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Control of cytochrome P_1-450 gene expression: Analysis of a dioxin-responsive enhancer system

(2,3,7,8-tetrachlorodibenzo-*p*-dioxin/glucocorticoid hormones/gene transcription/transfection/enzyme induction)

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ABSTRACT **We analyzed the function and sequence of a dioxin-responsive genomic element flanking the 5′ end of the cytochrome P_1-450 gene in high-activity variant mouse hepatoma cells. The element can regulate the mouse mammary tumor virus promoter. The element retains responsiveness to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) when the distance, the 5′ or 3′ position, and/or the 5′ or 3′ orientation with respect to the promoter are varied. The function of the element requires TCDD–receptor complexes. The element remains responsive to TCDD when transfected into cells from either a heterologous mouse tissue or a heterologous species (human). The DNA element and TCDD receptors together constitute a dioxin-responsive enhancer system.**

The cytochromes P-450 oxygenate lipophilic substrates and thereby contribute to many biological processes, ranging from steroid biosynthesis to chemical carcinogenesis. Several cytochromes P-450 are inducible, and enzyme induction often reflects an increase in the rate of cytochrome P-450 gene transcription. Thus, the cytochrome P-450 genes are potentially useful for studying the regulation of transcription in mammalian cells (1). We have shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) stimulates transcription of the cytochrome P_1-450 gene in mouse hepatoma cells (2, 3). The TCDD-induced increase in cytochrome P_1-450 gene transcription requires the binding of TCDD to an intracellular receptor and the accumulation of the inducer–receptor complexes in the nucleus (2, 4). A *cis*-acting genomic control element mediates the action of the TCDD–receptor complex (5). Here, we have analyzed the structure and function of the TCDD-responsive DNA domain from high-activity variant (HAV) mouse hepatoma cells (3, 5). Our findings indicate that this DNA region has the properties of a TCDD-responsive transcriptional enhancer.

MATERIALS AND METHODS

Materials. Materials were obtained as described (5).

Isolation of the TCDD-Responsive DNA Domain. We isolated the 2643-base-pair (bp) *Hin*dIII insert from the plasmid pHAVcat (5) using standard techniques. Deletion analyses (5) indicate that the TCDD-responsive domain is located within the 986-bp *Hin*dIII–*Bgl* II fragment shown as the stippled area in Fig. 1*A*. To isolate this region, we made the ends of the 2643-bp fragment blunt-ended, attached *Bgl* II linkers, digested with *Bgl* II, and isolated the appropriate fragment from an agarose gel. This fragment, designated "dioxin-responsive element," was inserted into other plasmids as described below.

Construction of Recombinant Plasmids. *pMcat5 and p0cat5 plasmids.* We isolated the 1306-bp *Hin*dIII insert from the plasmid Δ94 (6). This fragment contains a functional mouse mammary tumor virus (MMTV) promoter but lacks a glucocorticoid-responsive element (GRE). The fragment was inserted in the correct 5′–3′ orientation into the *Hin*dIII site in the plasmid pSV0cat (7), immediately upstream of the gene encoding bacterial acetyl CoA:chloramphenicol O^3-acetyltransferase (CAT; EC 2.3.1.28). The unique *Xho* I site, located 100 bp 5′-ward of the MMTV promoter, was converted to a *Bgl* II site by digestion with *Xho* I, addition of *Bgl* II linkers, digestion with *Bgl* II, and religation. This plasmid was designated pMcat5 and is shown in Fig. 1*B*. Functionally, it contains the CAT gene under the control of the MMTV promoter; in addition, it contains unique restriction sites (*Bgl* II, 5′-ward of the promoter, and *Bam*HI, 3′-ward of the CAT gene) into which the dioxin-responsive element can be inserted in either orientation. In addition, we constructed a similar plasmid (designated p0cat5) by converting the *Hin*dIII site in pSV0cat to a *Bgl* II site. This plasmid is analogous to pMcat5 except that p0cat5 lacks the insert containing the MMTV promoter.

