Molecular Cloning of Two cDNAs Encoding the Mouse Bilirubin/Phenol Family of UDP-Glucuronosyltransferases ($mUGT_{Br/p}$)[1]

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INTRODUCTION

Glucuronidation, catalyzed by UDP-glucuronosyltransferase (UGT),[5] is an important process in the metabolism of many drugs, carcinogens, xenobiotics, and endogenous substances such as bilirubin, steroids, and biogenic amines (1–3). UGT also plays an important role in the biosynthesis of proteoglycans, which confer important recognition properties on cell surfaces that influence cell adhesion and migration as well as the binding of growth factors and enzyme inhibitors (4). In humans, deficiency in glucuronidation has significant clinical and toxicological consequences. For example, inability to glucuronidate bilirubin may lead to its accumulation in plasma and tissues and result in jaundice. In more severe cases, patients may be at risk for bilirubin encephalopathy (kernicterus). These clinical sequalae have been observed in patients with Crigler–Najjer syndrome where there is an absence or partial deficiency in bilirubin UGT activity (5).

UGTs belong to a multigene family which comprises at least 21 distinct cDNAs in five mammalian species (5–19). Recent advances in biochemical and molecular biological approaches have given insight into the substrate specificity, function, and structure of UGT. Thus far, the human and rat bilirubin and phenol UGTs have been characterized. It was found that there are structural similarities between the bilirubin UGTs and the phenol UGTs between and within two species. Specifically, the 3′ ends of both the bilirubin and the phenol UGT are identical. To date, the structure of the mouse UGT gene is not known and so the question remains whether it will be similar to that in man. Since the mouse is one of the most widely utilized animal models for pharmacologic as well as toxicological testings of pharmaceutical compounds, it is relevant to study the mouse genes. It has been reported that there are species differences between the mouse and the rat in the regulation of UGT activity for bilirubin and phenolic substrates (20). Here, we report the cloning of two mouse liver cDNAs which encode bilirubin and phenol UGT. The first cDNA, designated $mUGT_{Br1}$, consists of 2216 base pairs (bp) and contains 1608-nucleotide open reading frame beginning at nucleotide 16, which encodes a 535-amino acid polypeptide of 60 kDa. The second cDNA, designated $mUGT_{p4}$, consists of 2158 bp and encodes a protein of at least 521 amino acid residues. The two cDNAs encode an identical C terminus but a variable N terminus. From the deduced amino acid sequence alignment, $mUGT_{Br1}$ shares 78, 65, 63, 61, 65, and 70% sequence homology with human *hBR1* (17), *hBr2* (17), *hPhl* (15), *hPh4* (18), and rat *rPh* (8) *rBr* (11), whereas $mUGT_{p4}$ shares 62, 61, 63, 74, 65, and 65%, respectively.

MATERIALS AND METHODS

Screening of a λgt11 cDNA Library

A mouse adult liver cDNA library in phage lambda gt11 (λgt11) and the host cells (*Escherichia coli* strain Y1090) were purchased from Clontech Lab. (Palo Alto, CA). Four hundred thousand plaques at a density of ~20,000 per 150-mm plate were screened using a 426-bp *KpnI/XbaI* fragment of the human 2351-bp full-length *HUG-Br1* cDNA (16) according to the methods established in our laboratory (21) and by Ausubel *et al.* (22). The probes were prepared by random hexamer primed synthesis (Prime-a-Gene Labeling System; Promega, Madison, WI) using [α-^{32}P]dCTP (3000 Ci/mmol, NEN Dupont; 1 Ci = 37 GBq) to generate a specific activity of 1–3 × 10^9 cpm/μg DNA (23). Positive plaques were replated and rescreened twice until two isolated positive plaques were used to purify phage DNA as described in (22), with minor modifications. Subfragments representing inserted cDNA in λgt11 were generated by digestion with *Eco*RI and ligated into *Eco*RI-digested, phosphatase-treated plasmid Bluescript SK+ (Stratagene, La Jolla, CA). The sequences of the inserts were determined on double-stranded DNA by the dideoxynucleotide chain termination method of Sanger *et al.* (24), modified as in Sequenase Version 2.0 protocols (U.S. Biochemical Corp., Cleveland, OH). It was found that the two clones were identical and contained 1202 bp without an open reading frame and therefore are partial cDNAs (25).

