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C/EBPα Is a Regulator of the UDP Glucuronosyltransferase *UGT2B1* Gene

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ABSTRACT

The rat UDP glucuronosyltransferase, UGT2B1, is expressed in the liver where it glucuronidates steroids, environmental toxins, and carcinogens. A region between −88 and −111 base pairs upstream from the *UGT2B1* gene transcription start site contains a CCAAT enhancer binding protein (C/EBP)-like element and was previously shown by Dnase I footprint analysis to bind to proteins in both rat liver and human hepatoma (HepG2) cell nuclear extracts. In this study, the importance of this region in the regulation of the *UGT2B1* gene was assessed by functional and DNA binding assays. Varying lengths of the *UGT2B1* gene promoter, with and without the C/EBP-like element, were fused to the chloramphenicol acetyltransferase reporter gene and transfected into HepG2 cells. Transcriptional activity of the UGT2B1 promoter construct containing the C/EBP-like element was strongly elevated in the presence of a cotransfected C/EBPα expression vector. In contrast, no change was observed when an expression vector encoding C/EBPβ was cotransfected with the UGT2B1 promoter constructs. Introduction of point mutations into the C/EBP-like element prevented any C/EBPα-mediated increase in chloramphenicol acetyltransferase activity. Gel shift analyses demonstrated that the C/EBP-like element binds a complex of nuclear proteins present in both HepG2 cells and rat liver. The presence of C/EBPα in this complex was confirmed by supershift analysis with antiserum to this factor. These data strongly suggest that the liver-enriched factor C/EBPα binds to, and activates, the *UGT2B1* gene promoter. The importance of C/EBPα in the regulation of the homologous mouse *UGT2B1* gene was also assessed *in vivo*. Transcripts homologous to UGT2B1 were detected in the livers of mice containing intact *c/ebp*α and *c/ebp*β genes and in mice containing a homozygous null mutation in the *c/ebp*β gene. In contrast, these transcripts were not detected in mice with a disrupted hepatic *c/ebp*α gene. These data extend the findings with the rat *UGT2B1* gene promoter and establish that C/EBPα, but not C/EBPβ, is an essential transcriptional regulator of the homologous *UGT2B1* gene in the mouse.

Glucuronidation is an important process that modifies the biological activity of endogenous and exogenous lipophilic chemicals and enhances their rates of excretion in the urine or bile (Bock, 1991; Mulder, 1992). The UGTs that catalyze this reaction have been classified into two families, designated UGT1 and UGT2 (Mackenzie, 1995; Mackenzie, *et al.*, 1997). The latter family has been further subdivided into UGT2A forms, which glucuronidate odorants, UGT2B forms, and a rabbit UGT2C form. The UGT2B subfamily consists mainly of rat (7 forms), human (5 forms), and rabbit (3 forms) members [reviewed in Mackenzie *et al.* (1997)]. Only one UGT2B form, UGT2B5, has been characterized to date from the mouse (Kimura and Owens, 1987). UGT2B1, which is the best characterized of the rat family 2B forms, is mainly expressed in the liver and glucuronidates numerous foreign chemicals, including morphine and metabolites of the carcinogens 2-acetylaminofluorene and benzo[α]pyrene (Mackenzie *et al.*, 1993; Pritchard *et al.*, 1994). As it is also highly active toward steroids and their hydroxylated derivatives, UGT2B1 may have an important physiological role in regulating levels of circulating steroids.

To understand the mechanisms that regulate the expression of UGT2B1, we have begun to analyze the rat *UGT2B1* gene promoter. The promoter is able to drive expression of the CAT and placental alkaline phosphatase reporter genes when transfected into both rat and human hepatoma cell lines (Mackenzie and Rodbourn, 1990; Hansen *et al.*, 1997). Moreover, the liver-enriched factor, HNF1α, was shown to bind to, and activate, the UGT2B1 promoter (Hansen *et al.*, 1997). DNase I footprinting of the proximal 205 bp of this promoter revealed other protein factors that bound within

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ABBREVIATIONS: UGT, UDP glucuronosyltransferase; CAT, chloramphenicol acetyltransferase; C/EBP, CCAAT enhancer binding protein; HNF1, hepatocyte nuclear factor 1; ds, double-stranded; PCR, polymerase chain reaction; bp, base pair(s).

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this region. In particular, a region between −88 and −111 (region C) was found to be protected. Comparison of region C with a database of eukaryotic regulatory elements showed that the sequence between −91 and −99 was similar to the C/EBP consensus binding site, RTTGCGYAAY (R = A, G; Y = C, T) (Osada *et al.*, 1996).

