for a high level of expression in hepatoma cells and its induction by TCDD.

NAD(P)H:quinone oxidoreductase (NQO_1) is a flavoprotein that catalyzes the two-electron reduction of different quinones, quinone imines, and azo dyes for further metabolism and excretion from the body (Talalay & Benson, 1982; Iyanagi, 1987). Evidence has been presented that NQO_1 may have a protective effect against carcinogenicity, mutagenicity, and other toxicities caused by quinones and their metabolic precursors (Lind et al., 1982; Di Monte et al., 1984a,b).

The NQO_1 activity is known to be inducible by 3-methylcholanthrene (Robertson et al., 1986; Williams et al., 1986), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) (Beatty & Neal, 1976; Jaiswal et al., 1988), and phenolic antioxidants (Prochaska et al., 1985). Our laboratory is interested in investigating the molecular mechanism of NQO_1 induction by PAHs and TCDD and in particular in determining if the aromatic hydrocarbon (Ah) receptor that mediates PAH- and TCDD-induced transcriptional activation of cytochrome P-450$\underline{1}$A1 (CYP$\underline{1}$A1) gene expression [reviewed in Nebert and Gonzalez (1987), Whitlock (1987) Gonzalez (1989), and Nebert and Jones (1989)] also plays a role in NQO_1 gene induction by the similar compounds. In the present report we have cloned the human NQO_1 gene, determined its exon–intron organization, and shown that the increase in the NQO_1 gene expression by TCDD in Hep-G2 cells is due to an increase in the transcription of the gene. We also show that the 1850 bp of 5′ flanking region of NQO_1 gene when attached to chloramphenicol acetyltransferase (CAT) gene and transfected into human Hep-G2 and mouse Hepa-1 cells increased the expression of CAT gene by severalfold upon treatment with TCDD. The sequence analysis of this 1850 bp region of NQO_1 gene revealed the presence of potential binding sites for the aromatic hydrocarbon (Ah) receptor (xenobiotic response element), as were also present in the regulatory region of the CYP$\underline{1}$A1 gene in response to TCDD (Nebert & Gonzalez,

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[‡]The nucleic acid sequence of the human NQO_1 gene reported in this paper has been submitted to GenBank under Accession Number J05348.

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1987; Whitlock, 1987; Gonzalez, 1989; Nebert & Jones, 1989), as well as binding sites for the AP1 and AP2 proteins, known for their involvement in 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induction of gene expression (Mitchell & Tjian, 1989). In addition, sequence analysis also showed the presence of consensus sequence of the antioxidant response element (ARE) identified recently in the upstream region of glutathione *S*-transferase ya subunit gene and rat NAD(P)H:quinone reductase gene (Rushmore et al., 1990; Rushmore & Pickett, 1990; Favreau & Pickett, 1991). Deletion mutagenesis studies on the 5′ flanking region of human NQO_1 gene have been performed to identify the DNA segments required for a basal level of expression in hepatoma cells and its induction by TCDD.

Materials and Methods

Cloning and Sequencing of the Human NQO_1 Gene. Human liver genomic DNA was isolated (Maniatis et al., 1982), partially digested with *Sau*3A, and fractionated on a 10–40% sucrose density gradient. Fragments of 15–25 kb were collected (Maniatis et al., 1982). The DNA fragments were inserted into the *Bam*HI site of λEMBL3 (Frishauf et al., 1983). The ligated DNA was packaged in vitro with extracts derived from *Escherichia coli* BHB2688 and BHB2690, as described (Maniatis et al., 1982; Hohn, 1979). Recombinant phage were then plated onto *E. coli* LE392, and approximately 1.5×10^6 plaque-forming units were screened (Jaiswal et al., 1985) by hybridization with human NQO_1 cDNA inserts that had been ^{32}P-labeled by nick translation (Rigby et al., 1977). Five positive plaques were purified by a minimum of three round of plaque purification. DNA was isolated by CsCl gradient centrifugation (Maniatis et al., 1982). Overlapping clones were aligned by restriction endonuclease mapping and Southern blot hybridization analysis using NQO_1-5′ (nucleotides 1–657) and NQO_1-3′ (nucleotides 658 to the first polyadenylation site) cDNA as the probes. All the positive clones showed a similar restriction enzyme overlapping pattern consistent with a single NQO_1 gene. One of the clones named λhNQO_1g-18 was found to contain the entire gene with flanking regions and was used for further studies. Various restriction fragments carrying the entire human NQO_1 gene and approximately 2 kb each of 5′ and 3′ flanking regions were excised out from the λhNQO_1g-18 clone and subcloned in pUC18 for further refined mapping and nucleotide sequencing.

