Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 8545–8549, November 1990
Biochemistry

Dioxin-inducible, Ah receptor-dependent transcription *in vitro*

(2,3,7,8-tetrachlorodibenzo-*p*-dioxin/cytochrome P450/gene expression/enhancer/protein–DNA interactions)

LONG-PING WEN*, NERVILLE KOEIMAN, AND JAMES P. WHITLOCK, JR.

Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

Communicated by Paul Talalay, August 2, 1990 (received for review July 2, 1990)

ABSTRACT **We have developed a homologous *in vitro* transcription system that requires (*i*) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (called TCDD or dioxin), (*ii*) the Ah receptor, and (*iii*) a dioxin-responsive enhancer for activity. Unfractionated nuclear extracts from mouse hepatoma cells contain an inhibitor and fail to direct transcription *in vitro*. However, following phosphocellulose chromatography and reconstitution, the fractionated nuclear extract directs accurate transcription *in vitro*, using as a template the promoter/enhancer region from the mouse cytochrome P_1-450 gene (*Cyp1a1*) linked to a "G-free cassette" (which generates a transcript with no guanosine residues). Extracts from TCDD-treated cells exhibit higher activity than extracts from untreated cells when transcribing a template containing both the promoter and enhancer but not when transcribing a template containing the promoter alone. Extracts from Ah receptor-defective cells fail to direct *in vitro* transcription in a TCDD-inducible fashion. A regulatory element that contains two binding sites for the liganded Ah receptor plus a truncated *Cyp1a1* promoter suffices to direct TCDD-inducible, Ah receptor-dependent transcription *in vitro*. The inducible, receptor-dependent, enhancer-dependent properties of this system make it appropriate for analyzing *in vitro* the mechanism of dioxin action and the function of the Ah receptor.**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (called TCDD or dioxin), a widespread and persistent environmental contaminant, elicits in experimental animals a diverse set of tissue- and species-specific responses, including epithelial metaplasia, tumor promotion, teratogenesis, immunosuppression, and enzyme induction (1, 2). Epidemiological studies have been inconclusive, and the health risk that dioxin poses to humans remains uncertain (3). An understanding of the mechanism of TCDD action may help in assessing the risk that the dioxin poses to man.

The induction of transcription of the mouse cytochrome P_1-450 gene (*Cyp1a1*) by TCDD constitutes a useful response for analyzing the mechanism of dioxin action (4). Using mouse hepatoma cells, we have shown that the transcriptional response requires the binding of TCDD to an intracellular protein (the Ah receptor), followed by the binding of the liganded receptor to a specific DNA recognition sequence present in multiple copies within a dioxin-responsive enhancer located upstream of the *Cyp1a1* gene. However, the mechanism by which the receptor–enhancer interaction activates gene transcription is not well understood. In addition, the structural and functional properties of the Ah receptor are largely unknown, because the protein has not been purified. As a step toward addressing these issues, we report here the development of a homologous, TCDD-inducible, receptor- and enhancer-dependent *in vitro* transcription system, which is suitable for analyzing the molecular aspects of dioxin action and the function of the Ah receptor.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Materials. 3′-*O*-Methyl-GTP was purchased from Pharmacia. [α-^{32}P]UTP (specific activity = 3000 Ci/mmol; 1 Ci = 37 GBq) was from Amersham. Molecular biological reagents were from Bethesda Research Laboratories, Boehringer Mannheim, New England Biolabs, and Promega Biotec.

