0026-895X/01/5903-405–414$3.00
MOLECULAR PHARMACOLOGY
Copyright © 2001 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 59:405–414, 2001

Vol. 59, No. 3
764/889357
Printed in U.S.A.

MINIREVIEW

Genetic Multiplicity of the Human UDP-Glucuronosyltransferases and Regulation in the Gastrointestinal Tract

ROBERT H. TUKEY and CHRISTIAN P. STRASSBURG

Departments of Pharmacology and Chemistry & Biochemistry, University of California, San Diego, La Jolla, California (R.H.T.); and Department of Gastroenterology and Hepatology, Hannover Medical School, Hannover, Germany (C.P.S.)

Received December 19, 2000; accepted December 19, 2000

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

ABSTRACT

The metabolism of ingested foods and orally administered drugs occurs in the hepato-gastrointestinal tract. This process is facilitated by several supergene families that catalyze oxidative metabolism as well as conjugation of the small molecular weight substances that enter the systemic circulation through resorption in the gastrointestinal tract. The catalytic action carried out by one of several conjugation reactions leads to the eventual elimination of the resultant metabolites from the cell. As early as 1959 (R. T. Williams, *Detoxification Mechanisms*) it was suggested that the detoxification of most agents is efficiently performed by the phase II conjugation reactions, because the addition of bulky, water-soluble groups to the target substrates facilitates the partitioning of these metabolites from the lipid into the aqueous compartments of the cell. The combined efforts of the phase II reactions provides remarkable redundancy in a biological system that seems to be designed to assure that many endogenously generated catabolic products as well as exogenous agents introduced through the surface tissues of the digestive tracts are efficiently removed through excretion to the bile or urine. In this review, we focus on recent findings that highlight the genetic multiplicity and regulatory patterns of the phase II superfamily UDP-glucuronosyltransferases (UGTs). Although much is known regarding the number of UGTs that make up the *UGT1* and *UGT2* gene families, as demonstrated after the characterization of expressed cDNAs, examples are also presented in which information obtained from the human genome project will aid in the final characterization of the genetic multiplicity. In addition, tools have now been developed and examples presented to identify the expression patterns of the UGTs in human tissues, paying particular attention to expression patterns of these genes in the hepato-gastrointestinal tract.

Eliminating from different tissues small molecules that may be present as steroids, heme byproducts, free fatty acids, environmental contaminants, xenobiotics, drugs, and dietary byproducts is performed most efficiently via the addition of glucuronic acid, a process that leads to detoxification of the original compound. The conversion of small lipophilic molecules to water-soluble glucuronides is catalyzed by the superfamily of UGTs (Tukey and Strassburg, 2000; Dutton, 1980). The UGTs are localized in the endoplasmic reticulum and utilize UDP-glucuronic acid (UDPGlcUA) as cosubstrate for the formation of β-D-glucuronides (Dutton and Storey, 1953). The chemical index of compounds that serve as substrates for the UGTs ranges into the thousands, and the reactive groups that are used have been observed to be alcoholic, phenolic, carboxyl, thiol, carbonyl, and amino linkages (Dutton, 1978). There are only rare examples that glucuronides retain biological activity; therefore, this pathway is regarded as a "detoxification" mechanism, as originally proposed by Dutton (1975). In any one species, the remarkable diversification in substrate specificity and the ability of glucuronidation to play such a significant role in the detoxification process can be attributed to two significant biological parameters. First, although there is some selectivity in substrate specificity between the different UGTs, there is also remarkable redundancy between the UGTs in their ability to accept similar compounds as potential substrates for glucuronidation

This work was supported in part by National Institutes of Health Grants GM49135 and CA79834 (R.H.T.) and Grant STR493/13–3 from the Deutsche Forschungsgemeinschaft (C.P.S.).

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; UDPGlcUA, UDP-glucuronic acid; kb, kilobase pair(s); RT, reverse transcriptase/transcription; PCR, polymerase chain reaction.

(Tukey and Strassburg, 2000). This assures that in any given tissue, adequate metabolic processes are in place to facilitate the process of detoxification. Second, the ability to form β-D-glucopyranosiduronic acid derivatives with the many different reactive groups can be attributed in part to the evolution of a large multigene family (Mackenzie et al., 1997). Like all multigene families involved in metabolism, selective pressure has led to gene duplication events (Gonzalez, 1989), followed by divergence of sequence, a process most likely aimed at accommodating the metabolic requirements of the host species.

