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Coordinate Regulation of the Human *UDP-Glucuronosyltransferase 1A8*, *1A9*, and *1A10* Genes by Hepatocyte Nuclear Factor 1α and the Caudal-Related Homeodomain Protein 2

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ABSTRACT

The human UDP-glucuronosyltransferases (UGT) -1A8 and -1A10 are exclusively expressed in extrahepatic tissues and primarily in the gastrointestinal tract, whereas UGT1A9 is expressed mainly in the liver and kidneys. We have demonstrated previously that the *UGT1A8* and *UGT1A10* genes, in contrast to the *UGT1A9* gene, are regulated via an initiator-like element in their proximal promoters. To determine the elements that contribute to the gastrointestinal expression of UGT1A8 and -1A10, we conducted deletion analysis of the *UGT1A8*, *-1A9*, and *-1A10* promoters in the colon-derived cell line Caco2. DNA elements contributing significantly to *UGT1A8*, *-1A9*, and *-1A10* promoter activity were found to reside primarily within 140 base pairs of the transcription start site. Within this region, putative binding sites for the intestine-specific transcription factor, caudal-related homeodomain protein 2 (Cdx2), and hepatocyte nuclear factor 1 (HNF1) were identified. Using gel shift and functional assays, HNF1α was demonstrated to bind to and activate the *UGT1A8*, *-1A9*, and *-1A10* promoters. In contrast, Cdx2 bound to and activated the *UGT1A8* and *-1A10* promoters but could not activate the *UGT1A9* promoter. A single base pair difference between the *UGT1A8* and *-1A10* promoters, three base pairs downstream of the consensus Cdx2 site, contributed to the observed difference in Cdx2 binding and Cdx2-mediated promoter activation of these two promoters. In addition, Cdx2 was shown to cooperate with HNF1α to synergistically activate the *UGT1A8*, *-1A9*, and *-1A10* promoters. These studies provide insight into the mechanisms controlling the extrahepatic expression of the *UGT1A8*, *-1A9*, and *-1A10* genes.

The liver plays a well-established role in the first-pass metabolism of drugs and other ingested xenobiotic compounds by phase I and phase II metabolic pathways (Dutton, 1980). Several enzymes involved in these pathways are expressed in the liver, including members of the cytochrome P450 (P450) and UDP-glucuronosyltransferase (UGT) superfamilies. However, the site of first contact for many ingested compounds, including drugs, dietary chemicals, and environmental mutagens, is the gastrointestinal tract, which comprises a large surface capable of metabolizing xenobiotics before they enter the systemic circulation. The gastrointestinal tract is known to variably express a wide range of P450 and UGT enzymes, which suggests that this organ may significantly contribute to extrahepatic first-pass metabolism (Tukey and Strassburg, 2001). Although, a large amount of research has focused on the importance of P450- and particularly CYP3A4-mediated biotransformation in the gastrointestinal tract, much less is known about the relative importance of glucuronidation in this organ.

Recent studies have revealed the expression of multiple isoforms of the UGT1A and UGT2B subfamilies throughout the tissues of the gastrointestinal tract, including the esophagus, stomach, small intestine, and colon (Strassburg et al., 1998a,b, 1999, 2000). Members of the UGT1A subfamily are encoded by a single complex gene locus located on chromosome 2 that spans more than 200 kilobases in length (Gong et al., 2001). Through a process of RNA splicing, eight functional UGT1A isoforms are generated with a unique exon 1 joined to an identical set of exons 2 to 5 (Ritter et al., 1992).

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P.A.G. and R.H.L. contributed equally to this work.

ABBREVIATIONS: P450, cytochrome P450; UGT, UDP-glucuronosyltransferase; bp, base pair; SI, sucrase isomaltase; LPH, lactase-phlorizin hydrolase; HNF1, hepatocyte nuclear factor 1; Cdx, caudal-related homeodomain protein; PCR, polymerase chain reaction; EMSA, electrophoretic mobility shift assay; wt, wild-type; m, mutant.

Each exon 1 is separated by 5′-flanking regions ranging from 5 to 23 kilobases in length, which are presumed to regulate independently the expression of each individual isoform. The *UGT1A* genes can be grouped into two clusters determined by their sequence identity, the *UGT1A7* to *-10* gene cluster being >70% similar in their first exon sequence and <60% similar to the other *UGT1A* genes (including the *UGT1A3* to *-5* cluster) (Gong et al., 2001). Three of the four isoforms comprising the *UGT1A7* to *-10* gene cluster, UGT1A7, -1A8, and -1A10, are the only UGTs known to be expressed exclusively in extrahepatic tissues, particularly those of the gastrointestinal tract (Strassburg et al., 1997, 1998a; Cheng et al., 1998; Mojarrabi and Mackenzie, 1998). The remaining functional UGT1A isoforms (UGT1A1, -1A3, -1A4, -1A6, and -1A9) are all expressed in the liver and variably in the gastrointestinal tract (Harding et al., 1988; Ritter et al., 1991; Wooster et al., 1991; Mojarrabi et al., 1996; Tukey and Strassburg, 2001). The unique pattern of expression of the *UGT1A7* to *-10* gene cluster suggests that these enzymes may have a specialized role in the gastrointestinal tract. Within the gastrointestinal tract, UGT1A7 expression is limited to the esophagus and stomach (Strassburg et al., 1997, 1999), whereas UGT1A8 and -1A10 expression is further extended to the small intestine and colon (Cheng et al., 1998; Mojarrabi and Mackenzie, 1998; Strassburg et al., 1998a, 2000). In contrast, UGT1A9 expression in the gastrointestinal tract is limited to the colon (Strassburg et al., 1998a). The variable expression of the *UGT1A7* to *-10* gene cluster within the gastrointestinal tract suggests that each isoform may be differentially regulated by tissue-specific factors acting upon promoter elements in the 5′-flanking region of their respective first exons.

Investigations into the mechanisms regulating the expression of two markers of intestinal differentiation, sucrase isomaltase (SI) and lactase-phlorizin hydrolase (LPH), have revealed that both genes are regulated by cooperative interaction of hepatocyte nuclear factor 1α (HNF1α) and the caudal-related homeodomain protein 2 (Cdx2) (Mitchelmore et al., 2000; Krasinski et al., 2001; Boudreau et al., 2002). HNF1α has previously been shown to be involved in the liver-specific regulation of several UGTs, including human UGT2B7, UGT2B17, UGT1A1, and rat UGT1A7 (Bernard et al., 1999; Gregory et al., 2000; Ishii et al., 2000; Metz et al., 2000). HNF1α is a homeodomain-containing protein that forms homodimers or heterodimers with the related-factor HNF1β and regulates gene transcription through the palindromic consensus sequence GTTAATNATTAAC (Mendel and Crabtree, 1991). Although HNF1α was originally discovered in the liver, it is also expressed in the kidney, pancreas, stomach, and intestine, in which it has been implicated in the regulation of a number of genes (Baumhueter et al., 1990; Mendel and Crabtree, 1991). In contrast to HNF1α, the expression of Cdx2 seems to be limited to the intestine (James et al., 1994; Suh et al., 1994). Cdx2 and Cdx1 are members of the mammalian homeobox gene family related to the *Drosophila melanogaster* caudal gene. Cdx2 has been demonstrated to play important roles in intestinal cell differentiation and proliferation (Suh and Traber, 1996), as well as in the regulation of several intestine-specific gene promoters (Suh et al., 1994; Drummond et al., 1996; Troelsen et al., 1997), primarily through binding to a TTTAT/C core consensus sequence.

To determine the mechanisms responsible for gastrointestinal expression of the *UGT1A7* to *-10* gene cluster, our group cloned and characterized the promoters of the *UGT1A8*, *-1A9*, and *-1A10* genes. In preliminary studies, we identified an initiator-like region that contributed to the regulation of the *UGT1A8* and *-1A10* genes, but not the *UGT1A9* gene, in the colon-derived cell line Caco2 (Gregory et al., 2003). Here, we have extended these studies to demonstrate that the intestine-specific transcription factor Cdx2 differentially binds to and regulates the *UGT1A8*, *-1A9*, and *-1A10* promoters in Caco2 cells. In addition, Cdx2 was shown to cooperate with HNF1α to synergistically activate the *UGT1A8*, *-1A9*, and *-1A10* promoters.