pMcat3.5. We modified pSV0cat by converting the unique *Nde* I site, located 51 bp 5′-ward of the *Hin*dIII site, to a *Bgl* II site by digestion with *Nde* I, addition of *Bgl* II linkers, digestion with *Bgl* II, and religation. The 1400-bp MMTV long terminal repeat (LTR) was isolated by digestion of the plasmid pCH110 (6) with *Hin*dIII, and the LTR was inserted in the correct 5′–3′ orientation into the *Hin*dIII site in the modified pSV0cat. This plasmid was designated pMcat3. The dioxin-responsive element was inserted in the correct 5′–3′ orientation into the *Bgl* II site of pMcat3, and the recombinant was designated pMcat3.5 (Fig. 1*C*). Functionally, this plasmid contains, in 5′-to-3′ order: the dioxin-responsive element, the MMTV GRE, the MMTV promoter, and the CAT gene. Plasmid pMcat3′ contains an unmodified *Nde* I site; it is functionally identical to pMcat3.

pMcat4.1. We inserted the 1400-bp MMTV LTR in the correct 5′–3′ orientation into the *Hin*dIII site in pSV0cat. We converted the *Xho* I site in the LTR to a *Bgl* II site as described above. We inserted the dioxin-responsive element in the correct 5′–3′ orientation into the *Bgl* II site. The recombinant was designated pMcat4.1 (Fig. 1*D*). Functionally, it contains, in 5′-to-3′ order: the MMTV GRE, the dioxin-responsive element, the MMTV promoter, and the CAT gene.

We isolated recombinants as ampicillin-resistant colonies in *Escherichia coli* HB101. We used two unique restriction sites in the dioxin-responsive element (*Bgl* I at −1923 and *Bam*HI at −1456) to determine the orientation of the insert in the recombinant plasmids. For transfection experiments, large-scale plasmid preparations were made by standard procedures (8).

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Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; CAT, chloramphenicol acetyltransferase; GRE, glucocorticoid-responsive element; LTR, long terminal repeat; MMTV, mouse mammary tumor virus; HAV, high-activity variant; bp, base pair(s).

