0026-895X/00/050940-08$3.00/0
MOLECULAR PHARMACOLOGY

MOL 57:940–947, 2000 /13097/816880

Octamer Transcription Factor-1 Enhances Hepatic Nuclear Factor-1α-Mediated Activation of the Human UDP Glucuronosyltransferase 2B7 Promoter[1]

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Received November 12, 1999; accepted January 21, 2000

This paper is available online at http://www.molpharm.org

ABSTRACT

The human UDP glucuronosyltransferase, UGT2B7, is expressed in the liver and gastrointestinal tract, where it catalyzes the glucuronidation of steroids and bile acids. In this study, the *UGT2B7* gene was isolated and its proximal promoter was analyzed. The *UGT2B7* gene consists of 6 exons and extends over 16 kilobases (kb). It does not contain a canonical TATA box but has a region (−2 to −40) adjacent to the transcription start site that binds nuclear proteins. This region contains a consensus hepatic nuclear factor-1α (HNF1α)-binding site and an overlapping AT-rich segment. Varying lengths of the *UGT2B7* gene promoter, with and without these sites, were fused to the firefly luciferase reporter gene and transfected into HepG2 cells. UGT2B7 promoter activity with the HNF1/AT-rich element was stimulated by cotransfection with HNF1α. Additional activation was observed when HNF1α and octamer transcription factor-1 (Oct-1) were cotransfected simultaneously. However, Oct-1 alone did not stimulate promoter activity and did not bind to the promoter in the absence of HNF1α. Deletion of the HNF1/AT-rich region, or mutations in this region, abolished *UGT2B7* gene promoter activity and prevented HNF1α-mediated increases in promoter activity. The presence of HNF1α and octamer transcription factor-1 (Oct-1) in the protein complex that bound to the HNF1/AT-rich region was demonstrated by gel shift analyses with antibodies specific to HNF1α and Oct-1 protein. These results strongly suggest that the liver-enriched factor HNF1α binds to, and activates, the *UGT2B7* gene promoter and that the ubiquitous transcription factor, Oct-1, enhances this activation by directly interacting with HNF1α. This interaction between HNF1α and Oct-1 may fine-tune UGT2B7 expression.

Glucuronidation is a major pathway of drug metabolism in mammals. Many xenobiotics, including drugs and environmental pollutants, as well as endogenous substances such as bilirubin and steroid hormones, are glucuronidated and then excreted via either the urine or the bile (Mackenzie, 1995). In general, the resulting glucuronides are end-products of metabolism that have lost the pharmacological activities of the original compound. However, some glucuronides such as the potent analgesic, morphine-6-glucuronide (Shimomura et al., 1971), are more active than their parent aglycones. The UDP glucuronosyltransferases (UGTs) that catalyze this reaction have been classified into two families, designated UGT1 and UGT2 (Mackenzie, 1995; Mackenzie et al., 1997). The UGT2 family has been subdivided into UGT2A forms, which are olfactory specific, UGT2B forms, and a rabbit UGT2C form. To date, the human UGT2B subfamily consists of six forms (reviewed in Mackenzie et al., 1997). Human UGT2B7 is a major form in liver, where it catalyzes the glucuronidation of bile acids, steroids, and many foreign compounds possessing a hydroxyl or carboxylic acid moiety such as nonsteroidal anti-inflammatory drugs (Ritter et al., 1990; Jin et al., 1993, 1997). UGT2B7 contributes to the glucuronidation of 3α-hydroxysteroids (Jin et al., 1997) and appears to be the main enzyme responsible for morphine-6-glucuronide formation in humans (Coffman et al., 1997). Interindividual differences in UGT2B7 mRNA levels have been observed (Chen et al., 1993). Because these differences may be a determinant of the pharmacological significance of morphine-6-glucuronide as a potent metabolite of morphine, it is important that they are identified. An understanding of the transcriptional regulation of the *UGT2B7* gene should help to identify these factors and should provide a framework to search for regulatory polymorphisms that affect UGT2B7 expression.

[1] This work was supported by the National Health and Medical Research Council of Australia and grants from the Flinders Medical Centre and Anti-Cancer Foundations of South Australia. Part of this work was presented at the IXth International Workshop on Glucuronidation and the UDP Glucuronosyltransferases (Brisbane, Australia, Oct 21–23, 1998). P.I.M. is a National Health and Medical Council Principal Research Fellow. Y.I. is a recipient of an Overseas Fellowship from the Japan Research Foundation for Clinical Pharmacology and the Bilateral Scientist Exchange Program of the Japan Society for the Promotion of Science with the Australian Academy of Science.

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ABBREVIATIONS: UGT, UDP glucuronosyltransferase; Oct-1, octamer transcription factor-1; HNF, hepatic nuclear factor; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pair(s); DCoH, dimerization cofactor of HNF1; CREB, cAMP-response element-binding protein.

As a first step toward understanding the mechanisms that regulate UGT2B7 expression, we have isolated and characterized cosmid clones encoding the *UGT2B7* gene. The transcription start site was mapped and binding sites for nuclear proteins in the proximal promoter of the *UGT2B7* gene were identified by DNase I footprint assay. The importance of one of these binding sites, termed region A (−2 to −42) in the regulation of the *UGT2B7* gene was assessed by functional and DNA binding assays.

