Tissue-specific, Inducible, and Hormonal Control of the Human UDP-Glucuronosyltransferase-1 (UGT1) Locus

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The human UDP-glucuronosyltransferase 1 (UGT1) locus spans nearly 200 kb on chromosome 2 and encodes nine UGT1A proteins that play a prominent role in drug and xenobiotic metabolism. Transgenic UGT1 (Tg-UGT1) mice have been created, and it has been demonstrated that tissue-specific and xenobiotic receptor control of the UGT1A genes is influenced through circulating humoral factors. In Tg-UGT1 mice, the UGT1A proteins are differentially expressed in the liver and gastrointestinal tract. Gene expression profiles confirmed that all of the UGT1A genes can be targeted for regulation by the pregnane X receptor activator pregnenolone-16α-carbonitrile (PCN) or the Ah receptor ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In addition, the selective induction of glucuronidation activity toward lamotrigine, ethinyl estradiol, chenodeoxycholic acid, and lithocholic acid by either PCN or TCDD in small intestine from Tg-UGT1 mice corresponded to expression of the locus in this tissue. Induction of UGT1A1 by PCN and TCDD is believed to be highly dependent upon glucocorticoids, because submicromolar concentrations of dexamethasone actively promote PCN and TCDD induction of UGT1A1 in Tg-UGT1 primary hepatocytes. The role of hormonal control of the UGT1 locus was further verified in pregnant and nursing Tg-UGT1 mice. In maternal 14-day post-conception Tg-UGT1 mice, liver UGT1A4, UGT1A4, and UGT1A6 were induced, with the levels returning to near normal by birth. However, maternal liver UGT1A4 and UGT1A6 were dramatically elevated and maintained after birth, indicating that these proteins may play a critical role in maternal metabolism during lactation. With expression of the UGT1 locus confirmed in a variety of mouse tissues, these results suggested that the Tg-UGT1 mice will be a useful model to examine the regulatory and functional properties of human glucuronidation.

The formation of β-glucopyranosiduronic acids by the multigene family of UDP-glucuronosyltransferases (UGTs) requires UDP-glucuronic acid to transform drugs and xenobiotics into hydrophilic glucuronides, facilitating their excretion into the bile or urine (1). Located in the cellular endoplasmic reticulum, the UGs play a vital role in the metabolism and detoxification of steroids, bile acids, hormones, environmental toxicants, carcinogens, and a multitude of drugs (2). In humans, the UGT1 and UGT2 gene families encode 19 RNA transcripts that have been identified from human tissues, and in vitro expression of these transcripts in tissue culture has aided in defining the substrate specificities of the UGTs (2). Although UGT1 and UGT2 proteins are involved in drug metabolism (3), it is believed that the UGT1 proteins are favored in the metabolism of a greater proportion of xenobiotic substrates. Both UGT1 and UGT2 proteins glucuronidate endobiotic substrates, with the UGT1 enzymes showing specificity for estrogens, whereas the UGT2 proteins exhibit a preference for androgens (4). Seven UGT2B genes and three UGT2A genes are encoded as individual structural genes on chromosome 4, and the UGT1 locus encodes nine UGT1A proteins (UGT1A1 genes) on chromosome 2 (5, 6).

The UGT1A gene products are generated by a strategy of exon sharing, resulting in a family of microosomal proteins in which each contains a divergent amino-terminal 280 amino acids and a commonly shared carboxyl terminus that encodes 245 amino acids (7, 8). The UGT1 locus spans more than 200 kb on chromosome 2 (9) and is structured with a series of divergent exon 1 sequences that are organized consecutively over 150 kb with each exon 1 sequence encoding ~280 amino acids of the amino-terminal portion. Located in the 3′ region of the locus are exons 2–5, which encode the conserved 245 amino acids of the carboxyl region. Flanking each of the exon 1 sequences are the necessary structural elements to ensure appropriate transcriptional activation as monitored by expression in human tissue of UGT1A RNA gene transcripts (2). Reports regarding UGT1A RNA expression profiles indicate that each tissue contains a selective complement of UGT1A gene products (2, 10–12) with the gastrointestinal tract serving as a rich source for UGT1A expression (13–15). Adding to the uniqueness of these expression patterns, regulation of the UGT1 locus is also targeted by a number of xenobiotic and steroid receptors. The xenobiotic receptors pregnenolone X receptor (PXR), pregnane X receptor, and constitutive androstane receptor (CAR) (16), the constitutive androstane receptor (16, 17), as well as the Ah receptor (18) have been shown in tissue culture to regulate UGT1A gene expression, promoting UGT1A1 protein induction. In addition, glucocorticoids work in a synergistic fashion to promote PXR and constitutive androstane receptor induction of the UGT1A1 gene (19), providing support for the theory that circulating hormones may play a crucial role in maintaining appropriate levels.
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of the UGTS in vivo. Exposure to selective environmental toxicants that activate the Ah receptor has been linked to transcriptional regulation of UGT1A6 and UGT1A9 (20–22). Other recent findings have also demonstrated that human variants of the PXR have been implicated in expression of UGT1A3 and UGT1A4 (23), whereas the peroxisome proliferator-activated receptors α and γ regulate UGT1A9 (50). Thus, regulation of the UGTI locus is believed to be controlled in a tissue-specific manner by hormones, in addition to induction following exposure to xenobiotics. Clearly, a viable animal model to investigate the in vivo events associated with regulation of the UGTI locus would promote a better understanding of the role of selective xenobiotic receptors on gene control.

One of the most important concepts in all of drug metabolism is an understanding of those events that control both infant and maternal drug metabolism during fetal and neonatal development. It is well known that levels of human glucuronidation gradually increase through development, including the weeks and months following birth. Yet it might be anticipated that the dramatic changes in the levels of circulating hormones that occur during pregnancy and lactation (24) may alter the levels of hepatic enzymes in maternal liver. In rodents, several studies indicate that maternal liver glucuronidation activity is lower during pregnancy (25, 26). However, in humans, selective glucuronidation activities during pregnancy are induced, as evidenced by increased oral clearance of paracetamol (27) and lamotrigine (28, 29). Lamotrigine is a quaternary amine that undergoes N-glucuronidation (30) by human UGT1A4 (31), whereas paracetamol is a phenolic compound that can be glucuronidated by UGT1A1 and UGT1A6 (32, 33). Thus, in an effort to study those events that link homeostatic control of the UGT1 locus with various aspects of human glucuronidation in adults as well as during fetal development and lactation, a mouse UGT transgenic model (Tg-UGT1) has been developed that expresses a bacterial artificial chromosome encoding the entire UGT1 locus.

Evidence is presented that each of the nine UGT1A genes is expressed in selective tissues in Tg-UGT1 mice. Expression of the UGT1 locus in transgenic mice provides not only a unique opportunity to examine the regulatory properties that control the tissue-specific and xenobiotic receptor-elicted expression patterns of the individual UGT1A genes but also enriches an understanding of how the UGT1 locus may be regulated at times where changes are apparent in the physiological levels of circulating hormones. Our results illustrate that the transgenic UGT1 mice will allow for investigation of gene control and protein expression and may advance our understanding of how this locus is regulated in humans.

