Biochemical and Biophysical Research Communications **286,** 55–60 (2001)
doi:10.1006/bbrc.2001.5352, available online at http://www.idealibrary.com on IDEAL®

Cooperative Regulation of *CYP3A5* Gene Transcription by NF-Y and Sp Family Members

Shunsuke Iwano, Tetsuya Saito, Yoshiki Takahashi, Ken-ichi Fujita, and Tetsuya Kamataki[1]
Laboratory of Drug Metabolism, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido 060-0812, Japan

Received June 21, 2001

The purpose of this study was to clarify the mechanism(s) responsible for the transcriptional regulation of the human *CYP3A5* gene. It was found that a region from nucleotides −90 to −40 was involved in the transcriptional activity of the *CYP3A5* gene by transfection of a series of 5′-truncated promoter–luciferase chimeric genes into human hepatoma HepG2 cells. A gel shift assay using nuclear extracts prepared from HepG2 cells showed that nuclear factor-Y (NF-Y) and specificity protein (Sp) 1 and Sp3 bound to CCAAT box (−78/−68) and a basic transcription element (BTE) (−67/−46) in the *CYP3A5* gene. Furthermore, introduction of mutations in the CCAAT box, the BTE, or both elements decreased the transcriptional activity to 10, 21, or 4% of that seen with the intact gene. Thus, we conclude that the transcription of the *CYP3A5* gene is cooperatively regulated by NF-Y, Sp1, and Sp3 in HepG2 cells. © 2001 Academic Press

***Key Words:* *cis*-acting element; CCAAT box; BTE; transcriptional initiation site; luciferase assay; gel shift assay; cap site hunting method.**

Cytochrome P450 (CYP) is a heme-containing enzyme that catalyzes the oxidation of a wide variety of endogenous and exogenous compounds, including drugs, carcinogens, and other xenobiotic chemicals (1). CYP3A is the most abundant CYP expressed in the human liver and is responsible for the metabolism of more than 50% of clinically used drugs, such as dihydropyridines, cyclosporine, erythtomycin and barbiturates (2). This family of CYP is also involved in the bioactivation of carcinogens including aflatoxin B1 (3).

The human CYP3A subfamily consists of at least four members: CYP3A4 (4, 5), CYP3A5 (6, 7), CYP3A7 (8, 9), and CYP3A43 (10, 11). CYP3A4 is expressed in human adult liver (5) and intestine (12). CYP3A7 is a CYP3A isoform detected in human fetal liver (8, 9) and kidney (13). CYP3A43 is a new member of CYP3A isoform highly expressed in human prostate (11). In comparison with other CYP3A, CYP3A5 is expressed in various tissues such as human adult liver, kidney, lung and peripheral blood (6, 7, 14, 15). Therefore, the expression mechanism of the *CYP3A5* gene appears to be different from that of other *CYP3A* genes. However, the molecular mechanism controlling the constitutive expression of the *CYP3A5* gene has been poorly understood. The 5′-flanking region of the *CYP3A5* gene was isolated and sequenced by Jounaidi *et al.* (16). Subsequent study by Jounaidi *et al.* (16) demonstrated that the *CYP3A5* promoter did not show any transcriptional activity in human hepatoma HepG2 cells, although CYP3A5 mRNA was constitutively expressed in HepG2 cells (17). However, a recent study on the sequencing of the entire human *CYP3A* locus revealed that the 5′-flanking region of the *CYP3A5* gene isolated by Jounaidi *et al.* was that of the *CYP3A5* pseudo gene (11).

In the present study, we isolated and characterized the proximal promoter of the *CYP3A5* gene. We found that the expression of *CYP3A5* gene was cooperatively regulated through the CCAAT box and the BTE in HepG2 cells, and that NF-Y, Sp1 and Sp3 were responsible for the cooperative regulation.

MATERIALS AND METHODS

Cell culture. Human hepatoma HepG2 cells were purchased from RIKEN (Tsukuba, Japan). HepG2 cells were maintained in Dulbecco's modified Eagle medium (Nissui Pharmacy, Tokyo, Japan) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville,

Abbreviations used: BTE, basic transcription element; C/EBP, CCAAT/enhancer-binding protein; CYP, cytochrome P450; EGTA, ethleneglycol bis(2-aminoethyl ether) tetraacetic acid; ER6, everted repeat 6; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; HNF-3, hepatocyte nuclear factor-3; NF-1, nuclear factor-1; NF-Y, nuclear factor-Y; Sp, specificity protein; TBE, Tris borate/EDTA.

[1] To whom correspondence should be addressed at Laboratory of Drug Metabolism, Graduate School of Pharmaceutical Sciences, Hokkaido University, N12W6, Kita-ku, Sapporo, Hokkaido 060-0812, Japan. Fax: +81-11-706-4978. E-mail: kamataki@pharm.hokudai.ac.jp.