DNA Templates. In order to link the *Cyp1a1* promoter with the "G-free cassette" (5), which generates a transcript with no guanosine residues, we introduced an *Nde* I site at the +3 position of the *Cyp1a1* gene by site-directed mutagenesis, as follows. A synthetic oligonucleotide primer with the sequence 5′-CCCTAACCCTCATATGGGTAGTTCTTGG-3′ (corresponding to nucleotides −7 to +21 of the *Cyp1a1* gene, except that the *Nde* I recognition sequence CATATG has replaced the *Cyp1a1* sequence from nucleotides +4 to +9) was used to mutagenize a single-stranded M13Mp18 plasmid containing a 459-base-pair (bp) *Hin*dIII–*Ppu*MI *Cyp1a1* fragment isolated from the deletion mutant D9 (6). The plasmid containing the desired mutation (identified by digestion with *Nde* I) was digested with *Nde* I and *Hin*dIII, and the resulting 418-bp fragment (corresponding to nucleotides −414 to +3 of the *Cyp1a1* gene, with an *Nde* I half-site at the 3′ terminus) was gel-purified, blunt-ended with T4 DNA polymerase, and subcloned into *Sac* I-digested and blunt-ended p$(C_2AT)_{19}$, which contains a 380-bp G-free cassette (5), to form the plasmid −414/G/Eco. The *Eco*RI site was then converted to a *Bgl* II site to form the template designated −414/G. This template contains the *Cyp1a1* promoter (but not the dioxin-responsive enhancer) linked to the G-free cassette.

To construct the template containing the dioxin-responsive enhancer, a 482-bp *Bgl* II fragment spanning nucleotides −820 to −1302 was isolated from plasmid pMCAT5.9 (7) and was inserted into the *Bgl* II site of −414/G to form the template designated −414/G/DRE. This template contains the dioxin-responsive enhancer and the *Cyp1a1* promoter linked to the G-free cassette.

To construct the template containing a truncated *Cyp1a1* promoter, the template −414/G was digested with *Bal* I, and the 450-bp fragment containing the G-free cassette and the *Cyp1a1* domain spanning nucleotides −50 to +3 was subcloned into *Sma* I-digested pUC19 to form template −50/G. This template contains a TATAAA sequence (but no other known functional *Cyp1a1* promoter domain) linked to the G-free cassette.

To add recognition motifs for the liganded Ah receptor, two tandem copies of the synthetic oligonucleotide

5′-GATCTCTTCT***CACGC***AACTCCGAG-3′
3′-AGAAGA***GTGCG***TTGAGGCTCCTAG-5′

(oligo I), which contains a single binding site (in boldface italic letters) for the Ah receptor, were inserted into the *Bam*HI site of template −50/G to form the template desig-

Abbreviation: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

*Present address: Institute of Molecular and Cellular Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Singapore.

nated $-50/G/I_2$. This template contains two binding sites for the liganded Ah receptor plus the truncated *Cyp1a1* promoter linked to the G-free cassette.

The structures of the DNA templates were verified by restriction analyses and/or DNA sequencing. The adenovirus major late template (designated AdML) has been described previously (8).

The templates are illustrated schematically in Fig. 1. Correctly initiated transcripts should be 383 bp in length for the *Cyp1a1* templates and 390 bp for the AdML template. The length of the *in vitro* transcripts was determined by electrophoresis and autoradiography and comparison to markers of known size. Supercoiled plasmids were prepared by centrifugation in cesium chloride as described (9). Use of the G-free cassette as a reporter sequence has several advantages for *in vitro* transcription studies. It provides a simple, rapid, and quantitative assay, which has a low background and which avoids artifacts associated with the use of linear or nicked DNA templates (5).

Cell Culture. Culture of wild-type and class II variant mouse hepatoma cells was as described (10). Confluent monolayers were treated either with 1 nM TCDD (in dimethyl sulfoxide) or with dimethyl sulfoxide alone for 2 hr before harvesting by trypsinization. HeLa cells were cultured under the same conditions as the mouse hepatoma cells.

Nuclear Extracts. Crude nuclear extracts from HeLa or hepatoma cells were prepared according to Dignam *et al.* (11), except that the final dialysis step was omitted. Instead, HeLa nuclear extracts were desalted by chromatography on a Bio-Rad Econo-Pac 10DG column and eluted with buffer E [20 mM Hepes (pH 7.9), 20% (vol/vol) glycerol, 0.2 mM EDTA, and 0.5 mM dithiothreitol] containing 0.1 M KCl (11). Nuclear extracts from hepatoma cells were adjusted to 0.3 M KCl in buffer E and were loaded onto a phosphocellulose mini-column (5 mg of protein per ml of bed volume) equilibrated with buffer E containing 0.3 M KCl. The column was washed with buffer E containing 0.3 M KCl, and the flow-through fraction was collected. The bound protein was eluted with buffer E containing 1 M KCl. Fractions from the 0.3 M wash and the 1 M elution that contained detectable amounts of protein were pooled separately and were desalted using Bio-Rad Econo-Pac 10DG columns, as described above. The fractions were divided into aliquots and stored at −80°C. These extracts remain transcriptionally active for up to 2 months.