Materials and Methods

Construction of UGT1A8, -1A9, and -1A10 Promoter-Luciferase Deletion Constructs. The *UGT1A8*, *-1A9*, and *-1A10* promoters were isolated from a lambda genomic library as described previously (Gregory et al., 2003). Fragments of the *UGT1A8* and *-1A10* promoters of approximately 2000, 1000, 400, 140, 80, and 25 base pairs in length were generated by PCR using the forward primers shown in Table 1 (primers are named by gene, size of promoter fragment, and then orientation) and the 1A8/10rev common reverse primer. Resulting PCR products were digested with KpnI and MluI (New England Biolabs, Beverly, MA) and cloned into the pGL3-basic reporter vector (Promega, Madison, WI). The only exception to the above procedure was for the *UGT1A10* 2000for-bp promoter, which was amplified using the 1A10-1000for and 1A10rev1 primers and cloned into the MluI and XmaI sites of pGL3-basic. A *UGT1A9* 2000-bp promoter fragment was isolated from a PstI digest of the *UGT1A9* lambda clone and cloned into the PstI site of pGL3+, which has been described previously (Gregory et al., 2003). The *UGT1A9* 1000- and 400-bp promoter fragments were generated by PCR using the 1A9-1000for and 1A9-400for forward primers and the 1A9rev1 reverse primer. Resulting PCR products were digested with *Pst*I and cloned into pGL3+ vector. The *UGT1A9* 140-, 80-, and 25-bp promoter-luciferase constructs were generated as described for *UGT1A8* and *-1A10* using the 1A9-140for, 1A9-80for, and 1A9-25for forward primers coupled with the 1A9rev2 reverse primer shown in Table 1.

Mutagenesis of HNF1 and Cdx2 Binding Sites in the UGT1A8, -1A9, and -1A10 Promoters. The HNF1 binding site was mutated in the context of the 140-bp *UGT1A8*, *-1A9*, and *-1A10* promoters using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) with the 1A8/10-HNF1mfor and 1A8/10-HNF1mrev (*UGT1A8* and *-1A10*) and 1A9-HNF1mfor and 1A9-HNF1mrev (*UGT1A9*) primer pairs shown in Table 1. The Cdx2 binding site was mutated in the context of the 140-bp *UGT1A8* and *-1A10* promoters using a two-step PCR protocol. Overlapping PCR fragments were generated from the 140-bp *UGT1A8* and *-1A10* promoter constructs using the 1A8-140for and 1A8-Cdx2mrev (UGT1A8 fragment 1), 1A10-140for and 1A10-Cdx2mrev (UGT1A10 fragment 1), 1A8-Cdx2mfor and 1A8/10rev (UGT1A8 fragment 2), and 1A10-Cdx2mfor and 1A8/10rev (UGT1A10 fragment 2) primer pairs shown in Table 1. Fragment 1 of each respective gene was mixed with fragment 2 and subsequently amplified using the 1A8-140for or 1A10-140for forward and the 1A8/10rev reverse primers. The resulting PCR products were digested with KpnI and MluI and cloned into pGL3-basic. A 140-bp *UGT1A8* promoter construct containing mutations in both the HNF1 and Cdx2 sites was generated using the UGT1A8-140 HNF1m construct in the above two-step PCR procedure. A 140-bp *UGT1A10* promoter construct in which the A-to-C nucleotide difference between the *UGT1A8* and *-1A10* Cdx2 sites is changed was generated as for the UGT1A10-140 Cdx2 mutant construct above, using the 1A10-140for and 1A10-Cdx2(A to C)mrev and

1A10-Cdx2(A to C)mfor and 1A8/10rev primer pairs (Table 1) to generate the fragments for overlapping PCR.

Cell Culture and Transfection. Caco2 cells obtained from the American Type Culture Collection (Manassas, VA) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, 0.1 mM mixture of nonessential amino acids (all from Invitrogen, Carlsbad, CA), and 80 μg/ml gentamycin at 37°C in 5% CO_2. Cells were plated into 24-well plates at a density of 7.5×10^4 cells/well and transfected the following day with 0.5 μg of promoter construct and 0.025 μg of the *Renilla reniformis* vector pRL-null (Promega, Madison, WI) using 2 μl/well LipofectAMINE 2000 according to the manufacturer's protocol (Invitrogen). For cotransfections, 0.25 μg of HNF1α, Cdx2, or both HNF1α and Cdx2 expression vectors was added to the above reaction mix and normalized to a total of 1 μg DNA with pCMV5 (empty expression vector) before incubation with 3 μl/well LipofectAMINE 2000. After 48 h, the cells were harvested in 100 μl of 1× passive lysis buffer and 20 μl assayed for luciferase and *R. reniformis* activity using the Dual-Luciferase Reporter Assay System (Promega). Luminescence was measured using a Packard TopCount luminescence and scintillation counter (Mt. Waverly, Victoria, Australia). The HNF1α and HNF1β and the Cdx2 expression plasmids were kind gifts from Dr. Gerald Crabtree (Stanford University, Stanford, CA) and Dr. Cathy Mitchelmore (University of Copenhagen, Copenhagen, Denmark), respectively.

Nuclear Extracts and Electrophoretic Mobility Shift Assay. Nuclear extracts were prepared from Caco2 cells essentially as described by Schreiber et al. (1989). Probes were prepared by annealing complementary sense and antisense oligonucleotides, followed by end-labeling with ^{32}P-ATP (PerkinElmer Life and Analytical Sciences, Rowville, VIC, Australia) using T4 polynucleotide kinase (New England Biolabs) and purification through G25 columns (Amersham Biosciences Inc., Piscataway, NJ). Electrophoretic mobility shift assay reactions consisted of 5 μg of Caco2 nuclear extract, 1 μg of poly(dI-dC), and unlabeled competitor probes (if needed) in a 15-μl reaction containing 25 mM Tris-HCl, pH 7.6, 100 mM KCl, 0.5 mM dithiothreitol, 5 mM $MgCl_2$, 0.5 mM EDTA, and 10% glycerol (all from Sigma Chemical Co., St. Louis, MO). After incubation on ice for 10 min, 50,000 cpm of probe (0.5–1 ng) was added, and the reaction was incubated for a further 30 min at room temperature. For supershift assays, ~2 μg of HNF1α, HNF1β (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and Cdx2 (Biogenex, San Ramon, CA) antibodies were added directly after the addition of the labeled probe. DNA-protein complexes were resolved in 0.5× Tris borate-EDTA on 4% nondenaturing polyacrylamide gels.

In Vitro Transcription and Translation. Cdx2 protein was synthesized using the TNT Quick transcription/translation kit according to the manufacturer's instructions (Promega). Briefly, 1 μg of the hamster pCM-Cdx2 plasmid was incubated with 0.02 mM methionine in 50 μl of the supplied reaction mix and transcribed/translated with T7 polymerase for 90 min at 30°C. The Cdx2 protein was diluted 1:5 and used in electrophoretic mobility shift assay reactions as described above.

TABLE 1

Primers used to create luciferase promoter constructs and mutants

UGT1A8, *-1A9*, and *-1A10* promoter fragments of approximately 2000, 1000, 400, 140, 80, and 25 base pairs in length were amplified by PCR using the forward primers (primers are named by gene, size of promoter fragment, and then orientation) in combination with either the 1A8/10rev, 1A10rev1, 1A9rev1, and 1A9rev2 reverse primers as described under *Materials and Methods*. The resulting PCR products were subsequently cloned into the pGL3-basic vector (see *Materials and Methods*). Mutations in the *UGT1A8*, *-1A9*, and *-1A10* HNF1 site were generated using the HNF1m forward and reverse primer pairs (primers are named by gene, mutation, and then orientation) according to the QuikChange site-directed mutagenesis kit (Stratagene). Mutations in the *UGT1A8* and *-1A10* Cdx2 site were generated through a two-step PCR procedure. Overlapping PCR fragments were amplified using the following primer pairs: 1A8-140for and 1A8-Cdx2mrev (UGT1A8 fragment 1), 1A10-140for and 1A10-Cdx2mrev (UGT1A10 fragment 1), 1A8-Cdx2mfor and 1A8/10rev (UGT1A8 fragment 2), 1A10-Cdx2mfor and 1A8/10rev (UGT1A10 fragment 2), 1A8-140for and 1A8-Cdx2(A to C)mrev (UGT1A10* fragment 1), and 1A10-Cdx2(A to C)mfor and 1A8/10rev (UGT1A10* fragment 2). Fragment 1 of each respective gene was mixed with fragment 2 and subsequently amplified using the 1A8-140for or 1A10-140for forward and the 1A8/10rev reverse primers (*, A-to-C mutation in Cdx2 site). Mutations are bold, and the restriction sites used for cloning are underlined.