0006-291X/01 $35.00

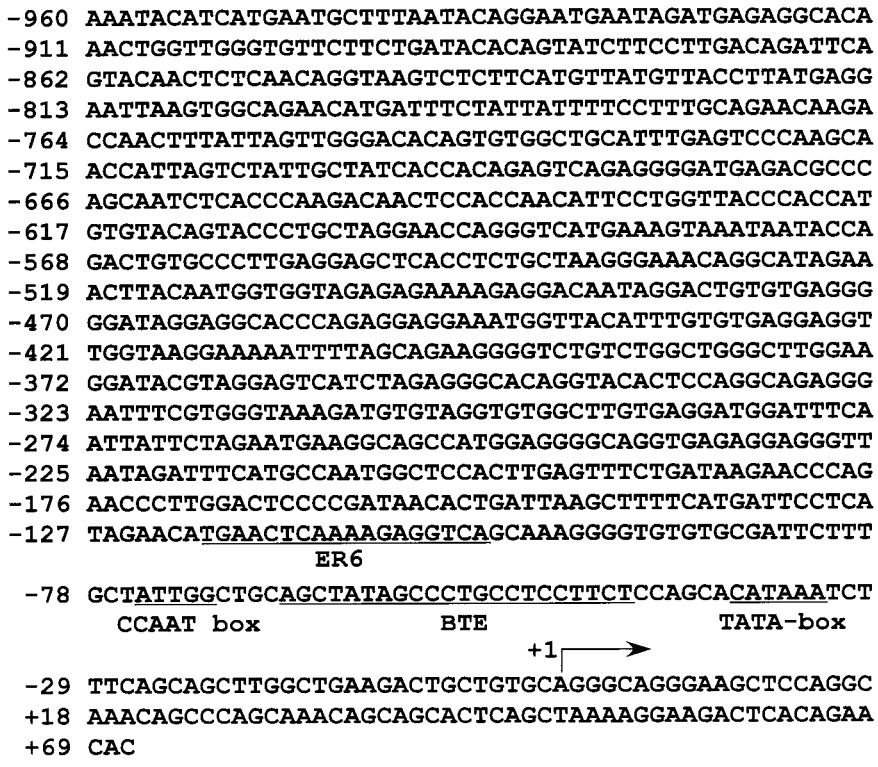

FIG. 1. Nucleotide sequence from −960 to +71 of the *CYP3A5* gene. Arrows indicate the transcriptional initiation site of the *CYP3A5* gene determined by the cap site hunting method. Underlines indicate ER6, CCAAT box, BTE, and TATA-box.

MD), nonessential amino acids (ICN, Aurora, OH) and 1 mM sodium pyruvate (Gibco BRL, Rockville, MD) in 5% CO_2 at 37°C.

Cloning of the 5′-flanking region of CYP3A5 gene. The 5′-flanking region of the *CYP3A5* gene was isolated by GenomeWalker kits (Clontech, Palo Alto, CA) following the protocol of the manufacturer. The human genomic DNA was purchased from Clontech. The primers used were as follows: 3A5GSP1, 5′-GCCAAATTTGGGATGAGGTCCATCGCC-3′; 3A5GSP2, 5′-GTGTTCTGTGAGTCTTCCTTTTAGCTGAG-3′. The PCR product was subcloned into the *Eco*RV site of the pBluescriptII KS vector (pBS-3A5) and sequenced by an Applied Biosystems Model 377A (Applied Biosystems, Foster City, CA).

Determination of transcriptional initiation site. The transcriptional initiation site in the *CYP3A5* gene was determined by using Cap site cDNA dT/human liver kits (Nippon Gene, Tokyo, Japan) following the method of the manufacturer. The first PCR was carried out with a primer specific for the *CYP3A5* gene, 5′-TTTAGGAACTTCTTAGTGCTC-3′, and a primer corresponding to a sequence of an oligo-DNA ligated to the 5′-end of cDNAs, 5′-GATGCTAGCTGCGAGTCAAGTC-3′. The PCR product thus obtained was then used as a template for the second PCR with a nested primer specific for the *CYP3A5* gene, 5′-GGAGTTGACCTTCATACGTTC-3′ and a nested primer corresponding to an oligo-DNA sequence ligated to the 5′-end of cDNAs, 5′-CGAGTCAAGTCGACGAGTGC-3′. The PCR product was inserted into a pGEM-T easy vector (Promega, Madison, WI) and sequenced with an Applied Biosystems Model 377A (Applied Biosystems, Foster City, CA).