***In Vitro* Transcription.** A typical *in vitro* transcription reaction (30 μl) contained 20 mM Hepes (pH 7.9), 10% (vol/vol) glycerol, 60 mM KCl, 6 mM $MgCl_2$, 1 mM dithiothreitol, 0.6 mM CTP, 0.6 mM ATP, 12 μM UTP, 30 μM 3′-O-methyl-GTP, 10 μCi of [α-^{32}P]UTP, 800 ng of DNA template, and various amounts of fractionated nuclear extract, as indicated in *Results*. The order of reagent addition was buffer, extract, DNA, and NTPs. After incubation at 30°C for 60 min, the reactions were terminated by the addition of 300 μl of stop buffer [20 mM Tris Cl (pH 7.5), 200 mM NaCl, 15 mM EDTA, 0.2% SDS, and 200 μg of yeast tRNA per ml]. The reaction mixture was extracted with an equal volume of phenol/chloroform, 1:1 (vol/vol), the aqueous phase was removed, and nucleic acid was precipitated at −80°C by addition of 2.5 volumes of ethanol. The nucleic acid was fractionated by electrophoresis on a 6% polyacrylamide/7 M urea gel. Autoradiography of the dried gels was performed at −80°C with an intensifying screen.

RESULTS

Mouse Hepatoma Cell Nuclear Extracts Contain a Transcriptional Inhibitor. Initially we were unable to establish *in vitro* transcription using nuclear extracts from mouse hepatoma cells, with either a *Cyp1a1* template or an AdML template; however, we noted that HeLa nuclear extracts transcribe both templates. Therefore, we suspected that the hepatoma cell extracts contain an inhibitor of transcription. To test this possibility, we performed mixing experiments, such as that described in Fig. 2. Our results reveal that addition of increasing amounts of hepatoma cell extract to the HeLa extract produces progressively greater inhibition of transcription. The inhibition occurs with both the adenoviral template and the *Cyp1a1* template, although it appears more severe with the latter (Fig. 2). The inhibition occurs whether or not the hepatoma cells have been treated with TCDD (data not shown). These observations imply that mouse hepatoma cells contain an inhibitor(s) of transcription and that the *Cyp1a1* template is more sensitive than the AdML template to its action. We have not characterized this inhibitor or its mechanism of action in detail.

***In Vitro* Transcription Using Fractionated and Reconstituted Nuclear Extract.** Other investigators have used phosphocellulose chromatography as an initial step in the fractionation of transcription factors (12). In order to minimize the effect

