The Human CYP1A2 Gene and Induction by 3-Methylcholanthrene

A REGION OF DNA THAT SUPPORTS AH-RECEPTOR BINDING AND PROMOTER-SPECIFIC INDUCTION*

(Received for publication, August 3, 1993, and in revised form, November 12, 1993)

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The gene for cytochrome P4501A2 is constitutively expressed in the liver of vertebrates and shows induced expression when an organism is exposed to polycyclic aromatic hydrocarbons and halogenated hydrocarbons. To identify DNA elements regulating transcription of the human CYP1A2 gene, transient transfection experiments were conducted in the human hepatoma cell line HepG2. Dissection of the 5'-flanking portion of the CYP1A2 gene identified two regions that contributed to the overall induction by 3-methylcholanthrene. One region located at −2259/−1987 contains an xenobiotic-responsive element-like sequence, termed X1, that binds a nuclear 2,3,7,8-tetrachloro dibenzo-p-dioxin-inducible protein in HepG2 and wild type mouse Hepa-1 cells, but not in the Ah receptor nuclear translocation defective mouse C− mutant e1 cells. In addition, deletion of this region of the CYP1A2 gene reduces the 3-methylcholanthrene (3-MC)-initiated induction of chloramphenicol acetyltransferase activity in both promoter- and enhancer-specific constructs. The second responsive region is located at −2259/−1987. This region of the gene contains a second xenobiotic-responsive element-like element, but this element does not associate with the nuclear Ah receptor. However, there does exist several potential AP1 binding sites and a conserved TATA box. A DNA fragment from −2259/−1970 that contains these elements was shown to function as an efficient eukaryotic promoter, in addition to supporting 3-MC-induced promoter activity. These results suggest that Ah receptor-specific and promoter-specific elements regulate the expression of the human CYP1A2 gene.

Cytochrome P450 represents a multigene family of hemoproteins that catalyze the metabolism of foreign compounds, as well as endogenous compounds, such as steroids, fatty acids, prostaglandins, and vitamins (1). The expression of many forms of cytochrome P450 is controlled by various external stimuli, such as drugs and chemicals. The CYP1 (2) genes, CYP1A1 and CYP1A2, represent a subfamily of cytochrome P450s that are induced in response to environmental pollutants, such as halogenated aromatic hydrocarbons (HAH)1 and polycyclic aromatic hydrocarbons (PAH) (3). The induction of cytochrome P4501A1 in mice and rats has been shown to be at the level of transcriptional regulation (4-6). The mechanism by which PAHs, such as 3-methylcholanthrene (3-MC) and halogenated aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), transcriptionally activate the CYP1A1 gene involves the binding of ligand to the Ah receptor (AhR). The ligand-bound AhR translocates to the nucleus (7-9), where it associates with enhancer elements in the 5'-flanking region of the CYP1A1 gene referred to as dioxin-responsive elements (DREs) or xenobiotic-responsive elements (XREs). These elements are conserved among mice, rat, rabbit, and humans with respect to sequence and location within the CYP1A1 gene (10, 11). Multiple copies of the consensus DRE sequence are found in the 5'-flanking region of the CYP1A1 gene and have been shown to be required for inducer-dependent transcription when used in DNA transfection experiments (10-14).

Alignment of the functional DRE sequences from the mouse CYP1A1 gene has resulted in the identification of a consensus DRE having the invariant core sequence of T-GCGTG, flanked by several conserved nucleotides (12, 13). These nucleotides important for AhR binding have also been defined through detailed studies using mutant DRE oligomers in gel mobility shift assays and methylation protection and interference experiments (14-17). In addition to defining the nucleotides required for high-affinity binding, these nucleotides required for function have also been defined (12).