MATERIALS AND METHODS

Reagents—2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was obtained from Wellington Laboratories (Guelph, Ontario, Canada). Pregnenolone-16α-carbonitrile (PCN), Trizma (Tris base), saccharo-1,4-lactone, dexa-methasone, UDP-glucuronic acid, and 1,4-bis-[2-(3,5-dichloropyridyloxy)] benzene (TCPPOB) were from Sigma. Lamotrigine was purchased from Toronto Research Chemicals Inc., Ontario, Canada, and the 2-N-glucuronide metabolite was a gift from GlaxoSmithKline. Both ethinyl estradiol-2 and ethinyl estradiol-3-glucuronide were obtained from Steraloids Inc. Solvents for chromatography were HPLC grade from Fisher. Ammonium acetate, HPLC grade, was from J. T. Baker Inc. A sample of human small intestinal microsomes, collected from three woman and three men, was purchased from BIOPREDIC International, UK. Human UGT1A1, UGT1A4, and UGT1A6 cDNAs expressed in baculovirus-infected insect cells were purchased from BD Biosciences.

Generation of the UGT1 Humanized Mouse—A bacterial artificial chromosome encoding the entire human UGT1 locus, described previously (18), was purified by CsCl banding and dialyzed against microinjection buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA, 30 μM spermine, 70 μM spermidine, and 100 mM NaCl). The purified DNA was microinjected into the pronucleus of CB6F1, (an F1 hybrid between BALB/c and C57BL/6N mice) mouse eggs and transplanted into the oviduct of pseudopregnant C57BL/6N mice. All procedures for the generation of the transgenic mice were carried at the University of California, San Diego, Superfund Transgenic Core Facility. For genotyping, DNA was isolated from tail clippings of 46 3-week-old mice, and a 366-bp region in exon 5 of the common region of the human UGT1 locus was identified by PCR in 12 founders using sense (5′-cataataataatcagcccag-3′), bases 187,423–187,443, GenBank™ accession number AF297093 and antisense (5′-cccttttaaaacacacaagg-3′), bases 187,789–187,809) primers. Each founder was further profiled by PCR using specific primers that encoded a portion of each of the unique exon 1 sequences (11). Five founders containing the entire UGT1 locus were bred into C57BL/6N mice from the Jackson Laboratory (Bar Harbor, ME).

The transgene UGT1 copy number in each of the Tg-UGT1 mice was determined from tail genomic DNA. Real time quantitative PCR was performed using the QuantiTect™ SYBR® Green PCR kit (Qiagen, Valencia, CA), and the reactions were run in an MX400 Multiplex QPCR machine. Each reaction contained varying concentrations of DNA and 0.2 μM of the UGT1A3 sense 5′-ttgtgaaatgtctctttgctca-3′ and antisense 5′-ccaatgaacagctggtgagc-3′ oligonucleotides. PCR was conducted in a 50-μl reaction, and the polymerase was activated at 95 °C for 10 min followed by 40 cycles of 95 °C for 30 s, 58 °C for 1 min, and 72 °C for 30 s, and a final cycle of 95 °C for 1 min. In a separate reaction, mouse β-actin primers were used with each sample of DNA, and the corresponding cycle threshold (Ct) values were used to determine the exact loading of DNA.

Total molecules of the cloned UGT1 locus in the BAC DNA was determined by generating Ct values over a dilution of BAC DNA from 10 to 200 pg, with each dilution evaluated in 100 ng of wild type mouse DNA. Because the BAC DNA is represented by 2.5 × 106 bases, the generated Ct values from the diluted BAC DNA were calculated to represent 0.5–100 copies of the UGT1 locus. This allowed for a direct comparison of the number of copies of the UGT1 locus per sample of human and mouse transgenic DNA. For these calculations, we assumed that human haploid DNA contains two copies of the UGT1 locus.

Preparation of Antibodies to Human UGT1A1, UGT1A4, and UGT1A6—The preparation of polyclonal antisera recognizing residues 29–159 of the human UGT1A1 protein has been described (34). Antisera recognizing human UGT1A4 and UGT1A6 were prepared using the same methodologies. Briefly, His6-α,β,γ,δ-tagged fusion proteins were expressed in E. coli strain SG13009 (QIagen) from pQE30 (QIagen)-based plasmid constructs containing the coding sequence for residues 30–160 of UGT1A4 (construct pQE30-h1A4) or 12–131 of UGT1A6 (construct pQE30-h1A6). Expression of each fusion protein was induced in log phase cultures of transformed bacteria by the addition of 1 mM isopropyl β-D-thiogalactopyranoside. After a 4-h induction, the cultures were harvested, and fusion proteins were purified by affinity chromatography using nickel-nitriilotriacetic acid–Sepharose affinity resin (QIagen). Immunizations were performed using 10 female B6C3F1 mice for each individual form. One week after the final booster injection, animals were anesthetized, and blood was collected by cardiac puncture. The protocol used for raising antisera followed National Institutes of Health guidelines for the care and use of laboratory animals and received the approval of the Virginia Commonwealth University Institutional Animal Care and Use Committee. Serum samples for each antisera were pooled and aliquoted (50 μl/tube) prior to storage at −80 °C.
Micromodal Protein Isolation from Transgenic Mouse Tissues—Using three animals per group, the liver and small and large intestinal tissues were collected from Tg-UGT1 and wild type mice. For the small and large intestine, the tissue was dissected lengthwise and the luminal surface gently rinsed in 1.15% KCl before freezing on dry ice. Tissue samples from each treatment group were combined and frozen in liquid nitrogen in a porcelain mortar and pulverized under liquid nitrogen. A sample of the pulverized tissue was added to 5 volumes of 1.15% ice-cold KCl, and the tissue was homogenized using a motorized glass Teflon homogenizer. The tissue homogenate was first centrifuged at 2,000 × g for 10 min at 4 °C, and the supernatant was collected. The supernatant was then centrifuged at 9,000 × g for 10 min at 4 °C, and this resulting supernatant was centrifuged at 100,000 × g for 60 min at 4 °C. The pellet was resuspended in buffer (50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride), and the protein concentration was determined by the Bradford method.

Immunoblot Analysis—All Western blots were performed using NuPAGE BisTris-polyacrylamide gels as outlined by the supplier (Invitrogen). Protein was heated at 70 °C for 10 min in loading buffer and resolved in 4–12% BisTris gels under denaturing conditions (50 mM MOPS, 50 mM Tris base, pH 7.7, 0.1% SDS, 1 mM EDTA) prior to transferring the proteins to polyvinylidene difluoride membrane using a semidyne transfer system (Novex). The membrane was blocked with 5% nonfat dry milk in 10 mM Tris-HCl, pH 7.4, containing 0.15% NaCl and 0.05% Tween 20 (Tris-buffered saline) for 1 h at room temperature, followed by incubation with primary antibodies (mouse anti-human UGT1A1, UGT1A4, or UGT1A6) in Tris-buffered saline overnight at 4 °C. Membranes were washed and exposed to horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Each membrane was washed again, and the conjugated horseradish peroxidase was detected using the ECL plus Western blotting detection system (Amersham Biosciences), and the proteins were detected following exposure to x-ray film.