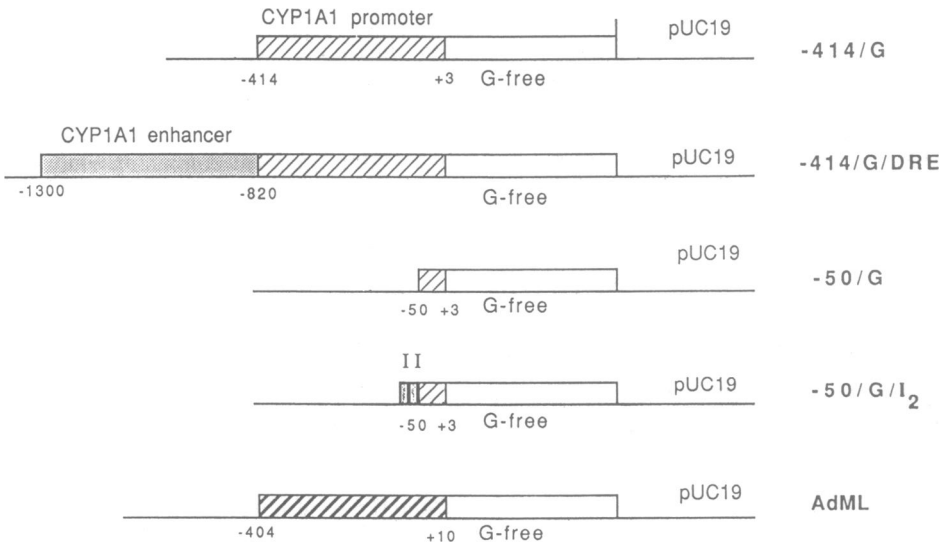

FIG. 1. DNA templates. See text for details of template construction. Numbers indicate the boundaries of the corresponding DNA domains, in nucleotides, from the transcription start site. G-free, guanine-free cassette, used as the reporter gene; I, synthetic oligonucleotide, containing a single recognition motif for the liganded Ah receptor; AdML, adenovirus major late promoter.

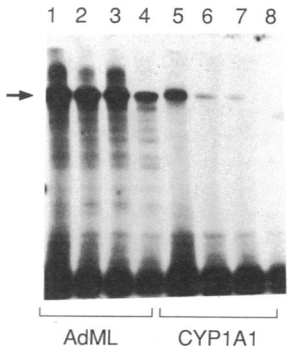

FIG. 2. Inhibition of *in vitro* transcription by nuclear extracts from mouse hepatoma cells. Transcription reactions containing 5 μl (40 μg of protein) of HeLa nuclear extract and either an adenoviral template (lanes 1–4) or the *Cyp1a1* template −414/G/DRE (lanes 5–8) were incubated with the indicated volumes of nuclear extract (3 μg of protein per μl) from TCDD-treated (1 nM for 2 hr) mouse hepatoma cells. Lanes: 1 and 5, 0 μl of nuclear extract; 2 and 6, 2 μl of nuclear extract; 3 and 7, 4 μl of nuclear extract; 4 and 8, 8 μl of nuclear extract. The arrow indicates the position of the correctly initiated transcripts.

of the transcriptional inhibitor, we used this chromatographic technique to separate mouse hepatoma cell nuclear extracts into two fractions, which were eluted from a phosphocellulose column at 0.3 M KCl and 1.0 M KCl, respectively. We assayed these two fractions, separately and together, for their ability to direct *in vitro* transcription from two *Cyp1a1* templates (Fig. 3*A*). Our findings reveal that the reconstituted extract is active in transcribing a template containing the *Cyp1a1* promoter plus the dioxin-responsive enhancer (Fig. 3*A*, lane 2) but is much less active when the template lacks the enhancer (Fig. 3*A*, lane 4). This observation indicates that the reconstituted extract is able to direct transcription from a *Cyp1a1* template *in vitro*. Our findings also imply that *in vitro* transcription of *Cyp1a1* templates requires the dioxin-responsive enhancer; therefore, we employed enhancer-containing templates in the studies described below.