Characterization of gene structure and the molecular cloning of cDNAs have identified 16 UGT gene products in humans. Based upon amino acid sequence relatedness and evolutionary divergence, these proteins fall into two gene families that have been classified as *UGT1* and *UGT2* (Mackenzie et al., 1997) (Fig. 1). The ability to clone the UGT cDNAs and express recombinant proteins with the aid of heterologous cell culture tools has allowed for the characterization of substrate specificities. To date, more than 350 known agents have been identified as substrates for the expressed UGTs (Tukey and Strassburg, 2000). This large classification of structurally divergent compounds spans many different chemical classes, including alcohols, flavones, coumarins, carboxylic acids, amines, opioids, and steroids. The reactive groups associated with these classes of agents are found in many products of our regular diet and in numerous pharmaceutical drugs, which are administered via an oral route requiring subsequent intestinal transport and possibly first pass metabolism before resorption and entry into the systemic circulation. Examining the expression profiles and functional activity of the UGTs in the gastrointestinal tract has been pursued to better understand the contribution these enzymes play in intestinal uptake and metabolism.

Characterization of the UGTS

The UGTs have evolved as a unique class of proteins that utilize UDPGlcUA as cosubstrate, are found exclusively in vertebrates, and are the only "credible" enzyme for adding glucuronic acid to aglycones (Dutton, 1975). The UGTs are, however, distantly related in amino acid sequence to vertebrate and invertebrate proteins that catalyze the addition of glycosyl groups from other UDP-sugars to small cellular molecules (Kapitonov and Yu, 1999). A consensus amino acid signature sequence (Prosite accession number PS00375) is found in the different classes of UDP-glycosyl transferases, mainly the UDP-glucuronosyltransferases, the putative UGTs from *Caenorhabditis elegans*, the mammalian 2-hydroxyacylsphingosine 1-β-galactosyltransferase, the plant flavonol *O*-(3)-glucosyltransferases, the baculovirus ecdysteroid UDP-glucuronosyltransferase, and the prokaryotic zeaxanthin glucosyltransferase (Mackenzie et al., 1997; Kapitonov and Yu, 1999). There are more than 470 protein sequences in the various databases worldwide that encode this signature sequence (an up-to-date inventory of the available sequences that recognize the UGT signature sequence can be obtained at *http://srs.ebi.ac.uk/* by searching with the accession number PS00375). It is predicted that this conserved amino acid sequence may link these proteins in function by participating in the acceptance of the UDP-sugar as cosubstrate in the active site of the transferase. Although only the UGTs utilize UDPGlcUA as cosubstrate, it seems that the biological process underlying the transfer of sugar molecules to nonlipid dependent cellular substrates is an ancient event that has evolved and diversified and is essentially conserved from the early prokaryotes to man.

The UGTs range in size from 526 to 533 amino acids and each is characterized by several uniquely conserved features (Fig. 2).

- The amino terminal region of all the UGTs (with the exception of UGT1A10) encodes an endoplasmic reticulum consensus NH_2-terminal signal sequence that facilitates the insertion of the protein during translation and is then removed as the protein is directed into the membrane. All of the UGTs have type I transmembrane topology, classi-