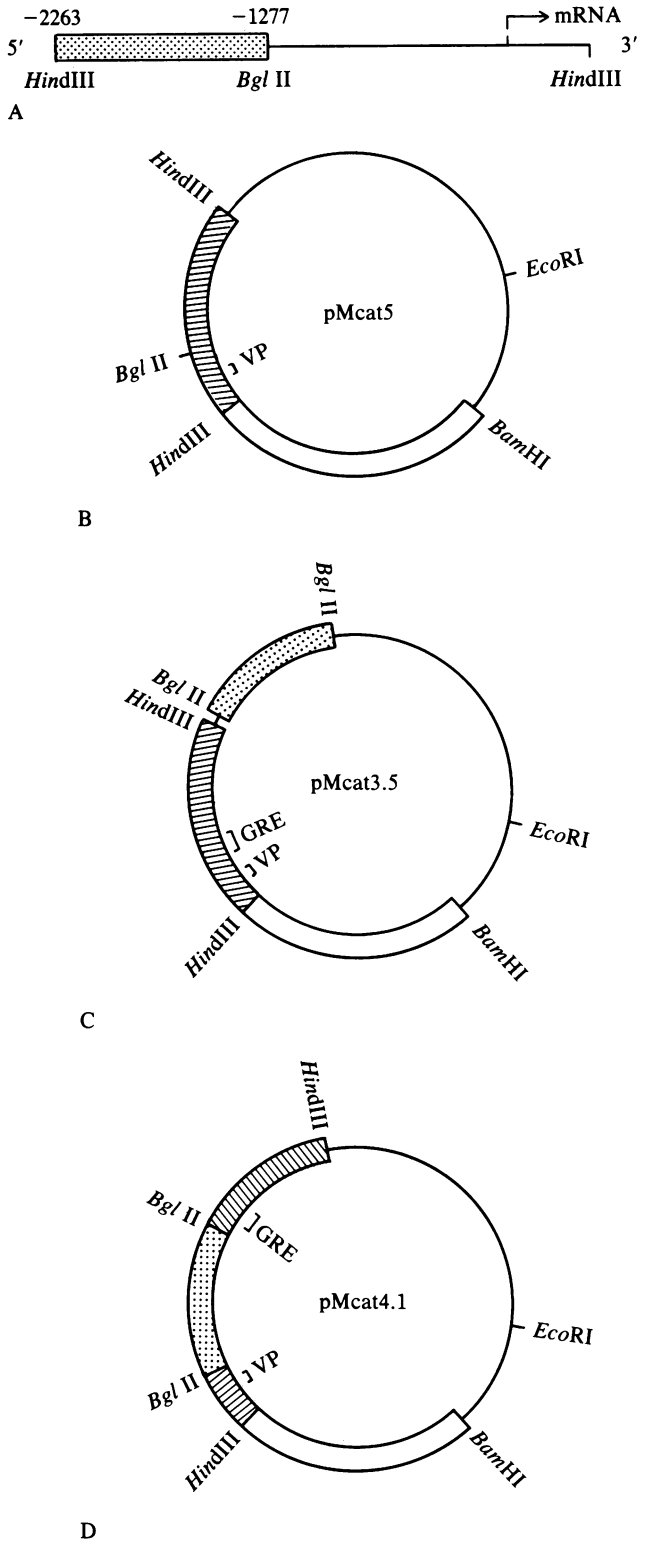

FIG. 1. Functional components of recombinant plasmids. (*A*) The DNA fragment from which the dioxin-responsive element was isolated. The fragment is the 2643-bp *Hin*dIII insert of the plasmid pHAVcat (15). The arrow indicates the start site for cytochrome P_1-450 gene transcription; nucleotides 5′-ward of the start site are denoted by consecutive negative numbers. ⊠, The 984-bp fragment designated as the dioxin-responsive element. (*B*) Schematic diagram of pMcat5. □, CAT gene; ▨, MMTV DNA; VP, viral promoter. The dioxin-responsive element was inserted into either the *Bgl* II site or the *Bam*HI site. (*C*) Schematic diagram of pMcat3.5. □, CAT gene; ▨, MMTV DNA; VP, viral promoter; ⊠, dioxin-responsive element, which is situated in the correct 5′–3′ orientation. (*D*) Schematic diagram of pMcat4.1. The components are identified as described for *C*.

DNA Sequencing. The 2643-bp *Hin*dIII insert was isolated from pHAVcat and digested with *Alu* I, *Hae* III, or *Sau*3A. The fragments were subcloned into the M13 mp18 and mp19 vectors (9) by standard techniques and were sequenced by the dideoxy chain-termination method (10).

Other Methods. Cell culture, transfections, induction with TCDD (1 nM) and CAT assays were as described (5). We have performed each experiment at least three times with consistent results.

RESULTS AND DISCUSSION

Function of the Dioxin-Responsive Element in Heterologous Cells. We transfected pHAVcat (5) into a mouse embryo fibroblast cell line (C3H 10T½) (11), a human mammary epithelial cell line (T47D) (12), and wild-type mouse hepatoma cells. The results (Fig. 2) demonstrate that TCDD induces CAT activity in all three cell lines. The CAT activity in uninduced cells is probably due to endogenous inducers in the culture medium (3). Thus, the cytochrome P_1-450 promoter and the dioxin-responsive element derived from mouse hepatoma cells are functional in cells from a heterologous mouse tissue and in cells from a heterologous species. These findings imply that the properties of TCDD receptors are very similar among different tissues and species. The functional conservation of the dioxin-responsive system is consistent with the idea that TCDD receptors may also function in cell proliferation or differentiation (13).

Function of the Dioxin-Responsive Element as an Enhancer. To test the dioxin-responsive element for enhancer activity, we inserted it in various locations and orientations into the plasmid pMcat5 (Fig. 1*B*) and transfected the recombinants into wild-type mouse hepatoma cells. The results in Fig. 3 *Upper* show that TCDD induces CAT activity in each instance. Control experiments (Fig. 3 *Lower*) indicate that both the dioxin-responsive element and the MMTV promoter are required for efficient CAT gene expression. Our results indicate that the dioxin-responsive element (*i*) can regulate a heterologous promoter from various distances and (*ii*) remains functional irrespective of its 5′–3′ orientation and 5′–3′ position with respect to the promoter. These properties are analogous to those of other viral and cellular enhancers (14). The dioxin-responsive element also requires TCDD receptors for its function (see below). Therefore, our findings imply that the dioxin-responsive element, together with TCDD receptors, constitute a TCDD-regulated enhancer system. The system presumably augments gene expression by increasing the frequency of transcription initiation (15). In addition, the system functions independently of (at least) two other regulatory domains, to which it is linked *in vivo* (5). This