For the most part, the molecular mechanisms that control the expression of the CYP1A2 gene are unknown. The regulation of the CYP1A2 gene by TCDD and 3-MC in mice (4, 5) and isolated rat hepatocytes (18) occurs through transcriptional activation. DNA transfection experiments demonstrated that the human CYP1A2 gene contains sequences within 3.2 kb of the 5'-flanking gene that are responsive to 3-MC in human hepatoma cells, suggesting transcriptional activation of this gene (19). DNA sequence analysis, however, has not identified DREs in the flanking sequences of the CYP1A2 gene from any species. Although both CYP1A1 and CYP1A2 are transcriptionally activated by PAHs, they are also controlled by other modes of regulation. For example, they differ in their pattern of expression in that 1A2 is found predominantly in the adult liver, whereas 1A1 is found in the liver, as well as in extrhepatic tissue of all age groups (20, 21). It has been shown recently that an auxiliary protein, ARNT (AhR nuclear translocator), is required for the nuclear translocation of the AhR and for AhR

* This work was supported in part by United States Public Health Service Grant GM36590 (to R. H. T.) and Grant 2300 from the Council For Tobacco Research, USA, Inc. (to L. C. &.I. The costs of publication of this article were defrayed in part by the payment with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: HAH, halogenated aromatic hydrocarbon; PAH, polycyclic aromatic hydrocarbon; 3-MC, 3-methylcholanthrene; AhR, dioxin Ah-receptor; CAT, chloramphenicol acetyltransferase; DRE, dioxin responsive element; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; XRE, xenobiotic-responsive element; kb, kilobase pairs.
binding to DREs (22, 23). It is plausible, based on the differences in regulatory patterns between 1A1 and 1A2, that other trans-activating factors (e.g. other ARNT-like proteins) act in concert with the AhR in mediating the induced expression of the CYP1A2 gene (24). Such interactions may result in an alternate binding site for the AhR at a responsive element. The present study was designed to localize the responsive element in the human CYP1A2 promoter and determine if related DRE sequences bind the AhR and if they are required for inducer-dependent transcriptional activation of the human CYP1A2 gene. The results of these experiments implicate the AhR as well as AhR independent mechanisms underlying the induction of the CYP1A2 gene.

MATERIALS AND METHODS

Plasmid Constructions—To obtain 5'-progressive deletions of the human CYP1A2 gene (19), sequences from −3201 to +53 (relative to the start of transcription), containing the promoter, exon one, and 5'-flanking sequences, were removed from a genomic clone with a KpnI restriction digest. The KpnI sites were made blunt ended and cloned into the EcoRV site of the plasmid pBSCAT, generating the plasmid p1A2CAT. Deletions were generated using exonuclease III and mung bean nuclelease digestion (Stratagene, San Diego, CA). The plasmid was first linearized at the KpnI site in the vector and then digested with XhoI to create a 5'-overhang for directional exonuclease III digestion. Following incubation with exonuclease III for several periods of time, mung bean nuclease was used to repair the ends and the plasmid was re-ligated. This technique allowed for the generation of deletion clones without having to subclone deleted sequences. Deletion end points were determined by double-stranded DNA sequencing (25).

To generate fragments to test for enhancer activity, the DNA containing the 5-MC-responsive element was removed from the human CYP1A2 gene by a KpnI (−3201) and PstI (−1595) restriction digest. This fragment was made blunt ended and cloned into the EcoRV site of pSVCAT-BS and is called p1A2SVCAT. This vector contains the SV40 enhancerless promoter driving the expression of the CAT gene. 5'-progressive deletions of this responsive element were generated as described above. Additional deletions were generated using the polymerase chain reaction.

Cell Culture, DNA Transfection, and CAT Assay—The human hepatoma cell line, HepG2, was obtained from ATCC, and the mouse wild type hepatoma cell Hepa-1c1c7 and the AhR nuclear translocation defective cell Hepa-1c1c7II were provided by Dr. Whitlock (Stanford University). All cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. The DNA transfections were performed by the calcium phosphate precipitation technique (27). Cells were transfected with 10 μg of plasmid DNA and in cotransfected experiments with 0.1 μg of pSV2LUC (28) as an internal control for transfection efficiency. Twenty-four hours after transfection, cells were treated with 10 μM 3-MC or 0.1% dimethyl sulfoxide, and cells were harvested 16–18 h after treatment.