Primer	Nucleotide Sequence
1A8-2000for	5′-TTTTGGTACCAGAGCTGAGTTCAGGTC-3′
1A8-1000for	5′-TTTTGGTACCTACGTTTAAACAACCAG-3′
1A8-400for	5′-TTTTGGTACCTTGGAACATAGGATACC-3′
1A8-140rev	5′-TTTTGGTACCTCAAAAAATGATACTC-3′
1A8-80for	5′-TTTTGGTACCATTTTTTTTTTTTTTATGAC-3′
1A8-25for	5′-TTTTGGTACCGTGCCTGTAGTTCTTCC-3′
1A9-1000for	5′-ACGCATCTGCAGGTTCTTGCCGAAGCCTTC-3′
1A9-400for	5′-ACGCATCTGCAGAGAGCATGAGTTGCCATC-3′
1A9-140rev	5′-TTTTGGTACCTCAGCAAAAGCTACTC-3′
1A9-80for	5′-TTTTGGTACCATTTTTTTTTATGAAAGGAT-3′
1A9-25for	5′-ACGCAGGGTACCGTGCTGGTATTTCTCCCA-3′
1A10-2000for	5′-CTATACGCGTGTATTAGGTTTGCTTGGT-3′
1A10-1000for	5′-CTGGGGTACCTGTACTGTCGTATAC-3′
1A10-400for	5′-CTGTGGTACCCCTGGAACATGAGATGCC-3′
1A10-140rev	5′-AGTAGGTACCTCAGCAAATGATACTC-3′
1A10-80for	5′-GTGAGGTACCTTTTTTTTTTTTTTTATGAA-3′
1A10-25for	5′-TTTTGGTACCGTGCCTGTACTTCTTCC-3′
1A8/10rev	5′-AGCCACGCGTGAACTGCAGCCCGAGCC-3′
1A10rev1	5′-CCACCCCGGGCGAGCCATGAGAGAACTG-3′
1A9rev1	5′-AGCCATCTCGAGCAGAGAACTGCAGCTGAGAGC-3′
1A9rev2	5′-AGCCATACGCGTCAGAGAACTGCAGCTGAGAGC-3′
1A8/10-HNF1mfor	5′-GTTCTTATGAGT**CGC**TCATTGGCAGTGAGTG-3′
1A8/10-HNF1mrev	5′-CACTCACTGCCTATGA**GCG**ACTCATAAGAAC-3′
1A9-HNF1mfor	5′-CTTGTTCTTTTGGGT**CGC**TCATTGTCAGTGACTG-3′
1A9-HNF1mrev	5′-CAGTCACTGACAATGA**GCG**ACCCAAAAGAACAAG-3′
1A8-Cdx2mfor	5′-TGTGATTTTTTTTTTTTT**GGC**TGACAGGATAAATACAC-3′
1A8-Cdx2mrev	5′-CCTGTCA**GCC**AAAAAAAAAAAAATCACACTCACT-3′
1A10-Cdx2mfor	5′-TGTGATTTTTTTTTTTTT**GGC**TGAAAGGATAAATACAC-3′
1A10-Cdx2mrev	5′-CCTTTCA**GCC**AAAAAAAAAAAAATCACACTCACT-3′
1A10-Cdx2(A to C)mfor	5′-TGTGATTTTTTTTTTTTTTATGA**C**AGGATAAATACAC-3′
1A10-Cdx2(A to C)mrev	5′-CCT**G**TCATAAAAAAAAAAAAAAATCACACTCACT-3′

Results

Elements between −140 and −25 Are Important for UGT1A8, -1A9, and -1A10 Promoter Activity. UGT1A8 and -1A10 have a unique pattern of tissue-specific expression in that they, in addition to UGT1A7, are the only UGTs to be expressed exclusively in extrahepatic tissues and mainly in the gastrointestinal tract (Strassburg et al., 1997, 1998a; Cheng et al., 1998; Mojarrabi and Mackenzie, 1998). In contrast, UGT1A9 is expressed primarily in the liver and kidneys, with its expression in the gastrointestinal tract being limited to the colon (Wooster et al., 1991; Strassburg et al., 1998a). Previously, we demonstrated that an initiator-like element and Sp1 site in the *UGT1A8* and *-1A10* proximal promoters plays a role in regulating these two genes, but not the *UGT1A9* gene (Gregory et al., 2003). To determine elements contributing specifically to the gastrointestinal expression of the UGT1A8 and -1A10, we have made promoter-deletion constructs of the *UGT1A8*, *-1A9*, and *-1A10* genes in the pGL3 basic vector and analyzed their activity in the colon-derived cell line Caco2. Figure 1A shows a comparison of the activity of *UGT1A8*, *-1A9*, and *-1A10* promoter constructs ranging from −2000 to −25 base pairs relative to the transcription start sites. The −2000 promoter constructs all had variable basal promoter activity, suggesting that different factors are responsible for their expression. When the promoters were deleted to −1000, the *UGT1A8* and *-1A9* promoter activities did not significantly vary from their respective −2000 constructs, but the *UGT1A10* promoter activity increased 2-fold. Deleting each promoter to −400 further increased *UGT1A10* promoter activity to 3-fold greater than the −2000 construct, but again it did not alter *UGT1A8* and *-1A9* promoter activity. Taken together, these data suggest that *UGT1A10* may be negatively regulated by elements present between −400 and −2000. Further deletion of the *UGT1A8*, *-1A9*, and *-1A10* promoters to −140 decreased *UGT1A10* promoter activity to a level similar to the equivalent *UGT1A8* construct and increased *UGT1A9* promoter activity by ~2-fold. This region between −400 and −140 may contain elements that differentially regulate the *UGT1A9* and *-1A10* genes. Deletion of the promoters to −80 decreased both the activity of *UGT1A8* and *-1A10* by 2-fold and slightly increased *UGT1A9* promoter activity. The −80 *UGT1A8*, *-1A9*, and *-1A10* promoter constructs were approximately equal in promoter activity, suggesting that the region between −140 and −80 is important in determining the differential activity of the three genes. In addition, the activities of the −140 and −80 *UGT1A8* and *-1A10* promoter constructs were almost identical between the two genes, indicating that elements in the −140 to −80 region may coordinately regulate their activity. On close inspection of the −140 to −80 region, we identified an HNF1α binding site, which was shared in the *UGT1A8* and *-1A10* promoters and differed in the *UGT1A9* promoter by 1 base pair (Fig. 1B). The HNF1 site was identified from sequence homology and a locality similar to that of a previously identified HNF1α binding site in the rat *UGT1A7* promoter (Metz et al., 2000). Deletion of each promoter to −25 decreased their activity to levels similar to background (pGL3 vector), implying that this region is universally essential for *UGT1A8*, *-1A9*, and *-1A10* promoter activity. In addition to the Sp1 binding site identified previously in this region (Gregory et al., 2003), closer inspection of the −80 to −25 region also revealed a putative binding site for the caudal-related protein Cdx2 in all three promoters (Fig. 1B). This Cdx2 site was identified after comparison of the *UGT1A8*, *-1A9*, and *-1A10* promoters with the claudin-2 promoter, which is expressed in the intestine and contains HNF1 and Cdx2 binding sites in close proximity (Sakaguchi et al., 2002). Cdx2 is a homeodomain containing protein specifically expressed in the gastrointestinal tract, which is an important regulator of several gastrointestinal-specific gene markers (Suh et al., 1994; Troelsen et al., 1997). In addition, Cdx2 has recently been shown to interact with

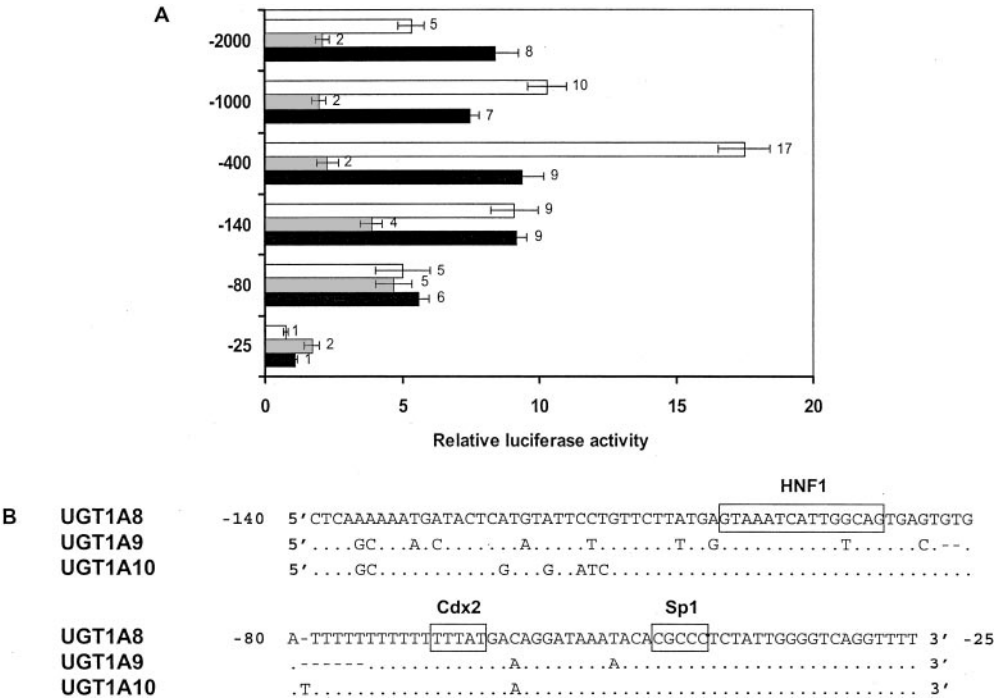

Fig. 1. Deletion analysis of the *UGT1A8*, *-1A9*, and *-1A10* promoter in Caco2 cells. A, deletion constructs (0.5 μg) containing varying lengths of the *UGT1A8* (■), *UGT1A9* (▨), and *UGT1A10* (□) promoters were transfected into Caco2 cells with 0.025 μg of the pRL-null vector as an internal control of transfection efficiency. Cells were harvested 48 h after transfection and were assayed for firefly and *R. reniformis* luciferase activities. Relative luciferase activities are expressed as a fold induction over the promoterless pGL3-basic vector (set at a value of 1). The results shown are a representative experiment of three individual experiments performed in triplicate (± S.D.). B, the proximal −25 to −140 base pairs (relative to the transcription start site) of the *UGT1A8*, *-1A9*, and *-1A10* promoters are aligned. Identical bases in the *UGT1A9* and *-1A10* genes compared with the *UGT1A8* gene are indicated with a period, nonidentical bases with the base change, and missing bases with a dash. The putative HNF1, Cdx2, and Sp1 binding sites are boxed.