Isolation and Treatment of Mouse Transgenic Primary Hepatocytes—Primary hepatocytes were isolated from 8- to 12-week-old mice. Mice were anesthetized by isoflurane inhalation. The portal vein was cannulated and perfused with Hanks' balanced salt solution (Ca²⁺-free and Mg²⁺-free) containing 0.1 mM EGTA and 10 mM Hepes, pH 7.4, for 5 min at the rate of 7 ml/min. As soon as the perfusion was started, the anterior vena cava was cut to allow continuous flow to proceed out of the liver. At this time, the perfusate was changed to a solution containing 20 µg/ml Liberase Blendzymes (Roche Applied Science) that dissolved in Hanks' balanced salt solution (with Ca²⁺ and Mg²⁺), and the perfusion continued for another 5 min. The liver was removed, and the hepatocytes were isolated by mechanical dissection followed by filtration through a sterile 70-µm filter. The cells were immediately collected by centrifugation at 50 × g for 30 s and washed in Dulbecco's modified Eagle's tissue culture media. Cell viability was examined by trypan blue exclusion, and experiments were conducted only if viability exceeded 90%. The hepatocytes were then cultured in 6-well collagen-treated plates (Discovery Labware, Bedford, MA) in 3 ml of Dulbecco's modified Eagle's medium containing penicillin/streptomycin and supplemented with 10% fetal bovine serum. Three hours after plating, the medium was replaced with fresh medium. The hepatocytes were treated with various chemicals 24 h after seeding for further studies. For analysis of proteins by Western blot, hepatocytes were collected and lysed in a buffer containing 0.05 M Tris-HCl, pH 7.4, 0.15% NaCl, 0.25% deoxycholic acid, and 1% Nonidet P-40 with a complement of protease and phosphatase mixture inhibitors (Sigma). After incubation of this mixture for 30 min on ice, the solubilized lysate was centrifuged for 20 min in a refrigerated Eppendorf centrifuge at 16,000 × g. The supernatant was collected and used directly for Western blot studies.

Determination of UGT Catalytic Activity—For the determination of lithocholic acid (LCA)-24 and chenodeoxycholic acid (CDCA)-24 glucuronide, microsomal proteins (10 µg) were incubated in the presence of LCA or CDCA (200 µM) and UDP-glucuronic acid (2 mM) for 1 h at 37 °C in a glucuronidation assay buffer as reported (4). Assays were ended by adding 100 µl of methanol with 0.02% butyraldehyde hydroxytoluene (Sigma), and the production of LCA- and CDCA-24G was subsequently ensured by liquid chromatography with electrospray ionization coupled to mass spectrometry. Briefly, 25 µl of analytical standards or assays were injected into an Alliance 2690 HPLC system (Waters, Milford, MA). Analytes were separated on a Synergi RP Hydro 100 × 4.6 mm, 4-µm column (Phenomenex, Torrance, CA) and eluted at a flow rate of 1.0 ml/min with a split ratio of 1:4. Initial conditions were 40% water, 1 mM ammonium formate, 60% methanol, 1 mM ammonium formate, followed by a linear gradient to 85% methanol, 1 mM ammonium formate in 3 min. This condition was maintained for 2 min, and the column was flushed with 95% methanol, 1 mM ammonium formate for 2 min and re-equilibrated to initial conditions for an additional 2 min. All analytes were quantified by multiple reactions in monitoring mode with a triple quadrupole mass spectrometer API 3000 (Applied Biosystems-Siex, Concord, Canada) equipped with a turbo ion spray source. Ion pairs detected were m/z 567.5 → 391.5 for CDCA-24G and 551.5 → 375.5 for LCA-24G, and the concentration of glucuronides produced in each assay was obtained by reporting the peak area ratios to the standard curves established with the corresponding purified conjugate.

For analysis of lamotrigine and ethinyl estradiol glucuronidation, glucuronidation reactions were started by the addition of 3 mM UDP-glucuronic acid and incubated for 30 min with lamotrigine and 20 min with ethinyl estradiol at 37 °C. Chilled acetonitrile (200 µl) was used to stop the reactions, followed by centrifugation at 13,000 rpm for 5 min. The supernatant was the centrifuged once through 0.2-µm nylon spin filters. The separation of lamotrigine-ß-glucuronide was carried out by injecting an aliquot (2 µl) on a Higgins Analytical Haisil C-18 column (100 × 2.1 mm, 5 µm, ChromTech, Apple Valley, MN) at room temperature. The gradient consists of 20–60% acetonitrile in 20 mM ammonium acetate buffer, pH 6.67, over a period of 6 min. The percentage of organic was held steady for 2 min before and after the gradient, followed by a 4-min re-equilibration step. The flow rate for the analysis was 0.3 ml/min. Retention time for lamotrigine-ß-glucuronide was 5.4 min. Data acquisition was achieved on liquid chromatography–mass spectrometry Solution Software® from Shimadzu Inc. Standard curves for lamotrigine-ß-glucuronide (added to blank pig liver microsomes) were developed with concentrations of 10–500 µM and showed an R² = 0.999. The coefficient of variation was <10% at all concentrations of lamotrigine-ß-glucuronide. Kinetic analysis was performed with Sigma Plot (Systat, Richmond, CA).

The HPLC separation of ethinyl estradiol-glucuronide was carried out by injecting an aliquot (2 µl) on a Higgins Analytical Haisil C-8 column (100 × 2.1 mm, 5 µm, ChromTech, Apple Valley, MN) at room temperature. The mobile phase was initially held steady at 20% acetonitrile, 80% 20 mM ammonium acetate buffer, pH 3.0, for 5 min. This was followed by a linear gradient from 20 to 80% acetonitrile in 20 mM ammonium acetate buffer, pH 3.0, over a period of 18 min. The percentage of organic was held steady for 4 min at 80%, with re-equilibration for 5 min. The flow rate for the analysis was 0.3 ml/min. Ethinyl estradiol-glucuronide was eluted at 17.5 min. Standard curves for ethinyl estradiol-glucuronide were developed with concentrations of 1–400 µM and showed an R² = 0.998. The coefficient of variation was <10% at all concentrations of ethinyl estradiol-glucuronide.
Total RNA Preparation and Analysis of RNA by Real Time RT-PCR—Primary hepatocytes attached to the collagen-coated plates were washed in cold phosphate-buffered saline once, followed by the addition of 1 ml of acidic phenol/guanidinium isothiocyanate solution (TRIzol, Invitrogen). After 3 min, the TRIzol was removed; 200 μl of chloroform was added, and the solution was vortexed for 15 s. The solution was centrifuged at 11,000 rpm in a refrigerated Eppendorf centrifuge for 15 min, and the water phase was removed. The RNA was precipitated by the addition of 500 μl of isopropanol and collected by centrifugation, followed by washing with 75% ethanol. Using Omniscript Reverse Transcriptase (Qiagen, Valencia, CA) according to the manufacturer’s protocol. For detection of human UGT1A1 RNA, the forward primer was 5'-aacaacgcttgctc-3' and the reverse primer was 5'-gctgCCAAGGATCCG-3' (MS7899). Analysis of the mouse β-actin RNA, the forward primer was 5'-agaactgctggctc-3' and the reverse primer was 5'-gctgCCAGGGAGACA-3'. The polymerase was activated at 95 °C for 10 min followed by 40 cycles of amplification that consisted of the following: 95 °C for 30 s and 63 °C for 1 min followed by 72 °C for 45 s. Amplification was followed by DNA melt at 95 °C for 1 min and a 41-cycle dissociation curve starting at 30 s and 63 °C for 1 min followed by 72 °C for 45 s. Amplification was followed by DNA melt at 95 °C for 1 min and a 41-cycle dissociation curve starting at 30 s and 63 °C for 1 min followed by 72 °C for 45 s. Amplification was followed by DNA melt at 95 °C for 1 min and a 41-cycle dissociation curve starting at 30 s and 63 °C for 1 min followed by 72 °C for 45 s. Amplification was followed by DNA melt at 95 °C for 1 min and a 41-cycle dissociation curve starting at 30 s and 63 °C for 1 min followed by 72 °C for 45 s and a final extension at 72 °C for 7 min. For UGT1A8 PCR amplification, the polymerase was activated at 95 °C for 15 min followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 7 min. Analysis of expressed RNA included an antisense oligonucleotide specific for the common region that encoded exon 2, whereas all of the sense primers encoded a highly specific segment of each exon 1 sequence that allowed for the unique identification of each UGT1A RNA. PCRs were carried out in a PerkinElmer Life Sciences GeneAmp DNA thermocycler PCR system. Ten microliters of each PCR product was resolved in a 1.5% agarose gel containing 1 μg/ml ethidium bromide and photographed using Polaroid 665 positive/negative film (Polaroid, Cambridge, MA).