The C/EBP family of proteins is comprised of bZIP (basic leucine zipper) proteins that have related DNA binding and dimerization properties. There are at least six members of the C/EBP family, C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, C/EBPϵ, and CHOP10 (Osada *et al.*, 1996). The liver expresses all of these isoforms and it is known that C/EBPα and C/EBPβ recognize similar DNA binding sites. C/EBPα was originally shown to be involved in regulation of liver-specific genes such as albumin, transthyretin, and α-fetoprotein (Lichtsteiner *et al.*, 1987; Costa *et al.*, 1989; Zhang *et al.*, 1991). C/EBPα is also essential for energy homeostasis as illustrated by studies in C/EBPα-null mice (Wang *et al.*, 1995). More recently, C/EBPα has been shown to be involved in expression of genes encoding drug metabolizing enzymes including cytochromes P450 (Yano *et al.*, 1992; Lee *et al.*, 1994; Tollet *et al.*, 1995) and the glutathione transferase Ya subunit (Pimental *et al.*, 1993).

The purpose of this study was to determine whether UGTs, in particular the *UGT2B1* gene, is also regulated by C/EBP transcription factors. Reporter constructs containing various lengths of the UGT2B1 promoter were transfected into HepG2 cells, together with expression vectors encoding C/EBPα and C/EBPβ. Interaction of the UGT2B1 C/EBP-like region with C/EBPα was also investigated by electrophoretic mobility shift assays. The data demonstrate that C/EBPα binds to, and activates, the UGT2B1 promoter. Furthermore, studies in C/EBPα- and C/EBPβ-deficient mice establish the primary importance of C/EBPα as a regulator of members of the *UGT2B* gene subfamily *in vivo*.

Experimental Procedures

Materials

Restriction enzymes, calf intestinal phosphatase, and Klenow enzyme were obtained from New England Biolabs (Beverly, MA). Poly(dI-dC) was purchased from Boehringer Mannheim (Indianapolis, IN), dNTPs and Dnase I from Pharmacia (Piscataway, NJ), [^{32}P]dCTP from Bresatec (Adelaide, Australia) and *Taq* polymerase from Perkin-Elmer (Norwalk, CT). The C/EBPα and C/EBPβ expression plasmids were a kind gift from Dr. Peter Johnson (NCl, Frederick, MD). Antibodies specific for C/EBPα and C/EBPβ were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Methods

Construction and expression of UGT2B1 promoter constructs. Constructs containing 5′ deletions of the −311/+14 UGT2B1 promoter fragment were generated by PCR. The following oligomers were used to define the 5′ ends of the deletion constructs: −122 (5′-TTCCATGCTTGTATTTACACA-3′); −70 (5′-TTGATGGTTTAAAAGTTATATATT-3′); and −41 (5′-TTGGGTGACTGAACTTTCAT-3′). The −122-UGT2B1 promoter construct contained region C (5′-TGTATTTACACATGGCGTAACATC-3′). An oligomer spanning −10 to +14 of the UGT2B1 gene (5′- GATATCTGTCGTTCATTGTAG-3′) was used in PCR to define the 3′ end of each of these UGT2B1 promoter deletion fragments. The region C mutant constructs were synthesized by PCR. The sequences of the forward primers containing *Hin*dIII sites (altered nucleotides underlined) was as follows: MutC1 (5′-CTTAAGCTTACACATGGCCATTCATCATT-3′), MutC2 (5′-CCATGCTTAAGCTTACACTACCGCATTCATCATT-3′). Alb-CAT was generated by PCR amplification of the promoter (− 174 to +22) of the mouse albumin gene. All fragments were subcloned into the pSV0ACAT plasmid vector that was previously restricted at the *Hin*dIII site and end-filled with Klenow enzyme. DNA sequencing was carried out on all constructs to ensure no undesired mutations had been introduced during DNA amplification by *Taq* polymerase. pSV232LUC containing the luciferase reporter gene driven by the SV40 minimal promoter, was employed as an internal control in all transfections. Conditions for transfection and for CAT and luciferase assays were as described previously by Liu and Gonzalez (1995).

Oligonucleotides. Complimentary oligonucleotides to the C/EBP-like region of the rat UGT2B1 promoter (−111 to −88) were synthesized by Life Technologies. These were designated region C (5′-TGTATTTACACATGGCGTAA-3′, 5′-GATGTTACGCCATGTGTAAAT-3′) and region C1 (5′-CACATGGCGTAACATC-3′, 5′-GATGTTACGCCATGTG-3′). A mutant oligomer to region C used in the gel shift assay was as follows: Cmut1 (5′-CACATGGCCATTCATC-3′; 5′-GATGAATGGCCATGTG-3′). Oligomers to the C/EBP consensus element (5′-TGCAGATTGCGCAATCTGCA-3′, 5′- TGCAGATTGCGCAATCTGCA-3′) were obtained from Santa Cruz Biotechnology.

Nuclear extract preparation. Nuclear extract was isolated from rat liver according to the method of Cereghini *et al.* (1987). Nuclear extracts were prepared from HepG2 cells using the method of Schreiber *et al.* (1989). Aliquots were frozen at −80° at a concentration of 1 μg/μl. Protein estimations were determined by the Bradford (1976) assay using bovine serum albumin as a standard.