Experimental Procedures

Materials

Restriction enzymes and calf intestinal phosphatase 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), [^{35}S]dATP from Amersham, [γ-^{32}P]ATP from Bresatec (Adelaide, Australia), *Taq* polymerase from Perkin-Elmer (Norwalk, CT), and eLONGase from Life Technologies (Rockville, MD). pGL3-basic, pGL3-control, pRL-TK, and the Dual-Luciferase detection kit were purchased from Promega (Madison, WI). The hepatic nuclear factor (HNF)-1α and Oct-1 expression plasmids were kind gifts from Dr. Gerald Crabtree (Stanford University, Stanford, CA) and Dr. Rick Sturm (University of Queensland, Brisbane, Australia), respectively. Antibodies specific for HNF1α, HNF1β, and Oct-1, and specific blocking peptide to each antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Methods

Isolation of the *UGT2B7* Gene. Two genomic clones, COS-16 and COS-17, containing the human *UGT2B7* gene were isolated from a lymphocyte genomic DNA library constructed in the Spcos2 cosmid vector (Kimura et al., 1989) with ^{32}P-labeled UGT2B7 variant cDNA (Jin et al., 1993) as a probe. The cosmid clones were analyzed by restriction mapping. All *UGT2B* genes characterized to date have the same exon/intron structure. On the basis of this knowledge, oligonucleotides to UGT2B7 cDNA sequence were designed to amplify across putative UGT2B7 introns. The sizes of the polymerase chain reaction (PCR) products were determined on agarose gels by comparison with appropriate DNA size markers, and exon/intron boundaries were identified by sequencing the PCR products. A 1.7-kb *Hin*dIII fragment of COS-17 that contained the proximal promoter was subcloned into pBluescriptII-SK(+) (Stratagene, La Jolla, CA) and sequenced.

Determination of the UGT2B7 Transcription Start Site. The transcription start site was determined by RNase protection and primer extension analyses. RNase protection analysis was performed according to the RPA II RNase Protection Assay Kit protocol (Ambion AS, Austin, TX). Human liver polyA(+) RNA was used with and without the addition of RNase A. After denaturing at 80°C, reaction products were separated on a 6% denaturing polyacrylamide gel, together with products of known UGT2B7 DNA sequence, which were used as marker. After electrophoresis for 3 h at 60 W, gels were processed for autoradiography.

Footprint Analysis. Footprint analysis was performed according to the method of Kroeger and Abraham (1997), by using Dynabeads M-280 Streptavidin-coated beads (Dynal Inc., Oslo, Norway) and biotinylated primers. A 229-base pair (bp) fragment of the UGT2B7 promoter (−171 to +58 bp) was generated by PCR with primer sets for both the sense (5′-GCACTCATAAAGATAAAAGG-3′, biotinylated-5′-CTTGGTGCAATGCAATGCTT-3′) and anti-sense (biotinylated-5′-GCACTCATAAAGATAAAAGG-3′, 5′-CTTGGTGCAATGCAATGCTT-3′) orientations. The transcription factor binding regions were analyzed by DNase I footprint assay with human hepatoma (HepG2) cell nuclear extracts, by using 50,000 cpm of ^{32}P-labeled sense or anti-sense probe. Nuclear extracts were prepared as previously described (Hansen et al., 1997) and aliquots were stored at −80°C before use. Samples were treated with 1 U of DNase I for 5 min before isolation of the ^{32}P-labeled DNA/magnetic bead conjugate. After heating in formamide loading buffer, samples were separated on 6% acrylamide gels. Sequencing ladders were generated with the same sense and anti-sense primers used in the synthesis of the footprinting probes and run in the same gel as footprint reactions. This enabled direct determination of the regions of sequence involved in DNA-protein interactions.

Construction and Expression of UGT2B7 Promoter Constructs. Constructs containing 5′ deletions of the −800/+58 UGT2B7 promoter fragment were generated by PCR. The following oligonucleotides with *Kpn*I sites (underlined) were used to define the 5′ ends of the deletion constructs: −275 (5′- CGGGGTACCAGATCTGTCACTGCTACTG-3′), −44 (5′-CGGGGTACCAAGGGTTACATTTTAACTTCTTG-3′) and −27 (5′-CGGGGTACCTTCTTGGCTAATTTATCTTTGG-3′). An oligonucleotide spanning +39 to +57 of *UGT2B7* gene (5′-CGGGGTACCTGGTGCAATGCAATGCTTG-3′) was used in PCR to define the 3′ end of each of these UGT2B7 promoter deletion fragments. The region A mutant constructs were synthesized by PCR. In the case of the −44 m construct, the forward primer contained a mutated HNF1 site (5′-CGGGGTACCAAGGGTTACATTTGCCCTTCTTG-3′). For the −275 m construct, the HNF1 site was similarly altered by using complementary oligonucleotides (5′-AAGGGTTACATTTGCCCTTCTTG-3′ and 5′-CAAGAAGGGCAAATGTAACCCTT-3′), and the mutations were introduced by sequential PCR steps (Cormack, 1991). The PCR was performed with eLONGase (Life Technologies, Rockville, MD). The oligonucleotide primers were synthesized by Bresatec (Adelaide, Australia). All fragments were digested with *Kpn*I and subcloned into pGL3-basic vector at compatible sites (Promega, Madison, WC). DNA sequencing was carried out on all constructs to ensure that no undesired mutations had been introduced during DNA amplification by eLONGase. The pRL-TK vector containing the Herpes simplex virus thymidine kinase gene promoter was used as an internal control in all transfection experiments. Transfections were performed as previously described (Hansen et al., 1997). Cells were harvested at 68 h post-transfection, and promoter activities in cell lysates were determined by the Dual-Luciferase Reporter Assay System (Promega) in 96-well plates with a Packard TopCount luminescense and scintillation counter.

