The aryl hydrocarbon receptor (AhR) repressor (AhRR) gene has been isolated and characterized from a mouse genomic library. The gene is distributed as 11 exons in a total length of about 60 kilobase pairs. Fluorescence in situ hybridization analysis has shown that the AhRR gene is located at mouse chromosome 13C2, at rat chromosome 1p11.2, and at human chromosome 5p15.3. The AhRR gene has a TATA-less promoter and several transcription start sites. In addition, putative regulatory DNA sequences such as xenobiotic responsive element (XRE), GC box, and NF-κB-binding sites have been identified in the 5′-upstream region of the AhRR gene. Transient transfection analyses of HeLa cells with reporter genes that contain deletions and point mutations in the AhRR promoter revealed that all three XREs mediated the inducible expression of the AhRR gene by 3-methylcholanthrene treatment, and furthermore, GC box sequences were indispensable for a high level of inducible expression and for constitutive expression. Moreover, by using gel mobility shift assays we were able to show that the AhR/Arnt heterodimer binds to the XREs with very low affinity, which is due to three varied nucleotides outside the XRE core sequence. We have also shown that Sp1 and Sp3 can bind to the GC boxes. Finally, both transient transfection analysis and gel mobility shift assay revealed that the AhRR gene is up-regulated by a p65/p50 heterodimer that binds to the NF-κB site when the cells have been exposed to 12-O-tetradecanoylphorbol-13-acetate, and this inducible expression was further enhanced by cotreatment of 12-O-tetradecanoylphorbol-13-acetate and 3-methylcholanthrene.

The aryl hydrocarbon receptor repressor (AhRR) is a member of the growing superfamily of bHLH/PAS transcription factors and has recently been found to work as a repressor of Ah receptor (AhR) function. AhR is an intracellular mediator of the xenobiotic signaling pathway and resides as a complex with HSP90 (1, 2), XAP2 (3, 4), and p23 (5, 6) in the cytoplasm. Xenobiotics such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3-methylcholanthrene (3MC) bind to the AhR with an extremely high affinity, and the receptor complex subsequently translocates to the nucleus, where it switches its partner molecule from HSP90 to the AhR nuclear translocator (Arnt) protein (7, 8). In the nucleus the formed AhR/Arnt heterodimer binds to the xenobiotic responsive element (XRE) sequences, which are enhancer DNA elements present in the 5′-flanking region of target genes. In addition to genes for a series of xenobiotic metabolizing enzymes, and cell cycle and growth-related factors (9), the AhRR gene has recently been identified as a target gene for the AhR/Arnt transcription factor (10).

Accordingly, AhR and AhRR constitute a regulatory loop of xenobiotic signal transduction where the liganded AhR in a heterodimer with Arnt activates expression of the AhRR gene, and the expressed AhRR, in turn, inhibits the function of AhR (10). For instance, in normal human skin fibroblasts, cytochrome P450Ia1 is not up-regulated by AhR ligands, which is probably due to the expression of AhRR (11). Besides the well-known function of the induction of drug-metabolizing enzymes, functional analyses of AhR knockout mice have revealed that AhR is involved in teratogenesis such as cleft palate and hydrenephrosis induced by TCDD (12), chemical carcinogenesis caused by benzo(a)pyrene (13), immunosuppression due to thymic involution, and hepatotoxicity caused by TCDD (14). It is also possible that AhR is involved in adverse biological effects of environmental endocrine disruptants. Actually, liganded AhR is reported to interfere with transcriptional activation of the cathepsin D gene which is regulated by the estrogen receptor (15). Despite the extensive efforts to elucidate the functional role of AhR in these biological effects, our knowledge is still far from being complete.