Construction of reporter plasmids. The 5′-flanking regions of the *CYP3A5* gene from −960 to +71, from −710 to +71, from −460 to +71, from −340 to +71, from −200 to +71, from −90 to +71 and from −40 to +71 relative to the transcriptional initiation site were obtained by PCR with respective sense primers 3A5-*Bgl*II-S, 3A5-710 *Bgl*II, 3A5-460 *Bgl*II, 3A5-340 *Bgl*II, 3A5-200 *Bgl*II, 3A5-90 *Bgl*II and 3A5-40 *Bgl*II, and an antisense primer 3A5-*Bgl*II-AS, and pBS-3A5 as a template. The oligonucleotide primers used for the synthesis of DNA fragments were as follows: 3A5-*Bgl*II-S, 5′-CCGGAGATCTAAAATACATCATGAATGC-3′; 3A5-710 *Bgl*II, 5′-AAGCAGATCTTAGTCTATTGCTATCACC-3′; 3A5-460 *Bgl*II, 5′-GATAAGATCTACCCAGAGGAGGAAATGG-3′; 3A5-340 *Bgl*II, 5′-ACAGAGATCTTCCAGGCAGAGGG-3′; 3A5-200 *Bgl*II, 5′-TGGCAGATCTTGAGTTTCTGAATAAGAAC-3′; 3A5-90 *Bgl*II, 5′-GCAAAGATCTGTGTGCGATTCTTTGC-3′; 3A5-40 *Bgl*II, 5′-TTCTAGATCTCATAAATCTTTCAGCAGC-3′; 3A5-*Bgl*II-AS, 5′-TAAGAGATCTGTGTTCTGTGAGTCTTCC-3′. The respective DNA fragments thus synthesized were digested with *Bgl*II and then inserted into the *Bgl*II site of a luciferase reporter plasmid, Basic Vector 2 (Toyoinki, Tokyo, Japan), to construct reporter plasmids p3A5/-960, p3A5/-710, p3A5/-460, p3A5/-340, p3A5/-200, p3A5/-90 and p3A5/-40.

Mutations in the CCAAT box, the BTE and both of the elements in p3A5/-960 were generated by a PCR-based site-directed mutagenesis (18) using sense primers 3A5CCAAT-S, 3A5BTE-S and 3A5C/B-S, and antisense primers 3A5CCAAT-AS, 3A5BTE-AS and 3A5C/B-AS to obtain reporter plasmids, p3A5CCAATmutant, p3A5BTEmutant and p3A5C/Bmutant, respectively. The oligonucleotide primers used for site-directed mutagenesis were as follows: 3A5CCAAT-S, 5′-GCGATTCTTTGCTATGAGCTGCAGCTA-3′; 3A5BTE-S, 5′-CTATAGCGAGGCCTCCTTCT-3′; 3A5C/B-S, 5′-GCGATTCTTTGCTATGAGCTGCAGCTATAGCGAGGCCTCCTTCT-3′; 3A5CCAAT-AS, 5′-TAGCTGCAGCTCATAGCAAAGAATCGC-3′; 3A5BTE-AS, 5′-AGAAGGAGGCCTCGCTATAG; 3A5C/B-AS, 5′-AGAAGGAGGCCTCGCTATAGCTGCAGCTCATAGCAAAGAATCGC-3′.

Transient transfection and dual-luciferase assay. HepG2 cells (2×10^6 cells) were plated onto 60-mm dishes, and the cells were then transfected with a reporter plasmid (5 μg) and the pRL-SV40 (0.1 μg) (Promega, Madison, WI) as an internal control by using the methods of calcium phosphate coprecipitation (19). Four hours after the DNA transfection, cells were treated with 20% glycerol for 1.5