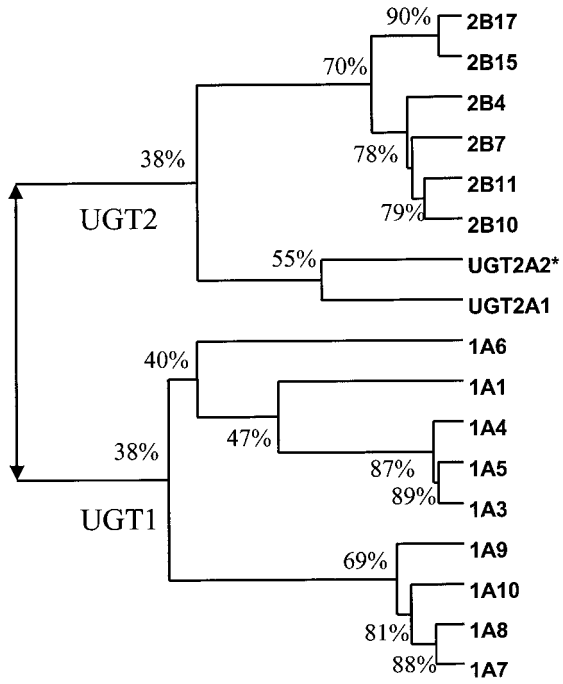

Fig. 1. Phylogenetic tree of the human UGTs. Shown in this dendrogram are those sequences encoding only the exon 1 regions of the proteins. The displayed proteins were aligned with Clustal X (obtained from *ftp://ftp.ebi.ac.uk/pub/software/dos/clustalw/clustalx*) and the dendrogram generated with the average linkage clustering method (UPGMA) from the Phylip program (*http://evolution.genetics.washington.edu/phylip/getme.html*). The results of the UPGMA sorting was drawn with TreeView (*http://taxonomy.zoology.gla.ac.uk/rod/treeview*). All of the sequences were obtained from Swisspro or Genbank, and their accession numbers are indicated by "ac". UGT2B17-acO75795 (Beaulieu et al., 1996); *UGT2B15*-acP54855 or P23765 (Coffman et al., 1990; Chen et al., 1993); *UGT2B4*-acP06133; P36538; O60731 (Jackson et al., 1987; Ritter et al., 1992a; Chen et al., 1993; Jin et al., 1993a; Levesque et al., 1999); *UGT2B7*-acP16662 (Ritter et al., 1990; Jin et al., 1993b); *UGT2B11*-acO75310 (Beaulieu et al., 1998b); *UGT2B10*-acP36537 (Jin et al., 1993a); *UGT2A1*-acQ9Y4X1 (Jedlitschky et al., 1999); *UGT1A6*-acP19224 (Harding et al., 1988; Ritter et al., 1992b); *UGT1A1*-acP22309 (Ritter et al., 1992b; Sutherland et al., 1992); *UGT1A3*-acP35503 (Mojarrabi et al., 1996); *UGT1A4*-acP22310 (Ritter et al., 1991); *UGT1A5*-acP35504 (Ritter et al., 1992b); *UGT1A7*-acO00473 (Strassburg et al., 1997b); *UGT1A8*-acO14928 (Mojarrabi and Mackenzie, 1998; Strassburg et al., 1998a); *UGT1A9*-acP36509 (Wooster et al., 1991); *UGT1A10*-acO00474 (Meech and Mackenzie, 1998; Strassburg et al., 1997b). UGT2A2-the sequence encoding this protein was identified on clone 401_E_05, as011254. Note that the cDNA and protein encoding UGT1A5 has not been identified in any human tissues. It is possible that the promoter for *UGT1A5* is not functional.

fied with the N terminus and catalytic domain inside the endoplasmic reticulum (Meech and Mackenzie, 1997).

- The feature of each protein most likely responsible for the unique recognition of the many different structurally divergent compounds is the region flanking the amino terminal recognition sequence (amino acid 35) and that of the conserved carboxyl region (amino acid 290). This stretch of the amino terminus is approximately 260 amino acids. When the amino-terminal components of the UGTs are used to generate phylogenetic relationships in the form of a dendrogram (Fig. 1), the branch points and similarities of each UGT to the others are identical to the patterns observed if the full-length proteins were used (for comparison, see Tukey and Strassburg, 2000). This region is the most divergent of the proteins. Several laboratories have performed experiments designed to examine the functional domains of these regions. By exchanging the divergent regions of the amino terminus among different UGT cDNAs, catalytic activity analysis of the chimerics has indicated that this region is most likely to be responsible for the unique functional diversity that is observed with each protein (Mackenzie, 1990; Li et al., 1997).
- The carboxyl half of each protein, approximately 245 amino acids, is highly conserved between UGT1 and UGT2 proteins. This region is identical in all of the UGT1A proteins (Wooster et al., 1991; Ritter et al., 1992b). Within this region has been identified the characteristic signal sequence or Prosite that is located approximately at amino acids 369 to 407.
- Each UGT contains a hydrophobic stretch of amino acids at the distal end of the carboxyl region that has been predicted to span the membrane (Iyanagi et al., 1986).
- Positioned in close proximity to the carboxyl-end, hydrophobic membrane spanning region are a cluster of highly charged amino acids, that in combination with the membrane spanning region has been predicted to serve as a classical anchor sequence securing the protein to the membrane (Iyanagi et al., 1986).