Determination of Exon–Intron Junctions. To find the nucleotide sequences encompassing the exon–intron junctions of NQO_1 gene without sequencing the whole gene, we synthesized several oligonucleotides 15–17 bp in length selected at random from the corresponding NQO_1 cDNA sequence and used these as primers for sequencing the NQO_1 gene fragments subcloned into pUC18 plasmid as published (Sal et al., 1989). This process of sequencing continued until we recovered all of the cDNA sequences from the corresponding gene.

Determination of 5′ and 3′ Flanking Sequence of the NQO_1 Gene. A 3-kb *Pvu*II fragment from NQO_1 gene containing 5′ flanking region, the first exon and a portion of the first intron was excised out by digestion of the λhNQO_1g-18 clone with *Pvu*II restriction enzyme and subcloned into pUC18 at the *Sma*I site. The insert was removed by digestion with *Hin*dIII and *Eco*RI, separated by agarose gel electrophoresis, and used to make a sonication shotgun library in M13mp10 (Deninger, 1983). Sequencing was carried out by standard M13 cloning protocols and the dideoxy sequencing method (Sanger et al., 1977; Messing et al., 1981). Each strand of DNA was sequenced at least five times. Wherever necessary, we also used oligonucleotides synthesized from known sequence as primers to sequence the pUC18 plasmid containing the 5′ flanking DNA of NQO_1 gene. Nucleotide sequence alignments and analysis of nucleotide and protein sequence data were carried out by standard computer programs. By the use of a synthesized oligonucleotide from the 3′ end of NQO_1 cDNA, we were able to obtain 67 bp of sequence in the 3′ flanking region of the NQO_1 gene.

Determination of Start Site of Transcription for the NQO_1 Gene. We used the primer extension method to determine the start site of transcription in the NQO_1 gene. Twenty-three base pair long synthetic oligonucleotide selected from the 5′ leader sequence of coding as well as noncoding strands of NQO_1 cDNA was used as primers. The oligonucleotide selected from coding strand worked as control. The primers after end labeling with ^{32}P were hybridized to total RNA isolated from TCDD-treated (100 nM for 48 h) and untreated Hep-G2 cells and then extended using AMV reverse transcriptase (Bhat et al., 1990). The primer-extended fragments of 61 bp were detected when oligonucleotide from noncoding strand was used. Nothing was detected with oligonucleotide primer selected from the coding strand because of the similar sequence to that of the NQO_1 mRNA.

Nuclear Run-On Transcription. Nuclear run-on experiments were performed using nuclei isolated from TCDD-treated (100 nM for 48 h) and untreated Hep-G2 cells (McKnight & Palmiter, 1979). The human B-actin DNA and human CYP1A1 cDNA were used as controls.

Construction of NQO_1–CAT Recombinant Plasmids. The 1960 bp DNA segment was excised out by digesting the pUC18 plasmid carrying the 3-kb *Pvu*II fragment of the NQO_1 gene with *Nco*I restriction enzyme. This fragment contained 1850 bp of the 5′ flanking region and 110 bp of first exon. The *Nco*I fragment was first treated with mung bean nuclease to remove the initiation of translation codon for NQO_1 protein, followed by treatment with Klenow polymerase, and then cloned into the pBLCAT3 vector (Luckow & Schutz, 1987) after adding *Bgl*II linkers. The resulting recombinant plasmid was called pNQO_1CAT1.85. The deletion mutants pNQO_1CAT1.55 and -0.13 were made by digestion of pNQO_1CAT1.85 plasmid with *Hin*dIII and *Pst*I restriction enzymes in separate experiments. The *Hin*dIII enzyme created a deletion of 307 bp from the 5′ end of the NQO_1 gene promoter and was named pNQO_1CAT1.55. Similarly, the *Pst*I enzyme created a deletion of 1720 bp from the 5′ end of the NQO_1 promoter and was called pNQO_1CAT0.13. The 475 bp (*Sau*3A to *Bgl*II) fragment of DNA containing 365 bp of 5′ flanking region and 110 bp of the first exon of the human NQO_1 gene was isolated by digestion of the 1960 bp *Bgl*II fragment with *Sau*3A and subcloned into the *Bgl*II site of the pBLCAT3 vector to give rise to plasmid pNQO_1CAT0.365. The recombinant plasmids pNQO_1CAT1.34, -0.837, and -0.786 were created by controlled digestion of linearlized pNQO_1CAT1.55 plasmid with nuclease *Bal*31 and subsequent cloning of different lengths of DNA fragments at the *Bgl*II site of vector pBLCAT3 after adding *Bgl*II linkers (Davis et al., 1986). The 1013 bp *Bgl*II–*Rsa*I fragment from the 5′ end of the NQO_1 gene promoter (1960 bp *Bgl*II fragment) was isolated and blunt ended and after adding *Sal*I linkers was inserted into the *Sal*I site of the plasmid pNQO_1CAT0.365 to create the recombinant plasmid pNQO_1CATΔ0.837–0.365 with an internal deletion of the DNA segment in the region −837 to −365. The *Sau*3A segment of DNA (in the region −780 to −365) of the NQO_1 gene promoter was cloned at the *Bam*HI site of the vector pBLCAT2 (a derivative of pBLCAT3, containing the basal tk promoter, designed to study