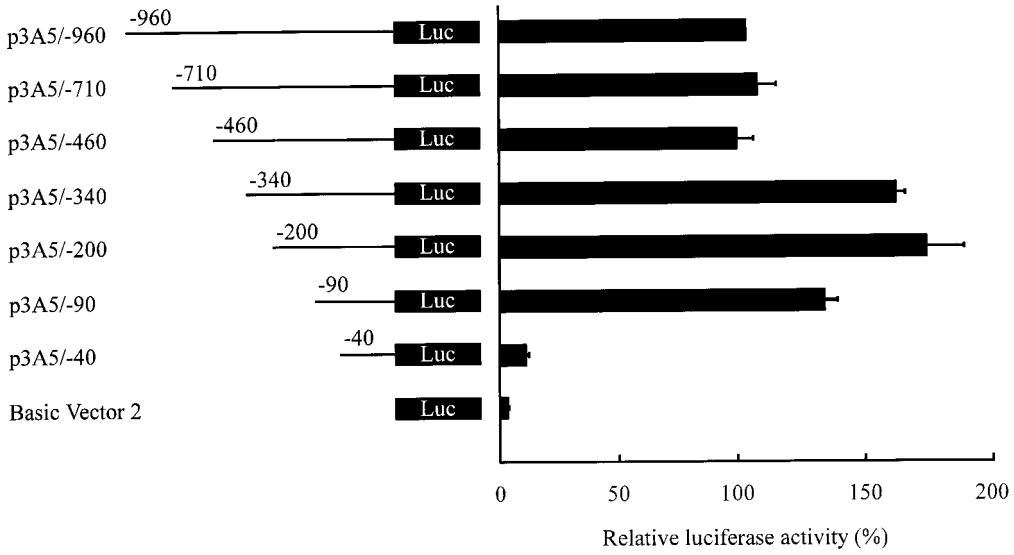

FIG. 2. Transcriptional activity of the 5′-flanking region of the *CYP3A5* gene. The methods to construct the deletion mutants are described under Materials and Methods. HepG2 cells (2×10^6 cells) were transiently transfected with a reporter plasmid (1 μg) by the calcium phosphate coprecipitation method. Cells were harvested 36 h after the DNA transfection, and luciferase activity was measured. The numbers given to deletion mutants indicate the 5′-ends of the 5′-flanking sequence of the *CYP3A5* gene counted negatively from the transcriptional initiation site. The values represent the mean ± SD of at least three independent experiments. The mean value obtained with p3A5/-960 was defined as 100%.

min. After 36 h, the cells were washed with phosphate-buffered saline, followed by a dual-luciferase assay according to the manufacturer's instructions (Promega, Madison, WI).

Gel shift assay. Nuclear extracts were prepared from HepG2 cells according to the method of Dignam *et al.* (20). The gel shift assay was performed with double-stranded synthetic oligonucleotides labeled with [γ-^{32}P]ATP (Amersham Pharmacia Biotech, Uppsala, Sweden) and T4 polynucleotide kinase (Takara, Tokyo, Japan). The binding reaction was carried out with a reaction mixture (10 μl) containing 25 mM Hepes (pH 7.9), 4% Ficoll, 40 mM KCl, 0.5 mM dithiothreitol, 0.1 mM EGTA, 1 mM $MgCl_2$, 5% glycerol, poly(dI–dC) (0.5 μg), nuclear extracts (10 μg) and a ^{32}P-labeled probe DNA (5 fmol). The mixture was incubated at 24°C for 30 min. The DNA-protein complexes formed in the mixture were resolved on a 4% non-denaturing polyacrylamide gel in 0.5× TBE at 100 V at room temperature, and visualized by autoradiography. Antibodies to Sp1 and Sp3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to NF-YA and NF-YB were kindly provided by Dr. Roberto Mantovani (Universita di Milano, Milan, Italy). Antibodies to NF-YC were a kind gift from Dr. Hiroyoshi Ariga and Dr. Takahiro Taira (Hokkaido University, Sapporo, Japan). The super-shift assay was performed using these antibodies according to the method described below. After the incubation of probe DNAs with nuclear extracts, antibodies were added to the reaction mixture and incubated at 24°C for 1 h. The products were then analyzed by a gel shift assay. Oligonucleotides used as probes were as follows: 3A5CAT, 5′-GCGATTCTTTGCTATTGGCTGCAGCTA-3′; 3A5BTE, 5′-CTATAGCCCTGCCTCCTTCT-3′; 3A5CATmut., 5′-GCGATTCTTTGCTATGAGCTGCAGCTA-3′; 3A5BTEmut., 5′-CTATAGCGAGGCCTCCTTCT-3′; HNF-3, 5′-TCTGATTATTGACTTAGTCAAG-3′; NF-Y, 5′-AAATATTTTTCTGATTGGCCAAAGAGTAAT-3′; NF1, 5′-TATTTTGGATTGAAGCCAATATGATA-3′; C/EBP, 5′-TGCAGATTGCGCAATCTGCA-3′; Sp1, 5′-ATTCGATCGGGGCGGGGCGAGC-3′.

RESULTS AND DISCUSSION

The 5′-flanking region from −960 to +71 of the *CYP3A5* gene was isolated by a genomic walking method. The nucleotide sequence of the clone was 100% identical to that of the *CYP3A5* gene reported previously (11). The transcriptional initiation site located at +31 bp downstream of the TATA-box was determined by a cap site hunting method using mRNA prepared from human adult livers (Fig. 1).

To examine whether or not a possible element(s) responsible for the transcriptional regulation of the *CYP3A5* gene existed in the region from nucleotides −960 to +71 of the *CYP3A5* gene, human hepatoma HepG2 cells were transiently transfected with a p3A5/-960 plasmid carrying a region from −960 to +71 of the *CYP3A5* gene (Fig. 2). Luciferase activity seen with the p3A5/-960 was 25-fold higher than that seen with a control plasmid (Fig. 2), indicating that a possible *cis*-acting element(s) may be present in the region from −960 to +71 of the *CYP3A5* gene. To further identify a region responsible for the transcriptional regulation of the *CYP3A5* gene, the 5′-flanking sequence of the *CYP3A5* gene was successively deleted. The deletion from −90 to −40 resulted in the decrease of the luciferase activity to 11% of that seen with p3A5/-960, suggesting that a positive regulatory element(s) existed in the region from −90 to −40 of the *CYP3A5* gene.