To understand better the AhR-mediated xenobiotic signal transduction system, it is important to characterize the AhRR gene and to elucidate the regulatory mechanisms of its expression. In the present paper, we describe the entire structure of the AhRR gene, and we have also determined its chromosomal localization.
**Structure and Expression of AhRR Gene**

location at 13C2 for the mouse, at 1p11 for the rat, and at 5p15.3 for the human gene. Transient DNA transfection experiments using a reporter gene containing the AhRR promoter sequence revealed that at least three different regulatory sequences, XRE, GC box, and NF-κB-binding site, function as enhancers and work cooperatively to augment the expression of AhRR mRNA. Moreover, gel mobility shift assay (GMSA) demonstrated that AhRR/Arm heterodimer, Sp1, Sp3, and NF-κB work as transactivation factors on their cognate DNA sequences.

**EXPERIMENTAL PROCEDURES**

**Library Screening**—A 129SV mouse genomic library (Stratagene) was screened with a full-length mouse AhRR cDNA as probe. The probe was labeled with [α-32P]dCTP by the random priming method (16). Hybridization was carried out at 65 °C for 12 h in hybridization buffer (1 × NaCl, 50 mm Tris-HCl (pH 7.5), 1 mm EDTA, 0.1% sodium N-dodecylsulfosuccinate, 1° Denhardt’s solution (17), 10 μg/ml denatured salmon sperm DNA). Filters were washed with 2 × SSC at 65 °C, as described previously (17), and subsequently exposed to x-ray film. Twenty eight positive clones were isolated from [1 × 10^6] plaques. Restriction mapping of genomic AhRR sequences in a AFKII vector was carried out using BamHI, HindIII and SacI. A part of the intron 3 that was missing in the cloned DNAs was amplified by the PCR method using the LA Taq kit (Takara) and a pair of primers mRRg-1, 5'-CAT TGC ACA GTT GGC CTA GTT GGG CTG ATG-3', and mRRg-2, 5'-TTC CTG CAC GGG GAA GCT TCT TG-3', as probes. The probe sequence revealed that at least three different regulatory sequences, XRE, GC box, and NF-κB sequence are involved. XRE, GC box, and NF-κB were used as enhancers and work cooperatively to augment the expression of AhRR mRNA.

**Gel Mobility Shift Assay (GMSA)**—Nuclear extracts were prepared from HeLa cells according to the method described previously (19) and used for GMSA. For supershift analyses, monoclonal antibodies against Sp1, Sp3, p65, and p50 (Santa Cruz Biotechnology) were separately preincubated with nuclear extracts at 0 °C for 1 h in a total volume of 10 μl of binding buffer (10 mm HEPES (pH 7.0), 100 mm NaCl, 0.1 mm EDTA, 1 mm MgCl₂, 0.1 mm leupeptin, 0.1 mg/ml bovine serum albumin) and followed by incubation with 1 μl of [32P]-labeled oligonucleotide probe (described below) at 15 °C for 20 min. The following double-stranded oligonucleotides were used as probes: XRE1 (5'-gact CGG CTC GGC TGG TGG GGT GG-3'); XRE2 (5'-gact GAG GAC CAC GCT AAG TCG TG-3'); XRES (5'-gact GAC GGC ACA CCT TAA TC); GCho(1) (5'-gact GTG CTG GAG GGC CTT CC); GCho(2) (5'-gact GTG CTG GAG GGC CTT CC); GCho(3) (5'-gact GTG CTG GAG GGC CTT CC); and human NF-κB sequence (5'-gact GTG CTG GAG GGC CTT CC) as probes. The probe sequence was labeled with [α-32P]dCTP and used as a probe. The probe was labeled with biotin-16-dUTP using nick translation and hybridized to denatured metaphase chromosomes at a final concentration of 25 ng/ml probe. Filters were washed with 2 × SSC, 0.2 mg/ml Cot-1 DNA, 2 mg/ml salmon sperm DNA, and 2 mg/ml Escherichia coli tRNA. Hybridized signals were detected with fluorescein isothiocyanate-avidin (Roche Molecular Biochemicals). Cells were counterstained with 4',6-diamidino-2-phenylindole, and slides were examined through a Nikon epifluorescence microscope equipped with a CCD camera (Photometrics, Tucson, AZ). Images were captured with Quips (Vysis) software and processed with Adobe Photoshop 3.0 software.