HNF1α to regulate gastrointestinal-specific gene expression (Mitchelmore et al., 2000; Krasinski et al., 2001; Boudreau et al., 2002). Therefore, we investigated whether the gastrointestinal-specific expression of UGT1A8 and -1A10 was regulated through the putative HNF1 and Cdx2 binding sites.

The Putative HNF1 and Cdx2 Binding Sites Are Important for UGT1A8 Promoter Activity. To determine whether the putative HNF1 and Cdx2 binding sites were important for *UGT1A8* promoter activity, mutations in these sites were introduced in the context of a −140 *UGT1A8* promoter construct and transfected into Caco2 cells. Figure 2 shows that mutation of either the HNF1 (1A8-140 HNF1m) or Cdx2 (1A8-140 Cdx2m) site did not significantly decrease promoter activity compared with the wild-type construct. However, when both sites were mutated in the same construct (1A8-140 HNF1m/Cdx2m), a 2-fold decrease in promoter activity was observed. These results suggest that the HNF1 and Cdx2 binding sites are important for *UGT1A8* promoter activity and that the loss of one site may be compensated by the presence of the other.

HNF1α, -β, and Cdx2 Bind to the Putative HNF1 and Cdx2 Sites in the UGT1A8 Promoter. Gel shift assays with labeled double-stranded oligonucleotide probes to the putative *UGT1A8* HNF1 and Cdx2 sites were used to investigate whether HNF1 and Cdx2 proteins present in Caco2 nuclear extracts could bind to these regions. The addition of Caco2 nuclear extracts to the *UGT1A8* HNF1 probe (UGT1A8-HNF1wt) (Fig. 3A) resulted in the formation of a major DNA-protein complex consisting of several distinct bands (Fig. 3A, lane 2). This complex is typical of HNF1α and HNF1β homo- and heterodimers binding to the HNF1 site (Blumenfeld et al., 1991). The HNF1-like complex was not formed when a mutant probe (UGT1A8-HNF1m) (Fig. 3A) was used in place of the UGT1A8-HNF1wt probe (lane 1), indicating that complex formation was specific to the intact HNF1 site. To confirm that HNF1α and HNF1β were present in the HNF1-like complex, antibodies to these factors were added to the reactions after the addition of labeled probe. The HNF1α antibody decreased the formation of at least two of the bands and resulted in the formation of a larger supershifted complex (lane 3). The HNF1β antibody decreased the formation of the two lower bands (lane 4). Taken together, these results demonstrate that both HNF1α and HNF1β are able to bind to the *UGT1A8* HNF1 site. This HNF1 site is conserved in the *UGT1A10* promoter and differs by 1 bp in the *UGT1A9* promoter (Fig. 1B); however, this difference does not affect the binding of HNF1 proteins (data not shown). Therefore, HNF1α and HNF1β were considered to bind equally to the *UGT1A8*, *-1A9*, and *-1A10* HNF1 sites.

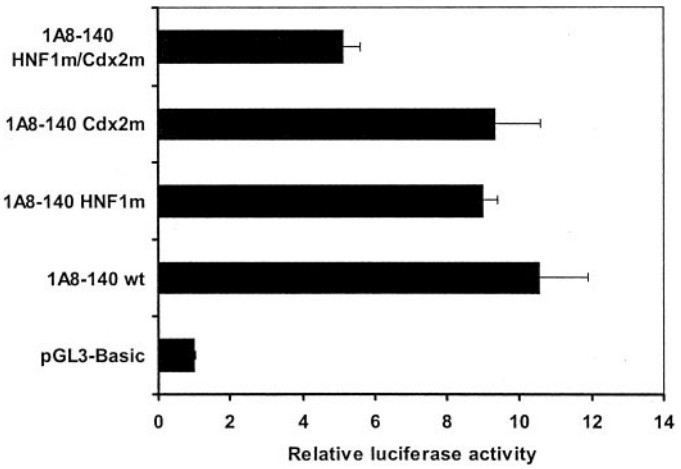

Fig. 2. Mutational analysis of the putative HNF1 and Cdx2 binding sites in the *UGT1A8* promoter. Wild-type and HNF1, Cdx2, and HNF1/Cdx2 mutant *UGT1A8*-190 promoter constructs (0.5 μg) were transfected into Caco2 cells with 0.025 μg of the pRL-null vector as an internal control of transfection efficiency. Cells were harvested 48 h after transfection and assayed for firefly and *R. reniformis* luciferase activities. Relative luciferase activities are expressed as a fold induction over the promoterless pGL3-basic vector (set at a value of 1). The results shown are a representative experiment of three individual experiments performed in triplicate (± S.D.).

The addition of Caco2 nuclear extracts to a labeled double-stranded oligonucleotide probe containing the putative *UGT1A8* Cdx2 binding site (UGT1A8-Cdx2wt) (Fig. 3B) resulted in the formation of two major complexes (A and B) as shown in Fig. 3B (lane 1). Both complexes were effectively competed with by the addition of a 50-fold molar excess of the wild-type probe (lane 2), but only complex A was competed with using the same amount of the UGT1A8-Cdx2m probe (Fig. 3B, lane 3). This suggests that complex B represents specific binding to the Cdx2 site. To confirm the presence of Cdx2 in complex B, supershift assays were performed with a monoclonal Cdx2-specific antibody. Addition of the Cdx2 antibody supershifted complex B but not complex A formed with the 1A8-Cdx2wt probe, indicating that complex B contains Cdx2 (lane 4). Furthermore, complex B does not form when the 1A8-Cdx2mut probe is used (lane 5), and Cdx2 antibody was unable to supershift complex A formed with this mutant probe (lanes 6). Taken together, these results suggest that Cdx2 specifically binds to the *UGT1A8* Cdx2 site and that an unidentified complex A also binds to this region in the absence of an intact Cdx2 site.

HNF1α and Cdx2 Synergistically Activate the UGT1A8, -1A9, and -1A10 Promoters. Having established that HNF1α, HNF1β, and Cdx2 bind to their respective sites in the *UGT1A8* promoter, we decided to test whether these factors could activate the *UGT1A8*, *-1A9*, and *-1A10* promoters in transfection assays. Expression vectors containing the above factors were cotransfected into Caco2 cells with the *UGT1A8*, *-1A9*, and *-1A10* −140 wild-type (wt) constructs. As shown in Fig. 4A, HNF1α and Cdx2 are both able to increase the promoter activity of the 1A8-140wt promoter by ~3-fold. In contrast, cotransfection with HNF1β had no significant effect on promoter activity (data not shown). When both HNF1α and Cdx2 were cotransfected together with the 1A8-140wt promoter, a further increase in promoter activity of ~7-fold was observed. This suggests that HNF1α and Cdx2 are likely to be working cooperatively to regulate the *UGT1A8* promoter. To determine whether this cooperation can occur in the absence of the HNF1 and Cdx2 sites, cotransfections were also performed with the UGT1A8-140 constructs containing mutations in the HNF1 and Cdx2 sites. Mutation of the HNF1 site prevented activation of the promoter by HNF1α but not by Cdx2, which activated the 1A8-140 HNF1m promoter by the same amount as the wild-type promoter (Fig. 4A). However, the HNF1 mutation prevented cooperative activation of the promoter by HNF1α and Cdx2, indicating that the HNF1 site is needed for cooperation to occur. Mutation of the Cdx2 site prevented activation of the promoter by Cdx2, but not by HNF1α, which activated the 1A8-140 Cdx2m promoter by an amount similar to that of the

wild-type promoter (Fig. 4A). In this case, however, HNF1α and Cdx2 were still able to cooperatively activate the 1A8-140 Cdx2m promoter, albeit to a slightly lower level than the wild-type construct (~6-fold above basal). Taken together, these data suggest that HNF1α and Cdx2 activate the *UGT1A8* promoter through their respective sites but that their cooperative effect requires the presence of an intact HNF1 site. As a negative control, the 1A8-140 HNF1m/Cdx2m double mutant construct was also cotransfected with HNF1α and Cdx2 (Fig. 4A). This construct was only marginally responsive to HNF1α and Cdx2 cotransfection (<2-fold), confirming that the sites tested are the only HNF1- and Cdx2-responsive sites in the 1A8-140 promoter.