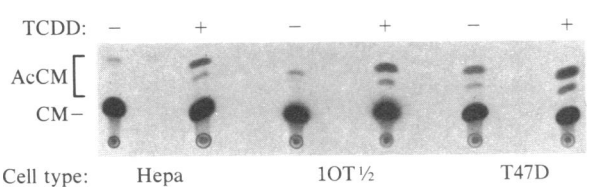

FIG. 2. Function of the dioxin-responsive element in heterologous cells. Wild-type mouse hepatoma cells (Hepa), C3H mouse embryo fibroblast cells (10T½), and human mammary epithelial cells (T47D) were transfected with pHAVcat, and CAT activities were measured in extracts from uninduced (−TCDD) and induced (+TCDD) cells. The autoradiogram shows the conversion of [^{14}C]chloramphenicol (CM) to its acetylated products (AcCM). From left to right, the conversion of CM to AcCM (measured by scintillation counting and expressed as a percentage of CM converted) was 0.3%, 1.3%, 0.3%, 2.3%, 0.9%, and 4.9%.

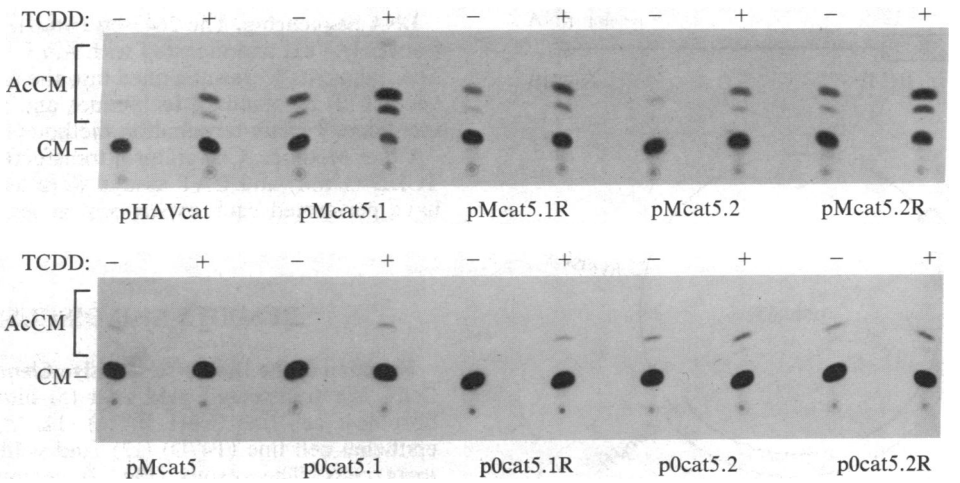

FIG. 3. Function of the dioxin-responsive element as an enhancer. Wild-type mouse hepatoma cells were transfected with the indicated plasmid, and CAT activities were measured in extracts from uninduced (−TCDD) and induced (+TCDD) cells. The autoradiogram shows the conversion of [^{14}C]chloramphenicol (CM) to its acetylated products (AcCM). (*Upper*) Experiments with pMcat5. In plasmid pMcat5.1, the dioxin-responsive element is inserted into the *Bgl* II site of pMcat5 in the correct 5′–3′ orientation; in pMcat5.1R, it is inserted into the *Bgl* II site in the reverse 3′–5′ orientation. In plasmid pMcat5.2, the dioxin-responsive element is inserted into the *Bam*HI site of pMcat5 in the correct 5′–3′ orientation; in pMcat5.2R, it is inserted into the *Bam*HI site in the reverse 3′–5′ orientation. From left to right, the conversion of CM to AcCM (expressed as a percentage of CM converted) was 0.4%, 7.0%, 10%, 92%, 3.0%, 12%, 0.5%, 4.8%, 6.3%, and 55%. (*Lower*) Control experiments. Plasmid pMcat5 lacks the dioxin-responsive element. Plasmid p0cat5 lacks the MMTV promoter but contains the dioxin-responsive element, inserted as described for pMcat5 above. From left to right, the conversion of CM to AcCM (expressed as a percentage of CM converted) was 0.3%, 0.3%, 0.4%, 1.1%, 0.2%, 0.8%, 1.2%, 1.8%, 1.2%, and 1.8%.