Gel Shift Assay. Complementary oligonucleotides corresponding to the HNF1/AT-rich element in region A of the *UGT2B7* gene (Fig. 2, −48 to −23, 26 bp) were synthesized by Life Technologies. These were as follows: UGT2B7 HNF1/AT (5′-TTATAAGGGTTACATTTTAACTTCTT-3′, 5′-AAGAAGTTAAAATGTAACCC-TTATAA-3′). The remaining oligonucleotides containing the consensus binding sites for HNF1, Oct-1 and cAMP-response element-binding protein (CREB), were synthesized by Bresatec (Adelaide, Australia) as follows: HNF1con (5′-TCAGGTTAATCATTAACGATCT-3′, 5′-AGATCGTTAATGATTAACCTGA-3′), Oct-1con (5′-TGTCGAATGCAAATCACTAGAA-3′, 5′-TTCTAGTGATTTGCATTCGACA-3′), Oct-1 m with a mutated Oct-1 binding site (5′-TGTCGAATGCAAGCCACTAGAA-3′, 5′-TTCTAGTGGCTTGCATTCGACA-3′), and CREB (5′-AGAGATTGCCTGACGTCAGAGAGCTAG-3′, 5′-CTAGCTCTCTGACGTCAGGCAATCTCT-3′). Double-stranded oligonucleotides were end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP after annealing of complementary oligonucleotides. Conditions for gel shift assay were as described previously by Hansen et al. (1998) with the use of 6-μg cell nuclear extracts. For supershifts, either 1 or 2 μl of antibody was added after addition of ^{32}P-labeled oligonucleotide, and samples were incubated for 20 min at room temperature. Each blocking peptide to the antibody was added before addition of ^{32}P-labeled oligonucleotide and was preincubated with nuclear extracts for 5 min at room temperature.

Results

Characterization of the *UGT2B7* Gene And Its Proximal Promoter. Cosmid clones that hybridized to ^{32}P-labeled UGT2B7 cDNA were isolated from a human genomic DNA library. Restriction mapping and sequencing was per-

formed on these clones. One genomic clone, COS-17, which contained the UGT2B7 coding region, was selected for additional analysis. Exon/intron boundaries and intron sizes were determined by PCR and sequencing to show that the *UGT2B7* gene consists of 6 exons and extends over 16 kb as depicted in Fig. 1.

A 1.7-kb restriction fragment from COS-17 that contained exon 1 of the *UGT2B7* gene was generated by *Hin*dIII digestion, subcloned into pBluescript, and sequenced. The sequence of the 0.9 kb of 5′-flanking DNA in this fragment is shown in Fig. 2. The transcription start site was mapped to an area 59 bp 5′ to the translation initiation codon by RNase protection by using human liver mRNA (Fig. 3). Although a cluster of sites was observed, the site corresponding to the more abundant protected fragment was designated the transcription start site. The position of this site was also confirmed by primer extension (data not shown) and was 2 bases 5′ to the start of the UGT2B7v cDNA (Jin et al., 1993). The *UGT2B7* gene promoter did not contain a canonical TATA box or any known transcription initiator-type elements in the proximity of the transcription start site.

Because some elements responsible for tissue-specific regulation of genes encoding drug-metabolizing enzymes have been found within 0.2 kb of the transcription start site, the possible occurrence of transcription factor binding sites in the UGT2B7 promoter (−171 to +58 bp) was assessed by DNase I footprint assays with human hepatoma (HepG2) cell nuclear extracts. Three footprints termed region A (−2 to −40), region B (−68 to −81), and region C (−89 to −114) in the UGT2B7 proximal promoter were detected (Fig. 4, underlined in Fig. 2). Region A contains overlapping HNF1 (indicated in Fig. 2) and an AT-rich, potential Oct-1 binding site as assigned by MatInspector (Quandt et al., 1995), with a core similarity setting of 0.75 and a matrix similarity setting of 0.80. Regions B and C contain possible Myb and NF-1 binding sites. The unusual position of a HNF-1 binding site so close to the transcription start site and the lack of known sequences for the binding of the transcription initiation complex prompted us to focus on region A and its importance in regulating *UGT2B7* gene promoter activity.

Functional Analysis of *UGT2B7* Gene Promoter Activity In HepG2 Cells. To determine whether the HNF1/AT-rich element in region A contributed toward *UGT2B7* gene promoter activity, various lengths of the *UGT2B7* gene promoter were prepared by PCR and subcloned into the promoterless pGL3-basic vector. The promoter constructs were designated 2B7 −275/+57 (HNF1/AT-rich element in region A, regions B and C present), 2B7 −275 m/+57 (point mutations introduced into the HNF1-like element in region A), 2B7 −44/+57 (HNF1/AT-rich element in region A present), 2B7 −44 m/+57 (point mutations introduced into the HNF1-like element in region A) and 2B7 −27/+57 (HNF1/AT-rich element absent; Fig. 5A). The ability of these constructs to drive the firefly luciferase reporter gene was tested by transfection into human liver hepatoma HepG2 cells, which contain HNF1 and Oct-1 transcription factors. The UGT2B7 constructs containing region A were active in the HepG2 cell line (Fig. 5B). The longer *UGT2B7* gene promoter (−275/+57) was about 12-fold more active than the shorter promoter (−44/+57). When region A was absent, as in the 2B7 −27/+57 construct, or mutated, as in the 2B7 −44 m/+57 and −275 m/+constructs, *UGT2B7* gene promoter activity was comparable to or less than that of the promoterless control, pGL3-basic.

Cotransfection of a HNF1α expression vector with the 2B7 −44/+57 construct containing the HNF1/AT-rich element, elevated UGT2B7 promoter activity 3-fold (Fig. 5C). In contrast, no increase in promoter activity was observed when an expression vector encoding Oct-1 was cotransfected with the 2B7 −44/+57 construct. However more than 10-fold stimulation of the promoter activity was observed when HNF1α and Oct-1 were cotransfected simultaneously with the 2B7 −44/+57 construct. Introduction of point mutations into the HNF1 element prevented any significant HNF1α-mediated increase or additional Oct-1-mediated activation in reporter expression. As expected, cotransfection of the 2B7 −27/+57 construct which lacked the HNF1 and Oct-1-like elements with either HNF1α or Oct-1 expression vectors had no effect on luciferase activity. Cotransfection of HNF1β, a transcription factor related to HNF1α, also had no effect on UGT2B7 gene promoter activity (results not shown).