The CAT assays were performed by thin layer chromatography as described by Gorman et al. (29). The quantitation was done by cutting the chromatogram corresponding to the acetylated and nonacetylated [14C]chloramphenicol and counting by liquid scintillation. CAT activity is expressed as the percentage of conversion calculated as the ratio of the acetylated form to the total. The -fold induction refers to the ratio of CAT activity of induced cells to the uninduced cells. Protein concentrations were measured by the method of Bradford (30).

Isolation of Nucleolar and Cytoplasmic Protein Extracts and Gel Mobility Shift Assays—Nuclear extracts were prepared from control and TCDD-treated HepG2, mouse Hepa 1c1c7 (wild type), and Class II variant cells as described by Denison (31). Cells were treated with 10 μM TCDD for 2 h prior to isolation of nuclear extract. Mouse Hepa cell cytosol was prepared in ice-cold EGPM buffer (10 mM EDTA, 20 mM potassium phosphate, 10% (w/v) glycerol, and 2 mM 2-mercaptoethanol, pH 7.2) as described by Wilhelmsson (32). For gel mobility shift assays, 10 μg of nuclear extract or 20 μg of activated cytosol were used. Cytosol was activated by the addition of 10 μM TCDD for 4 h at 28 °C. Gel mobility shift assays were performed as described by Denison (31), with the exception that gel electrophoresis was conducted using 1 X TBE buffer (0.089 M Tris borate, 0.089 M boric acid, 0.002 M EDTA). The DNA probes, double-stranded oligonucleotides, were labeled with T4 poly-nucleotide kinase and [γ-32P]ATP. Sequences of probes are as follows: mouse DRE3, 5' GAGCTCGAGGTGCTGAGGAGG 3'; human 1A2, X1, 5' GAGACCTGACCCACCATCTCTTA 3'; human 1A2, X2, 5' CATTAGTAAAAGCGAGGGAATCT 3'. The competitor for competition studies was a fragment of DNA from the SV40 promoter or the human α-antitrypsin gene.

RESULTS

Identification of cis-Acting Regulatory Elements in the Human CYP1A2 Promoter and 5'-Flanking Sequences—In previous work (19), we reported that there existed cis-acting elements within approximately 3 kb of the human CYP1A2 gene that were responsible for 3-MC-induced transcription of heterologous gene promoters. To further characterize these sequences, the plasmid, p1A2CAT, containing the human CYP1A2 promoter and 3.2 kb of 5'-flanking sequences was used in transient transfection experiments in HepG2 cells to determine the dose-dependent induction by 3-MC. As shown in Fig. 1, increasing concentrations of 3-MC resulted in increased expression of CAT. A concentration of 10 μM resulted in approximately 8-fold induction of CAT expression, and this concentration was used in subsequent experiments. CAT constructs containing the CYP1A2 promoter and flanking sequences in an orientation opposite to the transcription of the reporter gene did not show CAT activity above background in transfected control and treated cells, providing further evidence that the observed induced CAT activity was directed by trans-activation of CYP1A2 sequences.

To identify the region responsible for the 3-MC-inducible expression of the human CYP1A2 gene, the 5'-flanking region of the gene was dissected by progressively deleting sequences in the 5' to 3' direction of the p1A2CAT plasmid. The ability of these sequences to drive the expression of CAT was determined by transient transfection experiments in HepG2 cells. Progressive 5'-deletions resulted in a decrease of induced CAT activity (Fig. 2A). Deletions to −2195 kb (relative to the CYP1A2 gene transcriptional start site) resulted in an approximately 40–50% decrease in induced CAT expression, whereas further deletions to −1716 kb completely abolished both constitutive and induced expression. These results confirmed our earlier observation that a 3-MC-inducible element was contained between −3.2 and −1.6 kb (19).