**RESULTS**

**Structure of the mAhRR Gene**—To isolate genomic clones of the AhRR gene, a mouse genomic library, using full-length AhRR cDNA as a hybridization probe. Twenty eight positive clones were isolated from [1 × 10^6] plaques. Restriction mapping and sequencing analyses revealed that the isolated 28 clones were mainly classified into two groups that contained 5'-half and 3'-half of the AhRR gene, respectively. Representative clones (RRG15, RRG10, RRG30, and RRG26) that covered almost the entire sequence of the AhRR gene are presented in Fig. 1. To fill in the gap between the two groups of genomic clones, the PCR method was used to amplify a unique 9-kb fragment in intron 3. The PCR product was subcloned into the pBlueScript SK(+) vector and, subsequently, subjected to sequence analysis. It was clearly demonstrated that sequences from 5' and 3' ends of the 9-kb fragment overlapped with the 3' end of the RRG10 insert and the 5' end of the RRG30 insert (data not shown), respectively, which confirmed that the amplified 9-kb fragment filled the gap between RRG10 and RRG30. The precise locations of exon-intron boundaries were determined by sequencing subcloned DNA fragments. Our data conclude that the AhRR gene is 60 kb long and consists of 11 exons split by 10 introns (Fig. 1).

**Southern Blot Analysis of Mouse Genomic DNA**—To confirm the structure of the mouse AhRR gene in chromosomal DNA, genomic DNA was digested with two different restriction enzymes and fractionated by agarose gel electrophoresis, followed by Southern blot analysis using full-length mouse AhRR cDNA as a probe. Except for the 9.6-kb band that was detected in all three digestions and probably is due to undigested DNA, only DNA fragments that were expected from the restriction map of the determined AhRR gene sequence were observed (Fig. 2). For overlapping fragments and the 1.6-kb band of two fragments.

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2 J. Mimura and Y. Fujii-Kuriyama, unpublished data.
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**FIG. 1.** Restriction map and structural organization of the mouse AhRR gene. For the genomic structure, filled and open boxes represent coding regions and 5′- and 3′-untranslated regions, respectively. For the cDNA structure, open boxes and solid lines represent the coding region, and the 5′- and 3′-untranslated regions, respectively. Characteristic structural motifs are indicated below the cDNA structure. Numbers above the exons indicate the exon number counted from the 5′ terminus. Genomic DNA clones that contain a part of AhRR gene (RRG15, RRG10, RRG30, and RRG26) are shown at the top. The gap between isolated genomic DNAs was linked by PCR method and is indicated by a broken line. The cleavage sites of restriction enzymes are indicated by HindIII (H) and BamHI (B).

**FIG. 2.** Southern blot analysis of genomic DNA. Genomic DNA isolated from 129SV mouse tail (10 μg) is cleaved with two different restriction enzymes, BamHI (lane 1), HindIII (lane 2), BamHI and HindIII (lane 3), as described under “Experimental Procedures.” The digested DNAs were electrophoresed on 1% agarose and blotted onto nylon membrane. The blotted membrane was hybridized with a 32p-labeled full-length cDNA probe as described under Experimental Procedures. The hybridized filter was then exposed to x-ray film. Molecular weights are indicated in kb to the left of the figure.

In lanes 1 and 3, the hybridization signal bands could be roughly explained by the restriction map. These results suggest that the mouse genome contains a unique gene for AhRR, which is consistent with the result of the chromosome localization (described below), and that the cloned gene reflects the intact structure for the chromosomal AhRR gene.