Cotransfection of the *UGT1A9* −140 promoter (1A9-140wt) with HNF1α revealed a level of activation similar to that observed with the equivalent *UGT1A8* construct (~2.5-fold) (Fig. 4B). In contrast, Cdx2 was not able to activate the 1A9-140wt promoter, suggesting that the Cdx2 site is not functional in the context of this promoter. However, when both HNF1α and Cdx2 were cotransfected, the 1A9-140 promoter was synergistically activated by ~5-fold. This synergism was likely to occur through the HNF1 site, as was the case for the *UGT1A8* promoter. To confirm this suggestion, cotransfections were performed with a 1A9-140 construct containing an HNF1 site mutation (1A9-140 HNF1m). Mutation of the HNF1 site did not significantly decrease the basal activity of the promoter, but it virtually abolished activation by either HNF1α alone or in combination with Cdx2, indicating that the HNF1 site is critical for *UGT1A9* promoter activation by HNF1α and synergistic activation by HNF1α and Cdx2 (Fig. 4B).

Cotransfection of the *UGT1A10* −140 promoter (1A10-140wt) with HNF1α revealed a level of activation equivalent to that observed with the *UGT1A8* construct (~3-fold) (Fig.

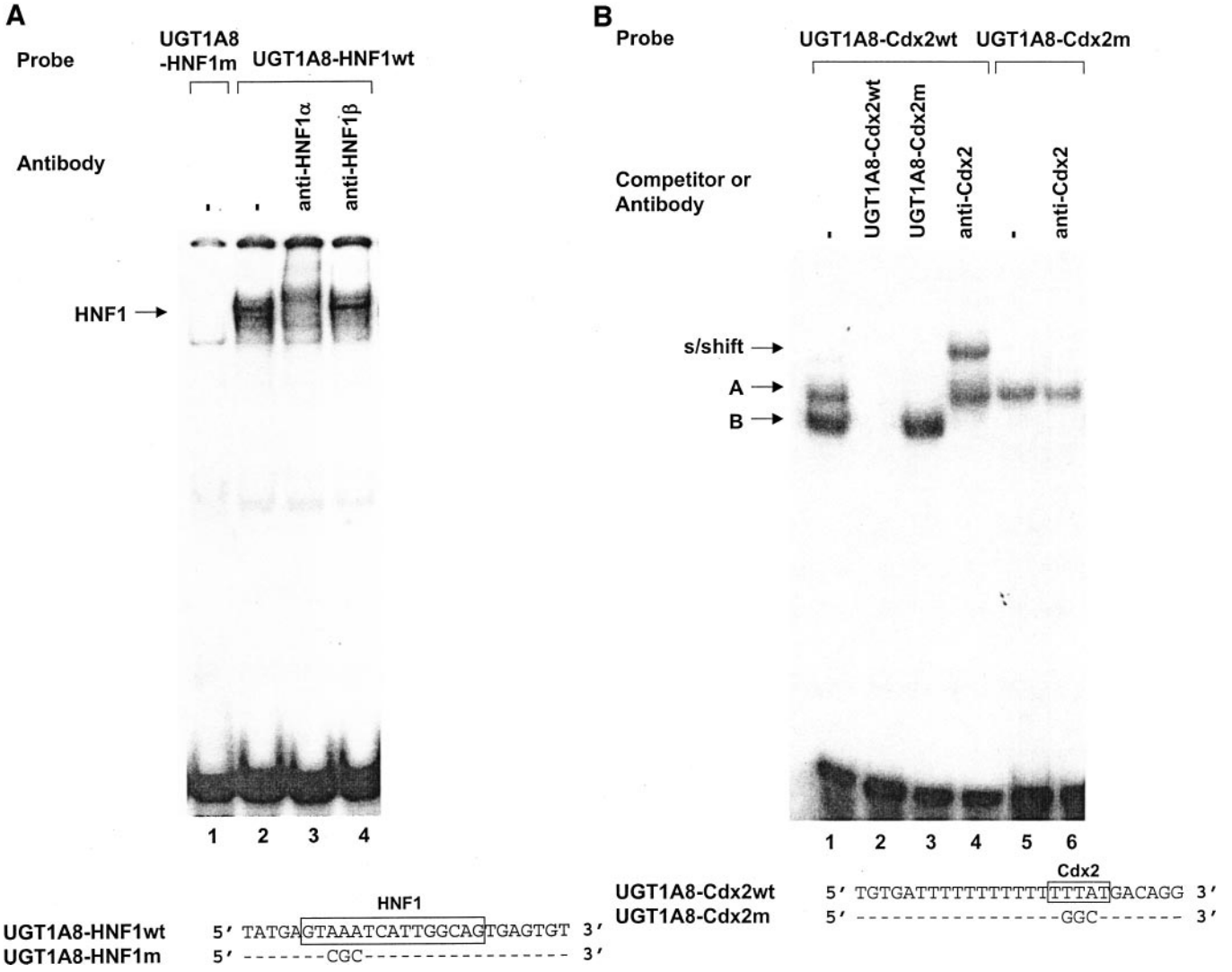

Fig. 3. HNF1α and -β and Cdx2 bind to the HNF1 and Cdx2 sites in the *UGT1A8* promoter. A and B, double-stranded oligonucleotide probes (50,000 cpm) corresponding to wild-type (wt) and mutant (m) HNF1 and Cdx2 sites in the *UGT1A8* promoter were incubated with 5 μg Caco2 nuclear extracts and resolved on a 4% nondenaturing polyacrylamide gels. Unlabeled competitor oligonucleotides were added at 50-fold molar excess before the addition of labeled probe. For supershift assays, the HNF1α, HNF1β, and Cdx2 antibodies were added after the addition of labeled probe and incubated for 30 min before electrophoresis. Arrows, the major HNF1 and Cdx2 (complex B) complexes and supershifted bands. An unidentified complex binding to the UGT1A8-Cdx2 wild-type and mutant probes is shown as complex A. Sequences of the sense strand of the oligonucleotide probes used are shown below the gels. Identical bases are marked with a dash, and mutations are indicated by the appropriate nucleotide change. The HNF1 and Cdx2 sites are boxed.

4C). However, cotransfection with Cdx2 was only able to activate the 1A10-140wt promoter slightly (<2-fold), indicating that the *UGT1A10* Cdx2 site may be less efficient at binding Cdx2 than the *UGT1A8* Cdx2 site. When HNF1α and Cdx2 were cotransfected, a level of activation (~6-fold) similar to that obtained with the equivalent *UGT1A8* construct was observed. This suggests that the *UGT1A10* HNF1 site, as well as the *-1A8* and *-1A9* HNF1 sites, are more important in determining the synergistic effect of HNF1α and Cdx2 than the Cdx2 sites. Mutation of the *UGT1A10* HNF1 (1A10-140 HNF1m) and Cdx2 (1A10-140 Cdx2m) sites did not significantly decrease the basal activity of the *UGT1A10* promoter (Fig. 4C). When the 1A10-140 HNF1m construct was cotransfected separately with HNF1α and Cdx2, only Cdx2 was able to slightly activate the promoter (<2-fold), with no synergistic activation being observed upon the addition of both factors (Fig. 4C). In contrast, HNF1α, but not Cdx2, was able to activate the 1A10-140 Cdx2m promoter and synergistically activate in combination with Cdx2 to levels similar to those observed with the wild-type construct (~3.5-fold and ~5-fold, respectively) (Fig. 4C). These results demonstrate that only the HNF1 site is required for *UGT1A10* promoter activation by HNF1α and synergistic activation by HNF1α and Cdx2 (Fig. 4C). Taken together, the cotransfection experiments demonstrate that HNF1α is equally able to activate the *UGT1A8*, *-1A9*, and *-1A10* promoters through the conserved HNF1 site. Cdx2 is also able to synergistically activate the three promoters in combination with HNF1α, with the level of promoter activation being of similar magnitude for each and dependent only on the presence of the conserved HNF1 site. Cdx2, in the absence of cotransfected HNF1α, efficiently activates the *UGT1A8* promoter, weakly activates the *UGT1A10* promoter, and cannot activate the *UGT1A9* promoter through the conserved Cdx2 site. This suggests that the relative affinity of Cdx2 for the respective sites may vary between the *UGT1A8*, *-1A9*, and *-1A10* promoters because of their context. A recent report by Di Guglielmo et al. (2001) indicated that the nucleotides surrounding the core Cdx2 binding site are important in determining its binding affinity. Therefore, we investigated the influence of the C-to-A nucleotide difference between *UGT1A8* and *UGT1A9*/*1A10* promoters (located 3 bp downstream of the indicated Cdx2 site) (Fig. 1B) on the relative binding affinity of Cdx2 for its site.