RESULTS

UGT1 Locus Expression in Transgenic Mice—The entire UGT1 locus was isolated from a human BAC genomic library and characterized by restriction enzyme mapping and DNA sequence analysis of the open reading frames (18). The locus extends in the 5′-direction, encoding all of the functional exon 1 sequences (1A1 through 1A10) as well as the conserved exons 2–5 (18). The BAC clone was purified and microinjected into fertilized FVB/N mouse eggs, and transgenic mice were produced. Genotype analysis from tail clippings identified founders carrying exons 1 sequences UGT1A1 through UGT1A10 in addition to the 3′-noncoding region of exon 5 (Fig. 1). We arbitrarily selected five founders identified as Tg-UGT1a1, Tg-UGT1b1, Tg-UGT1c1, Tg-UGT1d1, and Tg-UGT1e1 for breeding experiments, and all transmitted the UGT1 locus into F1 progeny. Southern blot analysis of genomic DNA from each of the transgenic lines showed hybridization signals that were the same as human genomic DNA, indicating that the linear arrangement of the UGT1 locus was structurally intact (data not shown). Although the copy number of the UGT1 locus in each of these founders was calculated to be ~2 (Tg-UGT1a1), 1 (Tg-UGT1b1), 6 (Tg-UGT1c1), 4 (Tg-UGT1d1), and 5 (Tg-UGT1e1), the integration of such a large fragment of DNA, often in multiple copies, did not affect fertility, and upon gross pathological examination they were indistinguishable from wild type litter mates.

Examination of the constitutive expression patterns of UGT1A genes was characterized by Western blot analysis to assess the expression of UGT1A1, UGT1A4, and UGT1A6 in microsomal preparations from liver and small and large intestine. These experiments were performed with antibodies prepared against human UGT1A1 (34), UGT1A4, and UGT1A6. The relative levels of each UGT were compared with both recombinant proteins as well as those levels expressed in human liver microsomes. The polyclonal antibody to UGT1A1 has been shown previously not to react with rat liver microsomes (34), and it does not

FIGURE 1. Identification of the UGT1 exons in mouse tail DNA by PCR. The top drawing is a representation of the UGT1 locus and the organization of the unique 5′-exon 1 sequences and the conserved 3′-exons. The black boxes represent the unique exon 1 sequences (A1 through A10) that are spliced to common exons 2–5 that reside at the 3′ region of the locus. UGT1A13, UGT1A12, UGT1A11, and UGT1A12 are pseudogenes, and they are represented as open bars. PCR analysis of the human UGT1A sequences using tail DNA from Tg-UTG11032 mice is shown in the ethidium bromide-stained gel following amplification of the sequences using human-specific oligonucleotides that identify each of the exon 1 sequences (A1 through A10), as well as exon 5.
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In founders Tg-UGT1c-/-, UGT1a, and UGT1a6, the expression patterns of UGT1a, UGT1a4, and UGT1a6 in liver and gastrointestinal tract microsomes were compared with expression patterns observed in human liver microsomes as well as expressed UGTs prepared from SF-9 insect cells. With each of the founders, limited expression of human UGT1a and UGT1a4 was observed in liver, although UGT1a4 was detected in Tg-UGT1c. In comparison to human liver, the levels of UGT1a6 in Tg-UGT1c liver were similar to those in human liver, but UGT1a6 was also found expressed in Tg-UGT1d and Tg-UGT1e. Examination of microsomes from gastrointestinal tissue demonstrated that UGT1a was expressed in both the small and large intestine, with the relative abundance being significantly higher in the small intestine (Fig. 2). The anti-human UGT1a antibody identified an expression pattern in the small intestine from each founder that was similar to expression that was observed for UGT1a1, indicating that these genes may be under similar modes of regulation in this tissue. Expression of UGT1a4 in Tg-UGT1c appeared to be comparable with levels observed in human liver microsomes. Unlike UGT1a1 and UGT1a4, the expression of UGT1a6 was difficult to detect in small intestinal microsomes but was adequately expressed in colon microsomes from four of the Tg-UGT1 founders. Because UGT1a1, UGT1a4, and UGT1a6 were abundantly expressed in either the small or large intestine from founder Tg-UGT1c, we elected to proceed with a more thorough characterization of gene and protein expression in this founder line.

UGT1 Expression Patterns in Tissues from Tg-UGT1c Mice—In experiments using human tissues, it has been demonstrated by RT-PCR that the UGT1 locus generates a pattern of gene expression that is unique for each tissue (2, 5). To illustrate the patterns of UGT1a expression in transgenic mouse tissues, a presentation of the RNA transcript patterns from Tg-UGT1c are shown in Fig. 3. Low levels of UGT1a1, UGT1a3, UGT1a4, and UGT1a9 are observed in liver tissue with UGT1a6 being the most prominent. The more intense UGT1a6 gene transcript in liver corresponds to observed protein expression (Fig. 2). Although these five gene expression patterns have also been documented in human liver, the low levels of UGT1a1 and UGT1a4 gene transcripts in transgenic liver may explain the lack of protein expression found in this tissue. In colon and small intestine, UGT1a1, UGT1a3, and UGT1a4 gene transcripts are abundant when assayed at linear PCR rates. Likewise, the greater concentrations of UGT1a1 and UGT1a4 PCR products in the small intestine correspond to protein expression patterns (Fig. 2). A similar relationship exists for UGT1a6 expression, where a strong RNA transcript signal was found in colon and UGT1a6 was detected in colon microsomes. The weaker UGT1a6 RNA transcript identified in small intestine was concordant with a lack of protein expression. When we quantitated the expression of UGT1a1 RNA by real time RT-PCR, the fold expression when compared with brain matched the intensity of the banding patterns observed by RT-PCR. UGT1a1 RNA was found more abundantly in small intestine and colon than the other tissues, with slightly elevated levels found in stomach and heart (see quantitative levels in legend to Fig. 2).