Gel shift assay. After annealing of complimentary oligomers, 5′ extensions were end-filled using Klenow enzyme, dATP, dGTP, dTTP, and [^{32}P]dCTP for 20 min at room temperature. Alternatively, single-stranded oligonucleotides were end-labeled with polynucleotide kinase and [γ-^{32}P]ATP, followed by an annealing reaction. Gel shift assays were performed with 5 μg of nuclear extract in a 15-μl reaction mixture of 25 mM Tris·HCl, pH 7.6, 100 mM KCl, 0.5 mM dithiothreitol, 5 mM $MgCl_2$, 0.5 mM EDTA, 10% glycerol, and 2 μg of poly(dI-dC) together with 20,000 cpm of labeled probe (0.5–1 ng). Reactions were carried out for 20 min at room temperature. Competition assays were performed by adding 20- or 50-fold molar excess of unlabeled ds oligomer to the reaction and preincubating 5 min before adding the labeled probe. Supershifts using 1 μl of antibody were performed by incubation of the reaction at room temperature for 45 min. The reactions were resolved on 4% polyacrylamide gels in 0.5 × Tris/boric acid/EDTA (1× = 90 mM Tris/65 mM boric acid/2.5 mM EDTA, pH 8.3) at 250 V.

Analysis of mRNAs. Total RNA was isolated from mouse liver with the Ultraspec RNA reagent, according to the manufacturer's protocol (Biotec, Houston, TX). Northern analysis of the RNA (10- or 20-μg aliquots) was carried out as describe previously (Lee *et al.*, 1997a, 1997b). The hybridization probes were UGT2B1 (Mackenzie, 1986), C/EBPα, C/EBPβ, and actin cDNAs (Lee *et al.*, 1997a), and the albumin oligonucleotide 5′-CACTACAGCACTTGGTAACATGCTCACTC (Lee *et al.*, 1997a). Under the conditions of hybridization stringency used in this experiment [washes with 0.1 × standard saline/phosphate/EDTA (1× = 0.18 M NaCl, 10 mM NaH_2PO_4 and 1 mM EDTA, pH 7.7) and 1% SDS at 65° for 1 hr], the UGT2B1 cDNA probe is most unlikely to recognize UGT2B5, the only mouse UGT2B form identified to date (Kimura and Owens, 1987) as it is only 65% similar in sequence to UGT2B1. This mouse form is more similar in sequence (>80%) to the other known rat liver forms, UGT2B2, 3, 6, and 12 (Mackenzie *et al.*, 1997).

The animals used were: homozygous *c/ebp*α-loxP mice (*c/ebp*$\alpha^{fl/fl}$; *fl*, flanked by *loxP* sites); these mice contain *loxP* sites flanking both *c/ebp*α alleles and in the absence of *cre*, are indistinguishable from their wild-type counterparts (Lee *et al.*, 1997b): homozygous *c/ebp*α-loxP mice that have been infused with recombinant adenovirus carrying the *cre* gene; these mice have more than 80% of the *c/ebp*$\alpha^{fl/fl}$

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alleles specifically deleted in their livers and less than 10% of the normal C/EBPα levels (Lee *et al.*, 1997b), and *c/ebp*β−/− and +/− mice, which are homozygous and heterozygous for a null mutation at the *c/ebp*β locus (Lee *et al.*, 1997a, Sterneck *et al.*, 1997). The *c/ebp*β heterozygous animals have hepatic C/EBPβ levels similar to their wild-type littermates (Sterneck *et al.*, 1997).

Results

C/EBPα activates the *UGT2B1* gene promoter in HepG2 cells. To determine whether the C/EBP-like region contributes toward constitutive activity of the *UGT2B1* gene, 5′ deletions of the −311/+14 fragment were prepared using PCR and subcloned into the pSV0ACAT vector. These were designated −122/+14 UGT-CAT (region C present), −70/+14 UGT-CAT (region C absent) and −41/+14 UGT-CAT (regions C and B absent) (Fig. 1). The ability of these constructs to drive the CAT reporter gene was tested by transfection into human liver hepatoma HepG2 cells. In addition, a construct in which the proximal 174 base pairs of the rat albumin promoter was inserted upstream from the CAT reporter gene was used as a positive control in these experiments.

Transfection of the −41/+14 UGT-CAT, −70/+14 UGT-CAT and −122/+14 UGT-CAT constructs into HepG2 cells all resulted in weak but measurable CAT activities (Fig. 1). By comparison, HepG2 cells transfected with the albumin promoter (−174/+22) inserted upstream of the CAT reporter gene had about 4-fold greater CAT activity than that of the −122/+14 UGT-CAT construct.

