Transcriptional Regulation of the Human CYP1B1 Gene

EVIDENCE FOR INVOLVEMENT OF AN ARYL HYDROCARBON RECEPTOR RESPONSE ELEMENT IN CONSTITUTIVE EXPRESSION*

(Received for publication, August 2, 1999, and in revised form, December 9, 1999)

Stacey E. Shehin‡, Ryan O. Stephenson, and William F. Greenlee§

From the Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical School, Worcester, Massachusetts 01655-0126

The cytochrome P450 1B1 gene (CYP1B1) is expressed constitutively and is inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the human breast adenocarcinoma cell line MCF-7 but not in the human hepatoma cell line HepG2. Genomic DNA isolated from both cell lines was digested with the methylation-sensitive restriction enzyme isoschizomers Mspl and HpaII, and subjected to Southern analysis with a probe for the CYP1B1 promoter/enhancer region. Although differences were observed in methylation patterns for the CYP1B1 gene from MCF-7 and HepG2 cells, treatment with the demethylating agent 5-azacytidine (10 μM for 6 days) did not activate CYP1B1 mRNA expression in HepG2 cells. Furthermore, treatment with the histone deacetylase inhibitor trichostatin A (100 nM for 24 h) did not activate CYP1B1 mRNA expression in HepG2 cells. Comparative analysis of the constitutive expression of luciferase/B1 reporter constructs containing a series of deletions in the 5′ enhancer region indicated that in MCF-7 cells the region from –987 to –732 (relative to the transcription start site) was necessary for maximal levels of activity. Mutation of the aryl hydrocarbon receptor response elements (dioxin response elements) in this region showed that the dioxin response elements located at –833 is essential for constitutive gene expression in MCF-7 cells. In HepG2 cells, reporter gene activity was at least equal or greater than the activity observed in MCF-7 cells, which is in marked contrast to the expression of the native CYP1B1 gene. Taken together these findings indicate that the observed cell-specific differences in CYP1B1 constitutive expression are not mediated by DNA promoter/enhancer methylation, but are likely due to either 1) inaccessibility of the 5′-enhancer region in HepG2 cells to transcriptional activators due to a higher order chromatin structure that does not involve histone acetylation, or 2) the action of a repressor protein at cis-elements located outside of the –2296 to +25 region examined with the CYP1B1 reporter constructs. Furthermore, at least one of the dioxin response elements in the enhancer region is required for constitutive expression of CYP1B1.

* This work was supported by grants from the Susan G. Komen Foundation and the Research Foundation for Health and Environmental Effects. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Current address: The Procter & Gamble Co., Winton Hill Technical Center, 6100 Center Hill Ave., Cincinnati OH 45224. Recipient of National Research Service Award Fellowship GM18734.

§ To whom correspondence should be addressed: Chemical Industry Institute of Toxicology, 6 Davis Dr., Research Triangle Park, NC 27709-2137. Tel.: 919-558-1200; Fax: 919-558-1400; E-mail: Wgreenlee@ciit.org.

The human cytochrome P450 1B1 (CYP1B1) is differentially expressed between tissues, with the highest constitutive levels of mRNA detected in extrahepatic tissues such as kidney, mammary, and prostate (1, 2), and is implicated in the mediation of physiological functions as well as in bioactivation of procarcinogens (3, 4). CYP1B1 also is induced by polycyclic aromatic hydrocarbons, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).1 TCDD binds and activates the aryl hydrocarbon receptor (AhR), a member of the basic helix-loop-helix family of transcription factors. In its ligand-bound form the AhR interacts with a second basic helix-loop-helix protein, the aryl hydrocarbon receptor nuclear translocator (ARNT), and the resultant AhR/ARNT heterodimer activates gene transcription by binding to core recognition motifs contained within dioxin response elements (DREs) (5–7). Previous studies from this laboratory have identified a 190-bp enhancer region of the CYP1B1 gene promoter containing three DREs that is responsible for the maximal TCDD induction response (8). In addition, we have mapped 5′-enhancer regions of the CYP1B1 gene in the human keratinocyte cell line SCC12(c12c2) that are involved in regulation of constitutive expression (9).