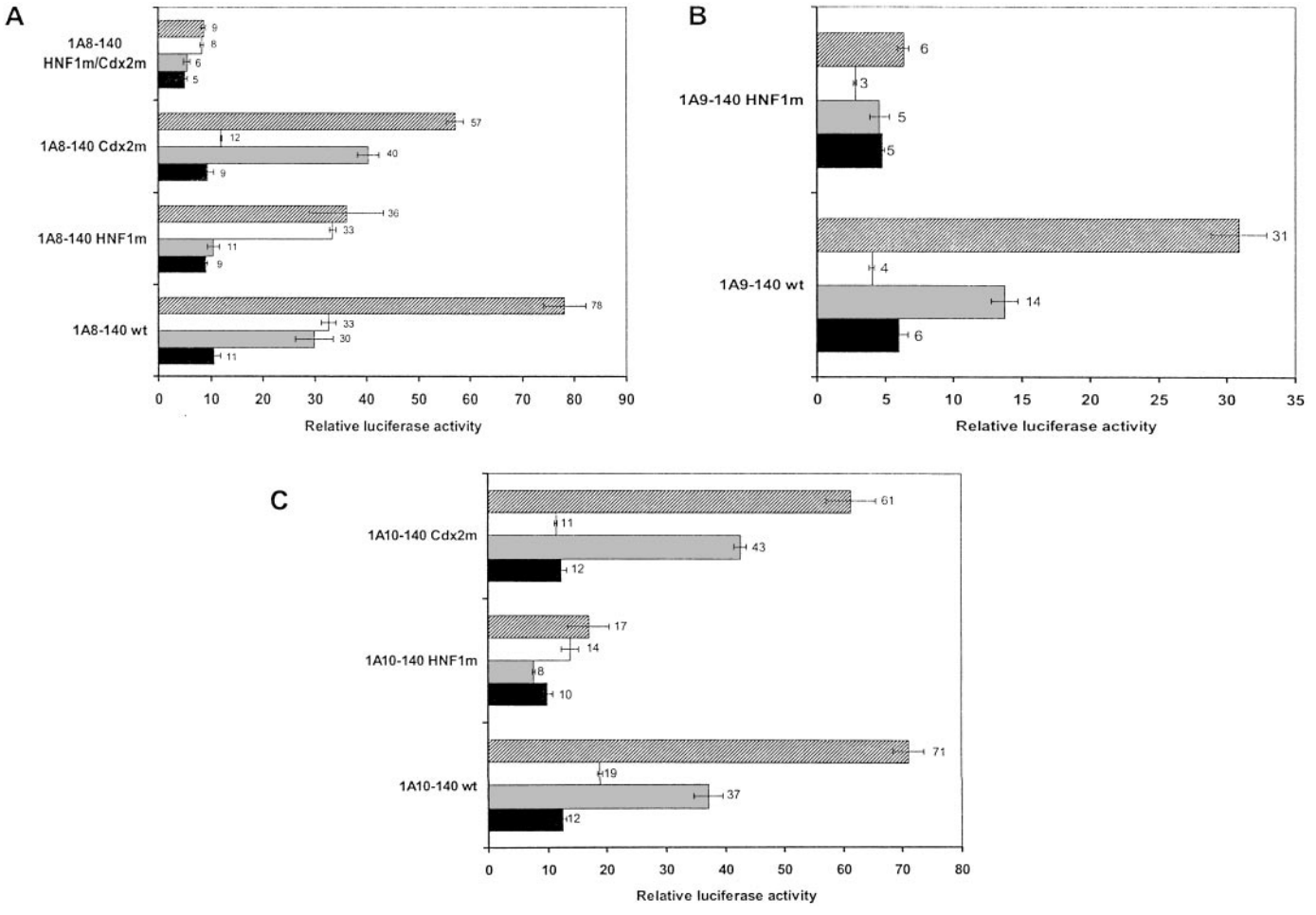

Fig. 4. HNF1α and Cdx2 synergistically activate the *UGT1A8*, *-1A9*, and *-1A10* promoters. A, B, and C, wild-type and HNF1, and Cdx2 and HNF1/Cdx2 mutant *UGT1A8* (A), *UGT1A9* (B), and *UGT1A10* (C) −140 promoter constructs (0.5 μg) were cotransfected into Caco2 cells with 0.25 μg of pCMV5 (empty expression vector; ■), HNF1α (▨), Cdx2 (□), or a combination of HNF1α and Cdx2 (▧) expression vectors. 0.025 μg of the pRL-null vector was added as an internal control of transfection efficiency, and the total amount of transfected DNA was normalized to 1 μg with pCMV5. Cells were harvested 48 h after transfection and were assayed for firefly and *R. reniformis* luciferase activities. Relative luciferase activities are expressed as a fold induction over the promoterless pGL3-basic vector (set at a value of 1). The results shown are a representative experiment of three individual experiments performed in triplicate (± S.D.).

Cdx2 Binds with Greater Affinity to the UGT1A8 Cdx2 Site Than to the UGT1A10 Cdx2 Site. Gel shift assays were used to compare the relative binding affinity of the *UGT1A8* and *-1A10* Cdx2 sites for Cdx2 using Caco2 nuclear extracts and in vitro transcribed/translated Cdx2 protein. The sequence of the sense strand of each oligonucleotide probe used (with the C/A base difference indicated in boldface type) are shown in Fig. 5. The addition of Caco2 nuclear extracts to the UGT1A8-Cdx2 probe resulted in the formation of an intense DNA-protein complex (labeled Cdx2; Fig. 5, lane 1) which was supershifted using the monoclonal Cdx2 antibody (lane 2) as observed previously. In contrast, the UGT1A10-Cdx2 probe only weakly bound the same Cdx2 complex (lane 3), as indicated by the supershifting of this complex with the Cdx2 antibody (lane 4). The only nucleotide differences between the UGT1A8-Cdx2 and UGT1A10-Cdx2 probes are the presence of an extra T in the poly(T) region

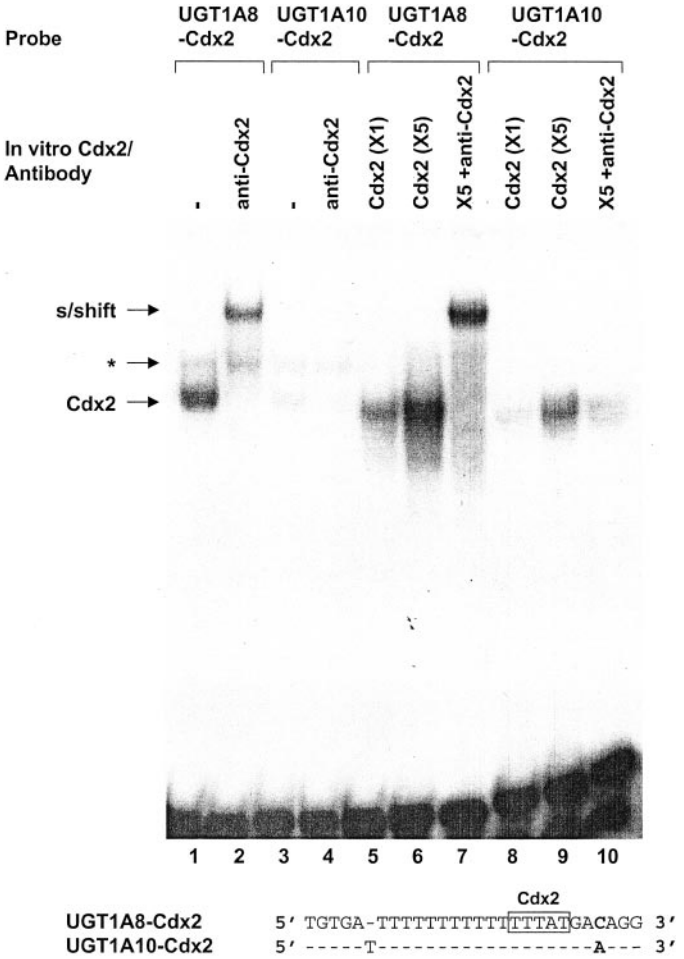

Fig. 5. Comparison of Cdx2 binding to the *UGT1A8* and *UGT1A10* Cdx2 binding sites. Double-stranded oligonucleotide probes (50,000 cpm) corresponding to *UGT1A8* and *UGT1A10* Cdx2 sites were incubated with either 5 μg of Caco2 nuclear extracts or varying amount (X1 or X5) of in vitro-transcribed/translated Cdx2 protein and then resolved on a 4% nondenaturing polyacrylamide gel. For supershift analysis, the Cdx2 antibody was added after the addition of labeled probe and incubated for 30 min before loading. Arrows, the Cdx2 complexes and supershifted bands. ⋆, the unidentified complex binding to the *UGT1A8* and *UGT1A10* Cdx2 probes with Caco2 nuclear extracts. Sequences of the sense strand of the oligonucleotide probes used are shown below the gel. Identical bases are marked with a dash, and base differences are indicated by the appropriate nucleotide change. The C/A base difference is indicated with boldfaced bases. The Cdx2 site is boxed.