In examination of other tissues, UGT1a10, which was found expressed exclusively in extrahepatic tissues in human (11), is expressed in the gastrointestinal tract (small intestine, colon, and stomach) of Tg-UGT1c mice as well as in heart and lung tissue. UGT1a7, originally recognized mouse Ugt proteins from liver microsomes. The UGT1a1, UGT1a4, and UGT1a6 antibodies are specific for these human isozymes as determined by Western blot analysis against each of the expressed proteins previously prepared in this laboratory (data not shown).

FIGURE 2. Western blot analysis of human UGT1a1, UGT1a4, and UGT1a6 identified in microsomes from liver, small intestine, and large intestine from five Tg-UGT1 founders. Three mice representing each founder line along with wild type litters (wt) were used to prepare microsomes. Samples (20 μg) of microsomal protein from liver, small intestine (SI), and large intestine (Colon) were separated by SDS-PAGE and transferred to nitrocellulose membranes. Standards (Std) for each blot included 0.5 μg of human expressed UGT1a1, UGT1a4, or UGT1a6 from baculovirus-infected insect cells, along with 20 μg of microsomes prepared from human liver. Specific UGT1a1-, UGT1a4-, and UGT1a6-antibodies were used to identify expressed protein in the Tg-UGT1 tissues. The transgenic UGT1 founders are identified in the figure as Tg-UGT1a (1a), Tg-UGT1b (1b), Tg-UGT1c (1c), Tg-UGT1d (1d), and Tg-UGT1e (1e). S represents 0.5 μg of the respective expressed standard; HLM is human liver microsome.

FIGURE 3. Differential regulation of the UGT1 gene locus in tissues from Tg-UGT1c mice. UGT1 gene expression in different tissues was identified using isoform-specific RT-PCR. RNA from each tissue was isolated from a pool of three tissues that were combined and pulverized in liquid nitrogen before RNA isolation in TRIzol (“Materials and Methods”). The ethidium bromide-stained gels show isoform-specific RT-PCR products co-amplified in the presence of β-actin primers as a control. Approximately 2 μg of RNA was used in each reverse transcription reaction before diluting the sample for each PCR. In this experiment, PCR was conducted at 30 extension cycles, and the intensities of the bands were quantitated for analysis on a 1.5% agarose gel containing ethidium bromide. S.I., small intestine.
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FIGURE 4. Immunoblot analysis and gene expression profiles of UGT1A1, UGT1A4, and UGT1A6 in Tg-UGT1c intestinal tissue following treatment with either pregenolone 16x-carbonitrile (PCN) or TCDD. Three Tg-UGT1c or wild type (WT) mice were treated by intraperitoneal injection every 24 h with either Me2SO (DMSO) (D), TCDD (T) (16 μg/kg), or PCN (P) (10 mg/kg) for 3 days. After 72 h, the small intestines and the large intestines from each treatment group were combined and the tissues pulverized under liquid nitrogen. A sample of each tissue was then used to prepare microsomes or to isolate total RNA. Upper, Western blot analysis of small and large intestinal microsomal protein using UGT1A1-, UGT1A4-, or UGT1A6-specific antibodies. Included as control is a sample of each protein generated from the expression of cDNAs in stably transfected HEK293 cells. Lower, RNA prepared from the same samples of tissue were used in RT-PCR studies, and the isoform-specific products were identified in ethidium bromide-stained agarose gels. Each PCR reaction was performed at linear amplification conditions, which was either 20, 25, or 30 cycles.

identified in human gastric epithelium (11), is found in transgenic stomach tissue but is also abundantly expressed in lung. Expression in kidney from Tg-UGT1c mice is very selective with UGT1A6 and 1A9 RNA being the dominant forms identified, which also represent the expression patterns found in whole brain. Like those results found in human colon (13), gene transcripts representing each of the UGT1A proteins are detected in transgenic large intestine, with UGT1A1, -1A3, -1A4, -1A6, and -1A10 representing the most abundant transcripts.

Induction of the UGT1 Locus by Ah Receptor and PXR Activators in the Gastrointestinal Tract—Several human UGTs have been shown to be regulated by activators of the Ah receptor (18, 20) and the PXR (16, 23). To look selectively at the induction of the UGT1 locus in Tg-UGT1 mice, Tg-UGT1c mice were bred, and three mice per group were selected for treatment with the either TCDD (16 μg/kg) or PCN (100 mg/kg). For both TCDD and PCN, the administration was by the intraperitoneal route, and each mouse was treated every 24 h over a 3-day period. Tissues from three mice were then pooled and pulverized under liquid nitrogen, and samples were used for microsomal preparation as well as for the isolation of total RNA.

When we examined the levels of expressed UGT1A proteins in the gastrointestinal tract, defined induction patterns were observed. In small and large intestinal microsomal preparations, UGT1A1 was inducible by both TCDD and PCN (Fig. 4), demonstrating that the Ah receptor and PXR are functional in this tissue. This was consistent with previous findings demonstrating that UGT1A1 could be regulated by Ah receptor ligands (18, 21). Identification of Ah receptor enhancer sequences and evidence that the Ah receptor drives UGT1A1 transcription have been described (18). Also identified in the enhancer region of the UGT1A1 gene were binding motifs that recognized PXR, which can be activated in rodents by PCN (16). In the gastrointestinal tract, UGT1A4 and UGT1A6 are differentially regulated, with UGT1A4 inducible in the small and large intestine by both TCDD and PCN, whereas UGT1A6 appears to be predominantly regulated only in large intestine (Fig. 4).

When we examined gene expression profiles, induction of all of the UGT1A gene transcripts was noted following treatment with either TCDD or PCN. Induction of UGT1A1 by TCDD and PCN in small and large intestine correlates with Western blot analysis of UGT1A1 in these tissues. Similar correlations can be made for both UGT1A4 and UGT1A6 in these tissues, although the abundance of UGT1A6 in small intestine as detected by immunoblot is not a good reflection of transcriptional activation. Most interestingly, TCDD can be seen to induce all of the gene transcripts in either small or large intestine. Expression of UGT1A3 and UGT1A10 is particularly susceptible to induction in small intestine, whereas expression of UGT1A5 and UGT1A7 is induced in the large intestine.

Induction of UGT Activity in Small Intestinal Microsomes from Tg-UGT1c—To determine whether expression of UGT1A1 gene products in Tg-UGT1c mice are active, UGT activity was determined in gastrointestinal microsomes from the small intestine prepared from untreated, PCN-treated, and TCDD-treated WT and Tg-UGT1c mice (TABLE ONE). In the selection of substrates, it has been shown previously that ethinyl estradiol is an excellent substrate for UGT1A1 (37), whereas bile acids are glucuronidated at the 24-carboxyl position by UGT1A3 (38). Lamotrigine forms a quaternary ammonium glucuronide (30), and it has been demonstrated that quaternary ammonium glucuronides are predominantly catalyzed by human UGT1A4 (39).