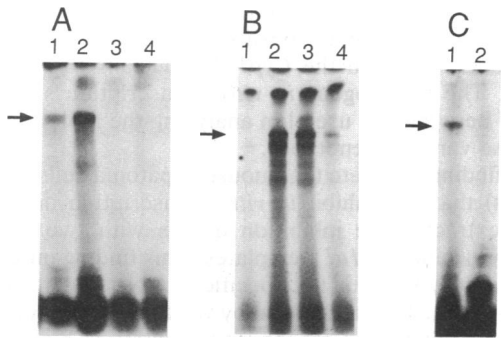

FIG. 3. *In vitro* transcription by nuclear extracts from mouse hepatoma cells. (*A*) Requirements for transcription. The 0.3 M KCl fraction (2.5 μl, ≈5 μg of protein) and the 1.0 M KCl fraction (10 μl, ≈8 μg of protein), prepared from nuclear extracts of TCDD-treated (1 nM for 2 hr) mouse hepatoma cells, were used, alone or in combination, to transcribe the −414/G/DRE template (lanes 1–3) or the −414/G template (lane 4). Lane 1, 1.0 M KCl fraction alone; lane 2, 0.3 M KCl and 1.0 M KCl fractions together; lane 3, 0.3 M KCl fraction alone; lane 4, 0.3 M KCl and 1.0 M KCl fractions together. The arrow indicates the position of the correctly initiated transcripts. (*B*) Reconstitution experiments. The 1.0 M KCl fraction (7 μl, ≈6 μg of protein) was combined with the indicated amounts of the 0.3 M KCl fraction (≈2 μg of protein per μl), prepared from nuclear extracts of TCDD-treated (1 nM for 2 hr) mouse hepatoma cells, and used to transcribe the −414/G/DRE template. Lane 1, 0 μl of the 0.3 M KCl fraction; lane 2, 2 μl of the 0.3 M KCl fraction; lane 3, 4 μl of the 0.3 M KCl fraction; lane 4, 8 μl of the 0.3 M KCl fraction. The arrow indicates the position of the correctly initiated transcripts. (*C*) Inhibition by α-amanitin. The 0.3 M KCl fraction (2.5 μl, ≈6 μg of protein) and the 1.0 M KCl fraction (10 μl, ≈8 μg of protein), prepared from nuclear extracts of TCDD-treated (1 nM for 2 hr) mouse hepatoma cells, were used to transcribe the −414/G/DRE template. Lane 1, no α-amanitin; lane 2, α-amanitin (1 μg/ml). The arrow indicates the position of the correctly initiated transcripts.

More detailed reconstitution studies (Fig. 3*A*) reveal that the 0.3 M fraction by itself generates no detectable transcription *in vitro* (Fig. 3*A*, lane 3) and that the 1.0 M fraction contains low activity (Fig. 3*A*, lane 1). However, combining the two fractions in about a 1:1 weight ratio (μg of protein:μg of protein) produces a synergistic effect on transcription (Fig. 3*A*, lane 2). Thus, each of the two fractions contains a factor(s) that is required for maximal transcription of the *Cyp1a1* template *in vitro*.

The ratio of the two fractions that yields maximal rates of transcription varies among different extracts and must be determined empirically (for example, see Fig. 3*B*). Our findings from nine experiments indicate that the optimal ratio of the 1 M fraction to the 0.3 M fraction is between 1:1 and 2:1 (μg of protein:μg of protein). On a relative protein basis, this ratio represents a 5- to 10-fold enrichment for the 1 M fraction relative to the 0.3 M fraction. Thus, the *in vitro* transcription reaction is likely to be enriched for the liganded Ah receptor. In addition, increasing the amount of the 0.3 M fraction beyond the optimum produces a decrease in transcription (Fig. 3*B*). This latter finding implies that the 0.3 M fraction contains (most of) the transcriptional inhibitor present in the hepatoma cell nuclear extracts.

The results in Fig. 3*C* reveal that α-amanitin, at a concentration of 1 μg/ml, abolishes the activity of the reconstituted system. This observation implies that RNA polymerase II directs the transcription of the *Cyp1a1* template *in vitro*.

Inducible, Receptor-Dependent Transcription *in Vitro*. Transcription of the *Cyp1a1* template *in vitro* requires the dioxin-responsive enhancer (Fig. 3*A*). This observation implies that transcription also requires the Ah receptor, the liganded form of which binds specifically to enhancer DNA. To test this hypothesis, we prepared nuclear extracts from both uninduced and TCDD-induced hepatoma cells and tested the reconstituted fractions for their ability to transcribe *Cyp1a1* templates *in vitro*. As shown in Fig. 4*A*, the reconstituted extract from TCDD-induced cells (Fig. 4*A*, lane 2) exhibits a higher level of transcription than does the extract from uninduced cells (Fig. 4*A*, lane 1). Thus, the transcriptional activity is TCDD-inducible. The results of multiple experiments indicate that pairs of extracts (i.e., uninduced and induced) vary in activity and that extracts from TCDD-induced cells consistently exhibit a 2- to 10-fold higher transcriptional activity than extracts from uninduced cells (data not shown). Induction also requires a template containing the dioxin-responsive enhancer, because it is not observed when templates containing only the *Cyp1a1* promoter are used (data not shown). Mixing experiments reveal that the 1 M fraction is largely responsible for the increased activity of extracts from TCDD-induced cells (Fig. 4*A*, lanes 3 and 4). Gel retardation analyses indicate that the 1 M fraction contains most of the liganded Ah receptor (data not shown); together, these findings imply that the liganded receptor contributes to TCDD-inducible transcription *in vitro*.