**Chromosomal Localization of the AhRR Gene**—By using the entire mouse cDNA as a probe, fluorescence in situ hybridization was carried out on metaphase chromosomes from mouse, rat, and human cells. Clear fluorescent signals were observed on mouse chromosome 13C2 and rat chromosome 1p11.2 (Fig. 3a), where conserved linkage homology has been identified between the two species (23–25). A single localization of the fluorescent signal confirmed the result using genomic Southern blot analysis of the mouse AhRR gene. In addition, the human AhRR gene was mapped to chromosome 5p15.3 by fluorescence in situ hybridization on the metaphase chromosomes (Fig. 3b) and by DNA blot hybridization using an isolated human AhRR genomic clone.3 The human chromosomal region carrying the AhRR gene is known to be syntenic to mouse chromosome containing 13C2.

**Promoter Sequence of the AhRR Gene**—The sequence of the promoter region of the mouse AhRR gene was determined and is shown in Fig. 4a. By using a pair of primers as described under “Experimental Procedures,” the 5′-RACE method assigned transcription start sites to the AhRR mRNA at three positions (Fig. 4a). Of the five clones isolated, one was uppermost, three were intermediate, and one lowestmost as shown. Examination of the sequence around these transcription start sites revealed that the AhRR gene lacks a TATA box sequence, which has been reported for other bHLH-PAS factor genes. Instead, multiple GC box sequences are present and that is often the case with TATA-less genes. We numbered the positions of the nucleotides by defining the position of most upstream transcription start site as +1. Survey of the promoter sequence revealed that there exist multiple potential enhancer DNA elements as follows: three XRE sequences at the 5′ terminus, and the 5′- and 3′-untranslated regions, respectively. For the cDNA structure, open boxes and solid lines represent the coding region, and the 5′- and 3′-untranslated regions, respectively. Characteristic structural motifs are indicated below the cDNA structure. Numbers above the exons indicate the exon number counted from the 5′ terminus. Genomic DNA clones that contain a part of AhRR gene (RRG15, RRG10, RRG30, and RRG26) are shown at the top. The gap between isolated genomic DNAs was linked by PCR method and is indicated by a broken line. The cleavage sites of restriction enzymes are indicated by HindIII (H) and BamHI (B).

**Promoter Activity of the AhRR Gene**—To assess the promoter activity of the AhRR gene, we constructed a reporter gene...

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(ahrr) by fusing the 5'-flanking 3.2-kb sequence of the ahrr gene with the 5' end of the luciferase structural gene. this reporter gene was transiently transfected into heLa cells, and the transfected gene displayed an enhanced expression of luciferase activity by ~7-fold compared with the control plasmid that was lacking the promoter sequence (fig. 5a, 1st and 2nd lanes), and the expression was further enhanced by 4-fold after addition of the ahrr ligand, 3mc. moreover, the inducibility (ratio of the luciferase activities with and without the inducer) was further increased about 12-fold by cotransfection of ahrr and arnt expression plasmids (fig. 5b, 2nd lane). from these results we postulate that the promoter sequence of the ahrr gene contains most, if not all, of the necessary dna elements for regulatory expression of the gene.

to determine the transcriptional activity of the putative enhancer sequences, point mutations were introduced into each of the xre sequences, and the mutated reporter gene constructs were transfected into heLa cells (fig. 5c). a mutation in each of the xre sequences caused a similar reduction in the enhanced expression of luciferase activity; moreover, the promoter that contained mutations in all three xre sequences markedly reduced the luciferase expression, although it displayed some remaining inducibility. it remained to be seen whether some remaining inducibility of all three xre mutant promoters is significant or not, because a control reporter pgl3 also displayed some inducibility for unknown reasons. to confirm the result from the mutation experiments, deletion mutants of the ahrr promoter were constructed (fig. 5b). the deletion constructs were transfected into heLa cells together with expression plasmids for ahrr and arnt, and luciferase activity was measured. the expressed activities were gradually decreased, depending on loss of the enhancer elements (fig. 5b), which confirms that all three xre sequences function as inducible enhancers.