In small intestinal microsomes isolated from WT and Tg-UGT1c mice, ethinyl estradiol and lamotrigine glucuronidation activity was detected and induced when mice were treated with either PCN or TCDD. The levels of ethinyl estradiol glucuronidation activity in transgenic mice were 3–5 times higher than those detected in WT mice, indicating that the elevated expression of human UGT1A1 underlies these values. Lamotrigine is glucuronidated in WT mice, and the activity is mildly induced following TCDD and PCN treatment. However, small intestinal Tg-UGT1c lamotrigine glucuronidation activity is nearly 10-fold those values detected in WT mice, reflecting the increased expression of UGT1A4. Whereas TCDD treatment leads to mild induction of lamotrigine UGT activity in transgenic mice, PCN treatment resulted in a 7-fold induction of activity, indicating the gene is a target for the PXR receptor. It is interesting to note that lamotrigine glucuronidation in transgenic microsomes is nearly 10-fold higher than...
observed in human small intestinal microsomes. Because the human small intestinal microsomes used in these studies are a combination of tissues from six individuals, these data would suggest that UGT1A4 is not significantly expressed in human gut.

For analysis of bile acid metabolism, carboxylic acid-specific glucuronidation of CDCA and LCA was quantitated. There was limited glucuronidation of either substrate in untreated WT small intestinal microsomes. In contrast, we detected significant activity with both substrates from human small intestinal microsomes. Untreated transgenic microsomes. In contrast, we detected significant activity with both substrates from human small intestinal microsomes. Untreated transgenic mice displayed about 30% the activity identified in human microsomes, and the glucuronidation activity was substantially induced in transgenic mice treated with TCDD. The elevated levels of bile acid glucuronidation in untreated and TCDD-treated Tg-UGT mice clearly demonstrate that human UGT1s are expressed and inducible in this tissue. The TCDD-induced bile acid glucuronidation activity in transgenic mice corresponds to induction of UGT1A3 by TCDD in the small intestine.

**Induction of the UGT1 Locus in Liver by PCN and TCDD**—When Tg-UGT mice were treated with either TCDD or PCN, induction of microsomal UGT1A1, UGT1A4, and UGT1A6 was observed (Fig. 5). When we examined gene expression profiles of the UGT1 locus by RT-PCR in Tg-UGT mouse liver, UGT1A1 RNA was present in untreated mice, but the antibody was unable to identify UGT1A1 protein in these mice. However, significant induction of UGT1A1 RNA was apparent following both TCDD and PCN treatment, a result that corresponded to induced UGT1A1 protein. The anti-UGT1A4 antibody recognized an endogenous protein in liver microsomes that migrates at approximately the same Rf value as human UGT1A4, but two bands can be seen in the sample isolated from PCN-treated Tg-UGT mice. It is apparent that the intensity of the antibody-recognized bands in Tg-UGT-untreated and TCDD-treated mouse is more intense than those in WT mice. An increase in UGT1A4 RNA is also visible in those samples taken from TCDD- and PCN-Tg-UGT mice. The anti-UGT1A6 antibody recognizes a faster migrating protein in liver microsomes from WT mice that is clearly induced following TCDD treatment, and this protein may correspond to the mouse Ugt1a6. The induction pattern observed by RT-PCR confirms that UGT1A6 RNA is induced in Tg-UGT mice by PCN and TCDD, yet the levels of UGT1A6 protein are significantly greater in TCDD treated Tg-UGT mice.

In a human liver, a strict pattern of UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 RNA expression has been observed (11, 14). Analysis of UGT1A gene transcripts in Tg-UGT liver demonstrates that both TCDD and PCN induce expression of each of these genes (Fig. 5), indicating that they are targets for activated Ah receptor and PXR. This process is selective, because TCDD is shown to also induce UGT1A10 (Fig. 5). The expression of UGT1A10 is not found constitutively in human liver, a finding that suggests that environmental exposure to Ah receptor ligands will lead to induction of this gene in liver. Because UGT1A10 is expressed in many extrahepatic tissues (11), its regulation is controlled by factors not present in liver. However, activation of the Ah receptor is sufficient to promote enhancer activity and transcriptional activation of the gene.

**Regulation of the Human UGT1 Locus in Transgenic Mice**—Expression of the UGT1 locus in liver demonstrates that both TCDD and PCN induce expression of each of these genes (Fig. 5), indicating that they are targets for activated Ah receptor and PXR. This process is selective, because TCDD is shown to also induce UGT1A10 (Fig. 5). The expression of UGT1A10 is not found constitutively in human liver, a finding that suggests that environmental exposure to Ah receptor ligands will lead to induction of this gene in liver. Because UGT1A10 is expressed in many extrahepatic tissues (11), its regulation is controlled by factors not present in liver. However, activation of the Ah receptor is sufficient to promote enhancer activity and transcriptional activation of the gene.

**TABLE ONE**

| Substrate | Human | Wild type mice treatment | Tg-UGT mice treatment |
|-----------|-------|--------------------------|-----------------------|
|           |       | Control | TCDD | Control | TCDD | PCN |
| Ethinyl estradiol | 231 ± 11 | 15 ± 2 | 11 ± 5 | 48 ± 6 | 72 ± 8 | 211 ± 18 | 234 ± 30 |
| Lamotrigine | 162 ± 12 | 93 ± 3 | 155 ± 19 | 272 ± 9 | 1160 ± 11 | 1438 ± 64 | 7290 ± 487 |
| LCA-24G | 2653 ± 112 | ND | ND | 88 ± 4 | 370 ± 51 | 1833 ± 21 | 346 ± 29 |
| CDCA-24G | 1954 ± 171 | 20 ± 4 | 16 ± 1 | 236 ± 5 | 222 ± 31 | 1156 ± 27 | 208 ± 28 |

For analysis of bile acid metabolism, carboxylic acid-specific glucuronidation of CDCA and LCA was quantitated. There was limited glucuronidation of either substrate in untreated WT small intestinal microsomes. In contrast, we detected significant activity with both substrates from human small intestinal microsomes. Untreated transgenic mice displayed about 30% the activity identified in human microsomes, and the glucuronidation activity was substantially induced in transgenic mice treated with TCDD. The elevated levels of bile acid glucuronidation in untreated and TCDD-treated Tg-UGT mice clearly demonstrate that human UGT1s are expressed and inducible in this tissue. The TCDD-induced bile acid glucuronidation activity in transgenic mice corresponds to induction of UGT1A3 by TCDD in the small intestine.