Identification of the 3-MC-Responsive Element in the 5'-Flanking Human CYP1A2 Gene—To search for responsive elements, a fragment of the human CYP1A2 gene from −3201 to
\(-3201\) (\(\text{PstI/KpnI}\)), encompassing the region determined from deletional analysis of the promoter to be 3-MC-responsive, was cloned into an enhancer vector in which the CAT gene is under the control of the SV40 enhancerless promoter (Fig. 2B). Having shown that the region from \(-3201\) to \(-1595\) was responsive to 3-MC, we generated both 5\(^{-}\)-to 3\(^{-}\)- and 3\(^{-}\)-to 5\(^{-}\)-progressive deletions. As shown in Fig. 2B, 5\(^{-}\)-deletions to \(-2532\) did not change the induced response, but deletions to \(-2423\) resulted in approximately a 40\% decrease in inducible CAT activity. Results of these deletional clones are consistent with the deletional data from promoter constructs (Fig. 2A) that showed a similar decrease in induced CAT activity at about 2.2 \(k\). A further decrease in sequences to \(-1987\) completely eliminated induction of CAT activity by 3-MC. These data establish the importance of sequences that lie within the region between \(-2532\) and \(-1987\) and suggest that two domains may be involved in 3-MC-mediated CAT activity.

To determine if sequences within the 3\(^{-}\)-end of the responsive element are also required for inducer activity, deletions were generated from the 3\(^{-}\)-end, keeping the 5\(^{-}\)-region constant. Results of these deletions are also shown in Fig. 2B. Deletion of the 3\(^{-}\)-end to \(-1762\) had no effect on the inducible CAT activity. Deletions to \(-2259\) resulted in approximately 60\% decrease, whereas further deletions to \(-2847\) completely abolished activity. These deletions are consistent with the promoter and other 5\(^{-}\)-enhancer deletions, demonstrating that two regions appear to be required for full inducer activity. One region encompasses the sequences defined by the end points \(-2532\) to \(-2423\) and the other region by the end points defined from the promoter construct \(-2195\) (Fig. 2A) to \(-1987\) which is defined by the enhancer construct (Fig. 2B).

The 3\(^{-}\)-region was further characterized by generating several additional DNA fragments by polymerase chain reaction and cloning these fragments into the SV40CAT plasmids. As shown in Fig. 2B, a DNA fragment from bases \(-2752\) to \(-1987\) exhibits a 5.6-fold increase in CAT activity following treatment with 3-MC, whereas a fragment from \(-2752\) to \(-2259\) exhibits only a 1.9-fold increase. When we generated a fragment from \(-2546\) to \(-2095\), a consistent 2.0-fold increase in CAT activity was observed. Since inducible CAT activity drops from nearly 6-fold to 2-fold by eliminating bases from \(-2095\) to \(-1987\), these data indicate that DNA within this region (\(-2095\) to \(-1987\)) is important for 3-MC-inducible activity.

When the DNA sequence was analyzed from \(-2546\) to \(-1987\), two XRE-like sequences were identified in this region. These sequences, referred to as X1 and X2, are shown in comparison to the mouse DRE\(_3\) (12) and human XRE1 (10) sequences and the consensus XRE (Fig. 3). The X1 sequence is 84\% similar to the consensus XRE and the sequence X2 is 79\% similar. In addition, X1 is 75\% similar to the functional consensus (12) and 100\% similar to the consensus binding sequence (17), whereas X2 is 88 and 71\% similar, respectively.

**Gel Mobility Shift Assays of XRE-like Sequences**—The fact that both XRE-like sequences appear in the 3-MC-responsive element suggested that X1 and X2 may be recognized by the AhR and that both sequences may be required for inducer-mediated activation of CAT. To determine if either of the XRE-like sequences were able to serve as a target sequence for the AhR, gel mobility shift assays were employed. Initial experiments were conducted to determine if these two XRE-like se-

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**FIG. 2. Analysis of deletion mutants.** Shown in A is the p1A2CAT gene construct and several 5\(^{-}\)-deletions. These clones were used in transient transfection experiments. CAT assays were performed and the -fold induction indicated on the right. The -fold induction refers to the ratio of CAT activity of induced cells to uninduced cells. The control pCATBS vector showed no induction of CAT in the presence of inducer. B, various regions of the CYP1A2 gene were examined for enhancer activity. A series of plasmids containing the 3-MC-responsive element (-3201/-1595) in various lengths were generated and used in transient transfection experiments. Values are the average of three to four independent experiments performed in duplicate.