as shown in fig. 4, an nf-kb-binding sequence is present near the transcription start sites. we were interested to examine whether this sequence functions as an inducible enhancer, and to do that, we treated transfected heLa cells with tpa. the expression of the reporter gene activity was enhanced slightly, but significantly, about 2-fold over the control level when tpa was added to the medium (fig. 6a). in a parallel experiment, 3mc treatment increased the luciferase expression by 3–4-fold. interestingly, simultaneous addition of 3mc and tpa enhanced markedly the expression of the reporter gene, indicating that the nf-xb site and the xre worked more than additively as inducible enhancers. these results were confirmed by a mutation experiment, showing that when the nf-xb sequence was mutated, the luciferase expression was not induced by tpa treatment (fig. 6a).

to confirm that the expression of endogenous ahrr mRNA is also induced by 3mc treatment, we performed rt-pcr analysis using RNAs extracted from treated cells (fig. 6b). consistent with both the results from the reporter gene analysis and the previous report (10), 3mc treatment induced a significant increase of ahrr mRNA compared with the control, and cotreatment of 3mc and tpa further enhanced the ahrr mRNA level in heLa cells. these results suggested that the nf-xb site and the xre site worked together for the inducible expression of the ahrr gene.

we next wanted to investigate the role of the involvement of the gc box sequences in regulation of the ahrr gene. we found that in absence of inducer the expression of the rr-3137Luc reporter gene was increased ~9-fold compared with the pGL3 control (fig. 7a, lanes 1 and 2). this constitutive expression of the reporter gene activity was considered to be contributed by the gc box sequences. to investigate this, we introduced point mutations into the gc boxes of rr-3137Luc either singly or in combination. when the mutant reporter genes, GCbox1 mut Luc and GCbox3 mut Luc containing base replacement on the gc box 1 and gc box 3, respectively, were transfected into heLa cells, a marked decrease in the constitutive luciferase expression was observed (fig. 7a). the reduction of the constitutive promoter activity due to point mutations in the gc box sequences also resulted in a lowered level of inducible expression by 3mc, although the induction ratio was not affected much (fig. 7b). these results indicate that the xre and the gc box sequences work cooperatively as enhancer elements and that the constitutive expression of the ahrr gene is mainly regulated by the gc box 1 and the gc box 3 sequences.

gel mobility shift analysis of various enhancer sequences—To investigate the proteins that bind to the regulatory DNA elements, gmsa was performed. as shown in fig. 8a, all the xre sequences, xrec1, xrec2, and xrec3 in the ahrr gene and the xre in the cypla1 gene, gave rise to shifted bands
with nuclear extracts from 3MC-treated HeLa cells (indicated by asterisks in Fig. 8a, lanes 5–8), and these shifted bands disappeared by competition experiments using their respective cold oligonucleotides (Fig. 8a, lanes 9–12). The shifted bands with XRE probes from the AhRR gene were rather weak (shown by asterisks) compared with the XRE from the CYP1A1.
gene. Since nuclear extracts from non-treated cells did not show any shifted bands with any of the XRE sequences (Fig. 8a, lanes 17–19), the shifted bands observed are considered to be specific for 3MC treatment. The binding of the XRE sequences of the AhRR gene with AhR/Arnt heterodimer was also confirmed in the GMSA using bacterially expressed AhR and Arnt (data not shown). GMSA using an anti-AhR antibody indicated that the shifted bands in HeLa cell nuclear extracts contained the AhR (Fig. 8a, lanes 13–16); in addition, the shifted band was also affected by an anti-Arnt antibody (data not shown). Taken together these results suggested that the AhR/Arnt heterodimer binds to the XRE sequences of the AhRR gene with a lower affinity compared with the XRE sequence in the CYP1A1 gene. From the results of the competition experiments using
AhRR XRE1 and CYP1A1 XRE as competitors (Fig. 8a, lanes 22–27), the binding affinity of AhR/Arnt to AhRR XRE1 was estimated to be one-tenth or less than the binding of the heterodimer to the CYP1A1 XRE (compare lanes 21 and 27). Since all the core sequences of these XREs matched the consensus of XRE, the nucleotide sequences outside the consensus may affect the binding affinity of the AhR/Arnt heterodimer, and this inference was found to be true. Replacement of three nucleotides immediately upstream of the AhRR XRE core sequence, according to the sequence of the CYP1A1 enhancer, remarkably augmented the binding activity to the AhR/Arnt heterodimer (Fig. 8b, lane 7). Interestingly, we found that three Ts outside the consensus are important for binding of the AhR/Arnt heterodimer to the XRE.