To test more definitively the idea that the Ah receptor participates in *Cyp1a1* transcription *in vitro*, we compared the activities of nuclear extracts from wild-type and receptor-defective variant mouse hepatoma cells. In the variant cells, the liganded receptor fails to bind to DNA, and the *Cyp1a1* gene does not respond to TCDD (13, 14). The results in Fig. 4*B* reveal that nuclear extracts from wild-type cells exhibit a TCDD-inducible increase in transcriptional activity (Fig. 4*B*,

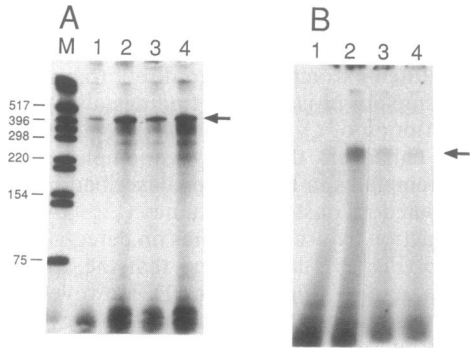

FIG. 4. Inducibility and Ah receptor dependence of *in vitro* transcription. (*A*) Induction by TCDD. Nuclear extracts were prepared from uninduced and TCDD-induced (1 nM for 2 hr) mouse hepatoma cells; the 0.3 M KCl and 1.0 M KCl fractions were used in the indicated combinations to transcribe the −414/G/DRE template. Lane 1, 0.3 M KCl fraction (uninduced) plus 1.0 M KCl fraction (uninduced); lane 2, 0.3 M KCl fraction (TCDD induced) plus 1.0 M KCl fraction (TCDD induced); lane 3, 0.3 M KCl fraction (TCDD induced) plus 1.0 M KCl fraction (uninduced); lane 4, 0.3 M KCl fraction (uninduced) plus 1.0 M KCl fraction (TCDD induced); lane M, 1-kilobase DNA ladder (BRL). The arrow indicates the position of the correctly initiated transcripts. (*B*) Ah receptor-dependence. Nuclear extracts were prepared from uninduced and TCDD-induced (1 nM for 2 hr) wild-type and receptor-defective variant mouse hepatoma cells. The 0.3 M KCl fractions and 1.0 M KCl fractions were combined and used to transcribe the −414/G/DRE template. Lane 1, uninduced wild-type cells; lane 2, TCDD-induced wild-type cells; lane 3, uninduced variant cells; lane 4, TCDD-induced variant cells. The arrow indicates the position of the correctly initiated transcripts.

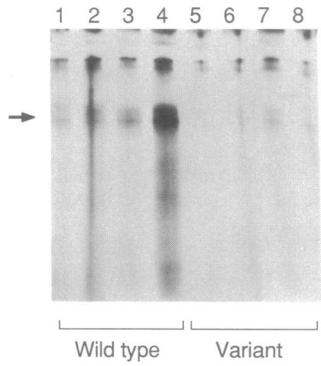

FIG. 5. *In vitro* transcription using truncated templates. Nuclear extracts were prepared from uninduced and TCDD-induced (1 nM for 2 hr) wild-type and receptor-defective variant mouse hepatoma cells. The 0.3 M KCl fractions and 1.0 M KCl fractions were combined and used to transcribe the −50/G template (lanes 1, 2, 5, and 6) and the $-50/G/I_2$ template (lanes 3, 4, 7, and 8). Lanes 1 and 3, uninduced wild-type cells; lanes 2 and 4, TCDD-induced wild-type cells; lanes 5 and 7, uninduced variant cells; lanes 6 and 8, TCDD-induced variant cells. The arrow indicates the position of the correctly initiated transcripts.

lanes 1 and 2), whereas extracts from the variant cells do not (Fig. 4*B*, lanes 3 and 4). Therefore, we conclude that a functional Ah receptor is required for TCDD-inducible transcription of the *Cyp1a1* template *in vitro*.