To investigate the effect of C/EBP on the UGT2B1 promoter region using CAT as the reporter, expression plasmids encoding the C/EBPα and C/EBPβ transcription factors were cotransfected with the UGT-CAT constructs, −41/+14, −70/+14, and −122/+14 UGT-CAT into HepG2 cells. Cotransfections were also performed with the control plasmid containing the albumin promoter (−174/+22) inserted upstream of the *CAT* gene, as this is known to be responsive to both C/EBPα and C/EBPβ. Transfections of the −41/+14 and −70/+14 constructs with the C/EBPα expression vector both resulted in small increases in CAT activity. When the length of the promoter was increased to 122 bp and included region C, the extent of induction increased dramatically to 35-fold. By contrast, cotransfection with the C/EBPβ vector did not affect activity of either the −41/+14, −70/+14, or the −122/+14 UGT2B1 promoter CAT constructs. In the same experiments, cotransfection of the C/EBPα and C/EBPβ expression plasmids with the −174/+22 albumin promoter CAT construct resulted in increased activities of 24-fold and 7-fold, respectively.

To further delineate the sequence involved in mediating transcriptional activation of the UGT2B1 promoter by C/EBPα, point mutations were introduced into the C/EBP-like element within region C. The resultant −122/+14 fragment was subcloned into pSV0ACAT and transfected into HepG2 cells. The mutant construct, mutUGT −122/+14, contained substitutions in one half-site of the C/EBP-like element (wild-type, ATGGCGTAAC; mutant, ATGGCCATTC). When this construct was cotransfected with C/EBPα, no increase in CAT activity was observed. A second mutant construct in which both half-sites were altered was similarly cotransfected with C/EBPα and yielded the same result (not shown). These data indicate that the stimulatory effect of C/EBPα was abrogated in the −122/+14 UGT2B1 construct after sequence alteration of the region C element.

C/EPBα binds to region C of the *UGT2B1* gene promoter. As demonstrated above, the promoter activity of the −122/+14 construct containing the C/EBP-like binding site was strongly enhanced after cotransfection of an expression vector encoding C/EBPα. Moreover, in our previous work we demonstrated that the region between −83 and −111 bp upstream from the transcription start site binds nuclear proteins from both rat liver and HepG2 cells (Hansen *et al.*, 1997). To establish whether these observations correlated with binding of C/EBP proteins to region C, gel shift assays were carried out using double stranded oligomers synthesized to this region.

A ^{32}P-labeled ds region C oligomer containing the entire −111 to −88 sequence bound a complex of proteins from HepG2 nuclear extracts (Fig. 2A). Binding of the labeled ds

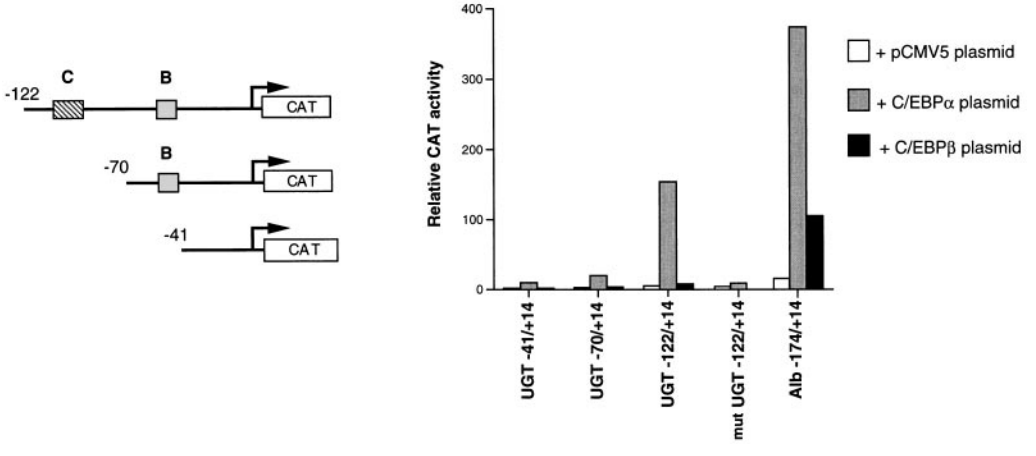

Fig. 1. Relative CAT activities of transfected UGT2B1 promoter deletion constructs with and without cotransfected expression plasmids for C/EBPα and C/EBPβ. Ten micrograms of each of the indicated UGT2B1 promoter deletion fragments, subcloned into pSV0ACAT, were transfected into HepG2 cells using calcium phosphate precipitation. These were cotransfected with 10 μg of either pCMV5 (□) or with expression plasmids encoding either C/EBPα (▨) or C/EBPβ (■). The −174/+22 Alb-CAT construct was employed for comparison of the effect of the cotransfections. Relative CAT activities are calculated by assigning the −41/+14 UGT-CAT construct a value of 1. The UGT-CAT cotransfections are the mean of two experiments. The Alb-CAT cotransfections are the means of three experiments. Values are normalized to luciferase activity. The UGT-CAT constructs are represented diagrammatically. *B*, the previously characterized HNF1-binding site; *C*, the C/EBP-like region C.