As shown in Fig. 4, the GC box 1 sequence from the AhRR gene contains an NF-κB sequence that overlaps in part with the GC box. GMSA experiments using HeLa cell nuclear extracts and the GC box 1 sequence as a probe gave rise to four major bands. The band with the highest mobility is considered to be nonspecific (indicated by an arrowhead), because nucleotide replacements in the specific enhancer element did not affect the binding (Fig. 9a). The band with the 2nd lowest mobility was assigned to be a complex containing NF-κB, p65, and p50, since this band disappeared by treatment with an
anti-p65 and an anti-p50 antibodies (Fig. 9a, lanes 6 and 7). Furthermore, a mutation in the NF-κB element specifically lost the binding activity as shown in the competition experiment (Fig. 9b, lane 7). The band with the lowest mobility was assigned to be a complex with Sp1, whereas Sp3 was included in the band that has the 2nd highest mobility (Fig. 9a, lanes 4 and 5), judging from analyses with anti-Sp1 and anti-Sp3 antibodies, respectively. From the immunological reactivity of the shifted bands, binding of Sp1, Sp3, and NF-κB to the GC box 1 sequence appeared to be reciprocal, probably because the two DNA elements are too closely arranged for simultaneous binding of the two factors. As shown in Fig. 9a, the GC box 2,3 sequence was also recognized by Sp1 and Sp3 with an apparently lower affinity than the GC box 1, which was estimated from the intensity of the bands. Both anti-Sp1 and anti-Sp3 antibodies erased their respective bands, indicating that the close arrangement of the GC box 2 and 3 sequence only allowed either of them to be bound by Sp1 or Sp3.

The NF-κB-binding site in the human AhRR gene differs in 2 of 10 bases compared with the mouse gene. To investigate whether NF-κB could bind to the human sequence, GMSA was performed with HeLa cell nuclear extracts that had been treated

![Diagram](image_url)
with TPA. As shown in Fig. 9c, the human sequence gave rise to a shifted band at the same position as with the mouse sequence, albeit in a lower intensity (Fig. 9c, lane 4). Moreover, this band was supershifted by treatment with anti-p65 or anti-p50 antibody.

DISCUSSION

Chromosome mapping and sequence analyses have shown that the AhRR gene is split into 11 exons in a total length of about 60 kb and that it is located on the mouse chromosome 13C2 (Figs. 1 and 3). It is also revealed that the rat AhRR gene is localized on chromosome 1p11.2 using mouse cDNA, confirming the recent results of the syntenic region between the two species (23). The human AhRR gene is mapped to chromosome 5p15.3 which is known to be syntenic to the mouse chromosome 13C2 (23). The structure of the AhRR gene is similar to other bHLH-PAS superfamily members such as AhR, HIF-1α, and Sim but different from Arnt and Arnt2. The exon-intron junctions of the AhRR gene are almost consistent with that of the AhR gene. These results suggest that the AhRR was first branched with the AhR from the Arnt groups and then diverged from the AhR gene during the evolutionary process.

No TATA box could be found in the upstream region of the AhRR gene, but instead, multiple GC box sequences were detected, which is the case of other bHLH-PAS transcription factor genes. In addition, several other regulatory elements were identified as shown in Fig. 4a. These regulatory DNA elements were mostly conserved in the promoter region of the human AhRR gene (Fig. 4b), suggesting their functional importance. Transient DNA transfection experiments using the AhRR reporter gene demonstrated a role for these regulatory elements, and we postulate that the constitutive activity of the AhRR gene is dependent on these three GC boxes. As shown in Fig. 7b, the reporter gene containing point mutations in all three GC box sequences was markedly reduced in the constitutive expression. The constitutive promoter activity of the three GC boxes was about 10-fold higher than the pGL3 control but appeared to be much weaker compared with GC boxes of other genes (data not shown). This phenomenon could be due to divergent GC box sequences of the AhRR gene. Moreover, this observation is in agreement with the fact that the AhRR mRNA level is very low under normal cell conditions.