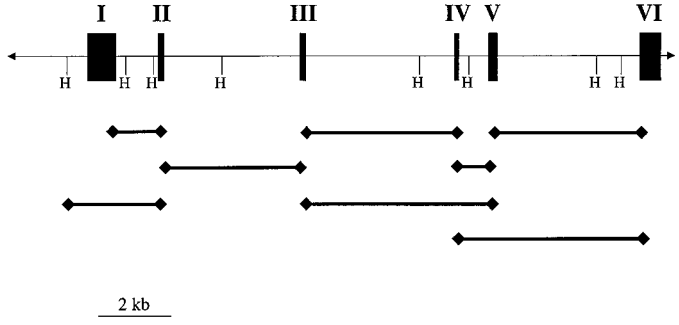

Fig. 1. Organization of the human *UGT2B7* gene. The position and sizes of the 6 exons (black boxes) and introns (connecting lines) are depicted. The PCR products that were generated using COS 17 as template and oligonucleotides to the ends of exons (black diamonds) are indicated. The length and partial sequences of these PCR products were used to determine intron lengths and intron/exon boundaries. *Hin*dIII restriction sites are denoted.

5′AAGCTTGGTTTACTTTTCTCTTTTTCTTTCTTTATCTTTGATTACTTTAAC
AAAGTTTAAAAAGATAGTGTCATCTTGAAACTCTCAATATCTTACATGATGGT
TGTAAGAATCAACATGTATACGCTATATCATAAATGAACCTTTAAAATATTAT
GTTACATAAAAGAAGCCAGTCACAAAATACCACATATTGTATGATTCTATGCA
TTAAAAATTGTCCAGAATAGGCAAATCTATAGAGATAGAAATTCAATTAGCAA
TTACCTAGGACAGGGGAAATGCAGAAACTAGGGGTGGAGAGAAAAAGGCTAAG
GACTATAGGGCTTATTTTGGGGAGATAAAAGGGCTCTCCAACTGATTGTTATG
GTAGATGCACAATTCTGTGAATATACTATGAAAACATTAAATTTTACACAATA
ATTGATAAATAGTATGATATTTAAATTACATGTCAACAAAGTTTACAAAAATA
TGTGGACCATGTTTAGTCATTTAATCTTTAGTTTTGTGTCAATGGACTGCAGA
ACAAGATCTGTCACTGCTACTGTTCTGGACACTCTTCTAAAATATATTGCATA
AGACAGATGGCATGTCCATACAAGATCCTTGATATTAGCTGAAGGATAGCACT
CATAAACATAAAACGGGAAATTAATCACATCTGTGTGAACAGATCATTTACCT
TCATTTGTCTCTTTGCCATCCACATGCTCAGACTGTTGATTTAATGATATTGT
Region C
Region B
ATGTACTTTGACTTATAAGG*GTTACATTTTAACTTCTTGGCTAATTTATCTTT*
Region A
GGACATAACCATGAGAAATGACAGAAAGGAACAGCAACTGGAAAACAAGCATT
GCATTGCACCAGGATGTCTGTGAAATGGAC-3′

Fig. 2. Nucleotide sequence of the *UGT2B7* gene proximal promoter. The three footprints (regions A, B, and C) are underlined. The translation start codon is boxed. The designated transcription start site is shown as the enlarged base. The HNF1-binding site in region A is shown in italics.

HNF1α Binds to Region A of the UGT2B7 Gene Proximal Promoter. As shown above, the HNF1/AT-rich element in region A of the human *UGT2B7* gene promoter binds nuclear proteins and is necessary for HNF1α/Oct-1-mediated enhancement of UGT2B7 promoter activity. To determine whether this region binds HNF1 specifically, gel shift analysis was carried out using ^{32}P-labeled double-stranded oligonucleotide containing the HNF1/AT-rich sequence (UGT2B7 HNF1/AT) and HepG2 nuclear extracts. Nuclear proteins that bound specifically to this oligonucleotide were detected as a doublet on autoradiographs (Fig. 6, arrow). Binding of the labeled oligonucleotide to the complex was substantially reduced, with excess unlabeled UGT2B7 HNF1/AT or HNF1-consensus oligonucleotides. In contrast, the binding was not significantly reduced in the presence of Oct-1-consensus oligonucleotide (up to 50-fold molar excess) or oligonucleotide containing point mutations (Hansen et al. 1997) that interrupted the HNF1-binding site (data not shown).

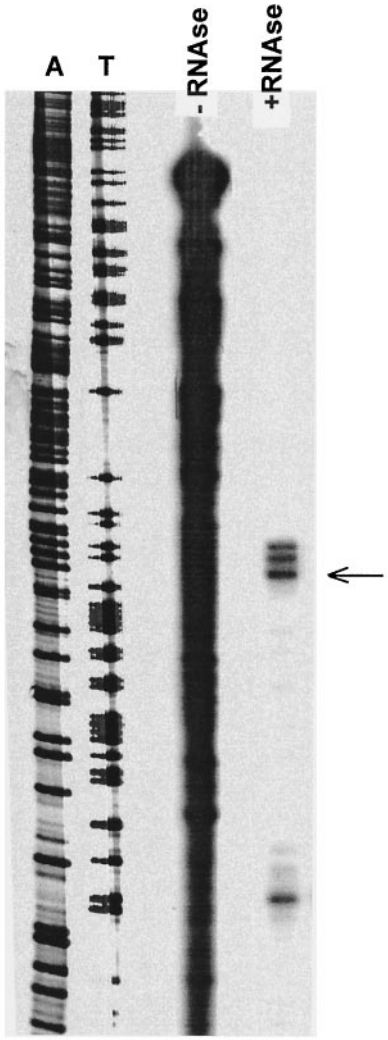

Fig. 3. RNase protection analysis of UGT2B7 RNA. Human liver poly(A+) RNA (5 μg) was incubated with ^{32}P-labeled UGT2B7 RNA transcript generated from a recombinant plasmid containing the region −45 to +166 of the *UGT2B7* gene. The sample was untreated (−) or treated with RNase A (+) and the products were separated by electrophoresis. A sequencing ladder generated from the corresponding region of the *UGT2B7* gene was used as a size marker. The protected fragment corresponding to the position of the UGT2B7 transcription start site is indicated by the arrow.