and the C to A base change (3 bp downstream of the indicated Cdx2 site) in the UGT1A10-Cdx2 probe. To confirm that the difference in Cdx2 binding was not caused by the influence of possible accessory factors present in the Caco2 nuclear extracts, Cdx2 protein was generated by in vitro transcription/translation and used in gel shift assays. When a small amount of synthesized Cdx2 protein (X1) was used in place of Caco2 nuclear extracts, a DNA-protein complex of moderate intensity was observed (Fig. 5, lane 5). Increasing the amount of in vitro Cdx2 protein added by 5 times (X5) strongly increased the intensity of this complex (lane 6). The relative mobility of this complex was slightly higher than the complex generated with Caco2 nuclear extracts but was confirmed to contain Cdx2 by the ability of the Cdx2 antibody to supershift this complex (lane 7). In contrast to the UGT1A8-Cdx2 probe, the UGT1A10-Cdx2 probe only bound Cdx2 weakly with the X1 amount (lane 8) and moderately with the X5 amount (lane 9) of in vitro synthesized Cdx2 protein. This UGT1A10-Cdx2 complex was also able to be supershifted with the Cdx2 antibody (lane 10). These results imply that the *UGT1A8* Cdx2 site has a higher binding affinity for the Cdx2 protein than does the *UGT1A10* Cdx2 site. Competition studies with the UGT1A8-Cdx2 probe confirmed the above results by demonstrating that a 5-fold molar excess of unlabeled UGT1A8-Cdx2 probe could significantly decrease complex formation, whereas a 25-fold molar excess of unlabeled UGT1A10-Cdx2 was needed to decrease complex formation by the same amount (data not shown). Given that *UGT1A9* and *UGT1A10* both share the C-to-A nucleotide difference with *UGT1A8* in their Cdx2 site and that both promoters are weakly responsive the Cdx2 cotransfection, we decided to perform this nucleotide change in the UGT1A10-140 promoter and test its effect in transfection assays.

The A-to-C Nucleotide Change in the UGT1A10 Cdx2 Site Increases Promoter Activity. The 1A10-140 (A to C mutation) promoter, containing the A-to-C nucleotide change in the Cdx2 site was transfected into Caco2 cells with comparison to the 1A10-140wt promoter. As shown in Fig. 6, the 1A10-140 (A to C mutation) promoter was significantly higher in basal activity (~50%) than the wild-type promoter. Small increases in promoter activity were also observed after cotransfection with Cdx2 and with Cdx2 in combination with HNF1α but not with HNF1α alone. These increases in activity can most likely be attributed to an increase in Cdx2 binding to the altered *UGT1A10* Cdx2 site. These results suggest that the C-to-A nucleotide difference in the *UGT1A8* and *UGT1A10* Cdx2 sites is important in determining the activity of these promoters. The effect of the additional T in the poly(T) of the *UGT1A10* promoter compared with the *UGT1A8* promoter was also tested in transfection assays but was found to not significantly alter promoter activity (data not shown).

Discussion

The UDP-glucuronosyltransferase family of enzymes possesses a remarkable diversity in substrate specificity and tissue-specific expression, which facilitates their role in the metabolism and subsequent elimination of a wide range of endobiotic and xenobiotic compounds. The site of first contact for many xenobiotic compounds is the gastrointestinal tract, which variably expresses UGT isoforms of the UGT1A and

UGT2B subfamilies. Two UGT1A isoforms in particular, UGT1A8 and UGT1A10, are expressed exclusively in extrahepatic tissues including the small intestine and colon (Strassburg et al., 1997, 1998a; Cheng et al., 1998; Mojarrabi and Mackenzie, 1998). These two enzymes have been shown to glucuronidate a range of dietary flavanoids (Cheng et al., 1999), environmental carcinogens (Mojarrabi and Mackenzie, 1998; Nowell et al., 1999), phenolic compounds (Cheng et al., 1999), and drugs (Mackenzie, 2000; Watanabe et al., 2002) and may also have specialized functions in the intestine. In this study, we demonstrated that the intestine-specific transcription factors Cdx2 and HNF1α cooperatively regulate the *UGT1A8*, *-1A9*, and *-1A10* promoters in the colon-derived cell line Caco2. These results are the first to define tissue-specific factors controlling the extrahepatic expression of the *UGT1A8* and *UGT1A10* genes.

The *UGT1A8*, *-1A9*, and *-1A10* promoters share a high degree of sequence similarity (>75% over the first kilobase) in their proximal promoters but are differentially regulated through several regions. We have demonstrated previously that an initiator-like site strongly contributed to *UGT1A8* and *-1A10* but not *-1A9* promoter activity (Gregory et al., 2003). Furthermore, an upstream Sp1 site, located between −25 and −80 bp of the proximal promoter (Fig. 1B), was shown to be essential for promoter activation through the *UGT1A8/1A10* initiator-like site (Gregory et al., 2003). On closer inspection of the *UGT1A8*, *-1A9*, and *-1A10* promoters by deletion analysis, we confirmed that promoter activation through the initiator-like site requires the presence of upstream factors. The −25 bp *UGT1A8*, *-1A9*, and *-1A10* promoter constructs, which included the overlapping Sp1/initiator-like site, had virtually no promoter activity above background levels (Fig. 1A). Extending the promoters to −80 bp in length equally stimulated the activity of each promoter by approximately 5-fold, presumably because of the addition of functional Sp1 and Cdx2 binding sites (Fig. 1, A and B). The initiator-like site is not present in the *UGT1A9* promoter, and therefore the activity of this −80 promoter may be caused by the upstream Sp1 site acting through a different mechanism. Interestingly, increasing the *UGT1A8*, *-1A9*, and *-1A10* promoters to −140 bp in length differentially affected *UGT1A8/1A10* and *UGT1A9* promoter activity (Fig. 1A). The *UGT1A8*, *-1A9*, and *-1A10* promoters all contain an HNF1 binding site between −80 and −140 (Fig. 1B); however, mutation of this site in each promoter only slightly diminished promoter activity (Fig. 4). Therefore, the differential activity of the −140 *UGT1A8/1A10* and *UGT1A9* promoters is likely to be caused by other differences in *cis* elements in the −80 to −140 region or by a combinatorial effect of HNF1 or these other *cis* elements with downstream promoter elements. In support of the latter hypothesis, mutation of the *UGT1A9* −140 promoter to generate an equivalent *UGT1A8/1A10* initiator-like site increased promoter activity to levels similar to the *UGT1A8/1A10* −140 promoters (data not shown).

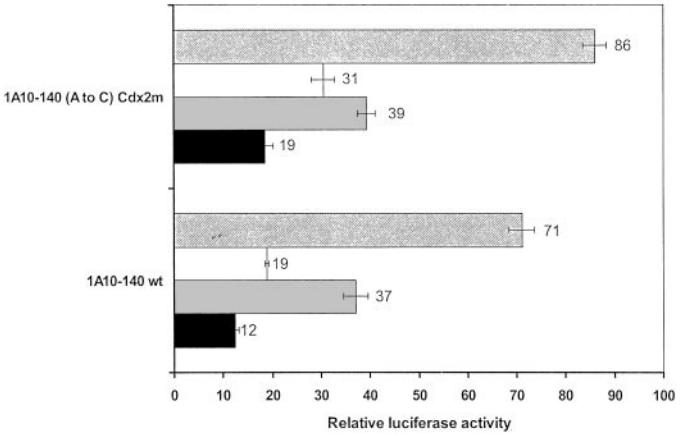

Fig. 6. A single nucleotide difference between the *UGT1A8* and *-1A10* Cdx2 sites influences basal promoter activity and Cdx2-mediated promoter activation. *UGT1A10* −140 wild-type and *UGT1A10* −140 (A to C) Cdx2 mutant promoter constructs (0.5 μg), in which the A-to-C nucleotide difference between the *UGT1A8* and *-1A10* Cdx2 sites is changed, were cotransfected into Caco2 cells with 0.25 μg of pCMV5 (empty expression vector; ■), HNF1α (▨), Cdx2 (□), or a combination of HNF1α and Cdx2 (▧) expression vectors. A 0.025-μg sample of the pRL-null vector was added as an internal control of transfection efficiency, and the total amount of transfected DNA was normalized to 1 μg with pCMV5. Cells were harvested 48 h after transfection and assayed for firefly and *R. reniformis* luciferase activities. Relative luciferase activities are expressed as a fold-induction over the promoterless pGL3-basic vector (set at a value of 1). The results shown represent a triplicate experiment (± S.D.).