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**A)**

- -3201
- -2195
- -1762
- -1415
- -1079
- -225

**B)**

- -3201
- -2532
- -2423
- -1987
- -2259
- -2546
- -2095

| Fold Induction | SV40 CAT 4.9 ± 0.2 | SV40 CAT 4.0 ± 0.2 | SV40 CAT 2.5 ± 0.2 | SV40 CAT 1.9 ± 0.1 | SV40 CAT 2.0 ± 0.2 |
|----------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| N.D.           | SV40 CAT 4.9 ± 0.2 | SV40 CAT 4.0 ± 0.2 | SV40 CAT 2.5 ± 0.2 | SV40 CAT 1.9 ± 0.1 | SV40 CAT 2.0 ± 0.2 |
| N.D.           | SV40 CAT 4.0 ± 0.2 | SV40 CAT 2.5 ± 0.2 | SV40 CAT 1.9 ± 0.1 | SV40 CAT 2.0 ± 0.2 |
| N.D.           | SV40 CAT 2.5 ± 0.2 | SV40 CAT 1.9 ± 0.1 | SV40 CAT 2.0 ± 0.2 |
| N.D.           | SV40 CAT 1.9 ± 0.1 | SV40 CAT 2.0 ± 0.2 | SV40 CAT 1.9 ± 0.1 |
| N.D.           | SV40 CAT 2.0 ± 0.2 | SV40 CAT 1.9 ± 0.1 | SV40 CAT 2.0 ± 0.2 |

**Notes:**

- SV40 CAT 4.9 ± 0.2 refers to the ratio of CAT activity between induced and uninduced cells.
- SV40 CAT 4.0 ± 0.2
- SV40 CAT 2.5 ± 0.2
- SV40 CAT 1.9 ± 0.1
- SV40 CAT 2.0 ± 0.2

**Results:**

- SV40 CAT 4.9 ± 0.2
- SV40 CAT 4.0 ± 0.2
- SV40 CAT 2.5 ± 0.2
- SV40 CAT 1.9 ± 0.1
- SV40 CAT 2.0 ± 0.2

**Conclusions:**

- SV40 CAT 4.9 ± 0.2
- SV40 CAT 4.0 ± 0.2
- SV40 CAT 2.5 ± 0.2
- SV40 CAT 1.9 ± 0.1
- SV40 CAT 2.0 ± 0.2

**Figure 2A:**

- **A)**
  - X1, X2, TATA1
  - N.D.
  - SV40 CAT 4.9 ± 0.2
  - SV40 CAT 4.0 ± 0.2
  - SV40 CAT 2.5 ± 0.2
  - SV40 CAT 1.9 ± 0.1
  - SV40 CAT 2.0 ± 0.2

- **B)**
  - SV40 CAT 4.9 ± 0.2
  - SV40 CAT 4.0 ± 0.2
  - SV40 CAT 2.5 ± 0.2
  - SV40 CAT 1.9 ± 0.1
  - SV40 CAT 2.0 ± 0.2

**Figure 2B:**

- **A)**
  - X1, X2, TATA1
  - N.D.
  - SV40 CAT 4.9 ± 0.2
  - SV40 CAT 4.0 ± 0.2
  - SV40 CAT 2.5 ± 0.2
  - SV40 CAT 1.9 ± 0.1
  - SV40 CAT 2.0 ± 0.2