To demonstrate the binding of HNF1 protein to region A in the UGT2B7 gene promoter, gel shift analysis was performed in the presence of antibodies to HNF1α and HNF1β (Fig. 7). In the presence of antibody specific for HNF1α, a supershift in the protein complex was observed. This supershift was abolished in the presence of the HNF1α peptide that was used as an antigen to generate the HNF1α-specific antibody. However, supershifts were not affected by the HNF1β peptide. Furthermore, a supershift was not observed with the HNF1β-specific antibody. These data indicate that HNF1α but not HNF1β binds to region A and are in accord with the demonstration that only HNF1α transactivates the UGT2B7 gene proximal promoter.

Oct-1 Is Part of the Protein Complex That Binds to Region A in the *UGT2B7* Gene Proximal Promoter. As demonstrated by the functional assays described previously, Oct-1 only activates the UGT2B7 promoter in the presence of HNF1α. To determine whether Oct-1 is part of the protein complex that binds to region A, supershift analysis with specific antibody to Oct-1 was performed. Specificity of the Oct-1 antibody was first demonstrated by its capacity to recognize the protein binding to an oligonucleotide containing a consensus Oct-1 binding site and its inability to recognize protein that binds to an unrelated site, a consensus cAMP response element binding site (Fig. 8). This antibody caused a supershift in part of the protein complex that binds

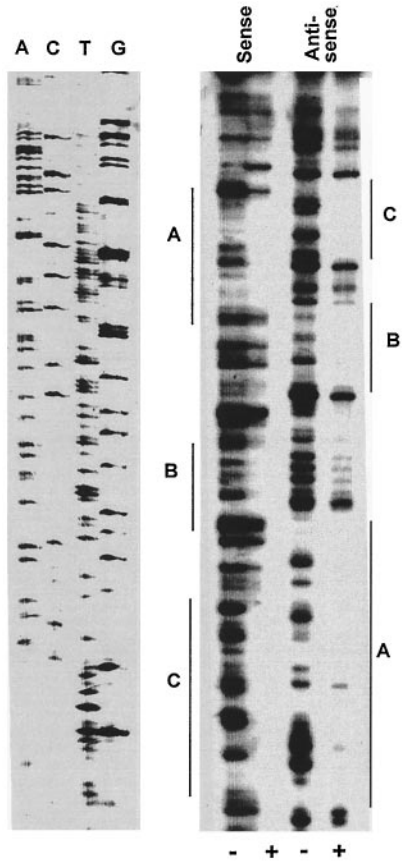

Fig. 4. DNase I footprint analysis of the *UGT2B7* gene promoter. Incubation of ^{32}P-labeled sense or anti-sense probe was performed in the presence of nuclear extracts from HepG2 cells (50 μg protein) with (+) and without (−) added DNase I. Regions resistant to DNase I digestion (regions A, B, and C) are denoted. A sequencing ladder generated with the sense primer employed in PCR of the footprinting probes is also shown. Sequence of the footprinted regions A, B, and C is denoted in Fig. 2.

to radiolabeled UGT2B7 HNF1/AT oligonucleotide (Fig. 9, A and B). The supershift was abolished in the presence of peptide that was used to generate the Oct-1-specific antibody but was not affected by peptide to the related factor, Oct-2 (Fig. 9A) or by HNF1α peptide (Fig. 9B). As shown in Fig. 6, the oligonucleotide containing the Oct-1 consensus binding site does not compete with binding of nuclear proteins, including HNF1α and Oct-1, to the UGT2B7 HNF1/AT rich site. This suggests that Oct-1 does not bind directly to this site. This was further confirmed by gel shift analyses with LNCaP cells, which do not contain HNF1α (Fig. 10). Nuclear extracts from LNCaP cells did not bind to the UGT2B7 HNF1α/Oct-1 site (Fig. 10, lanes 4–6), even though Oct-1 was present in these cells, as shown by the capacity of the Oct-1 consensus oligonucleotide to bind a protein recognized by the Oct-1 specific antibody (Fig. 10, lanes 9 and 10). However, as described above, nuclear proteins containing HNF1α and Oct-1 in HepG2 cells bound to the UGT2B7 HNF1/AT-rich site (Fig. 10, lanes 1–3). These data indicate that Oct-1 stimulates the activity of the UGT2B7 proximal promoter via interactions with HNF1α or other proteins in the complex rather than by direct binding to DNA.