Rescreening of a λZAP cDNA Library

The partial cDNA obtained above was used to rescreen a mouse liver λZAP cDNA library (Stratagene). The strategy

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[5] UGT(s) is used to denote UDP-glucuronosyltransferase protein(s) and italic *UGT* with a subscript, e.g., UGT_{Br1}, indicates the gene that encodes the protein.

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Fig. 1. Nucleotide and deduced amino acid sequences of mouse UGT_{B1} (bottom) and UGT_{P4} (top) cDNAs. The stop codon is designated ***. Putative N-terminal signal insertion sequence, membrane-anchoring peptides, and predicted asparagine-linked glycosylation sites (for UGT_{B1}) are indicated by the double line, the solid line, and the filled diamonds, respectively. Consensus polyadenylation signal is denoted by boldface italics.

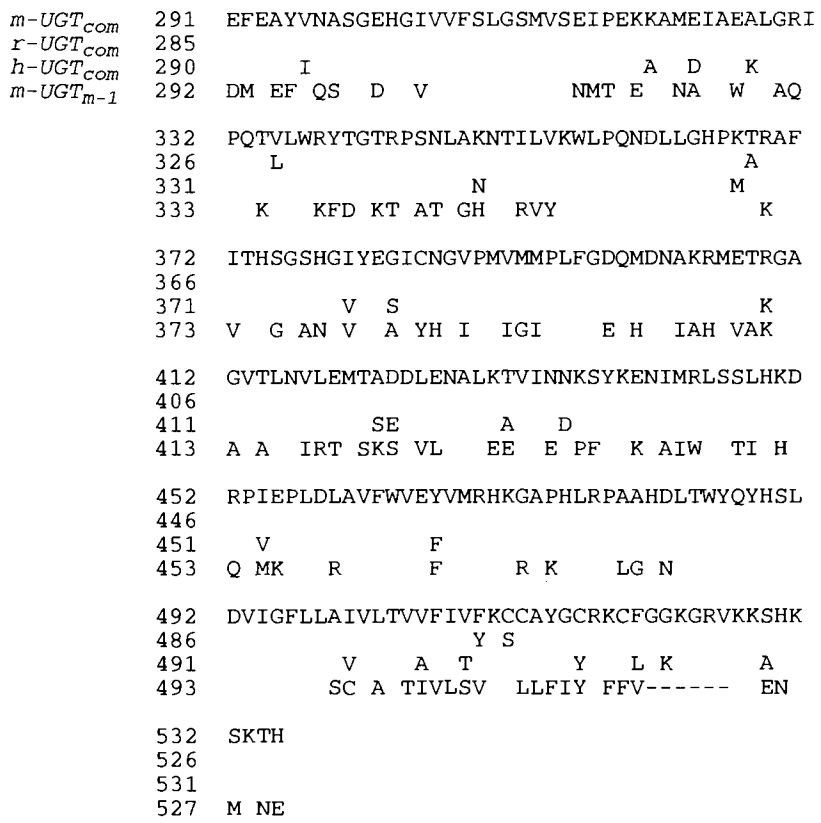

Fig. 2. Alignment of the deduced amino acid sequences of mouse UGT_{com}, rat UGT_{com}, human UGT_{com}, and mouse UGT_{m-1}. Differences in amino acid residues from mouse UGT_{com} are indicated for each gene.

was identical to that described above. A total of 10 plaques was obtained during primary screening. The plaques were replated and rescreened twice. After the third round, five plaques were selected and *in vivo* excision of pBluescript SK vector with the cDNA inserts using R408 helper phage was performed, according to the protocols provided by the manufacturer (Stratagene). The complete nucleotide sequences of both strands of two of the clones (9-1 and A-1) were determined by dideoxy sequencing as described above.