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oligomer to this complex was reduced when competed with 50-fold molar excess of the same unlabeled region C oligonucleotide. To further delineate the part of region C involved in binding C/EBPα, a shorter oligonucleotide derived from the region C sequence (Fig. 2B) and containing the entire C/EBP-like element was used in gel shift assays. A less complex pattern of bound proteins, which comigrated with the species of higher molecular mass detected by the C oligomer, was observed with this shorter oligonucleotide probe. Binding was entirely competed out with a 50-fold molar excess of either unlabeled region C or the shorter C1 oligomer.

To investigate whether the complex of proteins bound by the ds oligonucleotide contained C/EBP, excess cold oligomer containing the C/EBP consensus sequence (5′- TTGCGCAA-3′) was used in competition assays with the labeled C1 probe. As can be seen in Fig. 2B, the C/EBP oligonucleotide was as effective as the unlabeled C1 oligomer in competing for protein binding to the labeled C1 oligomer. These results indicate that the C1 oligomer binds a complex in HepG2 nuclear extract that has a high affinity for the C/EBP consensus oligomer.

To determine whether proteins bound by region C contain C/EBPα, supershift assays were performed using specific antiserum to this factor. In experiments with HepG2 nuclear extracts, the inclusion of C/EBPα antiserum decreased the mobility of some of the region C binding proteins (Fig. 3). A similar decrease in mobility was also observed when the C1 oligonucleotide was used in supershift assays. The complex binding to labeled oligomer containing the C/EBP consensus element was also supershifted (result not shown). These assays indicate that C/EBPα is present in the nuclear proteins that bind to region C.

As *UGT2B1* is a rat gene, we sought to confirm the results obtained with HepG2 nuclear extracts using extracts from rat liver nuclei. In Fig. 4, the C1 oligomer bound rat liver nuclear proteins that contained C/EBPα as assessed by supershift analysis. The extent of this binding was specifically decreased when competed with excess cold C1 and C/EBP oligomers. In addition, excess Cmut oligomer containing the same sequence as that employed in the transfection assays (see Fig. 1) failed to compete for binding to the C1 complex at concentrations that were 500-fold in molar excess. These results indicate that C/EBPα interacts with the UGT2B1 region C promoter element in both HepG2 and rat liver cells.

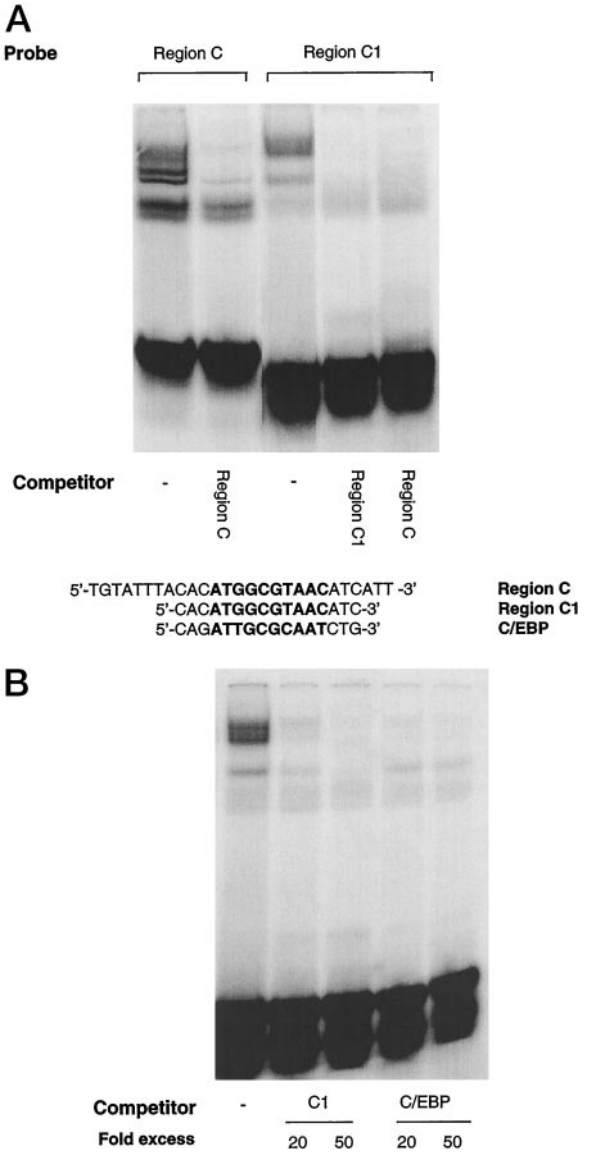

Fig. 2. A, Competition gel shift assay with the region C oligomers. ^{32}P-labeled ds oligomer probes (20,000 cpm) for each of the indicated regions were incubated with 5 μg of nuclear extract from HepG2 cells. Competition assays were performed using 20-fold molar excess of cold ds oligomers for either region C or C1. Sequences of these probes are shown. B, ^{32}P-labeled region C ds oligomer (20,000 cpm per reaction) was incubated with 5 μg of nuclear extract from HepG2 cells. Competition assays were performed using 20- and 50-fold molar excess of the indicated cold ds oligomers.