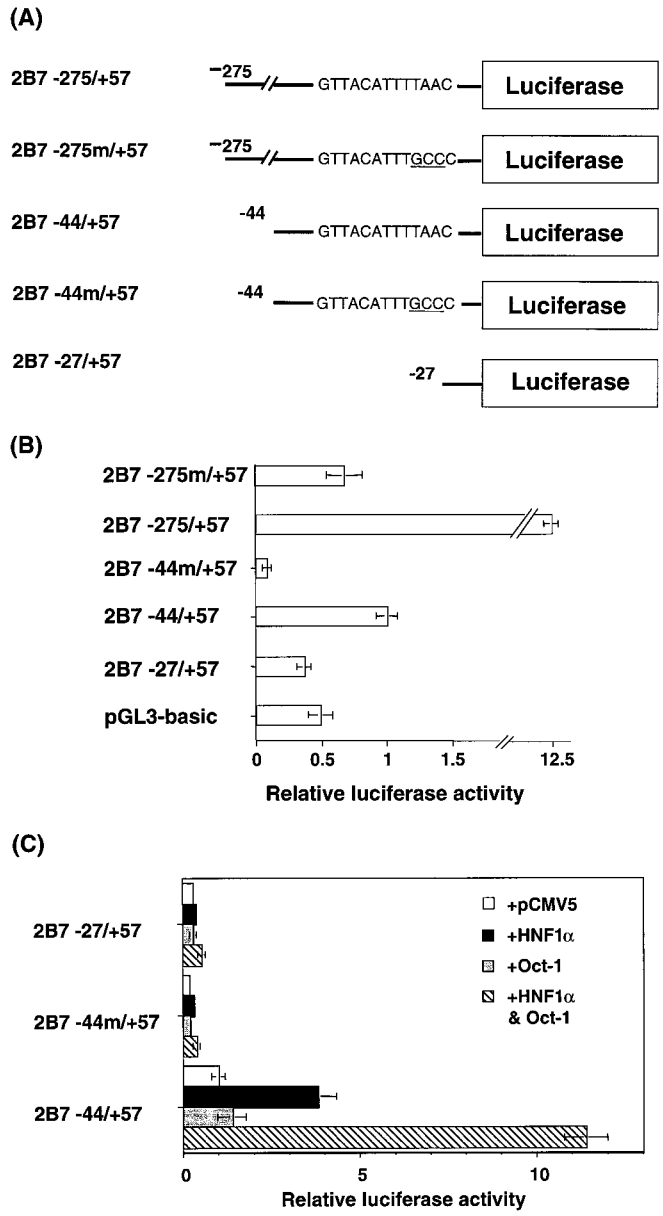

Fig. 5. Relative luciferase activity of transfected UGT2B7 promoter deletion constructs. In A, UGT2B7 deletion and mutation constructs used in transfection analysis are shown diagrammatically. The location of the HNF1/AT-rich site where transcription factors bind is indicated. The point mutations introduced are shown (underlined). In B, the relative strength of the UGT2B7 promoter with and without the HNF1/AT-rich region is demonstrated. The promoterless pGL3-basic is included as a reference. In C, 2 μg of the UGT2B7-firefly luciferase constructs were cotransfected with 40 ng of *Renilla* luciferase reporter plasmid (internal control) in the presence of 0.2 μg of pCMV5 (control) or 0.2 μg of HNF1α and/or Oct-1 expression vectors. Relative firefly luciferase activities were calculated as the mean of triplicate assays normalized to *Renilla* luciferase activities with S.E. Comparisons were made with the UGT2B7 −44/+57 construct. The promoter activity of the UGT2B7 −44/+57 construct cotransfected with HNF1α and Oct-1 expression vectors was 18% of a firefly luciferase reporter plasmid containing the SV40 promoter and enhancer (positive control). The results from a typical experiment are shown.

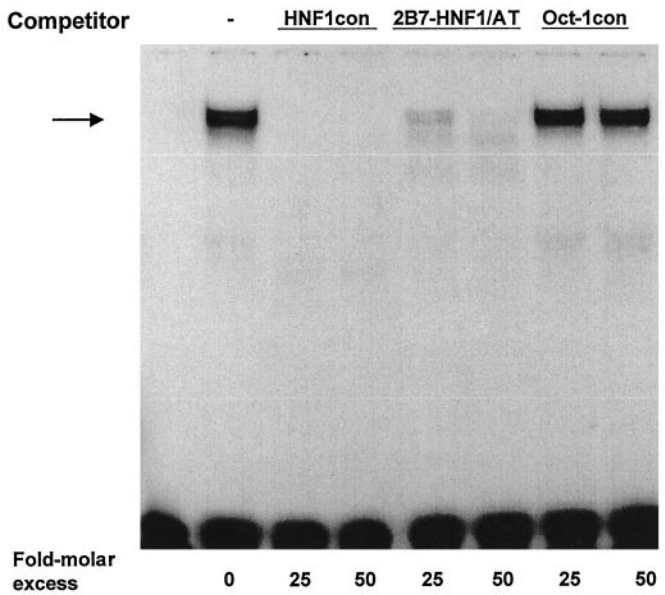

Fig. 6. Gel electrophoresis mobility shift assay of the UGT2B7-HNF1/AT element with various competitors. ^{32}P-labeled oligonucleotide (35,000 cpm per lane) was incubated with 6 μg of HepG2 nuclear extracts followed by electrophoretic resolution on a 4% nondenaturing polyacrylamide gel. The competition studies were performed using 25- and 50-fold molar excess of unlabeled double-stranded oligonucleotides for either HNF1 consensus (HNF1con), UGT2B7-HNF1/AT, or the Oct-1 consensus sequence. The sequences of the oligonucleotides are described in *Methods*.

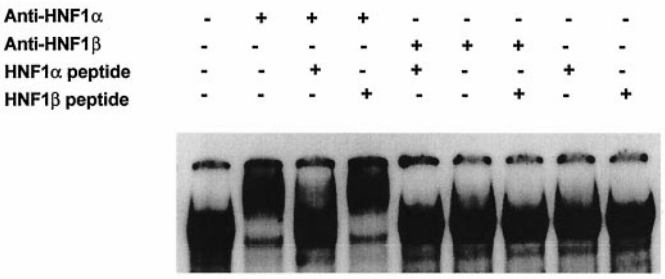

Fig. 7. Gel electrophoresis mobility and supershift assay of the UGT2B7 HNF1/AT element with HepG2 nuclear extracts. ^{32}P-labeled UGT2B7-HNF1/AT oligonucleotide (50,000 cpm per lane) was incubated with 6 μg of nuclear extract followed by electrophoretic resolution on a 3.5% nondenaturing polyacrylamide gel. Supershifts were performed in the presence of anti-HNF1α (2 μg) or anti-HNF1β (1 μg) anti-peptide antibody. The competition studies were performed with specific blocking peptide (2 μg) for each antibody.