We found that the region from −90 to −40 of the *CYP3A5* gene contained the inverted CCAAT box and the BTE (Fig. 1). To identify a nuclear factor(s) binding to the CCAAT box and the BTE of the *CYP3A5* gene, a gel shift assay was performed by using nuclear extracts prepared from HepG2 cells. A shifted band A (complex A) appeared with the CCAAT box of the *CYP3A5* gene

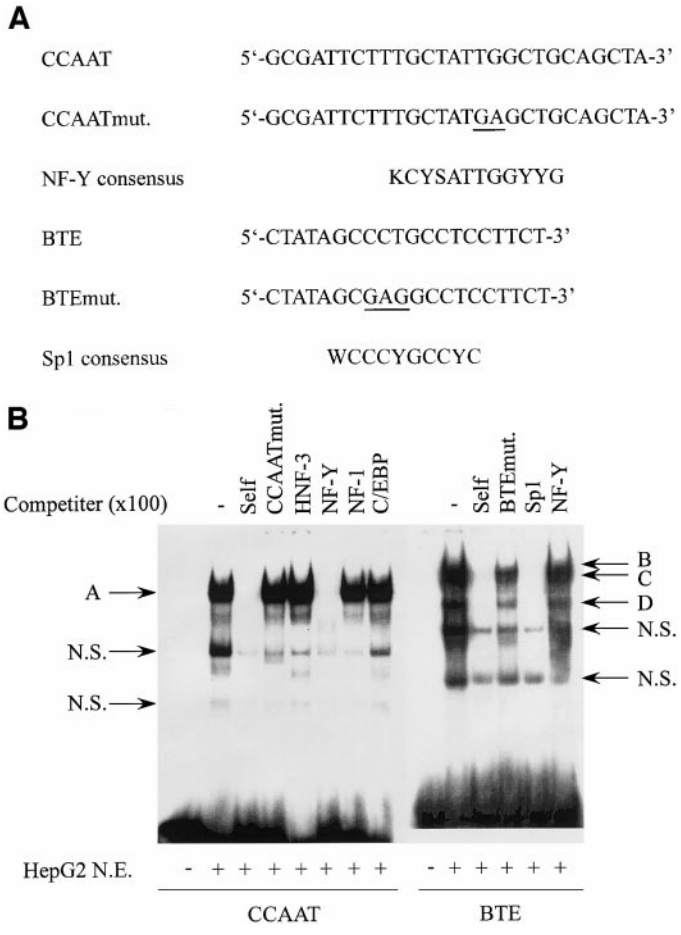

FIG. 3. Nuclear factors binding to the CCAAT box and BTE of the *CYP3A5* gene. (A) The alignment of the CCAAT box and the BTE of the *CYP3A5* gene. The sequence of the CCAAT box in the *CYP3A5* gene contains 12-base-pair domain KCYSAATTGGYGG, which is a consensus binding motif for NF-Y (from TFSEARCH). The sequence of the BTE in the *CYP3A5* gene is aligned with 10-base-pair domain WCCCYGCCYC, which is a consensus binding motif for Sp1 (from TFSEARCH). Mutations in CCAAT box and BTE are underlined. (B) Identification of a factor(s) binding to CCAAT or BTE. ^{32}P-labeled CCAAT and BTE were incubated with nuclear extracts (10 μg) prepared from HepG2 cells in the presence or absence of various competitors. The DNA complexes are shown by arrows. N.S., nonspecific band.

as a probe (defined this probe as CCAAT) (Fig. 3). Complex A disappeared by the addition of the 100-molar excess amount of the CCAAT, while the formation of complex A was not affected by the addition of the 100-molar excess amount of CCAATmut, which had two mutations within the CCAAT core sequence. This indicates that the CCAAT box is required for the formation of complex A. The CCAAT box is known to be recognized by several transcription factors such as NF-Y, NF-1 and C/EBP (21–23). Therefore, the NF-Y, NF-1, C/EBP or HNF-3 consensus site was added to a reaction mixture as a competitor. The results showed that only the NF-Y consensus site inhibited the formation of complex A, suggesting that NF-Y or a related factor(s) is bound to the CCAAT box of the *CYP3A5* gene.

Using the BTE of the *CYP3A5* gene as a probe (this probe defined as BTE), three shifted bands B, C and D were detectable (Fig. 3). In previous studies, we clarified that the BTE of the *CYP3A4* or *CYP3A7* gene was recognized by Sp family members (unpublished data). Accordingly, we hypothesized that the BTE of the *CYP3A5* gene might also be recognized by the Sp family members. Therefore, the Sp1 consensus site was added as a competitor. As was expected, three shifted bands, B, C, and D, formed in the presence of the BTE of the *CYP3A5* gene disappeared by the addition of the Sp1 consensus sequence. On the other hand, the BTE-mut, possessing three mutations within the putative Sp1 recognition site in the BTE, did not abolish the formation of the complexes, B, C, and D. The NF-Y consensus site did not eliminate the formation of the complexes, B, C, and D, indicating that members of the Sp family interact with the BTE of the *CYP3A5* gene.