RESULTS AND DISCUSSION

Plaque screening of the mouse liver λgt11 cDNA library (4×10^5 independent clones) with radiolabeled human *HUGBr1* cDNA probes identified two UGT cDNA clones. Both clones were sequenced from both ends and found to be identical. The clone contained 1202 bp without an open reading frame (OPR) and therefore is a partial cDNA. Using this partial cDNA as a probe, we rescreened another mouse liver cDNA library and obtained two cDNA clones (9-1 and A-1), designated $mUGT_{Br1}$ and $mUGT_{p4}$, respectively. Figure 1 shows the nucleotide sequence together with its predicted amino acid sequence for both clones. The partial cDNA obtained initially lacked 331 amino acid residues of the amino terminus (as indicated by the star at nucleotide 994). Clone 9-1 was found to have 2219 bp with a 1608-nucleotide open reading frame (ORF) encoding a 535-amino acid polypeptide of 60.1 kDa. The deduced amino acid sequence of $mUGT_{Br1}$ contains a characteristic hydrophobic signal peptide (amino acids 10 through 30) for protein transport across the endoplasmic reticulum (26). Two potential N-linked glycosylation sites exist at amino acid asparagine (Asn) 89 and 297. A putative transmembrane hydrophobic region is located at the carboxyl terminus, which is followed by a positively charged lysine (27). The translation stop codon, TGA, is located 5′ of the consensus sequences for polyadenylation (AATAAA) and a short segment of poly(A)$^+$ tail is shown in Fig. 1. Clone A-1 had 2158 bp, encoding a protein of at least 521 amino acid residues. However, it lacked the first few amino acids that correspond to the signal insertion peptide region. The two clones have identical 3′ end which encode an identical C terminus (Fig. 1). Alignment of $mUGT_{Br1}$ with other transferase clones shows overall identities of 78% to *hBr1* (17), 65% to *hBr2* (17), 63% to *hPh1* (15), 61% to *hPh4* (18), 65% to *rPh* (8), and 70% to *rBr* (11), at the amino acid level. If the N-terminal half of the deduced protein sequences is considered, the identities become 67, 43, 40, 38, 37, and 45%, respectively, indicating that $mUGT_{Br1}$ is orthologous to the bilirubin form of human *hBr1* (17). When $mUGT_{p4}$ is similarly aligned, the overall identities are 62, 61, 63, 74, 65, and 65%, and the identities become 38, 35, 38, 60, 37, and 37% (for the N terminus), respectively, indicating that $mUGT_{p4}$ is orthologous to the phenol form of human HlugP4 (18).

Figure 2 shows the alignment of the mouse carboxyl common domain ($mUGT_{com}$) of the deduced amino acid se-

quence of $mUGT_{Br1}$ (which is identical to $mUGT_{p4}$) with that of the same regions of the mouse UGT_{m-1} (19), the rat ($rUGT_{com}$) (8,11), and the human ($hUGT_{com}$) (15,17). Comparing the two mouse genes ($mUGT_{com}$ and UGT_{m-1}) shows only 54% identity (differs in 110 residues of 244; 39% identity overall), indicating that these two genes are from different families. This is consistent with the results from the rat and human, which show that there are multiple families of UGTs in each species (2,3,28).

When we examine UGT_{com}, we find that there is high homology across species. Of 244 amino acid residues, the mouse $mUGT_{com}$ differs in only four residues from the rat UGT_{com}, with 98.5% identity (95% identity at the DNA level). When UGT_{com} between the mouse and the human is compared (Fig. 2), only 22 residues are found to be different, with 91.1% identity (86% identity at the DNA level). The high homology of this carboxyl *common* terminus between human and rodents is indicative of evolutionary conservation of important biological functions across species.

Conjugation with glucuronide confers greater polarity and water solubility on the parent agents, thereby facilitating biliary and/or urinary excretion and detoxification. However, regulation of the UGT drug metabolizing enzymes also has important consequences in carcinogenesis (29,30) and toxicity (31). Questions remain as to which UGT isoenzyme(s) catalyzes such reactions and whether there is a genetic predisposition to such disease states. This can now begin to be addressed via recombinant DNA methodologies. To this end, we are in the process of establishing stable cell lines expressing the mouse UGTs.

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