Discussion

In this paper we have characterized the *UGT2B7* gene and shown that its proximal promoter is activated by the liver-enriched factor HNF1α and the ubiquitous factor, Oct-1. The UGT2B7 gene consists of 6 exons and extends over 16 kb. It is thus similar in arrangement to other *UGT2B* genes including *UGT2B1*, *UGT2B2*, *UGT2B4*, and *UGT2B17* (Mackenzie and Rodbourn, 1990; Haque et al., 1991; Beaulieu et al., 1997; Monaghan et al., 1997). However, in contrast to these genes, which have a TATA box approximately 30 bp upstream from the transcription start site, UGT2B7 does not have a canonical TATA box or other sequences that are known to specify the site of transcription initiation, such as the initiator element, a 17-bp element encompassing the transcription initiation site. We have, however, identified a region (region A) adjacent to the transcription start site that binds nuclear proteins and is most likely responsible for establishing the preinitiation complex. The 5′-end of region A contains an HNF1/AT-rich site. The position of this site just 27 bp upstream from the transcription start site is unusual, as genes containing a functional HNF1 site have the site positioned at least 40 bp or more from the transcription start site. These genes include the *UGT2B1* gene (Mackenzie and Rodbourn, 1990; Hansen et al., 1997); the bilirubin UDP glucuronosyltransferase gene, *UGT1A1* (Ueyama et al., 1997); the albumin, α-fetoprotein, and transthyretin genes (Courtois et al., 1988); the high affinity Na^+/glucose cotransporter (Rhoads et al., 1998); CYP2C1, CYP2C2 (Kim and Kemper, 1991), CYP2E1 (Liu and Gonzalez, 1995), and CYP3A2 genes (Legraverand et al., 1994); and the mouse guanylin precursor gene (Sciaki et al., 1994). The unusual position of the HNF/AT-rich site so close to the transcription start site suggests that it may be important in the recruitment of the general transcription machinery to the promoter. Indeed, if this site is removed or mutated, UGT2B7 promoter activity is lowered significantly. This is particularly evident when the longer promoter construct (−275/+57) is used in transfection experiments. The longer promoter is about 12-fold more active than the shorter promoter (−44/+57) and contains two other regions that bind transcription factors (regions B and C). These factors most probably contribute to the increased constitutive activity of the UGT2B1 promoter. Nevertheless, the activity of this longer promoter is all but abolished when the HNF/AT-rich site (region A) is mutated.

We have shown that the liver-enriched transcription factor, HNF1α, binds to the HNF1/AT-rich site and activates UGT2B7 promoter activity. Although this HNF1α-mediated activation is enhanced by Oct-1, Oct-1 alone does not activate the promoter or bind directly to the HNF1/AT-rich site. As supershift analysis demonstrates that Oct-1 is part of the complex that binds to the HNF1/AT-rich site, it is logical to assume that Oct-1 enhances promoter activity by directly interacting with HNF1α rather than binding to the DNA.

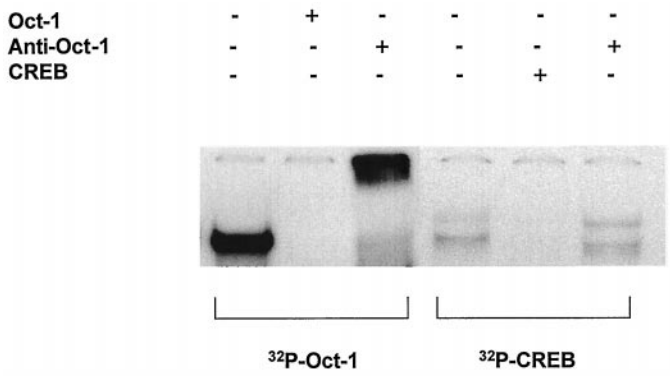

Fig. 8. Specificity of Oct-1 antibody in supershift assays. ^{32}P-labeled Oct-1 or CREB consensus oligonucleotide (35,000 cpm per lane) was incubated with 6 μg of HepG2 nuclear extract followed by electrophoretic resolution on a 3.5% nondenaturing polyacrylamide gel. Cold competitor oligonucleotide was included at 50-fold molar excess. Anti-Oct-1 (1 μg) antibody was added where indicated.