Template Elements Sufficient for Inducible, Receptor-Dependent Transcription *in Vitro*. The templates used in the studies described above contain about 480 bp of enhancer DNA and 415 bp of promoter DNA. These large templates are complex, and their use could unnecessarily complicate the molecular analysis of dioxin action. Therefore, we searched for simpler templates, which are sufficient to support TCDD-inducible, Ah receptor-dependent transcription *in vitro*. For example, we analyzed templates containing either a truncated *Cyp1a1* promoter alone or the truncated promoter linked to two recognition motifs for the liganded Ah receptor. [The only functional element within the truncated promoter is a TATAAA sequence (15). Also, our analyses of the dioxin-responsive enhancer reveal that increasing the number of receptor binding sites from one to two has a synergistic effect on enhancer activity (data not shown)]. The results in Fig. 5 reveal that extracts from wild-type cells direct TCDD-inducible transcription from the template containing the truncated promoter plus the receptor recognition motifs (Fig. 5, lanes 3 and 4) but not from the template containing the truncated promoter alone (Fig. 5, lanes 1 and 2). Furthermore, the inducible transcriptional activity is also Ah receptor-dependent because it does not occur when extracts from receptor-defective cells are used (Fig. 5, lanes 5–8). These findings indicate that a template comprised of a minimal promoter (containing a TATAAA sequence) and a minimal enhancer (containing two recognition motifs for the liganded Ah receptor) is sufficient to direct TCDD-inducible, Ah receptor-dependent transcription *in vitro*.

DISCUSSION

We have characterized a homologous *in vitro* transcription system that mimics the expression of the *Cyp1a1* gene *in vivo*; transcription is TCDD-inducible and Ah receptor-dependent and requires a dioxin-responsive enhancer. This system appears useful for studying several interesting aspects of dioxin action and *Cyp1a1* gene expression. For example, the system should provide a functional assay for the liganded Ah receptor during its purification. The system may also be useful for analyzing other factors that influence the induction of *Cyp1a1* transcription by TCDD. For example, we have described previously a class of high-activity variant mouse hepatoma cells that overtranscribe the *Cyp1a1* gene (16, 17); in addition, Hankinson and his colleagues (18, 19) have identified an Ah receptor-positive, dominant-negative class of variants, in which the *Cyp1a1* gene fails to respond to TCDD. The homologous *in vitro* transcription system described here may be useful in analyzing the molecular basis for these variant phenotypes.

Our findings indicate that mouse hepatoma cells contain a factor(s) that can inhibit *in vitro* transcription directed by HeLa extracts. The inhibition occurs with two unrelated (adenoviral and *Cyp1a1*) templates. This finding implies that the inhibitory factor is not specific to a particular transcriptional system. The mechanism by which the inhibitory factor acts is unknown. It might inhibit transcription by binding to a specific DNA sequence, or it may interfere with other protein components of the transcriptional machinery (see, for example, refs. 20 and 21 and references therein). Inhibitors may complicate the establishment of other homologous *in vitro* transcription systems. However, our studies indicate that the technical difficulties associated with an inhibitor(s) can be overcome, permitting the establishment of an *in vitro* transcription system that mimics gene expression *in vivo*.

A template that contains a truncated promoter plus two recognition motifs for the liganded Ah receptor is sufficient to support TCDD-inducible, receptor-dependent transcription *in vitro*. This observation imposes constraints upon models for the mechanism of dioxin action. For example, our findings suggest that the only DNA-binding proteins required for the induction of *Cyp1a1* gene transcription by TCDD are the liganded Ah receptor and the TATA-binding protein TFIID (22). Like receptors for steroid hormones, the liganded Ah receptor may contribute to the formation of stable preinitiation complexes (23–25). If so, our findings suggest that, with the exception of TFIID, other proteins participate in complex formation primarily by means of protein–protein interactions.