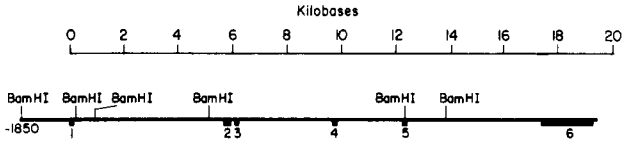


FIGURE 1: Human NAD(P)H:quinone oxidoreductase gene structure. Exons are denoted by black boxes and introns by the thick dark lines. The flanking regions, all the exons, and the second introns were completely sequenced. The sizes of all other introns were determined by restriction digestion and Southern hybridization.

the effect of a regulatory sequence on heterologous promoter, Luckow & Schutz, 1987) and was named $pNQO_1$tkCAT0.780–0.365.

Transfection and Expression of NQO_1–CAT Recombinant Plasmids into Hep-G2 and Hepa-1 Cells. The NQO_1–CAT chimeric DNAs were transiently transfected into the human Hep-G2 and mouse Hepa-1 cells by the calcium phosphate procedure (Parker & Stark, 1979). The CAT gene expression was monitored by measuring the CAT activity (Davies et al., 1980). The pBLCAT3 is a promoterless plasmid and was used as control. The RSV–βGal plasmid was cotransfected in each case to normalize the transfection efficiency by measuring the β-galactosidase activity (Guarente, 1983).

Results and Discussion

Exon–Intron Organization and Sequence Analysis of the 5′ Flanking Region of the Human NQO_1 Gene. The human NQO_1 gene structure is shown in Figure 1. The 5′ flanking sequences, exons, introns, and 3′ flanking sequences of NQO_1 gene have been determined (Figure 2). The human NQO_1 gene is approximately 20 kb in length and has six exons of 118, 165, 131, 114, 102, and 1883 bp interrupted by five introns of approximately 5500, 116, 3500, 2500, and 5000 bp in length. All the introns began with GT and ended with AG. Exons 2–5 are remarkably conserved in both nucleotide similarity and actual number of nucleotides between human NQO_1 gene cloned by us and recently reported rat quinone reductase gene (Bayney et al., 1989). The initiation of transcription for human NQO_1 gene was determined by primer extension analysis (data not shown) and is indicated at position +1 (Figure 2). From the primer-extended fragment of 61 bp we determined the size of the first exon as 118 bp. The first exon codes for two amino acids including the initiating methionine and provides one G for the first codon of the second exon. The sixth exon in human NQO_1 gene is 1883 bp in length compared to 907 bp in the corresponding rat gene (Bayney et al., 1989). Sequence analysis of the sixth exon in human reveals the presence of four potential polyadenylation signal sequence (AATAAA) and a single copy of human Alu repetitive sequence between the second and third polyadenylation signal site. In contrast, the sixth exon of the rat gene has only one polyadenylation signal sequence and no Alu repetitive sequence (Bayney et al., 1989). The sizes of introns in human and rat genes could not be compared as the same was not reported for the rat gene except the size of the second intron. The second intron in human NQO_1 gene is the smallest of all the introns (116 bp) and is 46 bp larger than the second intron of rat quinone reductase gene. The TATA box present in many eukaryotic genes and known for its role in correct initiation of transcription (Maniatis et al., 1987) was found located in the −37 to −32 bp region upstream from the cap site of the human NQO_1 gene. The position (distance measured from the initiation of translation codon and not from the cap sites) and nucleotide sequence of the TATA box region are highly conserved between human and rat genes. However, the first exon in human is 118 bp compared to 126 bp in the rat gene. Sequence analysis also showed the presence of one CAT box sequence (Maniatis et al., 1987) at nucleotide −649. The CAT box is found in many eukaryotic promoters and has been shown to increase the rate of transcription of the gene in which it is present (Maniatis et al., 1987; Mitchell & Tjian, 1989).