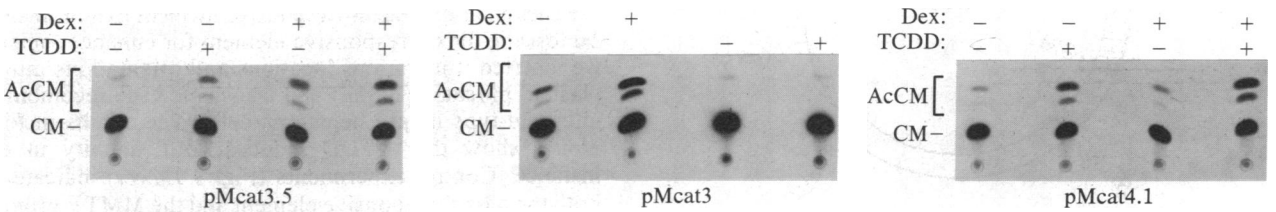

FIG. 4. Function of the dioxin-responsive element when linked to a GRE. Wild-type mouse hepatoma cells were transfected with the indicated plasmid, and CAT activities were measured in extracts from cells exposed to the indicated inducers. The autoradiograms show the conversion of [^{14}C]chloramphenicol (CM) to its acetylated products (AcCM). The concentration of dexamethasone (Dex) used was 0.5 μM (*Left*) and 1.0 μM (*Center* and *Right*). From left to right, the conversion of CM to AcCM (expressed as a percentage of CM converted) was: 0.2%, 1.3%, 2.4%, and 6.0% (*Left*); 1.2%, 13%, 0.2%, and 0.2% (*Center*); and 0.6%, 6.0%, 0.8%, and 23% (*Right*).

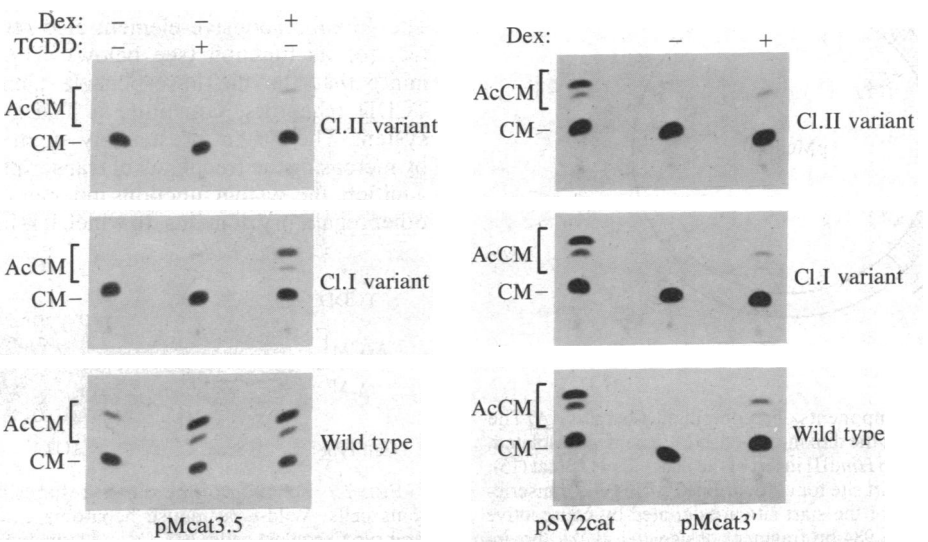

FIG. 5. Function of the dioxin-responsive element in enhancing the response to dexamethasone. Wild-type and variant mouse hepatoma cells were transfected with the indicated plasmid, and CAT activities were measured in extracts from cells exposed to TCDD or dexamethasone (Dex; 1.0 μM), as indicated. The autoradiograms show the conversion of [^{14}C]chloramphenicol (CM) to its acetylated products (AcCM). (*Left*) Cells transfected with pMcat3.5. The conversion of CM to AcCM (from left to right) was: 0.2%, 0.3%, and 2.0% [class (Cl.) II variant]; 0.2%, 1.2%, and 19% (class I variant); and 7.4%, 64%, and 63% (wild type). (*Right*) Cells transfected with pMcat3′ (a plasmid that is functionally identical to pMcat3). The conversion of CM to AcCM (from left to right) was: 0.3% and 1.3% (class II variant); 0.2% and 1.5% (class I variant); and 0.3% and 3.3% (wild type).

finding implies that other TCDD-responsive genes need not have combinations of regulatory elements identical to that of the cytochrome P_1-450 gene.