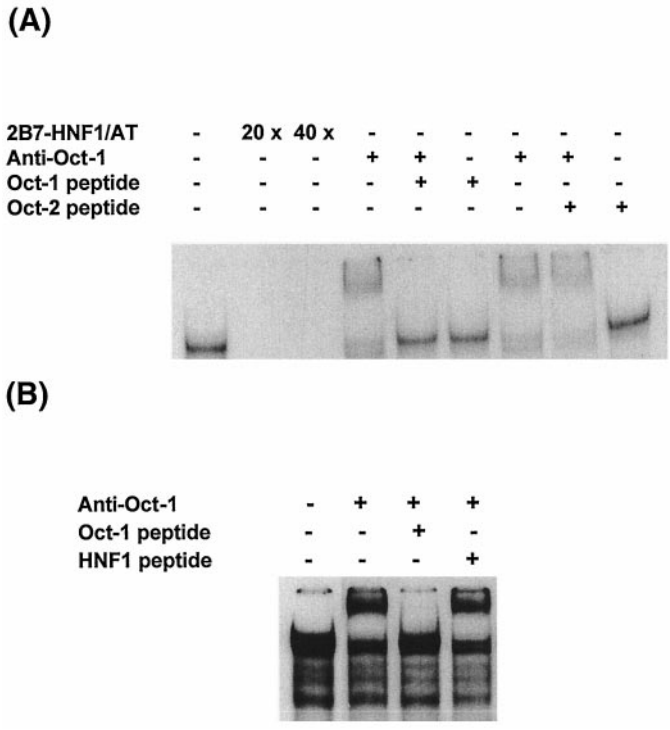

Fig. 9. Specificity of the interaction of Oct-1 with the UGT2B7 HNF1/AT element. ^{32}P-labeled UGT2B7 HNF1/AT oligonucleotide (35,000 cpm per lane) was incubated with 6 μg of HepG2 nuclear extracts followed by electrophoretic resolution on a 4% nondenaturing polyacrylamide gel. In A, the competitor oligonucleotide, UGT2B7-HNF/AT, was added as shown. The remaining reactions contained 1 μg of anti-Oct-1 or peptide competitor as indicated. In B, anti-Oct-1, alone or in combination with Oct-1 or HNF1 competitor peptides, was included in gel shift reactions.

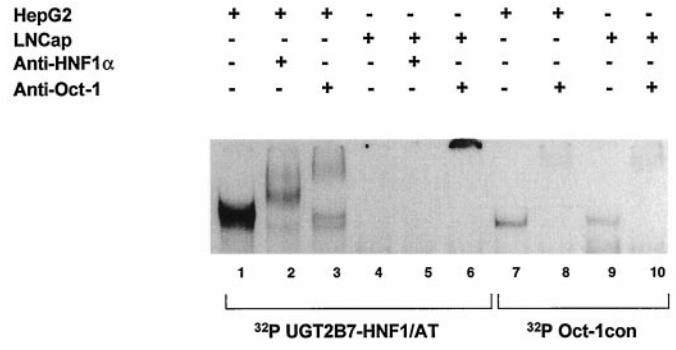

Fig. 10. Binding of HNF1α and Oct-1 to the UGT2B7 HNF1/AT element in liver versus prostate cell extracts. Either HepG2 (6 μg) or LNCaP (4 μg) nuclear extracts were incubated with 100,000 cpm of the indicated ^{32}P-labeled oligonucleotides containing the UGT2B7 HNF1/AT-rich region or the Oct-1 consensus binding site. Antibodies to either HNF1α (2 μg) or Oct-1 (1 μg) were included, as shown, for both extracts.

Oct-1 is known to bind to the basal transcription factor TFIIB (Nakshatri et al., 1995) and hence may act as the conduit for recruiting the preinitiation complex to the transcription start site. Although many genes are regulated by HNF1α and Oct-1, there is only one report demonstrating the interaction of these two factors in gene regulation. The interaction between HNF1α and Oct-1 occurred when their respective binding sites were adjacent to each other and was required for activation of the hepatitis B virus preS1 promoter (Zhou and Yen, 1991). The binding of HNF1α and Oct-1 in a complex to a single DNA binding site, as observed for the UGT2B7 proximal promoter, has not been described before.

HNF1α and Oct-1 are both homeodomain proteins of the Helix-Turn-Helix superclass of transcription factors. HNF1α binds as a homodimer or as a heterodimer with HNF1β to regulate gene transcription in the liver, kidney, and intestine (Frain et al., 1989; Rey-Campos et al., 1991; Cereghini et al., 1998). This binding may be stabilized via the direct interaction of a dimer of DCoH (dimerization cofactor of HNF1) with the HNF dimer to form a tetramer. The dimer of DCoH does not contact the DNA directly, but promotes the interaction of HNF1 with suboptimal DNA target sequences such as the α_1-antitrypsin gene TATA box region (Rhee et al., 1997). Oct-1 may have a role similar to that of DCoH as a coactivator by stabilizing the interaction of HNF1α with the UGT2B7 promoter and enhancing HNF1α-mediated transcription activation. Oct-1 has a bipartite DNA binding domain called the POU domain, which can bind as a monomer to DNA (Herr and Cleary, 1995). In contrast to HNF1α, Oct-1 can interact with several other factors including Oct-2 and Pit-1 (Herr and Cleary, 1995), the glucocorticoid receptor (Chandran et al., 1999), OBF-1 (Sauter and Matthias, 1998), MAT1 (Inamoto et al., 1997), VP16 (Babb et al., 1997), nuclear matrix proteins (Kim et al., 1996), Sp1 (Strom et al., 1996), NF1 (O'Connor and Bernard, 1995), the Vitamin D-3 Receptor (Liu and Freedman, 1994), and AP-1 (Pfeuffer et al., 1994) to regulate gene transcription. All these interactions involve the binding of Oct-1 to its cognate octamer binding site on DNA and do not depend on the recruitment of Oct-1 to other sites via an interaction with the factor bound at those sites, as is proposed in the regulation of the *UGT2B7* gene promoter.

In summary, we have shown that a region adjacent to the UGT2B7 transcription start site is necessary for promoter activity and that HNF1α binds to this region to activate the UGT2B7 proximal promoter. Furthermore, we show that Oct-1 acts as a coactivator to enhance the transcriptional activity of the UGT2B7 gene proximal promoter by HNF1α. This interaction between HNF1α and Oct-1 may fine-tune the expression of UGT2B7. Subsequent experiments will be directed toward identifying factors that bind to regions upstream of region A, (e.g., regions B and C) in the UGT2B7 promoter to obtain a more complete model of how this gene is regulated. However, it is apparent that these upstream activating factors that bind to regions B and C still require a functional interaction between HNF1α and region A for full activity. An understanding of the transcriptional regulation of the *UGT2B7* gene should provide new information and strategies to elucidate the basis of potential polymorphic variations in the expression of UGT2B7.

Acknowledgments

We thank Rikke Lewinsky, Philip Gregory, and Ceilidh Marchant for their excellent technical assistance and Behnaz Mojarrabi for help with DNA sequencing.

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