There are numerous mechanisms that contribute to the regulation of tissue-specific gene transcription. These mechanisms include, but are not limited to, DNA methylation, histone acetylation, and involvement of trans-acting factors (DNA-binding proteins) that bind to cis-acting DNA regulatory elements in a cell- or tissue-specific manner. In the case of DNA methylation, it has been demonstrated that hypermethylation of the genomic DNA 5′-ward of promoter elements can repress transcription (10). Higher order chromatin structure has long been suggested to be a critical component of transcriptional regulation (11). Over the recent years, many transcription coactivators and corepressors have been identified that produce local changes in chromatin structure through histone acetylation and deacetylation activities, respectively (12). Acetylation of histones can locally destabilize nucleosomes, thereby creating a permissive state for promoter activation (13). Cell-specific patterns of gene expression also depend on combinations of sequence-specific DNA-binding proteins that bind to cis-regulatory regions (14, 15). Recent studies suggest that a growing set of transcriptional cofactors mediate communication between diverse upstream regulatory proteins and the core RNA polymerase II transcription complex (16).

The mechanisms controlling the tissue-specific transcription of CYP1B1 are not known. In the present study, we used cell culture models that reflect the differential expression of 1 The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; CHX, cyclohexamide; DRE, dioxin response element; 3′-UTR, 3′-untranslated region; bp, base pair(s); kb, kilobase(s).
CYP1B1 in human tissues to investigate the role that DNA promoter/enhancer methylation and histone acetylation may play in transcriptional regulation of CYP1B1. The activities of CYP1B1 5′-deletion/luciferase-reporter constructs were used to identify the regions and putative mechanisms regulating both activation and repression of constitutive CYP1B1 expression. Most notably, by using site-directed mutation of cis-acting regulatory elements, we show that the DRE located at –833 of the CYP1B1 transcription start site is required for constitutive expression of the gene.

**EXPERIMENTAL PROCEDURES**

**Cells and Culture Conditions**—The human hepatoblastoma cell line HepG2 (American Type Culture Collection, Manassas, VA) was cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% (v/v) fetal bovine serum, 100 units of penicillin/ml, and 100 μg of streptomycin/ml. The human breast adenocarcinoma cell line MCF-7 (American Type Culture Collection) was cultured in Dulbecco’s modified Eagle’s medium supplemented with 5% (v/v) fetal bovine serum, 1 mM sodium pyruvate, 10 ng insulin/ml, 100 units of penicillin/ml, and 100 μg of streptomycin/ml. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO₂. The cells were subcultured (1:6 ratio) every 3–4 days. HepG2 cells were used in these studies at passages 78–84 and MCF-7 cells at passages 210–216.

For induction experiments, cells were treated with solvent vehicle (0.1% (v/v) DMSO (v/v)) or TCDD (final concentration, 10 nM). For inhibition experiments, cells were digested to completion overnight at 37 °C with methylation-sensitive restriction enzyme MspI or HpaII (1.5 units of enzyme/μg of DNA). The DNA samples were then fractionated by electrophoresis in horizontal 3% (w/v) NuSieve 3:1 agarose gels (FMC, Rockland, ME), capillary transferred under alkaline conditions to a positively charged nylon membrane (Nytran® Plus, Schleicher & Schuell), and cross-linked under UV light. The membranes were pre-hybridized, and Southern blot hybridization was performed using a radiolabeled, and cross-linked under UV light. The membranes were pre-hybridized, and Southern blot hybridization was performed using a radiolabeled probe (XhoI genomic fragment, described previously) was added to a 5-ml volume of hybridization solution at a concentration of 2 × 10⁶ cpm/ml and incubated with the membranes at 65 °C overnight. Following hybridization, the membranes were washed twice with 2 × SSC (1 × SSC contains 0.15 M sodium chloride and 0.015 M sodium citrate (pH 7.0)), 0.1% (w/v) SDS; once with 0.2 × SSC, 0.1% (w/v) SDS; and once with 1.0% SSC, 0.1% (w/v) SDS at 50 °C for 15 min each wash. The membranes were exposed to autoradiographic film with an intensifying screen at –80 °C for 96 h.

**Plasmid Constructs**—Progressive 5′ deletions of the CYP1B1 gene were made by restriction digestion of the plasmid subclone Xba 4 (described above), creating eight fragments: –2926 to +25, –1336 to +25, –987 to +25, –911 to +25, –732 to +25, –474 to +25, –101 to +25, and –47 to +25. After restriction digestion, the fragments were filled in using T4 DNA polymerase (New England Biolabs, Beverly, MA) to create flush ends and subsequently ligated into the Smal site of the luciferase (luc) reporter vector pGL3Basic (Promega, Madison WI). The orientation of the inserted fragments was verified by restriction digestion and/or DNA sequencing.