In general, it has been relatively difficult to establish *in vitro* transcription systems that respond to inducers, such as TCDD or hormones. Furthermore, several cytochrome P-450 genes are regulated at the transcriptional level (26); our observations demonstrate the potential usefulness of *in vitro* transcription for analyzing these systems.

This work was supported by Research Grant CA 32786 from the National Cancer Institute.

1. Poland, A. & Knutson, J. C. (1982) *Annu. Rev. Pharmacol. Toxicol.* **22,** 517–554.
2. Kimbrough, R. D. & Jensen, A. A., eds. (1989) *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins, and Related Products* (Elsevier, New York), 2nd Ed.
3. Lilienfeld, D. E. & Gallo, M. A. (1989) *Epidemiol. Rev.* **11,** 28–58.
4. Whitlock, J. P., Jr. (1990) *Annu. Rev. Pharmacol. Toxicol.* **30,** 251–277.
5. Sawadogo, M. & Roeder, R. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4394–4398.
6. Jones, P. B. C., Galeazzi, D. R., Fisher, J. M. & Whitlock, J. P., Jr. (1985) *Science* **227,** 1499–1502.
7. Fisher, J. M., Jones, K. W. & Whitlock, J. P., Jr. (1989) *Mol. Carcinogen.* **1,** 216–221.
8. Gorski, K., Carneiro, M. & Schibler, U. (1986) *Cell* **47,** 767–776.
9. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed., pp. 1.42–1.43.
10. Miller, A. G., Israel, D. I. & Whitlock, J. P., Jr. (1983) *J. Biol. Chem.* **258,** 3523–3527.
11. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
12. Matsui, T., Segall, J., Weil, P. A. & Roeder, R. G. (1980) *J. Biol. Chem.* **255,** 11992–11996.
13. Whitlock, J. P., Jr., & Galeazzi, D. R. (1984) *J. Biol. Chem.* **259,** 980–985.
14. Israel, D. I. & Whitlock, J. P., Jr. (1984) *J. Biol. Chem.* **259,** 5400–5402.
15. Jones, K. W. & Whitlock, J. P., Jr. (1990) *Mol. Cell. Biol.* **10,** 5098–5105.
16. Jones, P. B. C., Miller, A. G., Israel, D. I., Galeazzi, D. R. & Whitlock, J. P., Jr. (1984) *J. Biol. Chem.* **259,** 12357–12363.
17. Hirst, M. A., Jones, K. W. & Whitlock, J. P., Jr. (1989) *Mol. Carcinogen.* **2,** 40–46.
18. Hankinson, O. (1983) *Somatic Cell Genet.* **9,** 497–514.
19. Hankinson, O., Anderson, R. D., Birren, B. W., Sander, F., Negishi, M. & Nebert, D. W. (1985) *J. Biol. Chem.* **260,** 1790–1795.
20. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. (1990) *Cell* **61,** 49–59.
21. Ohkuma, Y., Horikoshi, M., Roeder, R. G. & Desplan, C. (1990) *Cell* **61,** 475–484.
22. Nakajima, M., Horikoshi, M. & Roeder, R. G. (1988) *Mol. Cell. Biol.* **8,** 4028–4040.
23. Freedman, L. P., Yoshinaga, S. K., Vanderbilt, J. M. & Yamamoto, K. R. (1989) *Science* **245,** 298–301.
24. Klein-Hitpass, L., Tsai, S. Y., Weigel, N. L., Allan, G. F., Riley, D., Rodriguez, R., Schrader, W. T., Tsai, M. J. & O'Malley, B. W. (1990) *Cell* **60,** 247–257.
25. Kalff, M., Gross, B. & Beato, M. (1990) *Nature (London)* **344,** 360–362.
26. Gonzalez, F. J. (1989) *Pharmacol. Rev.* **40,** 243–288.