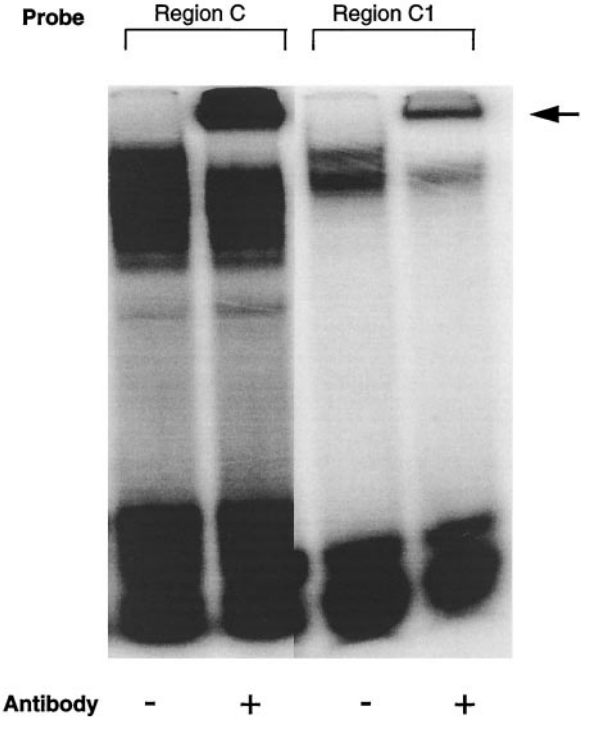

Fig. 3. Supershift assay with the region C oligomers. The ^{32}P-labeled region C and C1 oligomers (20,000 cpm) were incubated with either HepG2 extract alone, or with the addition of 1 μl of antibody to C/EBPα. Five micrograms of extract was used, and reactions containing antibody were incubated for 45 min at room temperature after addition of labeled ds oligomer. An *arrow* indicates the supershifted complex. All tracks originate from a single gel.

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C/EBPα is necessary for expression of UGT2B1 homologous transcripts in mice. Having established that C/EBPα, but not C/EBPβ, activates the *UGT2B1* gene promoter in transfected hepatoma cells, we sought to establish the importance of C/EBPα in regulating hepatic levels of transcripts homologous to UGT2B1 *in vivo*, using the mouse as a model. The hepatic expression of homologous UGT2B1 transcripts was measured in mice containing functional *c/ebpα* and *c/ebpβ* genes and in mice where these genes were disrupted. Mice with a functional *c/ebpα* gene as assessed by Northern analysis (Fig. 5A, *lanes 1–3*), also contained hepatic transcripts that hybridized to the rat UGT2B1 cDNA probe. However, mice in which the *c/ebpα* alleles were specifically disrupted in the liver by *cre*-mediated recombination after infusion of an adenovirus containing the Cre recombinase (Fig. 5A, *lanes 4–6*), had greatly diminished levels of these UGT transcripts. In contrast, the levels of albumin, C/EBPβ, and β-actin transcripts were not significantly altered by disruption of the *c/ebpα* gene (Fig. 5A). In parallel with results on transfected cells, disruption of the *c/ebpβ* gene did not alter the levels of homologous UGT2B1 transcripts in the liver (Fig. 5B).

Discussion

In this study we have shown that the rat UDP-glucuronosyltransferase 2B1 gene is specifically activated by C/EBPα and that this activation is correlated with the binding of C/EBPα to an element residing between −91 and −99 bp upstream of the *UGT2B1* gene transcription start site. Furthermore, we extend these findings to show that C/EBPα is essential for the expression of transcripts homologous to UGT2B1 in adult mouse liver.