Site-directed mutagenesis of the human CYP1B1 DNA regulatory elements were created in the –987 to –25 CYP1B1/luc reporter gene plasmid by oligonucleotide-directed polymerase chain reaction mutagenesis (QuikChange® site-directed mutagenesis kit, Stratagene, La Jolla, CA). The complimentary mutagenic primers are as follows (cis-acting element in boldface): DRE1 (–955 to –923), 5′-GAGCT-CCGTGAGAAG-3′; DRE2, (–870 to –842) 5′-GAGCT-CCGTGAGAAG-3′; and DRE1/E-box (–955 to –923), 5′-GAGCT-CCGTGAGAAG-3′.

**DNA Transfection and Transient Expression Assay**—Transfections were performed in triplicate in 60-mm dishes. 2.5 × 10⁴ MCF-7 or 3.5 × 10⁴ HepG2 cells were plated 24 h before transfection with 2.5 μg of CYP1B1/luc reporter gene plasmid and 2.5 μg of pCH110 (β-galactosidase expression vector) using 24 μg of Lipofectin in Opti-MEM reduced serum medium (Life Technologies). After 24 h, the medium was replaced with growth media containing 0.1% MeSO (v/v) or 10 nM TCDD and harvested after an additional 24 h. The cells were resuspended in reporter lysis buffer (Promega), and extracts were clarified by centrifugation (2 min at 12,000 × g) and assayed for β-galactosidase (23) and luciferase (24) activities. The protein concentration was quantitated using the BCA protein assay reagent (Pierce). Luciferase activity was analyzed by the test for means using a one-tailed analysis (Microsoft Excel).

**RESULTS**

Constitutive, TCDD-induced, and CHX-induced CYP1B1 Gene Expression in MCF-7 and HepG2 Cells—Previously, we...
reported that CYP1B1 displays a cell-specific pattern of constitutive and TCDD-inducible expression in two prototype human cell lines, HepG2 (nonresponsive) and ACHN (responsive) (25). Here, we confirm and extend these findings. CYP1B1 mRNA was not detected in HepG2 cells, either constitutively or after treatment with 10 nM TCDD (Fig. 1). In contrast, in MCF-7 cells, CYP1B1 mRNA was observed at low constitutive levels and was markedly induced by TCDD (Fig. 1). In MCF-7 cells, both constitutive and TCDD-induced levels of CYP1B1 mRNA were significantly enhanced in the presence of the protein synthesis inhibitor CHX, suggesting potential involvement of a labile transcriptional repressor in the regulation of this gene.

Co-treatment of HepG2 cells with TCDD and CHX resulted in the appearance of a CYP1B1 mRNA species migrating at a higher molecular weight than the 5.1-kb mRNA moiety seen in MCF-7 cells (Fig. 1). This larger mRNA species has been observed previously in these cells under the same treatment conditions and in liver tissue samples (1, 2, 25). These findings are consistent with a conclusion that the higher molecular weight species results from use of an alternate polyadenylation signal in the CYP1B1 3′-untranslated region (3′-UTR), resulting in a more stable transcript. In support of this position is the presence of four polyadenylation signals (AATAAA) in the 3′-UTR of the CYP1B1 mRNA and the previous observation that the CYP1B1 promoter in HepG2 cells displays low transcriptional activity that is not enhanced by treatment with TCDD (25).

**Characterization of the Influence of DNA Methylation and Histone Acetylation on CYP1B1 Expression**—The methylation status of the CYP1B1 promoter was analyzed by Southern blot hybridization using methylation-sensitive (HpaII) and methylation-insensitive (MspI) restriction enzymes. A 1.5-kb Xhol DNA probe was used that contained the sequence from −1311 to +216 relative to the transcription start site. In addition to the first 216 bp of the first exon (which is 371 bp in size), this region includes the 5′-regulatory flanking sequence containing the three functional DREs (from −1022 to −935) described previously (8). There are 27 consensus sites for MspI- and HpaII-digested lanes indicated that genomic DNAs were differentially methylated at these CCGG sites in MCF-7 and HepG2 cell lines (Fig. 2A).