The reliance of *UGT1A8*, *-1A9*, and *-1A10* promoter activity on multiple interacting elements is also demonstrated in the cooperative effect of HNF1α and Cdx2 on these promoters. Mutation of either the *UGT1A8* HNF1 or Cdx2 alone did not significantly alter promoter activity; however, mutation of both sites reduced promoter activity by 2-fold (Fig. 2). This result suggests that the loss of either the *UGT1A8* HNF1 or Cdx2 sites could be compensated by the presence of the other. Interestingly, mutation of the *UGT1A9* and *-1A10* HNF1 sites also did not significantly reduce promoter activity (Fig. 4), even though their Cdx2 sites bound Cdx2 with a much lower affinity that the *UGT1A8* Cdx2 site (Fig. 5). It is possible that the level of Cdx2 binding to the *UGT1A9* and *-1A10* sites is sufficient to promote basal levels of promoter activity or that other additional elements may compensate in the absence of the HNF1 site. Cotransfection studies showed that HNF1α and Cdx2 were able to activate the *UGT1A8*, *-1A9*, and *-1A10* promoters through their respective sites and, in combination, synergistically activate these promoters (Fig. 4). This synergistic effect only required the presence of a functional HNF1 site; however, a greater level of synergism was observed when the Cdx2 site was present. A similar mechanism of regulation has been shown with the intestine-specific SI and LPH genes. Mitchelmore et al. (2000) demonstrated that HNF1α and Cdx2 are able to physically interact and synergistically activate the porcine LPH promoter. The synergistic effect on the porcine LPH promoter occurred in the absence of a functional Cdx2 site, suggesting that binding to the HNF1 site was the only requirement for synergism. Cooperative binding of HNF1α and Cdx2 to the LPH HNF1 site could not be detected by gel shift assay, which is in agreement with our studies of the *UGT1A8*, *-1A9*, and *-1A10* promoters using Caco2 nuclear extracts (data not shown). This may be caused by the conditions of the gel shift assay or the lack of stabilizing factors that are present in vivo. However, HNF1α and Cdx2 were shown by Mitchelmore et al. (2000) to physically interact in vitro through their homeodomain regions using glutathione *S*-transferase pull-down assays. Cooperation between HNF1α and Cdx2 has also been shown to occur on the human SI and LPH promoters (Krasinski et al., 2001). These promoters contain HNF1 and Cdx2 binding sites located in close proximity to each other, as

observed with the *UGT1A8*, *-1A9*, and *-1A10* promoters. A recent report by Sakaguchi et al. (2002) has shown that that the claudin-2 promoter also has a structure similar to those of the above promoters and is synergistically activated by HNF1α and Cdx2 in Caco2 cells. These studies suggest that intestinal gene promoters may be regulated, in part, by a common mechanism involving the interaction of HNF1α and Cdx2. Although the presence of the Cdx2 site is dispensable, in some cases, for the synergistic effect of HNF1α and Cdx2, studies with the mouse SI promoter indicates that the SI Cdx2 site is essential for promoter activation in vivo (Boudreau et al., 2002). Several groups have also shown that a third family of accessory factors, GATA-4, GATA-5, and GATA-6, are also able to interact with HNF1α and Cdx2 to further enhance their activation (Krasinski et al., 2001; Boudreau et al., 2002; van Wering et al., 2002). It will be interesting to determine whether other intestinal gene promoters, including the *UGT1A8* and *-1A10* promoters, are also regulated by these GATA factors.

Gel shift assays indicated that Cdx2 bound with varying affinity to its respective sites in the *UGT1A8*, *-1A9*, and *-1A10* promoters. Nuclear extracts from Caco2 cells and in vitro-synthesized Cdx2 protein bound with greater affinity to the *UGT1A8* Cdx2 site than to the *UGT1A9/1A10* Cdx2 site (Fig. 5). These differences in Cdx2 binding correlated well with the ability of cotransfected Cdx2 to activate the respective promoters (Fig. 4). The Cdx2 binding site was identical in core sequence for all three promoters and matched the consensus binding site (TTTAT/C) (Suh et al., 1994). However, a single nucleotide difference (A to C), 3 bp downstream of the core Cdx2 binding site, between the *UGT1A8* and *UGT1A9/1A10* promoters was shown to be critical in determining the relative binding affinity of Cdx2 (Fig. 5). Analysis of the Cdx2 site in the guanylyl cyclase C promoter has also found that bases 3′ of the core Cdx2 sequence are important in enhancing the stability of Cdx2 binding (Di Guglielmo et al., 2001). Mutation of the nucleotide difference in the *UGT1A10* Cdx2 site (from A to C) was shown to be functionally important, increasing the basal level of *UGT1A10* promoter activity (Fig. 6). Interestingly, *UGT1A9*, which contains the same Cdx2 binding site as *UGT1A10*, could not be activated by cotransfected Cdx2 through this site. It is possible that the *UGT1A9* promoter lacks downstream promoter elements, such as the initiator-like site in *UGT1A8* and *-1A10*, which are necessary for Cdx2-mediated promoter activation. In support of this idea, Cdx2 was demonstrated to activate a *UGT1A9* promoter that was engineered to contain the *UGT1A8/1A10* initiator-like site (data not shown). The difference in Cdx2 binding affinity to the *UGT1A8* and *-1A10* promoters may become functionally significant when HNF1 levels are lower or absent. For example, during intestinal development, the level and distribution of HNF1α is known to vary significantly (Boudreau et al., 2002) in contrast to Cdx2 protein expression, which remains relatively unchanged (Silberg et al., 2000). The Cdx2 site may additionally serve a recruitment function, allowing Cdx2 to come into contact with HNF1α and promote higher levels of expression.

The demonstration that the *UGT1A8*, *-1A9*, and *-1A10* promoters are coordinately regulated by HNF1α and Cdx2, transcription factors with a tissue-specific distribution, has important implications for their tissue-specific expression. The *UGT1A7*, *-1A8*, and *-1A10* genes are unique among UGTs in that they are expressed exclusively in extrahepatic tissues. UGT1A8 and -1A10 expression has been detected throughout the various tissues of the gastrointestinal tract, including the small intestine and colon (Strassburg et al., 1997, 1998a; Cheng et al., 1998; Mojarrabi and Mackenzie, 1998), whereas UGT1A7 expression is limited to the stomach and esophagus (Strassburg et al., 1997, 1999). Strassburg et al. have further localized UGT1A family expression to the epithelial cells in both the crypt and villus of the intestinal mucosa. This localization correlates well with the expression of HNF1α and Cdx2, which are primarily found in the villus epithelial cells of the intestine (James et al., 1994; Silberg et al., 2000; Boudreau et al., 2002). Although HNF1α is also expressed in a wide range of other tissues throughout the body (Baumhueter et al., 1990), Cdx2 expression seems to be restricted to the intestine (James et al., 1994; Suh et al., 1994). The presence of a Cdx2 binding site within promoters such as *UGT1A8* and *-1A10* may therefore be a more important determinant of their intestinal expression than an HNF1 site. In support of this theory is the *UGT1A7* gene, whose promoter also contains an HNF1 binding site in the same position as *UGT1A8* and *-1A10* but lacks a comparable Cdx2 binding site (data not shown). The absence of this functional Cdx2 site may explain, in part, the lack of UGT1A7 expression in the small intestine and colon. In addition, Cdx2 is not expressed in the stomach (Silberg et al., 2000) where UGT1A7 is present, suggesting that a different mechanism contributes to the extrahepatic expression of this isoform. On the other hand, UGT1A9 is expressed in the colon (Strassburg et al., 1998a) and does not contain a functional Cdx2 binding site. It is possible that intestinal expression of UGT1A9 may be mediated through direct interaction of Cdx2 with HNF1α bound to its HNF1 site. Several other UGT isoforms, which are expressed in the intestine, also have a functional HNF1 site (Bernard et al., 1999; Ishii et al., 2000) and may be regulated in this tissue through similar interactions.

In summary, we have identified HNF1α and Cdx2 as important factors regulating the *UGT1A8*, *-1A9*, and *-1A10* promoters in extrahepatic cells. Cdx2 differentially binds to and regulates the *UGT1A8*, *-1A9*, and *-1A10* promoters and can synergistically activate the promoters through cooperation with HNF1α. These studies are the first to define factors that contribute to the extrahepatic expression of UGTs and provide insight into the mechanisms controlling intestinal expression of the *UGT1A8*, *-1A9*, and *-1A10* genes.

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