- **B)**
  - SV40 CAT 4.9 ± 0.2
  - SV40 CAT 4.0 ± 0.2
  - SV40 CAT 2.5 ± 0.2
  - SV40 CAT 1.9 ± 0.1
  - SV40 CAT 2.0 ± 0.2
sequences could compete for binding to the mouse DRE3. Using HepG2 nuclear extracts from control and TCDD-treated cells, it is demonstrated that only the X1 sequence competed for AhR binding (Fig. 4). An SV40 promoter fragment, used as a non-specific competitor, did not reduce binding of the inducible band to the DRE3 sequence. To show directly that this X1 sequence binds the AhR, gel mobility shift assays were performed using nuclear extracts prepared from wild type mouse Hepa-1c17 cells in addition to the Class II variant cells. Cells were treated for 2 h with 10 nM TCDD as described under "Materials and Methods." As shown in Fig. 5, a TCDD-inducible band formed with the X1 sequence. The X1-inducible band was specific in that it was eliminated by the presence of cold DRE in the reaction, but not by a nonspecific DNA, which lacks a DRE consensus sequence. In addition, in *vitro* transformation of the cytosolic AhR, followed by gel shift analysis, resulted in specific binding of the activated receptor to the X1 sequence (Fig. 6). Differences in the intensities of the TCDD-inducible bands between the DRE and X1 binding probably result from a lower affinity of the AhR for the X1 sequence, implying that nucleotides other than those described by Denison (17) contribute to tight binding. However, the presence of specific AhR binding at the X1 sequence is consistent with the functional response observed with the fragment of DNA that contains this element.

**Location of a Second TATA Box**—While the sequence spanning the X1 region is important for promoter and enhancer activity, DNA sequence between –2095 and –1987 also contributes to 3-MC induction. The DNA sequence from the X1 region to –1970 is shown in Fig. 7. The sequence from –2221 to –1970 shows several regions of DNA that have potential to associate with known transcriptional factors. There exists two potential AP1 binding sites which are characterized by the consensus sequence STGACTMA (33), a half-binding site for the liver-specific protein HNF1 (34) and a conserved TATAAA box (35). Interestingly, a DNA sequence search of GenBank to determine the fidelity of eukaryotic promoters identified the sequences within this region as a probable promoter. To examine the hypothesis that the second TATAAA sequence, identified as TATA2, can serve as an efficient promoter, an EcoRV/HaeIII digest was performed on the –3201 promoter CAT construct and the DNA from bases –2259 to –1970 cloned into the promoterless CAT construct (Fig. 7B). This DNA construct contains a fragment of DNA with the X1 sequence, the potential AP1 sites, and the TATA2 sequence. When this DNA was transfected into HepG2 cells, CAT activity was detected, which indicates that the DNA is able to support promoter activity. In addition, when the cells were treated with 3-MC, nearly a 4-fold increase in CAT activity was observed. However, when this element was placed in the opposite orientation, there was no constitutive or 3-MC-inducible CAT activity. This result indicates that this fragment supports promoter activity in an orientation-specific fashion and that other regions of the DNA enhance the promoter activity following treatment with 3-MC.
Double-stranded oligonucleotides for the mouse DRE and human and Methods. Following activation of the cytosol, 5'-end-labeled process of mouse DRE and the nonspecific DNA, α-antitrypsin the TCDD-induced protein-DNA complex. incubated prior to addition of probe. The results indicate the position of the TCDD-induced protein-DNA complex.

From -2546 to -2095 (Fig. 2B), which does not contain the TATA2 element, was cloned into the promoterless CAT construct, no expression or inducible activity was observed. This result suggests that the TATA2 sequence, in addition to other DNA sequences such as the AP1 sites, may play an important role in supporting inducible CAT activity.

DISCUSSION

The in vivo regulation of the mouse CyplA2 gene is controlled through transcriptional gene activation (5). Genetic studies, in which 1A2 expression cosegregates with the AhR, implicates the AhR as the mediator for the induced expression of 1A2 (36). Primary rat hepatocytes have been used to show by nuclear run-on studies that the rat CyplA2 gene is also transcriptionally activated by PAHs (18). Taken together, these data strongly suggest that the AhR binds sequences in the flanking gene to activate transcription.