Genetic Multiplicity

Within a short period of time, the complete DNA sequence of the human genome will be available and it will be possible to identify unambiguously the number and genetic composition of the *UGT* gene family. Although this project has recently been advertised as having been completed by the U.S. Human Genome Research Institute, the privately held Celera Genomics Corporation and other international centers participating in the human genome project, only remnants of chromosomal sequencing is presently available in DNA gene banks such as EMBL and GenBank. However, along with the cloning of cDNAs and gene mapping experiments, the gene bank data is starting to shed light on the multiplicity of the UGT supergene family.

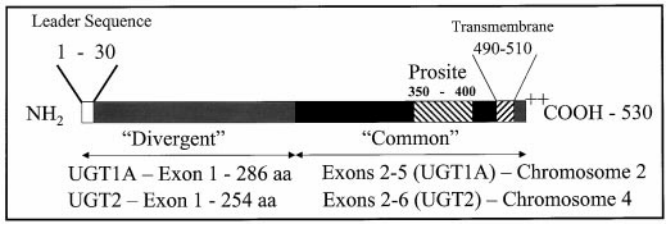

Fig. 2. Unique amino acid regions common to the UGTs. With the exception of UGT1A10, all of the UGTs contain an NH_4-terminal leader sequence as predicted by Von Heijne (1986). Both the UGT1 and UGT2 proteins contain a "divergent" region that is encoded by exon 1, and a "common" region encoded by five UGT2 exons and four UGT1 exons. Other salient features are a consensus Prosite (accession number PS300375), a transmembrane region and a highly charged carboxyl portion of the protein that facilitates anchoring the protein to the membrane (Iyanagi et al., 1986).

UGT2B Family. Evolutionary studies have indicated that although all of the UGT2 sequences in the different species are closely linked, selective evolutionary pressure has resulted in the generation of two subfamiles of *UGT2* genes whose function within each species is unique. For example, *UGT2B* gene products have been identified in species such as rodents, lagomorphs, and humans (Mackenzie et al., 1997), yet there does not seem to be a truly orthologous *UGT2B* gene product that shares similar catalytic function among these species. Experiments conducted on chimpanzees, the closest genetic relatives to humans, have resulted in the characterization of a unique panel of *UGT2B* genes (Belanger et al., 1997; Beaulieu et al., 1998a; Barbier et al., 1999a,b) that have little functional correlates with those identified in humans. This impressive segregation of structure and function indicates that the *UGT2B* genes have evolved as a result of selective pressure to deal with the removal of endogenous and exogenous substrates unique to the evolutionary constraints put upon each species. The lack of highly conserved *UGT2B* genes may well be the result of significant environmental differences and challenges confronting each species. The most dramatic of these could be diet, a factor that, over time, can define the biological makeup and influence the evolution of the enzymes needed for gastrointestinal and hepatic metabolism.

In humans, several of the UGT2 cDNAs have been mapped to chromosome 4-q13 or 4-q28 (Turgeon et al., 2000; Monaghan et al., 1994), and have been shown to be tightly linked within approximately 200 kb. The *UGT2* genes are composed of six exonic sequences as demonstrated by the characterization of UGT2B4 (Monaghan et al., 1997), *UGT2B7* (Carrier et al., 2000) and *UGT2B17* (Beaulieu et al., 1997; Belanger et al., 1998) (Fig. 3A). From the six UGT2B cDNAs, the ordered array of the genes indicates a mapping profile of *UGT2B7-UGT2B4-UGT2B15* (Monaghan et al., 1994), as illustrated in Fig. 3C. Using the exon 1 DNA sequence from any of the *UGT2B* genes to search the available shared genomic and DNA databases in a Blast N DNA sequence search, all of the current cDNAs can be identified, along with DNA sequence from several BAC clones (Fig. 3B). Each BAC clone in the figure shows regions represented by highly homologous UGT2 exon 1 sequences. At present, the DNA sequence of these genomic clones is not complete and the ordering of the fragments that were sequenced and deposited in the databases is still random, but the high homology of the UGT2B exon 1 sequences indicates that the *UGT2* gene family is significantly larger than represented by the available cDNA clones that have been characterized.