Nucleotide sequence analysis (Figure 2) of the 1850 bp of the NQO_1 gene promoter showed the presence of one copy of the sequence GCGTG, the five invariant bases of the xenobiotic response element (XRE) at position −740. The XRE sequences present in the regulatory region of the CYP1A1 gene are known for binding to the Ah receptor–inducer complex and increasing the expression of the CYP1A1 gene (Whitlock, 1987). The sequence analysis also showed the presence of a single copy of the antioxidant response element (ARE) at position −467 of the NQO_1 gene promoter. The ARE motif has recently been identified in the regulatory region of the ya subunit of the glutathione *S*-transferase gene (Rushmore et al., 1990; Rushmore & Pickett, 1990) and rat NAD(P)H:quinone reductase gene (Favreau & Pickett, 1991) and shown to mediate the basal expression and its induction in response to planar aromatic compounds (e.g., β-naphthoflavone) and phenolic antioxidants. The position and sequence of the rat ARE are highly conserved in the human NQO_1 gene (Figure 2).

We also found the consensus binding sites for the AP1 (at position −462) and AP2 (at position −156) proteins in the 5′ flanking region of the NQO_1 gene promoter. The AP1 and AP2 proteins have been shown to affect the transcription of many eukaryotic genes in the presence of TPA (12-*O*-tetradecanoylphorbol 13-acetate) (Mitchell & Tjian, 1989).

NQO_1 Gene Is Transcriptionally Activated in Response to TCDD. Nuclear run-on experiments performed using nuclei isolated from TCDD-treated and untreated Hep-G2 cells demonstrated that TCDD treatment increases the rate of transcription of NQO_1 gene by 3-fold (Figure 3). In the same experiment the human CYP1A1 gene transcription was found elevated 10-fold due to TCDD treatment as expected from previous studies (Cresteil et al., 1987). The low magnitude of the increase in transcription of the NQO_1 gene may be due to the high basal level of transcription of the NQO_1 gene in Hep-G2 cells (Figure 3).

Basal Expression and TCDD Induction of Human NQO_1 Gene Are Expected To Be Localized within a 415 bp Fragment of the DNA in the Upstream Region of the NQO_1 Gene. The NQO_1–CAT recombinant plasmids ($pNQO_1$CAT1.85, −1.55, −1.34, −0.837, −0.786, and −0.13) were transiently transfected into the human Hep-G2 and mouse Hepa-1 cells, and the CAT gene expression under the control of different lengths of promoter (as represented by numbers in kilobases) was monitored in the presence and absence of TCDD. As shown in Figure 4, the mouse Hepa-1 cells transfected with plasmid $pNQO_1$CAT1.85 expressed the CAT gene at high level, which increased in a dose-dependent fashion upon treatment with different concentrations of TCDD. In a related experiment our measurements showed that when we used plasmid $pNQO_1$CAT1.85 for transfection into Hep-G2 and Hepa-1 cells, the basal CAT activity was 31- and 111-fold higher compared to the promoterless plasmid pBLCAT3 (Figure 5). Treatment of the transfected Hep-G2 and Hepa-1 cells with 100 nM TCDD showed an increase of approximately 4- and 3-fold, respectively, in the expression of the CAT gene (Figure 5). An increase in the concentration of TCDD of more than 100 nM for treatment did not result in a further increase in fold induction of CAT activity in either cell type (Data not shown). Similar levels of basal and induced expression of CAT