Transient transfection studies in transformed human cell lines helped identify a region in the 5'-flanking gene of the CyplA2 gene that was responsive to 3-MC in the human hemato-ma cell line, HepG2, but not in other non-hepatic cell lines (19). The present study was designed to localize the sequences responsible for the induced response and to determine if they bind the AhR. Deletional analyses of CyplA2 promoter-CAT constructs and SV40 promoter-CAT constructs identified two regions in the 5'-flanking gene that appear to be required for full inducer activity. One region contained an XRE-like sequence (X1) that was able to bind a specific TCDD-inducible complex in receptor-competent cells, but not in receptor defective cells. Binding could be displaced by the DRE sequence, confirming that the inducible protein associated with X1 was the AhR. It is important to appreciate that binding of the AhR to X1 was much weaker than that observed for binding to the DRE element, which correlated with weak enhancer activity of DNA that contained the X1 sequence. However, when the X1 sequence was eliminated, promoter- and enhancer-specific transcriptional activation dropped up to 50%, indicating that the region of DNA containing X1 was important for the maximal 3-MC-induced activity of the CyplA2 gene.

A second region of DNA, which contained another XRE-like sequence that we have called X2, did not bind the receptor as determined by gel mobility shift assays. However, when DNA fragments were prepared that resulted in removal of the X1 sequence but not the X2 sequence, 3-MC was still able to initiate a 3-fold induction of CAT activity. DNA fragments that encompassed a region from -2259 to -1987 served as efficient enhancers in either orientation when directing 3-MC-induced transcription of the heterologous SV40 promoter CAT plasmids, demonstrating there existed cis-acting elements within this region that supported induction by PAHs. Interestingly, the X2 sequence, which resembles the known DRE sequence, does not bind the AhR and most likely plays little role in this induction process. However, there are two stretches of DNA that are similar to the consensus AP-1 or 12-O-tetradecanoyl-phorbol-13-acetate-responsive elements. These potential AP1 elements could play a significant role in CyplA2 induction by PAHs, since it has recently been demonstrated that an AP1 binding site is crucial for the β-naphthoflavone-induced transcriptional activation of the human NAD(P)H:quinone oxidoreductase gene (37). In addition, when we constructed by polymerase chain reaction a fragment of DNA that contained the X1 and X2 sequence, but not the 3' AP1 site (-2546 to -2095), a significant reduction in inducible CAT activity was observed. Since this suggests a complete assortment of proteins from the Jun and Fos family bind AP1 sites (38) and TCDD actually induces binding to AP1 sites, (39) it is very likely that the AP1 sites on this fragment of DNA contribute to the 3-MC-induced transcriptional activation that is observed.

During the course of these experiments, it became apparent that a second TATA box existed within the responsive region. When DNA containing the AP1 sites and the TATA box were cloned upstream of the promoterless CAT gene, the plasmid supported constitutive and 3-MC-induced CAT activity. It is unclear what role the second TATA box plays in the expression and inducibility of the human CyplA2 gene, but it is conceivable that along with the other regulatory elements located in the same region of the gene, this second TATA box participates in transcriptional control of the gene.

Combined, these results suggest that induction of the CyplA2 gene is controlled by a number of different regulatory factors. The X1-AhR binding region is important for the overall expression of the gene, but removal of this portion of DNA does not completely eliminate the induction response. Although the DRE elements that flank the CyplA1 gene serve as enhancer sequences and are responsible for most of the PAH- and TCDD-initiated transcriptional activation, additional regulatory elements appear to support PAH induced transcriptional activation of the CyplA2 gene. Similar results have been observed in both the NAD(P)H:oxidoreductase gene (37, 40) and the glutathione S-transferase Ya subunit gene (41-43), which contain both the AhR-specific XRE elements (12, 44) as well as the antioxidant-responsive elements that are encoded by the sequence 5'-ggTGACaaaGC-3' (42, 43). Since the antioxidant-responsive element reveals sequence similarity to the motifs that are recognized by the Jun and Fos family of proteins, experiments have been conducted to examine the contribution of these proteins toward gene regulation. These results indicate that these proteins are involved in the induction of the NAD(P)H:quinone oxidoreductase gene (37), but most likely do not participate in the induction of the glutathione S-transferase Ya.
subunit gene. Although there exists an antioxidant-responsive element at -1556/-1545 of the CYP1A2 gene, this region of the DNA is not involved in the 3-MC-initiated induction of the gene. Although an antioxidant-responsive element does not exist in the responsive region of the CYP1A2 gene, AP1-binding proteins may participate in the induction process.

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