To confirm the possibility of whether the nuclear factors bound to the CCAAT box or the BTE of the *CYP3A5* gene were NF-Y or members of the Sp family, a super-shift assay was performed by using antibodies raised against the three subunits of NF-Y complex, NF-YA, NF-YB and NF-YC, and the two isoforms of the Sp family, Sp1 and Sp3 (Fig. 4). The shifted band A that appeared with the CCAAT was supershifted by the addition of each antibody to NF-YA, NF-YB or NF-YC (Fig. 4), suggesting that the nuclear factor bound to the CCAAT box of the *CYP3A5* gene was

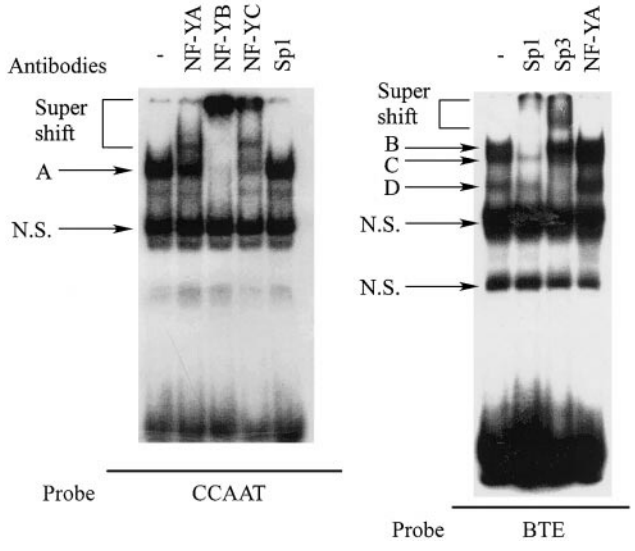

FIG. 4. A super shift assay using antibodies specific to the three subunits of NF-Y complex, Sp1, and Sp3. To identify a factor(s) binding to CCAAT or BTE, ^{32}P-labeled CCAAT or BTE was incubated with nuclear extracts (10 μg) from HepG2 cells in the presence or absence of various antibodies. The DNA–protein complexes are shown by arrows. Bracket indicates the supershifted band. N.S., nonspecific band.

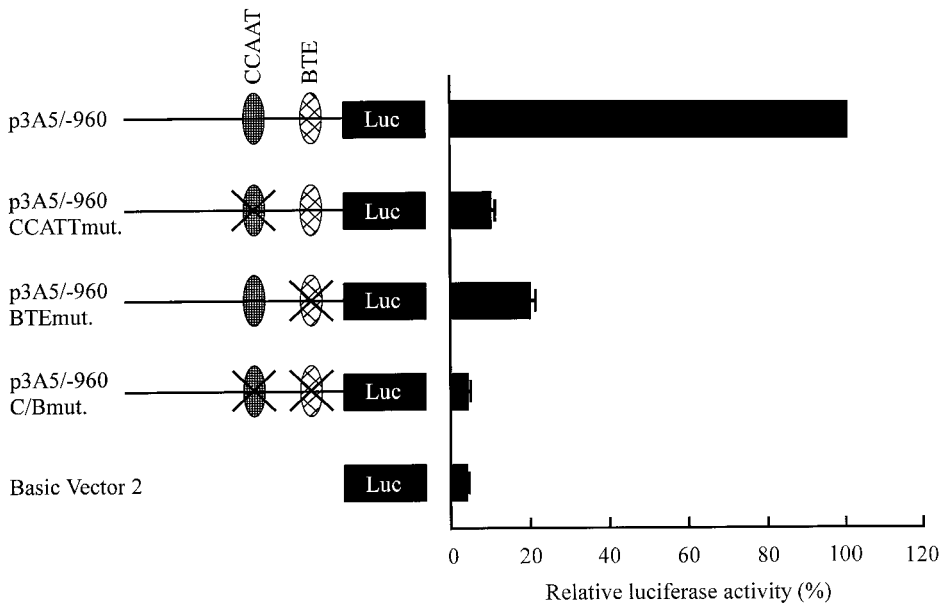

FIG. 5. Effects of mutations in CCAAT and BTE on the transcriptional activity of the *CYP3A5* promoter. The methods to construct the mutant plasmids are described under Materials and Methods. HepG2 cells (2×10^6 cells) were transiently transfected with respective reporter plasmid (1 μg) by the calcium phosphate coprecipitation method. The values represent the mean ± SD of at least three independent experiments. The mean value obtained with p3A5/-960 was defined as 100%.

NF-Y complex. When BTE was used as a probe, the addition of antibodies to Sp1 or Sp3 resulted in the super-shift of the complex B or the complexes C and D (Fig. 4), demonstrating that Sp1 and Sp3 interacted with the BTE of the *CYP3A5* gene in HepG2 cells.