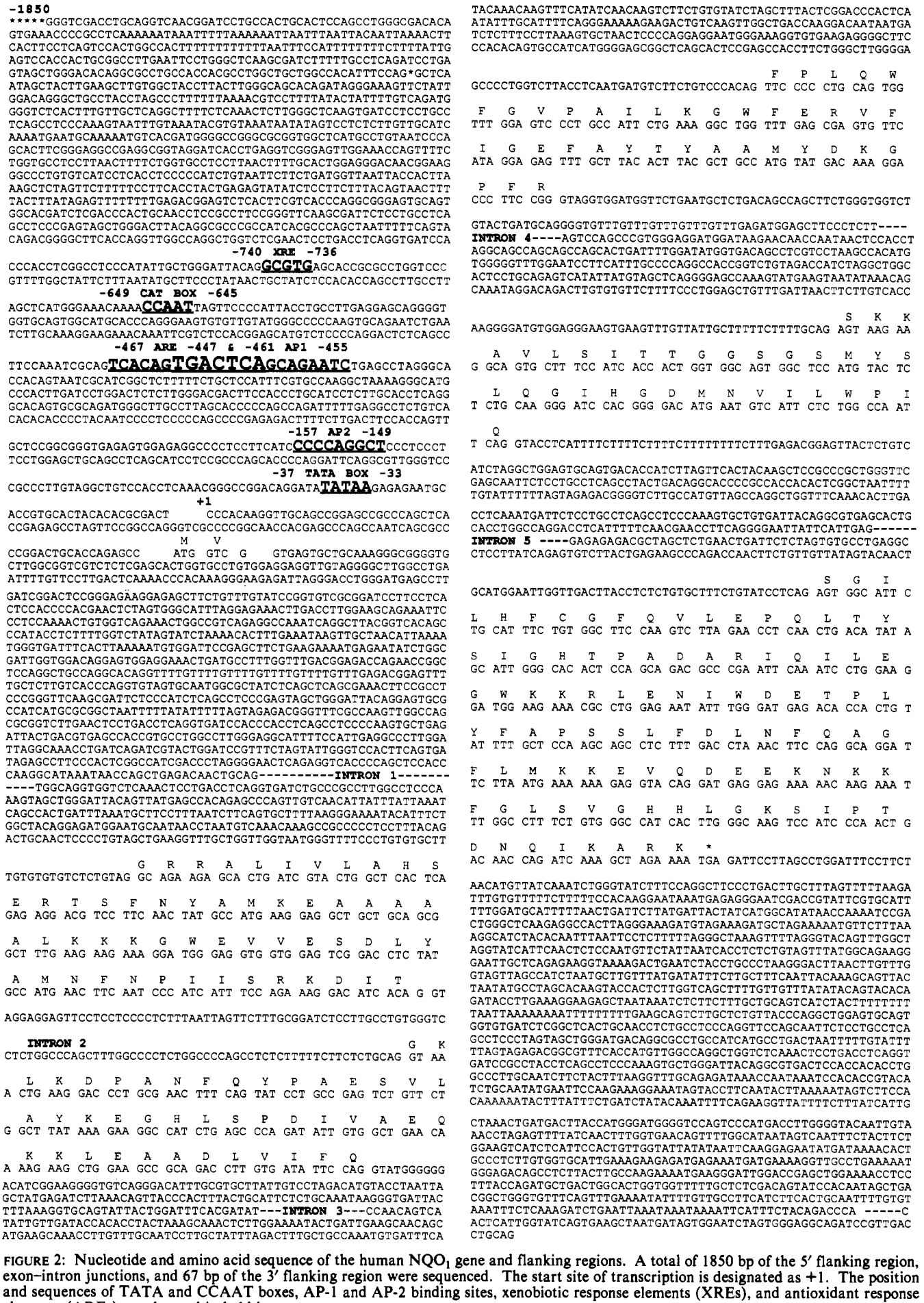


FIGURE 2: Nucleotide and amino acid sequence of the human NQO_1 gene and flanking regions. A total of 1850 bp of the 5′ flanking region, exon–intron junctions, and 67 bp of the 3′ flanking region were sequenced. The start site of transcription is designated as +1. The position and sequences of TATA and CCAAT boxes, AP-1 and AP-2 binding sites, xenobiotic response elements (XREs), and antioxidant response elements (AREs) are denoted in bold letters.