To determine whether the observed differences in the methylation status in the promoters of the CYP1B1-responsive (MCF-7) and nonresponsive (HepG2) cells lines was a determinant of transcriptional activity for this gene, Northern blot analysis for CYP1B1 mRNA was performed in HepG2 cells treated with the methyltransferase inhibitor 5-azacytidine (17). In the presence of 5-azacytidine under conditions in which the CYP1B1 DNA was demethylated (data not shown), no change in CYP1B1 expression was detected (Fig. 2B). The potential role of histone acetylation in transcriptional repression of CYP1B1 in HepG2 cells was assessed by Northern analysis performed on 10 μg of poly(A)+ mRNAs isolated from cells treated for 24 h with 0, 5, 50, and 100 nM trichostatin A (TSA) for 24 h. β-Actin was used as a loading control.

**Cell-specific Functional Characterization of the Human CYP1B1 Promoter**—To identify 5′-flanking regions involved in cell-specific regulation of CYP1B1 gene transcription, a series of luciferase reporter constructs containing various 5′-flanking fragments (from −2296 to −101; see under “Experimental Procedures”) were transfected into MCF-7 and HepG2 cells, which differ in CYP1B1 gene expression. In MCF-7 cells, there was a progressive increase in reporter activity with increasing size of upstream fragments, with the −987/+25 fragment showing the highest activity (Fig. 4A). This increase of activity may be due to the additional recruitment of enhancer elements necessary for maximal expression of the gene. The region between −987 and −732 contains at least six known transcription factor binding sites (Fig. 5), including three DREs that bind the ligand-activated AhR/ARNT heterodimer to activate gene transcription (5, 7). These three DREs were previously shown to be necessary and sufficient for TCDD-mediated induction of CYP1B1 (8). Surprisingly, in the CYP1B1 nonresponsive HepG2 cells,
constitutive activity of the CYP1B1/luc reporter constructs is equal to or greater than that observed in MCF-7 cells, with no progressive increase in luciferase activity with larger upstream fragments (Fig. 4A). The pattern of TCDD-inducible activity for the CYP1B1/luc gene constructs supported the involvement of the DREs located 5'ward of nucleotide –732 (Fig. 4B). These results suggest that a higher order of chromatin structure may play a role in transcriptional repression of the native CYP1B1 gene, independent of histone deacetylation (Figs. 3 and 4A). It is also possible that CYP1B1 repression is mediated by the action of a repressor protein at cis-elements outside the –2296 to +25 region examined with the reporter constructs.

**DISCUSSION**

The results of this study demonstrate that methylation of the 5' promoter/enhancer region does not play a role in the repression of CYP1B1 expression in liver cells. Also, histone acetylation does not appear to be a contributing factor regulating CYP1B1 constitutive expression. Transcription initiation on protein-encoding genes is mediated by a complex array of general initiation factors that function on diverse promoters and are influenced by gene- and cell-specific activators (28). Comparison of CYP1B1 5'deletion/luciferase reporter constructs be-
Fig. 5. 5′-Enhancer region of the CYP1B1 gene containing putative cis-acting elements. Identification of cis-regulatory elements in the region from –987 to –732 that exhibited the highest constitutive reporter gene activity in MCF-7 cells. The DRE and E-box elements containing the site-directed mutations are in boldface type.

tween CYP1B1 expressing and nonexpressing cells indicates a complex mechanism of transcriptional regulation likely involving trans-acting factors that function to both activate and repress transcription in a cell-specific manner. These factors may act by binding to cis-acting regulatory regions and functioning as transcriptional enhancers or repressors directly or by interacting with DNA or DNA-binding proteins that modulate higher order chromatin structure. These studies also demonstrate that chromatin structure likely plays a major role in transcriptional regulation of the human CYP1B1 gene, as has been suggested for the mouse gene (29, 30).