Function of the Dioxin-Responsive Element When Linked to a GRE. To examine the possible functional interaction between the dioxin-responsive element and another linked regulatory element, we constructed the plasmids pMcat3.5 and pMcat4.1 (Fig. 1 *C* and *D*). We transfected the plasmids into wild-type mouse hepatoma cells (which contain glucocorticoid receptors) and measured both TCDD-inducible and dexamethasone-inducible CAT activity in extracts from the transfected cells. In cells transfected with pMcat3.5, TCDD and dexamethasone each induce CAT activity (Fig. 4 *Left*). Thus, in this configuration, both the dioxin-responsive element and the GRE apparently function relatively independently of each other. As expected, cells transfected with pMcat3 exhibit dexamethasone-inducible CAT activity but no TCDD-inducible CAT activity (Fig. 4 *Center*). In contrast to the results with pMcat3.5, cells transfected with pMcat4.1 express TCDD-inducible CAT activity but virtually no dexamethasone-inducible CAT activity (Fig. 4 *Right*). Thus, the function of the GRE is influenced greatly by its position with respect to the dioxin-responsive element.

Our findings imply that the dioxin-responsive system functions (relatively) independently of a linked glucocorticoid-responsive system. However, the converse is not true; the GRE is less effective when positioned upstream of the dioxin-responsive element than when positioned downstream. Therefore, two inducer-regulated enhancer systems, which function independently when unlinked *in vivo*, may become interdependent when linked in a *cis* configuration. Taken together, our findings suggest that the transcription of (at least some) genes might reflect the influence of linked control elements, organized into combinations specific for each regulated gene (16). The enhancer properties of the dioxin-responsive system would allow its participation in such a multicomponent regulatory hierarchy.

Function of the Dioxin-Responsive Element and a Linked GRE in Variant Mouse Hepatoma Cells. To show that the regulation of the MMTV promoter by the dioxin-responsive element requires functional TCDD receptors, we transfected pMcat3.5 into variant mouse hepatoma cells. Class I variants