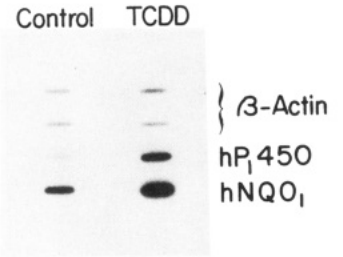


FIGURE 3: Nuclear transcription run-on experiment of the NQO_1 and hP_1450 (human CYP1A1) genes in TCDD-treated and untreated human hepatoblastoma (Hep-G2) cells. RNA transcripts from TCDD-treated and untreated Hep-G2 nuclei hybridizing to B-actin, hP_1450 (CYP1A1), and human NQO_1 cDNA inserts are shown. The hP_1450 and $hNQO_1$ cDNA inserts determine the rate of transcription of human CYP1A1 and NQO_1 genes, respectively. B-Actin gene transcription in Hep-G2 cells is unaffected by TCDD treatment.

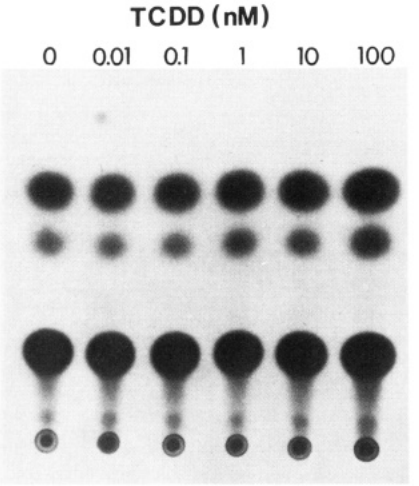
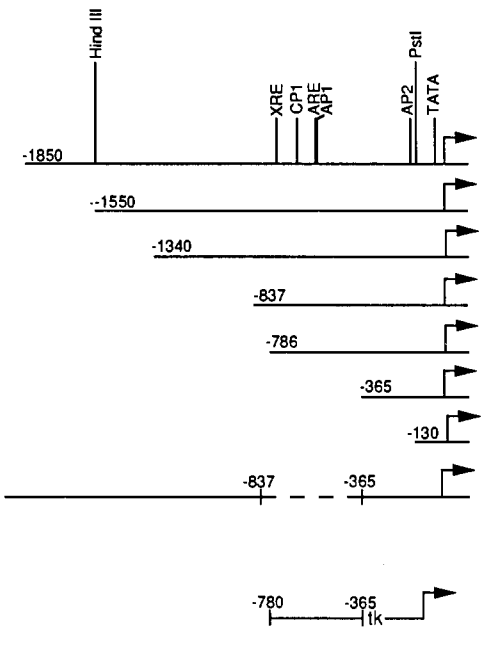


FIGURE 4: Transient expression and TCDD induction of $pNQO_1CAT1.85$ recombinant plasmid into mouse hepatoma (Hepa-1) cells. Ten micrograms of the $pNQO_1CAT1.85$ recombinant plasmid containing 1.85 kb of the 5′ flanking region of the human NQO_1 gene promoter was transiently transfected into mouse Hepa-1 cells by the calcium phosphate procedure. The CAT gene expression was monitored by measuring the CAT activity. RSV–βGal plasmid was used in each case to normalize the transfection efficiency. The transfected cells were treated with different concentrations of TCDD as shown on the top of each lane for 24 h.

gene were also observed when plasmids $pNQO_1CAT1.55$, −1.34, −0.837, and −0.786 were used for transfection (Figure 5). However, highly significant decrease in the basal levels of CAT gene expression and no induction upon treatment with TCDD were observed with plasmids $pNQO_1CAT0.0365$ and −0.13 (Figure 5). These results might indicate that the DNA segment between −0.837 and −0.365 kb of the NQO_1 gene promoter is essential for high basal expression of CAT gene and its induction by TCDD. To confirm this, we used the plasmid $pNQO_1CAT\Delta0.837–0.365$ (containing an internal deletion of the region between −0.837 and −0.365 from the initial 1.85-kb plasmid of the NQO_1 gene promoter) for transfection into Hep-G2 and Hepa-1 cells. As expected, this plasmid expressed very low levels of CAT activity which was not inducible by TCDD (Figure 5), indicating the need for the deleted fragment (−0.780 to −0.365) for high basal expression and its induction in response to TCDD.