Here we show in MCF-7 cells constitutively expressing CYP1B1 that at least one dioxin response element is necessary for transcriptional activation. The roles that AhR and ARNT play in regulating constitutive gene expression remain unclear. Studies of Cyp1B1 regulation in the mouse have shown a certain degree of cell specificity regarding the role of AhR. Primary bone marrow stromal cells prepared from AhR null mice constitutively express functional CYP1B1 (31), whereas fibroblasts from the same AhR+/− genotype do not express basal CYP1B1 (29). Recently, ARNT has been implicated as a repressor of Cyp1B1 basal expression in murine Hepa-1 cells, but the results are complicated by the finding that an ARNT-like protein may also be involved (30). Site-directed mutations of cis-regulatory elements that are known to interact with ARNT transcription factor heterodimers show that mutation of the E-box element alone decreased transcriptional activity, but when the 5′-flanking DRE was mutated in combination with the E-box, maximal reporter activity was observed both constitutively and upon TCDD induction (Fig. 6). These results suggest that there is a putative transcriptional activator or coactivator that interacts with the E-box motif and also imply that if there is an ARNT or ARNT-related repressor acting on the human CYP1B1 gene, it functions only in combination with the adjacent 5′-DRE. The next step will be to identify the proteins that bind to the DREs and E-box that mediate CYP1B1 expression and to determine the involvement of other cis-regulatory elements.

Although transcription initiation is a critical component in regulation of gene expression, the final product is not complete until the poly(A) tail is added and the mRNA is exported and translated into the cytoplasm. Changes in overall RNA processing efficiency or the effective strength of a particular splicing or polyadenylation site in a particular cell can serve as an important control point for gene expression in a tissue or developmental stage-specific manner. (32) Poly(A) site strength can directly influence the amount of cytoplasmic RNA produced from a transcript; therefore, changing polyadenylation efficiencies can have a profound effect on the amount and nature of a gene product (33). The cDNA for CYP1B1 contains an extraordinarily long 3′-UTR that contains multiple polyadenylation signals (1). Use of these multiple sites may be regulated or may instead reflect random use of signals with varying inherent strengths. This observation, plus the results of the Northern analysis of CHX/TCDD-treated HepG2 cells (Fig. 1), suggests that cell-specific alternative processing of the CYP1B1 mRNA may regulate the amount and/or ability of the final transcript to be translated.

The gene encoding CYP1B1 contains multiple polyadenylation signals in the 3′-UTR. The previously characterized CYP1B1 cDNA was isolated from a subclone of the human keratinocyte cell line SCC 12F (SCC12(c12c2)), which constitutively expresses CYP1B1, and its specific polyadenylation site has been identified (1). There are four additional polyadenylation splice signals in the CYP1B1 gene 3′-ward of the site identified in the cDNA clone, located at 72, 126, 459, and 509 bases more 3′-ward, respectively. It is possible that in the liver, alternative use of polyadenylation splice signals occurs. Tissue-specific alternative 3′-processing may result in an unstable mRNA that does not accumulate to detectable levels in Northern analysis using 10 μg of poly(A)+ RNA. In this regard, previous studies by this laboratory have shown that the CYP1B1 gene is transcriptionally active in HepG2 cells (at very low rates) and this rate is not significantly increased by the addition of TCDD (25). Therefore, it can be hypothesized that the observed mRNA detected in HepG2 cells treated with both TCDD and CHX may result from increased mRNA stabilization. Treatment with both an inducer and a protein synthesis inhibitor also suggests the involvement of a transcriptional repressor that is not overcome by CHX treatment alone. In addition, the magnitude of TCDD/CHX-induced activity is far less than that observed in MCF-7 cells.

This analysis of regulation of constitutive expression of the human CYP1B1 gene demonstrates that production of the final transcript is governed by cell-specific mechanisms acting at the level of transcriptional activation as well as at the level of mRNA processing. The results suggest these mechanisms likely involve protein-DNA and protein-protein interactions that affect transcription directly or by chromatin remodeling, and not by DNA methylation or histone acetylation. Because of the critical physiological and bioactivation roles of CYP1B1 (2, 4, 34, 35), it will be important to identify the factors responsible for transcriptional repression and/or cell-specific mRNA processing that may prevent transcript accumulation in liver. Knowledge of these mechanisms may allow for the use of the tissue-specific mechanisms regulating this gene to be used to activate or inactivate this gene or other genes with known tissue-type pathologies and also to enable improved prediction of target organ toxicity by metabolic products of CYP1B1.

Nucleosome positioning has been shown to repress the basal expression of Cyp1A1 in mouse hepatoma cells (36). In the transient transfections performed here, the –2296 to +25 re-
region of the CYP1B1 gene was removed from its native chromosomal position and placed into a different context of DNA/chromatin structure. This in itself may account for the high levels of reporter gene activity.