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-2300
                                        5' AAGCTTCGTCAAAG
-2250
    GAGTATTGTTAATCCTTTTCACCTCTGCATCAATCACCAGCATCCAGCAT
-2200
    AGTAGGAGCTTGGCGAATATTTGCACAATGCATGAGGGCGGCATGTAACT
-2150
    AGAGTGGGAGGTGAGGGGGTGGATGAAGTGTTGTTTTTTTTTCCTGGTCT
-2100
    TGGTCACAGAGCAGATATCAATGTTCTTTCTGGGCTCGGGAGCTCACAGG
-2050
    GAGGGCAGGTGAAGGTGTTAGTTAGGAACAGGTTGAGTTAGACACGCCAA
-2000
    GTTCAGATGTTCTTCTTACACTAATCTCACTCTGGGAGCACCCTTCAGGC
-1950
    CAGAGAGCACCTGCAAAACAGCCAGCTAGGCGTGCGGGTGGTGCTGCGTG
-1900
    TCCTGCCACTAAAAATGTGGACACACGCTGTTCAGGCCTTTGCTCTCAGG
-1850
    CAGAAGCGAGCATCGACGAAACCCGGCCCTTTGCACGTGGCGTGTCTTGT
-1800
    CGCGCCTTGCAAAGCATAGATATTTCTATCTCTTAAACCCACCCAACGCC
-1750
    AGGAGAGCTGGCCCTTTAAGAGCCTCACCCACGGTTCCCCTCCCCCAGCT
-1700
    AGCGTGACAGCACTGGGACCCGCGCCCGGTTGTGAGTTGGGTAGCTGGGT
-1650
    GGCTGCGCGGGCCTCCAGGCTCTTCTCACGCAACTCCGGGGCACCTTGTC
-1600
    CCCAGCCAGGTGGGGCGGAGACAGGCAGCCCGACCCTCTGCCCCCAGAGG
-1550
    ATGGAGCAGGCTTACGCACGCTAGCCTCAGGAACCTGTGTGCGTGCCAAG
-1500
    CATCACCCTTTGTAGCCCCAGACCCCCTCCTGCTGTCTCGCGTGGATCCT
-1450
    TCCTCCACCCTTTCCTCCACCATACTTAGATAGCTCTGCACCCGCCGCCC
-1400
    GACTTCTCTGTGTTGCCTGCTTCAGTATGTATGCACAACACATGCAAGCC
-1350
    CCGGGAGCCTACAGGGAGCTCGAAGCGGGGACTCCCGGGCTTGCAGCTTG
-1300                          -
    CCTAAGGTGACCCTTAGAGGTGAGATCTGCAAGCCTGCCATCCATCCCCA
-1250                                                 C
    CCCTCTAGATGAAGCAGCGCGAACTTCGGCCGATACCCAATTTGTGGGG-C
-1200
    ACAGAGTCAGTCCAATGGCGCCACTGGCCTTCCTGTCCTGTGACCTCTGG
-1150                          G
    GCTGGGGTCGTTGCGCTTCTCACGCGAGCTTG-GACTCAGTAATCCAGGGA
-1100T
    AGCAAAGTCACCACCCAGCTGTTCTCCCTCTACCAGCCTTTCCCGGGCAC
-1050
    CCATTGGCTTGTAGTAGGCAAGAGGATCTTACACACTGAATGTTTCAGGG
-1000
    GTGCAAAAATAGTGAAAAGGAATCCCTATGACCCGGAATGGAGGCCCCGA
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-950
   TACTTACTTTTTAGGTTACCCCAGAATTTTTCCTCAAACCCCTCCCTCAG
-900     C                    T
   TGGGATT-ATGCACTGTCCATGGAGCACCTTGAAAG-TGTGGGGGGTGGTGA
-850
   CCCCAACCTTTATTCTTTTTCTTTTCTTATGTTTTTTTGTGCCTGTACCT
-800
   ACATCAGGTATCCGGTATGGCTTCTTGCCTATCTCCCCCCTCCCCCCTGC
-750
   CTAGAGCACTCCCTAAGGCTGTCCCTCCCTCGGTCCCACCACTGGGCTCA
-700
   GATAAGGAGGCGTGGCCAACAGACACAGAGTCC TATAAA GGTGGTGGTGC
-650
   CTTCACCCTAACCCTGAAGGTGGTAGTTCTTGGAGCTTCCCCGATCCTCC
-600
   CTAGGGTCCTAGAGAACACTCTTCACTTCAGTCCCTCCTTACATGCCCAA
-550
   GGTGAGTGCTTGGGGGGGCTCCTGGATTGCCCCTCCCTCCCTCCCTCCCT
-500
   CCCTCCCTCCCTCCCAAGAAAAGGAAATTTTTGGTGAAGAGTTACTGGGT
-450
   AGCTCCTTCAAAACAGGGAACTACACTTTTATTTATTTATTTATTTATTT
-400
   ATTTAGTGGACCAGGTAAATTCCTTAAGGGAGAAACTGAGGCCAGAGGTA
-350
   TCAGCTATTTAGATAGAAATGAGATACCCTTCCACTTCAGGCCCCTCCCC
-300
   CTCCTCACTGACCTCCTAGGGCGTCTTCCCTGCACCTTTAGCCCCTTCCT
-250
   CAGGACTGCCAGTGGGGGTGAGGGCTCCTCTCTGGATGTGTAGGGTGGAC
-200
   AGTGAACATGCACTGAGCTTTTCTGGTGTTCTCTGTCTTATTTAAAAACT
-150
   ACCCCCAATAGCCTCTACCCTCCCAAAGGGCAGCATGTCAGAGCTGTTTC
-100
   TGACCTAGGCTAGAGAGGCTCCCTACCCCACTGACTGTCTCTTCTATCCT
 -50
   TGCTGGGATACCATCAGCTC CATAAA CTGAGAGCTTCAGGGAACTGGAAA
   1                                                  50
   CACCAGTTACATTTCCCTCGGCCCTTCCACCTTCCAAAGGTTAGGGTTCC
                                                     100
   TTAGGGACACCAATTATGAAGTGTGTTTTCAGCCCCTCCAGGAAGCCCCT
                                                     150
   CTGCTTCAAGCTGACTGTAGTATTTGGGAATTGTACATCTTCCTATCGTA
                                                     200
   TGTGCTTCAGATCTTCTCTTCCTAGGATCTGTACCCCTCTGAATTAAGCA
                                                     250
   ATCTTTCTTGTTGGCTTGTTTTGTTTTTTTGAGACAGGGTTTCTCTGTGT
                                                     300
   AGCCCTGGCTGTCCTGGAACTCACTCTGTAGACCAGGCTGGCCTCAAACT
                                                     350
   CAAGAGTTCCTCCTGCCTCTGTCTCCTAAGTGCTGGGGTTAAAGGTATGG
                                                     400
   CCAGTGCCTCCCTCCCAGATGAATTGGGCAAGCTT 3'
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FIG. 6. Nucleotide sequence of the 2643-bp insert isolated from pHAVcat. The start site of transcription (underlined at position −1) was determined previously by primer extension analysis (5). Nucleotides located 5'-ward of the transcription start site are designated by consecutive negative numbers; nucleotides located 3'-ward of the transcription start site are designated by consecutive positive numbers. The CATAAA sequence in the promoter domain is boxed. The six nucleotides that differ from the previously reported sequence in C57BL/6N mouse liver (19) are shown above the pHAVcat sequence. The TATAAA sequence (boxed) and the associated start site of transcription (underlined at position −637) reported for C57BL/6N mouse liver (19) are also indicated. For purposes of comparison, the *Bam*HI site at (−1456) corresponds to the *Bam*HI site at (−823) in the sequence reported previously for the cytochrome P_1-450 gene in mouse liver (19).