This is the first example of the regulation of a *UGT2B* gene by a member of the C/EBP transcription factor family, and adds to our previous finding that the *UGT2B1* gene promoter also interacts with and is activated by HNF1α (Hansen *et al.*, 1997). Both C/EBP and HNF1 also interact with the early promoter of the albumin gene (Fig. 1) (Lichtsteiner *et al.*, 1987). When these two factors are simultaneously overexpressed, there is a strong synergistic effect on transcription of this gene (Wu *et al.*, 1994). It was shown that a specific C/EBPα activation domain was required for this to occur, implying an interaction between these two factors. HNF1 and C/EBP also act synergistically on the expression of the

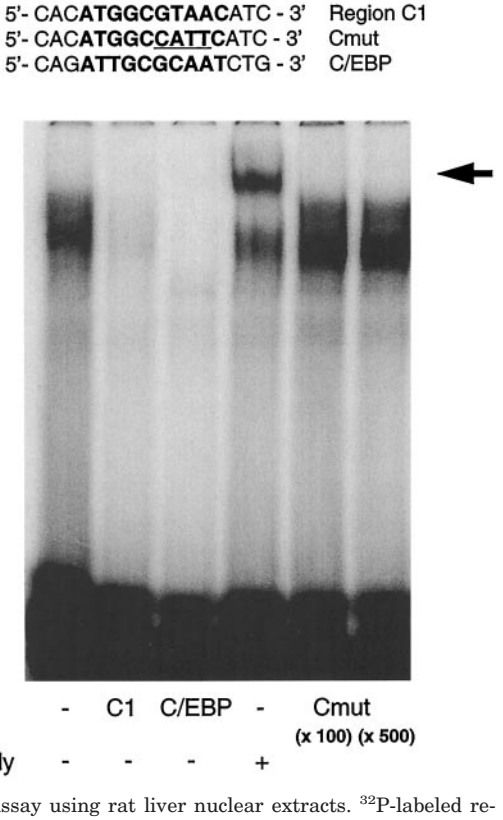

Fig. 4. Gel shift assay using rat liver nuclear extracts. ^{32}P-labeled region C1 oligomer (20,000 cpm) was incubated with rat liver nuclear extract (5 μg). Either 20-fold excess C1 ds oligomer, 20-fold excess ds C/EBP oligomer, or 100- or 500-fold cold ds Cmut oligomer (sequences as shown) were added as competitors of C1 binding. *Arrow*, complex supershifted using C/EBPα antibody.

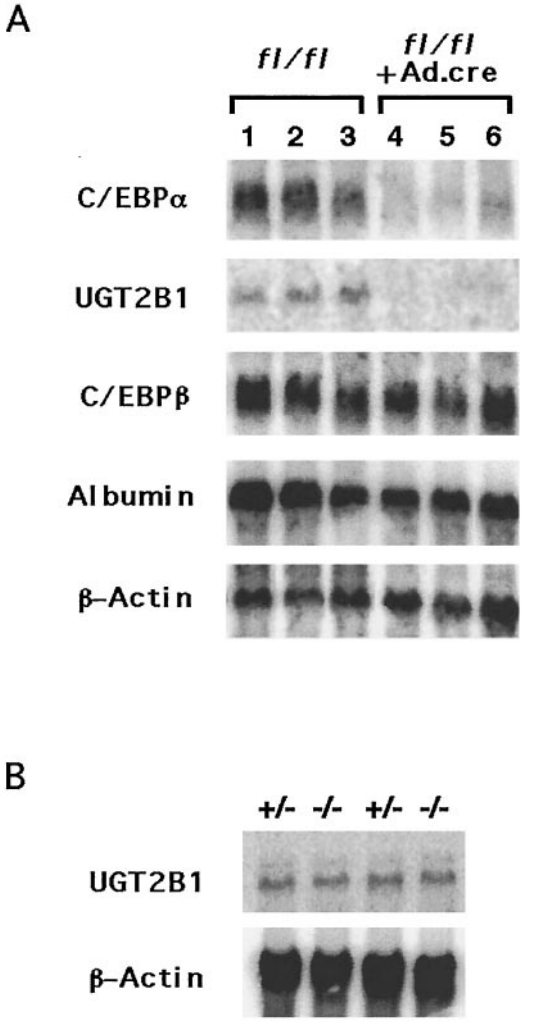

Fig. 5. Expression of homologous UGT2B1 transcripts in the livers of C/EBPα- and C/EBPβ-deficient mice. A, Total RNA (10 μg) was prepared from the livers of *c/ebpα*$^{fl/fl}$ adult mice 10 days after treatment with saline (*lanes 1–3*) or infusion with the recombinant adenovirus containing the *cre* gene (*lanes 4–6*). The RNA was denatured, electrophoresed on a formaldehyde-containing 1% agarose gel, blotted to nylon membranes, and probed with the indicated cDNA and oligonucleotide probes. B, Total RNA (20 μg) from the livers of adult mice homozygous for the *c/ebpβ* null allele (−/−) or their heterozygous littermates (+/−) was subjected to Northern analysis as indicated above. *Lanes*, RNA from individual animals.

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apolipoprotein B gene via an enhancer located within the second intron (Brooks and Levy-Wilson, 1992). However, we have not been able to observe any synergism between these two factors in the regulation of the UGT2B1 promoter in transfected HepG2 cells (Hansen AJ and Mackenzie PI, unpublished observations). Thus it is likely that one factor will not compensate for the absence of the other.