Because the –987 to –732 region of the CYP1B1 promoter showed maximal constitutive expression, this region was analyzed for known cis-acting DNA elements (Fig. 5). In addition, previous studies in this laboratory showed that reporter constructs containing the –1022 to +25 region of the CYP1B1 gene had the highest transcriptional activity in SCC12 (c12c2) cells (9). This region contains consensus sequences for potential transcription factor binding sites, including SP-1, SF-1, AP-2, and E-box. Also, this region contains the three critical DREs needed for TCDD induction. The exact nature of the involvement of these cis-acting elements in gene activation independent of exogenous aryl hydrocarbon ligand stimulation has not yet been determined, nor is it known whether the DREs functionally bind transcription factors other than the AhR/ARNT complex. There is much speculation about functional activity for the AhR in the absence of exogenous ligands. ARNT, on the other hand, is known to partner with other transcription factors and activate genes in an aryl hydrocarbon-independent manner (37, 38). It has been shown that complexes other than AhR/ARNT bind in vitro to two of the analogous DREs in this region in the mouse Cyp1B1 gene in two different cell types (29, 30). Therefore, because evidence suggests there may be a role for the DREs in constitutive gene expression, we analyzed the involvement of these elements in constitutive expression of the human CYP1B1 gene. To test the function of these elements, site-directed mutagenesis was utilized (see under “Experimental Procedures”). The mutations used in this study were previously shown to abolish binding of AhR/ARNT to the DRE (39) or the binding of ARNT with other protein partners to the E-box element (40).

In conclusion, the findings presented in this study in two human cell lines support a cell-specific regulation of CYP1B1 expression that does not involve DNA methylation or histone acetylation. Potential repressor mechanisms include changes in higher order chromatin structure not mediated by histone acetylation or the action of a repressor protein at cis-elements outside the –2296 to +25 region examined. The potential involvement of an AhR repressor, as reported recently in mice (41), cannot be ruled out.

REFERENCES
1. Sutter, T. R., Tang, Y. M., Hayes, C. L., Wo, Y.-Y. P., Jabs, E. W., Li, X., Yin, H., Cody, C. W., and Greenlee, W. P. (1994) J. Biol. Chem. 269, 13092–13099
2. Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., Guengerich, F. P., and Sutter, T. R. (1996) Cancer Res. 56, 2979–2984
Transcriptional Regulation of CYP1B1

3. Spink, D. C., Spink, B. C., Cao, J. Q., Gierthy, J. F., Hayes, C. L., Li, Y., and Sutter, T. R. (1997) J. Steroid Biochem. Mol. Biol. 62, 223–232
4. Hayes, C. L., Spink, D. C., Spink, B. C., Cao, J. Q., Walker, N. J., and Sutter, T. R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9776–9781
5. Denison, M. S., Fisher, J. M., and Whitlock, J. P. (1989) J. Biol. Chem. 264, 16478–16482
6. Reisz-Porszasz, S., and Hankinson, O. (1992) Science 256, 1193–1195
7. Denison, M. S., Fisher, J. M., and Whitlock, J. P. (1989) J. Biol. Chem. 264, 16478–16482
8. Reyes, H., Reisz-Porszasz, S., and Hankinson, O. (1992) Science 256, 1193–1195
9. Dolwick, K. M., Swanson, H. I., and Bradfield, C. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8566–8570
10. Bird, A. P. (1992) Cell 70, 5–8
11. Felsenfeld, G. (1996) Cell 86, 13–19
12. Chen, J. D., and Li, H. (1998) Crit. Rev. Eukaryotic Gene Expr. 8, 169–190
13. Wolfe, P., and Pruss, D. (1996) Curr. Biol. 6, 234–237
14. Mitchell, P. J., and Tjian, R. (1989) Science 245, 371–378
15. Maitani, T., Goodhourn, S., and Fischer, J. A. (1987) Science 236, 1237–1245
16. Mannervik, M., Nibu, Y., Zhang, H., and Levine, M. (1999) Science 284, 606–609
17. Creusot, F., Acs, G., and Christman, J. K. (1982) J. Biol. Chem. 257, 2941–2948
18. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
19. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
20. Strauss, W. M. (1995) Current Protocols in Molecular Biology, pp. 4.9.1–4.9.10, Wiley-Interscience, New York