In the feedback regulatory loop of the AhR/AhRR systems, all three XRE sequences in the 5′-flanking region of the AhRR gene were revealed to work as inducible enhancer shown by the deletion and point mutation experiments (Fig. 5). Although these XRE core sequences are in complete agreement with the XRE consensus sequence established by binding selection method (26), their affinity to nuclear extracts from HE.la cells treated with 3MC or to bacterially synthesized AhR/Arnt heterodimer was much weaker compared with the CYP1A1 XRE sequence (Fig. 8a). The base replacements outside the XRE consensus sequence demonstrate that not only the XRE core sequence but also three Ts outside the consensus are necessary for efficient binding of the AhR/Arnt heterodimer (Fig. 8b). For high level inducible expression of the AhRR gene, the GC box sequences are also important, because point mutations in the GC box sequences markedly reduced the level of the induced expression, whereas the inducibility of the gene (ratio of the level of the induced expression to the non-induced) appeared to be only moderately affected (Fig. 7b). These results indicate that enhancer effects of the XRE and the GC box sequences are more than additive in the AhRR gene. The cooperative interaction between Sp1 and AhR/Arnt heterodimer has been reported in the inducible expression of the CYP1A1 gene, which contains both XRE and GC box sequences in the promoter region (27). This cooperative enhancer effect is shown to be mediated by the physical interaction between the PAS domain of AhR/Arnt and the zinc finger domain of Sp1 (27). Interestingly, the promoter region of the AhRR gene also contains another enhancer sequence, the NF-κB site that works as inducible enhancer in response to TPA. TPA treatment of cells shows a modest increase of luciferase activity; however, when the cells were treated simultaneously with 3MC and TPA, the expression of the reporter gene was further enhanced (Fig. 6), suggesting a physical interaction between the transcription factors that bind to the XRE and the NF-κB site. A recent paper (28) has reported that AhR and RelA are capable of physical interaction, resulting in mutual suppression of transactivation of reporter genes driven by NF-κB and XRE sequences, respectively. The enhanced expression of the pNFκB-Luc reporter plasmid by a RelA expression vector was inhibited by activated AhR in the presence of the AhR ligand, β-naphthoflavone, whereas activated expression of an XRE reporter by AhR was inhibited by overexpression of RelA. The transactivation activities of AhR and NF-κB factors were mutually inhibited, probably due to sequestering through their interaction. In the case of the AhRR gene that contains both XRE and NF-κB sequences in the promoter, the simultaneous treatment with 3MC and TPA further enhanced expression of a reporter gene driven by the AhR promoter, which indicates that the NF-κB and the AhR/Arnt heterodimer interact with each other to enhance the gene expression. It is reported that many transcription factors, such as c-Jun, C/EBP, and Sp1 interact with NF-κB, which results in cooperative DNA binding and activation of gene expression, when their respective enhancer elements are correctly juxtaposed to the NF-κB site (29–32). In agreement, cooperative enhancement of the endogenous AhRR mRNA expression was actually observed in HE.la cells treated with 3MC and TPA (Fig. 6b). Finally, several cytokines such as interleukin-1β, tumor necrosis factor, and interferon-γ are reported to inhibit induced expression of CYP1A1 and CYP1A2 genes (33, 34). Since NF-κB is known to work as a downstream effector of these cytokines (35), the inhibitions of cytochrome P450 induction could be explained through the enhanced transactivation of AhR by NF-κB.

Acknowledgment—We are grateful to Hisaku Abe (Tohoku University) for technical assistance.

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J. Biol. Chem. 2001, 276:33101-33110.
doi: 10.1074/jbc.M011497200 originally published online June 21, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011497200

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