Finally, to further confirm that the CCAAT box and BTE played important roles for the transcriptional activity of the *CYP3A5* gene, mutations shown in Fig. 3 were introduced into the CCAAT box, the BTE or both elements of the 5′-flanking region of *CYP3A5* gene in the p3A5/-960 reporter plasmid (Fig. 5). The respective plasmids p3A5CCAATmut, p3A5BTEmut. and p3A5C/Bmut were then transfected into HepG2 cells. The introduction of mutations in the CCAAT box or the BTE caused 90 or 79% decrease in the transcriptional activity of the *CYP3A5* gene relative to that of the intact *CYP3A5* gene. Moreover, introduction of mutations in both the CCAAT box and the BTE further lowered the transcriptional activity by 96% compared to that seen with the intact *CYP3A5* gene. The results suggest that the transcription of the *CYP3A5* gene in HepG2 cells is cooperatively regulated through the CCAAT box and the BTE.

Thus, we conclude that the expression of the *CYP3A5* gene in HepG2 cells is cooperatively regulated by the CCAAT box and the BTE present in the proximal promoter, and that NF-Y and the Sp family members are involved in this cooperative regulation.

In the present study, we also isolated the 5′-flanking region of the *CYP3A5* pseudo gene. The nucleotide sequence of the 5′-flanking region of the *CYP3A5* pseudo gene cloned by us was identical to that reported by Gellner *et al.* (11). In accordance with the previous study by Shuetz *et al.* (17), the transcriptional activity of the 5′-flanking region of the *CYP3A5* pseudo gene was not observed in HepG2 cells (data not shown). Comparing the sequences of the CCAAT box and the BTE of the *CYP3A5* gene with those of the corresponding region of the *CYP3A5* pseudo genes, mutations were found in both the CCAAT box and the BTE of the *CYP3A5* pseudo genes. Thus, it may be possible that the binding affinity of the CCAAT box and the BTE of the *CYP3A5* pseudo genes for NF-Y, Sp family members or both factors is lower than that of *CYP3A5* gene, resulting in the low transcriptional activity of *CYP3A5* pseudo genes.

It has been reported that transcription of several genes, such as the major histocompatibility complex class II-associated invariant chain (Ii) and the thymidine kinase genes (24, 25), is cooperatively regulated by NF-Y and the Sp family members. A recent study has demonstrated that the direct protein–protein interaction between the NF-YA subunit and Sp1 is responsible for the cooperativity (24). Thus, the same mechanism may be involved in the transcriptional regulation of the *CYP3A5* gene.

As mentioned in the introduction, CYP3A5 is expressed in various tissues. The expression of CYP3A5 in various tissues may be accounted for by the following facts: (1) Both of the NF-Y and the Sp family members are ubiquitous transcriptional factors and (2) The NF-Y consensus site exists only in the *CYP3A5* promoter.

ACKNOWLEDGMENTS

We thank Dr. Mantovani of Universita di Milano for generously providing anti-NF-YA and anti-NF-YB antibodies, and Dr. Hiroyoshi Ariga and Dr. Takahiro Taira of Hokkaido University for anti-NF-YC antibodies. This work was supported in part by a grant-in-aid from the Ministry of Education, Science, Sports and Culture of Japan, and by Grant No. 99-2 from the Organization for Pharmaceutical Safety and Research (OPSR).