**FIGURE 5.** Protein and gene expression patterns of UGT1A1, UGT1A4, and UGT1A6 in liver from Tg-UGT mice treated with TCDD or PCN. Wild type and Tg-UGT mice were treated every 24 h with TCDD (16 μg/kg) or PCN (100 mg/kg) by intraperitoneal injection for 3 days, and the livers from three animals per group were combined and used to prepare microsomes or to isolate total RNA. Left, samples of liver microsomal protein (20 μg) were separated by SDS-PAGE, and immunoblot analysis was performed by using UGT1A1, UGT1A4, or UGT1A6 antibodies. A control, human liver microsomes (20 μg) were included in the blot. Right, total liver RNA (2 μg) was used in reverse transcription reactions followed by PCR analysis using isozyme-specific oligonucleotides. PCR reactions were conducted at 30 cycles. The transcripts were identified following electrophoresis in agarose gels stained with ethidium bromide.
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FIGURE 6. The role of glucocorticoids in the expression of UGT1A1 in primary hepatocytes from Tg-UGT1c mice. Top, primary hepatocytes from Tg-UGT1c mice were cultured in media that contained either 10 nM TCDD (T), 10 μM PCN (P), or 10 μM TCPOBOP (Tc). Control hepatocyte cultures contained only DMSO (D). The same combination of treatments was conducted when hepatocytes also contained 0.1 μM dexamethasone (DEX) or 1.0 μM β-estradiol. The top panel is an immunoblot of total cellular protein using the anti-UGT1A1-antibody. This is followed by a Western blot of the same extracts using a CYP1A1-antibody. The bottom panel is an RT-PCR analysis of RNA extracted from these samples using specific oligonucleotide primers to detect the expression of mouse Cyp3a11. Bottom, total RNA extracted from different treatment groups was used following reverse transcription for real time PCR analysis of UGT1A1. Tg-UGT1c hepatocytes treated only with DMSO, TCDD, PCN, or TCPOBOP are shown on the left, followed by analysis of hepatocytes co-treated with dexamethasone (DEX) and either TCDD, PCN, or TCPOBOP or hepatocytes co-treated with β-estradiol along with TCDD, PCN, or TCPOBOP.

Expression of the UGT1 Locus during Pregnancy—Considerable effort has been made to understand the role of glucuronidation in neonatal development (1), and it is well known in humans that bilirubin glucuronidation in newborns is induced immediately following birth. However, little information is known about the impact of fetal development or lactation on human glucuronidation. Because glucuronidation serves to detoxify and remove dietary and catabolic by-products, it might be anticipated that dramatic changes in the levels of circulating hormones and other humoral factors resulting from fetal development and early neonatal life may impact the regulation and expression of maternal proteins that participate in xenobiotic metabolism. To examine this possibility, we undertook a series of experiments to quantitate the levels of hepatic UGT1A1, UGT1A4, and UGT1A6 in maternal Tg-UGT1c mice at different stages during fetal development as well as during postnatal lactation and nursing.

Microsomes were prepared from pregnant Tg-UGT1c mice every 7 days following mating and 7 and 14 days following birth. Immunoblot analysis of UGT1A1, UGT1A4, and UGT1A6 demonstrates that each of these proteins is induced in liver microsomes at 14 days gestation (Fig. 7). The expression of UGT1A1 returns to nonpregnant Tg-UGT1c levels by birth, whereas the UGT1A4 and UGT1A6 levels remain slightly induced at 21 days. In maternal Tg-UGT1c mice that are nursing, there is little change in the relative levels of hepatic UGT1A1 from those found in nonpregnant Tg-UGT1c mice. However, tremendous induction of both UGT1A4 and UGT1A6 at 7 and 14 days following birth is demonstrated, indicating that hormonal balance during the period of lactation underlies this induction process. Combined, these data indicate that homeostatic control during fetal development and lactation plays a critical role in the control and expression of the UGT1 locus.
ing promoter regions has also been of value in attempting to determine in tissue culture those processes that might be important in controlling the tissue-specific and potentially inducible patterns of expression of the human UGT1 proteins. It is known that UGT1A1, UGT1A6, and UGT1A9 can be regulated by chemicals that promote activation of the Ah receptor (18, 20), whereas UGT1A1, UGT1A3, and UGT1A4 are targets for the xenobiotic receptors PXR or constitutive androstane receptor (16, 17, 23). However, whereas these studies are informative, an appropriate model to examine the tissue-specific and inducible properties of the UGT1 locus and the functional outcome of these expression patterns has been lacking. In this study, a transgenic animal model that expresses the UGT1 locus in a tissue-specific and inducible pattern has been created.

In the five founder strains that were examined, protein expression of UGT1A1, UGT1A4, and UGT1A6 was observed in liver and the gastrointestinal tract. Each of these proteins as well as their gene transcripts was found to be inducible by TCDD and PCN, demonstrating that glucuronidation in the liver and gastrointestinal tract can be subject to regulation by the Ah receptor and PXR. In liver and gastrointestinal tract, differences in the constitutive expression patterns of UGT1A4 and UGT1A6 were observed among the different founder lines. One possibility that may account for these differences in expression could be linked to the integration site of the BAC clone such that exposure of the chromatin to tissue-specific transcriptional factors is blocked. However, there was a consistent pattern of expression of UGT1A1 in both liver and the gastrointestinal tract in each of the founders. The inability to detect significant levels of UGT1A1 in liver microsomes may simply reflect minimal levels of protein expression, but detection of UGT1A1 RNA transcripts suggests that the UGT1A1 gene is regulated in liver.

The importance of UGT1A1 in liver is crucial, because bilirubin is conjugated exclusively by UGT1A1 in humans (2) and is excreted into the bile through the basolateral surface of the hepatocytes to the biliary canaliculi. The lack of abundant liver UGT1A1 expression in rodents may be a reflection of diet, which in humans is felt to play an important role in the control and expression of UGT1A1 (40, 41). Alternatively, it is now speculated that species differences in the structure of the ligand-binding domain of the PXR provides selectivity in activation by endogenous activators such as species-specific bile acids (42). It is conceivable that bile acid activation of rodent PXR is not sufficient to promote endogenous UGT1A1 transcriptional activation in Tg-UGT1 mice, but activation by other ligands may be sufficient to target gene induction of gene expression. There is support for this because activation of the rodent PXR can dominate transcriptional activation of UGT1A1 as demonstrated by PCN induction of UGT1A1 RNA in the liver of Tg-UGT1 mice (Fig. 5). Regardless, protein expression patterns in the liver and gastrointestinal tract indicate that the UGT1 transgenic mouse is a viable model to examine the expression patterns of the UGT1 locus in an intact animal model.

In liver, it was observed that UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 were each subject to induction by both PCN and TCDD when gene transcript levels were examined by RT-PCR. In the small and large intestine, PCN or TCDD treatment led to the induction of all nine of the UGT1A genes. The promotion of UGT1A gene transcription by TCDD in liver must require synergy with liver-specific transcriptional factors because UGT1A5, UGT1A7, and UGT1A8 are not regulated by TCDD in this tissue. This apparently is not the case in the induction of UGT1A10, where UGT1A10 is not expressed constitutively in Tg-UGT1c liver and yet is significantly induced by TCDD. Although the expression of UGT1A10 has been considered to be exclusively an extrahepatic protein (11), this finding indicates that environmental exposure to Ah receptor ligands such as polycyclic aromatic hydrocarbons may promote the induction of UGT1A10 in human liver.

Although a number of human tissues have been examined for the expression UGT1A5, this transcript has not been identified in humans (2). In Tg-UGT1c mice, UGT1A5 was found mildly expressed in small and large intestine and was also inducible following TCDD treatment. Induction of each of the UGT1A gene transcripts by TCDD links this process to activation of the Ah receptor, and must implicate binding of the Ah receptor–Arnt complex to enhancer xenobiotic receptor elements (XREs) (43). Ah receptor binding to XREs has been identified in the UGT1A1, UGT1A6, and UGT1A9 genes (18, 44), and it might be anticipated that conserved XRE sequences are present on each of the UGT1A genes. However, it is certainly possible that a limited distribution of XRE sequences such as those located on the UGT1A1, UGT1A6, and UGT1A9 genes are sufficient as enhancer sequences to promote transcriptional activation of each of the UGT1A genes, because induction of UGT1A1, -1A3, -1A4, -1A5, -1A6, -1A7, -1A8, -1A9, and -1A10 RNA has been observed following TCDD treatment (Figs. 4 and 6).