The *Sau*3A fragment of DNA (region between −0.780 and −0.365 kb containing the cis-acting DNA elements responsible for high levels of expression of the NQO_1 gene in hepatoma cells and its induction by TCDD) was attached to the basal thymidine kinase promoter (no enhancer) hooked to the CAT gene by cloning in the vector pBLCAT2, a derivative of pBLCAT3, to test if this segment of DNA can confer its effect on the heterologous promoter. This recombinant plasmid $pNQO_1tkCAT0.780–0.365$ upon transfection into Hep-G2 and Hepa-1 cells showed 10- and 57-fold higher levels of CAT activity, respectively, compared to the control plasmid pBLCAT2 which increased approximately 5-fold in case of both cell lines upon treatment with 100 nM TCDD (Figure 5), clearly establishing that the 415 bp *Sau*3A fragment indeed contains cis-acting DNA elements responsible for high levels of expression of NQO_1 gene in hepatoma cells and its induction by TCDD. The nucleotide sequence analysis of this 415 bp fragment of DNA indicated presence of one copy each of ARE and XRE. Because the ARE sequence is highly conserved between human and rat quinone oxidoreductase gene (Figure 6), it is possible that the human ARE, as in the case of rat, mediates the high level of expression of the NQO_1 gene in hepatoma cells, but this remains to be proven by further experiments. Interestingly, the sequence analysis of the human NQO_1 gene showed presence of a consensus binding site for AP1 protein contained within the ARE sequence (Figure 2). Extensive studies have clearly established that the AP1 protein forms homo- and heterodimers with itself and other members of the AP1 family of proteins (e.g., junB, JunD, fos, fra1, etc.) through leucine zipper motifs and plays an important role in transcriptional regulation by creating a variety of transcription factors with different functional properties [reviewed in Mitchell and Tjian (1989)]. Moreover, recently, an AP1 binding site located between nucleotides −58 and −65 in the regulatory region of the glutathione *S*-transferase π gene has been shown to be required for a basal level of expression into several different human cell lines including Hep-G2 (Xia et al., 1991). The presence of a perfect binding site for AP1 protein in human ARE might indicate its role in basal expression of the NQO_1 gene and remains to be investigated by further experiments. It is noteworthy that in the case of the rat ARE the first five bases of the consensus binding site for AP1 protein are conversed.

Involvement of Ah Receptor in the Induction of NQO_1 Gene Expression by TCDD. It is well-known that PAHs and TCDD induce the expression of a group of genes involved in the xenobiotic metabolism [reviewed in Nebert and Gonzalez (1987)]. Among these genes the best studied is the CYP1A1 gene induction by PAHs and TCDD (Nebert & Gonzalez, 1987; Whitlock, 1987; Gonzalez, 1989; Nebert & Jones, 1989). In brief, the TCDD and PAHs bind to the protein product of the Ah locus, the so-called Ah receptor. Following translocation of the inducer–receptor complex into the nucleus it recognizes and binds to xenobiotic/dioxin response elements (XREs) with five invariant bases, GCGTG, in the 5′ flanking region of the CYP1A1 gene, resulting in the transcriptional activation of the gene. Increased mRNA concentrations lead to increased levels of the CYP1A1 protein and consequently to an increased capacity to metabolize benzo[*a*]pyrene and other aromatic hydrocarbons. It should be noted that the induction of expression of CYP1A2 gene by TCDD is presumably not mediated by the Ah receptor because the induction of this gene in the rat is regulated primarily at the posttranscriptional level (Silver & Kraufer, 1988). Also, no XREs have been found in the mouse, rat, and human CYP1A2 genes (Ikeya et al., 1989), further supporting the notion that induction of the CYP1A2 gene is due to post-transcriptional modifications. Because both CYP1A1 and NQO_1 genes are induced by TCDD, it will be interesting to find out if these two genes are coordinately regulated by the same Ah receptor. The small quantity of data available in-



	Hep-G2		Hepa-1	
	Control	TCDD	Control	TCDD
pBLCAT3	1.7±0.1	1.8±o.1	2.4±0.2	2.6±0.4
pNQO1CAT1.85	52.9±3.8	212.4±5.6	267.1±9.9	698.0±18.3
pNQO1CAT1.55	55.8±4.9	265.9±19	195.0±29	960.9±40.6
pNQO1CAT1.34	56.9±3.9	289.6±20	205.2±27	990.6±41.7
pNQO1CAT0.837	49.8±2.9	215.5±6.8	159.6±31	750.8±11.9
pNQO1CAT0.786	47.5±2.2	221.3±14	160.9±6.9	670.5±11.2
pNQO1CAT0.365	5.3±0.3	6.3±0.3	20.4±5.6	28.4±3.2
pNQO1CAT0.130	1.2±0.2	1.5±0.4	8.9±2.3	9.1±0.6
pNQO1CAT Δ0.837-0.365	5.8±0.6	6.7±1.3	20.6±5.7	22.8±2.3
pBLCAT2	4.2±0.5	3.6±1.0	2.1±0.3	1.8±0.2
pNQO1tkCAT 0.780-0.365	40.3±3.9	209.6±13	120.6±12	749.9±23.6