REFERENCES

1. Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W., Gunsalus, I. C., and Nebert, D. W. (1996) P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6,** 1–42.
2. Chlerton, S., Daley, A. K., and Idle, J. R. (1992) The role of individual human cytochromes P450 in drug metabolism and clinical response. *Trends Pharmacol. Sci.* **13,** 434–439.
3. Shimada, T., and Guengerich, F. P. (1989) Evidence for cytochrome P-450NF, the nifedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. *Proc. Natl. Acad. Sci. USA* **86,** 462–465.
4. Molowa, D. T., Schuetz, E. G., Wrighton, S. A., Watkins, P. B., Kremers, P. G., Mendez-Picon, G., Parker, A., and Guzelian, P. S. (1986) Complete cDNA sequence of a cytochrome P-450 inducible by glucocorticoids in human liver. *Proc. Natl. Acad. Sci. USA* **83,** 5311–5315.
5. Beaune, P. H., Umbenhauer, D. R., Bork, R. W., Lloyd, R. S., and Guengerich, F. P. (1986) Isolation and sequence determination of a cDNA clone related to human cytochrome P-450 nifedipine oxidase. *Proc. Natl. Acad. Sci. USA* **83,** 8064–8068.
6. Schuetz, J. D., Molowa, D. T., and Guzelian, P. S. (1989) Characterization of a cDNA encoding a new member of the glucocorticoid-responsive cytochromes P450 in human liver. *Arch. Biochem. Biophys.* **274,** 355–365.
7. Aoyama, T., Yamano, S., Waxman, D. J., Lapenson, D. P., Meyer, U. A., Fischer, V., Tyndale, R., Inaba, T., Kalow, W., Gelboin, H. V., *et al.* (1989) Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J. Biol. Chem.* **264,** 10388–10395.
8. Kitada, M., Kamataki, T., Itahashi, K., Rikihisa, T., and Kanakubo, Y. (1987) P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16 alpha-hydroxylase of dehydroepiandrosterone 3-sulfate. *J. Biol. Chem.* **262,** 13534–13537.
9. Komori, M., Nishio, K., Fujitani, T., Ohi, H., Kitada, M., Mima, S., Itahashi, K., and Kamataki, T. (1989) Isolation of a new human fetal liver cytochrome P450 cDNA clone: Evidence for expression of a limited number of forms of cytochrome P450 in human fetal livers. *Arch. Biochem. Biophys.* **272,** 219–225.
10. Domanski, T. L., Finta, C., Halpert, J. R., and Zaphiropoulos, P. G. (2001) cDNA cloning and initial characterization of CYP3A43, a novel human cytochrome P450. *Mol. Pharmacol.* **59,** 386–392.
11. Gellner, K., Eiselt, R., Hustert, E., Arnold, H., Koch, I., Haberl, M., Deglmann, C. J., Burk, O., Buntefuss, D., Escher, S., Bishop, C., Koebe, H. G., Brinkmann, U., Klenk, H. P., Kleine, K., Meyer, U. A., and Wojnowski, L. (2001) Genomic organization of the human CYP3A locus: Identification of a new, inducible *CYP3A* gene. *Parmacogenetics* **11,** 111–121.
12. Watkins, P. B., Wrighton, S. A., Schuetz, E. G., Molowa, D. T., and Guzelian, P. S. (1987) Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. *J. Clin. Invest.* **80,** 1029–1036.
13. Schuetz, E. G., Schuetz, J. D., Grogan, W. M., Naray-Fejes-Toth, A., Fejes-Toth, G., Raucy, J., Guzelian, P., Gionela, K., and Watlington, C. O. (1992) Expression of cytochrome P450 3A in amphibian, rat, and human kidney. *Arch. Biochem. Biophys.* **294,** 206–214.
14. Wheeler, C. W., Wrighton, S. A., and Guenthner, T. M. (1992) Detection of human lung cytochromes P450 that are immunochemically related to cytochrome IIE1 and cytochrome IIIA. *Biochem. Pharmacol.* **44,** 183–186.
15. Janardan, S. K., Lown, K. S., Schmiedlin-Ren, P., Thummel, K. E., and Watkins, P. B. (1996) Selective expression of CYP3A5 and not CYP3A4 in human blood. *Pharmacogenetics* **6,** 379–385.
16. Jounaidi, Y., Guzelian, P. S., Maurel, P., and Vilarem, M. J. (1994) Sequence of the 5′-flanking region of CYP3A5: Comparative analysis with CYP3A4 and CYP3A7. *Biochem. Biophys. Res. Commun.* **205,** 1741–1747, doi:10.1006/bbrc.1994.2870.
17. Shuetz, J. D., Shuetz, E. G., Thottassery, J. V., Guzelian, P. S., Strom, S., and Sun, D. (1996) Identification of a novel dexamethasone responsive enhancer in the human *CYP3A5* gene and its activation in human and rat liver cells. *Mol. Pharmacol.* **49,** 63–72.
18. Higuchi, R., Krummel, B., and Saiki, R. K. (1988) A general method of *in vitro* preparation and specific mutagenesis of DNA fragments: Study of protein and DNA interactions. *Nucleic Acids Res.* **16,** 7351–7367.
19. Sambrook, J., and Russell, D. W. (2001) Introducing cloned genes into cultured mammalian cells. *In* Molecular Cloning (Crutis, S., and Zierler, M., Eds.), pp. 16.14–16.20, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
20. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11,** 1475–1489.
21. Osada, S., Yamamoto, H., Nishihara, T., and Imagawa, M. (1996) DNA binding specificity of the CCAAT/enhancer-binding protein transcription factor family. *J. Biol. Chem.* **271,** 3891–3896.
22. Zorbas, H., Rein, T., Krause, A., Hoffmann, K., and Winnacker, E. L. (1992) Nuclear factor I (NF I) binds to an NF I-type site but not to the CCAAT site in the human alpha-globin gene promoter. *J. Biol. Chem.* **267,** 8478–8484.
23. Didier, D. K., Schiffenbauer, J., Woulfe, S. L., Zacheis, M., and Schwartz, B. D. (1988) Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y box. *Proc. Natl. Acad. Sci. USA* **85,** 7322–7326.
24. Wrigh, K. L., Moore, T. L., Vilen, B. J., Brown, A. M., and Ting, J. P. (1995) Major histocompatibility complex class II-associated invariant chain gene expression is up-regulated by cooperative interactions of Sp1 and NF-Y. *J. Biol. Chem.* **270,** 20978–20986.
25. Sorensen, P., and Wintersberger, E. (1999) Sp1 and NF-Y are necessary and sufficient for growth-dependent regulation of the hamster thymidine kinase promoter. *J. Biol. Chem.* **274,** 30943–30949.