The results of gel shift assays indicate that nuclear proteins other than C/EBPα also bind to the region C sequence. Region C of the UGT2B1 promoter contains an element with 7 out of 10 nucleotides similar to the consensus C/EBP binding site. Because this includes the central CG dinucleotide seen in other bZIP protein binding sites (Vinson *et al.*, 1989; Johnson, 1993) it is likely that the C/EBP family isoforms other than C/EBPα can bind the region C element of the UGT2B1 promoter. Preliminary experiments with antisera to C/EBPβ suggest that this factor may bind to region C (Hansen AJ, unpublished observations). However, our results demonstrate that C/EBPβ does not *trans*-activate the UGT2B1 promoter in HepG2 cells and is not essential for expression of the homologous UGT2B1 transcript in the livers of mice. Genes such as albumin (Fig. 1) (Descombes *et al.*, 1990) and the human insulin-like growth factor II (Rodenburg *et al.*, 1995) in contrast, are activated by both the C/EBPα and β isoforms. The differential ability of the C/EBPα and β isoforms to *trans*-activate gene promoters has also been demonstrated in other studies. For example, only C/EBPβ was able to up-regulate activity of the CYP2D5 promoter after transfection into HepG2 cells (Lee *et al.*, 1994). This was because of a requirement for both the basic leucine zipper and the activation domains of this isoform to interact with Sp1. In a study of the α_1-acid glycoprotein gene promoter, C/EBPα was shown to occupy the acute phase response element site in control liver (Alam *et al.*, 1993). In the same study, C/EBPβ replaced C/EBPα in lipopolysaccharide-induced liver, in accordance with the increased expression of this isoform during the acute phase response. Thus, there seems to be preferential binding, and consequent modulation of transcription, by one or other of these C/EBP isoforms depending on the physiological state of the cell. Whether C/EBPβ activates the *UGT2B1* gene in a different physiological context remains to be established.

As mentioned above, synergism with HNF1 has not been detected. However, it is feasible that other non-bZIP factors may physically interact with C/EBP in modulating UGT2B1 gene transcription. For example, NF-κB was found to associate with C/EBPα, β, and γ (Stein *et al.*, 1993). This factor exerted a stimulatory effect on C/EBP activity, despite the absence of a NF-κB binding site. Similarly, the α_1-acid glycoprotein promoter is synergistically activated by C/EBPβ and the glucocorticoid receptor (Nishio *et al.*, 1993). Thus, it is possible that C/EBPα may interact with another factor(s) in the regulation of the *UGT2B1* gene and this regulation is mediated via region C.

As we had demonstrated transcriptional activation of the *UGT2B1* gene by C/EBPα in cultured hepatoma cells, we wished to confirm the importance of C/EBPα as a transcriptional regulator of UGT in the *in vivo* setting. Thus, we investigated the involvement of C/EBPα in the regulation of transcripts homologous to UGT2B1 in adult mouse liver. Using C/EBPα- and C/EBPβ-deficient mice, we demonstrated that C/EBPα, but not C/EBPβ is essential for expression of the UGT2B transcript. Thus other transcriptional factors, such as HNF1α, may not be capable of supporting UGT2B1 expression in adult liver in the absence of C/EBPα expression. Similarly, the transcript encoding the major bilirubin glucuronidating form, UGT1A1, is also greatly diminished in the livers of C/EBPα-deficient mice (Lee *et al.*, 1997b). These mice develop severe jaundice several days after infusion of the recombinant adenovirus carrying the *cre* gene as a result of elevated levels of unconjugated serum bilirubin (Lee *et al.*, 1997b). It is not known whether the early promoter of the mouse *UGT1A1* gene contains a C/EBP-like element similar to that found in rat *UGT2B1*.

These results on UGTs are in contrast to the effects on hepatic albumin and apolipoprotein D expression. Although both genes are transcriptionally activated by C/EBPα in transfected hepatoma cells, C/EBPα is not necessary for their expression *in vivo* (Fig. 5A) (Lee *et al.*, 1997b).

Studies of other genes encoding enzymes involved in the metabolism of foreign compounds have found a role for C/EBP in their regulation. The *CYP* genes shown to be regulated by C/EBP belong to the 2C and 2D subfamilies. Expression of rat CYP2C12 mRNA was shown to increase 10-fold after transfection of a C/EBPα expression vector into primary cultures (Tollet *et al.*, 1995). As described above, C/EBPβ was shown to interact directly with the transcription factor Sp1 in regulation of the rat *CYP2D5* gene (Lee *et al.*, 1994). In addition, albumin D site binding protein, a factor closely related to the bZIP family, binds with high affinity to, and *trans*-activates, the promoter of the rat *CYP2C6* gene (Yano *et al.*, 1992). Our results indicate that members of the drug detoxifying UDP glucuronosyltransferases of the 2B family can be added to the growing list of genes regulated by the family of C/EBP transcription factors.

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