FIGURE 5: Transient expression and TCDD induction of NQO_1–CAT recombinant plasmids in human hepatoblastoma (Hep-G2) and mouse hepatoma (Hepa-1) cells. The NQO_1–CAT chimeric plasmid DNAs from 1.85 to 0.13 contain different lengths of the NQO_1 gene promoter as represented by numbers in kilobase pairs in each construct. The plasmid $pNQO_1CAT\Delta0.837$–0.365 contains an internal deletion of −0.837 to −0.365 kb in the 1.85 kb of initial NQO_1 gene promoter. The *Sau*3A fragment (region between −0.780 and −0.365 kb) of the NQO_1 gene promoter was attached to the tk promoter hooked to the CAT gene to construct the plasmid $pNQO_1tkCAT0.780$–0.365. The pBLCAT3 is a promoterless plasmid, and pBLCAT2 is a derivative of pBLCAT3 containing basal tk promoter. The pBLCAT3 and pBLCAT2 plasmids were used as control plasmids. The various CAT plasmids were transiently transfected into the Hep-G2 and Hepa-1 cells, and their expression in the absence and presence of TCDD (100 nM for 24 h) was monitored by measuring the CAT activity. RSV–βGal plasmid was cotransfected in each case to normalize the transfection efficiency.

Rat ARE	**TCACAGTGACTTGGCAAAATC**
	: : : : : : : : : : : : : : : : : : : :
Human ARE	**TCACAGTGACTCAGCAGAATC**

FIGURE 6: Comparison of the nucleotide sequence of human and rat NAD(P)H:quinone oxidoreductase AREs. The AREs found in human and rat NAD(P)H:quinone oxidoreductase gene have been aligned to show the sequence homology. The sequence for rat ARE has been taken from recently published work of Favreau and Pickett (1991). The human ARE was identified by nucleotide sequence analysis of the human NQO_1 gene promoter. (:) denotes that the bases are identical.

dicates conflicting views on the involvement of Ah receptor in the induction of NQO_1 gene by PAHs and TCDD. It has been suggested (Kumaki et al., 1977) primarily on genetic grounds that the same Ah receptor mediated process involved in the CYP1A1 gene activation might be responsible for enhancing NQO_1 activity. However, an obligatory association of the induction of NQO_1 and CYP1A1 gene has not been established. More recently, an alternate mechanism has been suggested that first involves the metabolism of PAHs by increased CYP1A1 activity to electrophilic products which in turn provide a redox signal for increased expression of the NQO_1 gene (De Long et al., 1987). In this case the Ah receptor would be indirectly involved in the induction of NQO_1 gene expression by PAHs by interacting with sequences in the 5′ flanking region of CYP1A1 gene rather than the NQO_1 gene. Recently, a similar type of mechanism mediating through an antioxidant response element (ARE)/electrophile response element (EpRE) has been reported for the induction of expression of the ya subunit gene of glutathione *S*-transferase and the rat quinone reductase gene in response to polycyclic aromatic hydrocarbons and phenolic antioxidants (Rushmore et al., 1990; Rushmore & Pickett, 1990; Friling et al., 1990). However, Favreau and Pickett (1991) suggested an XRE-mediated mechanism of induction for the rat quinone reductase gene by TCDD. We, working on the regulation of human NQO_1 gene, have identified a 415 bp fragment of DNA (the region between −0.780 and −0.365) in the 5′ flanking region as responsible for induction of the NQO_1 gene expression in response to TCDD. The nucleotide sequence analysis of this fragment of DNA indicated presence of an XRE sequence at position −740 which is 5′ to the human ARE (Figure 2) compared to its 3′ location with respect to ARE in the rat quinone reductase gene (Favreau & Pickett, 1991). It remains to be investigated if this XRE sequence binds to Ah receptor and mediates induction of the NQO_1 gene expression by TCDD.

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