form few (i.e. 5–10% of wild type) TCDD–receptor complexes and exhibit decreased expression of the cytochrome P_1-450 gene in response to TCDD. Class II variants form TCDD–receptor complexes, which bind weakly to the nucleus; these variants fail to transcribe the cytochrome P_1-450 gene in response to TCDD (17, 18). In cells transfected with pMcat3.5, there is little TCDD-inducible CAT activity in the class I variants and no TCDD-inducible CAT activity in the class II variants (Fig. 5 *Left*). These results imply that the dioxin-responsive element requires TCDD–receptor complexes in order to function. Transfections with pSV2cat indicate that these results are not due to differences in transfectability between the cell types (Fig. 5 *Right*).

Even in uninduced cells, there is less CAT activity in the class I variants than in the wild-type cells, and there is no detectable CAT activity in the class II variants (Fig. 5 *Left*). These findings are consistent with the idea that the activity in "uninduced" cells reflects endogenous inducers that act via TCDD receptors. This observation allowed us to determine whether the "basal" activity of the dioxin-responsive element influences the response of the linked MMTV LTR to dexamethasone. In cells transfected with pMcat3.5, dexamethasone-induced CAT activity is lowest in the class II variants, is intermediate in the class I variants, and is highest in the wild-type cells (Fig. 5 *Left*). Control experiments indicate that when the dioxin-responsive element is not present, dexamethasone induces similar (low) levels of CAT activity in all three cell types (Fig. 5 *Right*). Dexamethasone does not interact with TCDD receptors (13, 18). Thus, the data indicate that the dioxin-responsive system can augment the response of the linked dexamethasone-responsive system. Again, these findings imply that the dioxin-responsive element, together with its inducer–receptor complex, functions as an enhancer system.

Nucleotide Sequence of the Regulatory Domains Located 5′-Ward of the Cytochrome P_1-450 Gene. Fig. 6 shows the nucleotide sequence of the 2643-bp insert isolated from the plasmid pHAVcat. The sequence from −1456 to +380 is virtually identical to that reported for the cytochrome P_1-450 gene from C57BL/6N mouse liver (19). The functional significance of the six single-nucleotide differences is unknown. There is a CATAAA sequence located between nucleotides −25 to −30 5′-ward of the start site of transcription in HAV cells. Other cytochrome P-450 genes also contain similarly modified "TATA boxes" (1). The promoter and transcription start site used in the HAV cells are located about 635 nucleotides 3′-ward of the promoter and transcription start site used in C57BL/6N mouse liver (19). The reason(s) for this difference is unknown. The dioxin-responsive element contains no sequences that exhibit major homologies to other viral or cellular enhancers.

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