In humans, the UGT1 locus is differentially regulated, with a unique complement of gene transcripts found in the different tissues (5). With the exception of liver and gastrointestinal tract, analysis of UGT1 expression patterns in other selective human tissues is lacking. When we examined expression patterns of the UGT1 locus in tissues from TG-UGT1c mice, several of the expression patterns were similar to those found in human tissue. Tissue from human gastric epithelium highlighted the expression of UGT1A7 (11), a property of expression that is found in transgenic stomach. Most interestingly, UGT1A7 is also found in abundance in transgenic lung tissue. This may be relevant because environmental toxicants such as polycyclic aromatic hydrocarbons present in tobacco smoke are substrates for UGT1A7-dependent glucuronidation (13, 45), indicating that this protein may play an important first pass role in detoxifying these carcinogens in the lung. Although analysis of human lung did not identify UGT1A7 (46), exposure to selective carcinogens such as polycyclic aromatic hydrocarbons and other Ah receptor activators may promote induction of the protein. All human liver samples that have been analyzed express UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 (11, 14), a pattern that is also maintained in transgenic liver. Human colon has been shown to express nearly all of the UGT1 gene transcripts (14), and this pattern is also maintained in transgenic large intestine. Certainly, the availability of a mouse model may be useful in predicting the expression patterns that may be found in human tissues. For example, TG-UGT1c heart tissue expresses an abundance of the UGT1A transcripts implicating an important role for glucuronidation in this tissue. We have identified UGT1A6 and UGT1A9 in whole brain, and this is relevant because it is known that selective neurotransmitters such as serotonin are subject to glucuronidation by UGT1A6 (47). The tissue-specific expression patterns indicate regulation of the locus is under selective transcriptional control, a process that may be influenced by homeostatic control through circulating humoral factors.

In Tg-UGT1c liver, UGT1A1 is induced following treatment with TCDD and PCN, indicating that cultured hepatocytes would be a viable tool to study the impact of UGT1A1 expression by xenobiotic receptor activation as well as the role of circulating hormones. When cultures of primary hepatocytes from Tg-UGT1c mice were treated with TCDD, UGT1A1 was induced, a property that was reflected in the activation of the Ah receptor and induction of mouse Cyp1a1. It is also apparent that PXR is activated following treatment of hepatocytes with PCN, because PXR-targeted expression of Cyp3a11 RNA is observed. However, limited induction of UGT1A1 is noted following Tg-UGT1c hepatocyte
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treatment with PCN, indicating that additional regulatory factors are needed to support PCN-elicited induction of this protein. Based upon the observation that glucocorticoids are weak activators of the PXR and may provide synergistic support for UGT1A1 expression, we noted that the addition of low concentrations (0.1 μM) of dexamethasone to the growth media facilitated PCN elicited induction of UGT1A1. Most notably, these low concentrations of dexamethasone supported over a 10-fold increase in TCDD induction of UGT1A1. The exaggerated induction of UGT1A1 by TCDD in the presence of glucocorticoids may be independent of Ah receptor function, because we did not observe a synergistic induction of Cyp1a1. This result also suggests that circulating humoral factors may also participate in the regulation of the UGT1 locus.

By having available the opportunity to examine UGT1A expression profiles in transgenic mice, we rationalized that the dramatic changes in steady-state levels of circulating hormones and steroid balance during pregnancy may provide an excellent opportunity to examine the impact of altered homeostatic control on maternal UGT1 expression. We observed that midway through gestation (day 14), expression of UGT1A1, UGT1A4, and UGT1A6 in liver was induced (Fig. 7), with the levels of expression returning to near normal levels just prior to birth. These results reflect findings that have been observed in clinical trials showing that drugs that are subject to glucuronidation by UGT1A4 and UGT1A6 are excreted at a greater rate during pregnancy (27,48). Most interestingly, these results are in contrast to findings in rats, where the levels of liver UGT1A1 were reduced in maternal liver during pregnancy (26). We can interpret these results to suggest that the human UGT1 genes are controlled by activated regulatory factors resulting from hormonal changes and are linked to the early stages of fetal development, but rodent UGT1A genes lack this ability to be regulated during pregnancy. The contrasting results between human and rodent glucuronidation during pregnancy may be a reflection of differences in evolutionary conservation of selective cis-acting regulatory sequences on the human UGT1 and rodent UGT1 locus. The sharp increase in UGT1A glucuronidation in maternal liver may also be a natural defense mechanism to facilitate detoxification or elimination of blood products resulting from catabolism during early embryogenesis.

The most dramatic UGT1A induction profile in maternal liver was observed with the induction of UGT1A4 and UGT1A6 following birth. Most interestingly, UGT1A1 was not induced relative to UGT1A4 and UGT1A6, indicating that selective humoral factors are modulating the regulation of UGT1A4 and UGT1A6 during lactation. Glucuronidation plays a critical role in the detoxification and removal of small lipophilic compounds, and the dramatic induction of UGT1A1 and UGT1A6 may represent an example of the natural defense system that is activated during lactation, ensuring that only the most essential nutrients are available to the nursing neonates. There is support for this possibility because it has been demonstrated that lactating rats exhibit enhanced hepatic \textit{p}-nitrophenol glucuronidation activity (49). We could also speculate that the induction of UGT1A4 and UGT1A6 during lactation is controlled through prolactin production, because it has been indicated that prolactin has been able to increase rat UGT1A6 but not rat UGT1A1 in ovariectomized rats (26). However, any one or a combination of the reproductive and metabolic hormones that are regulated during pregnancy and that impact mammary gland development and lactation (24) may underlie the dramatic induction of UGT1A4 and UGT1A6. Regardless, expression of the UGT1 locus in maternal tissue during pregnancy and lactation appears to undergo significant regulation, an observation that indicates that maternal glucuronidation plays a critical role in fetal and neonatal development. These findings suggest that one of the key actions of hormones or other humoral factors during pregnancy and neonatal development is to serve as a signal in the maternal circulation to provide a means for robust detoxification pathways.

Along with recent observations that the UGT1 locus is targeted for regulation by xenobiotics in combination with tissue-specific events, the Tg-UGT1 animal model may prove useful to study the impact of UGT1A1 metabolism on selective drugs as a function of induction and development. Whereas transgenic strains that express human xenobiotic metabolizing genes may be somewhat limited in usefulness because of drug metabolism from constitutive proteins, analysis of metabolism patterns using highly selective substrates that exhibit minimal metabolic activity by rodent proteins could prove to be useful in predicting human drug metabolism patterns, as recently demonstrated by the metabolism of midazolam in transgenic mice expressing CYP3A4 (36). We anticipate that the Tg-UGT1 mice will be particularly useful in evaluating the glucuronidation and clearance of drugs that form quaternary ammonium glucuronides, because formation of these \textit{N}-linked glucuronides do not occur efficiently in rodents. With the potential to genetically delete the rodent Ugt1 locus and remove all residual UGT activity originating from this locus, future experiments designed to humanize mice may prove valuable in examining the role of human glucuronidation patterns in drug metabolism and toxicity.

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