Shown in Fig. 3B is an example of the number of UGT2B exon 1-related sequences and the identification of the known *UGT2* genes. Blast N searches have identified BAC genomic clones that encode *UGT2B4*, *UGT2B10*, *UGT2B15*, and *UGT2A1* exon 1 sequences. Interestingly, BAC clone 401_EO5 encodes both *UGT2B4* and *UGT2A1*, confirming

these genes to be in close proximity. These data can be used with previous gene mapping experiments that position the orientation of *UGT2B7-UGT2B4-UGT2B15* genes (Monaghan et al., 1994) to demonstrate that the *UGT2A1* gene can be placed among the clustering of these UGT2B sequences.

The screening of the human genome sequences will aid in mapping and identifying those sequences that might encode additional UGT genes. Figure 3B demonstrates there exist many potential genetic elements that might contribute to the UGT supergene family. As previously indicated with the partial characterization of BAC clones that encode *UGT2B15* and *UGT2B17*, some of these homologous sequences are pseudogenes (Turgeon et al., 2000), as evident from the interruption of the open reading frames with termination sequences. Yet analysis of these highly homologous sequences will inevitably lead to the discovery of new genes.

An example of this power is evident from the identification of a new UGT2A sequence on BAC clone 401_E05. This sequence encodes an exon 1 sequence that is 58% identical in amino acid sequence to UGT2A1 (Fig. 4) and thus could be provisionally identified as UGT2A2. In this figure, we show an example of a simple experiment to demonstrate how this information can be applied to analyze the functionality of these new genes. Analysis of RNA expression by RT-PCR using primers specific to the UGT2A2 DNA sequence demonstrates that *UGT2A2* is expressed in a number of tissues such as liver and the small intestine (Fig. 4). This analysis confirms that the UGT2A family of proteins, like the UGT2B family, is highly diversified with regard to multiplicity and tissue expression. Although *UGT2A1* and *UGT2A2* have been identified on the same BAC clone, significant diversification in exon 1 sequence and the regulatory regions of the genes has occurred. For example, UGT2A1 was identified and shown to be expressed primarily in nasal mucosa tissue with little RNA detected in the hepatic-gastrointestinal tract (Jedlitschky et al., 1999), whereas in these experiments, UGT2A2 is clearly expressed in the hepatic-gastrointestinal tract. Although our understanding of the genetics and multiplicity of the UGT2 family has arisen primarily out of characterization of expressed cDNAs, a comprehensive picture of this Supergene family will emerge from the information made available as a result of the human genome project.

UGT1A Family. Unlike the UGT2 family, the UGT1 family of proteins are highly conserved in function and have been found in several vertebrates (Iyanagi et al., 1989, 1991; Ritter et al., 1992b; Li et al., 2000). The genes encoding the UGT1 proteins have undergone unusual evolutionary events compared with other supergene families. Experiments conducted by Ritter et al. (1992b) and Wooster et al. (1991) demonstrated that the carboxyl terminal region of several UGT1A proteins were identical, leading them to speculate that these proteins are processed by an alternative RNA splicing event. However, characterization of the *UGT1A* locus (Ritter et al., 1992b) led to the discovery of a unique biological structure that encoded the UGT1A proteins. Structural analysis of the *UGT1A* locus indicates that the processing of UGT1A RNA occurs through classical RNA splicing events and is not the result of alternative RNA splicing (Fig. 5)

The *UGT1A* locus is located on chromosome 2-q37 (Harding et al., 1990) and spans approximately 200 kb (Fig. 5). There are two primary domains important for the evolution of the multiple UGT1A proteins. First, each of the UGT1A proteins is encoded by five exons, with exons 2 to 5 conserved in all of the UGT1A proteins. The DNA sequence encoding exons 2 to 5 is located at the 3′ portion of the locus. Second, the sequences that encode the exon 1 portions of the UGTs are composed of blocks of DNA that exist as cassettes and are basically aligned in series upstream of exon 2. Identification of exons 1 through 6 were characterized by gene walking experiments conducted by Ritter et al. (1992b). Exons 7 through 13 were identified in Ida Owens' laboratory and the information was deposited into GenBank (accession number AF29703). Each functional exon 1 cassette is composed of a conventional transcriptional start site and a 5′ consensus spliceosome recognition sequence at the 3′-end of the cassette. The cassettes are separated from each other by 15 to 25 kb; flanking each cassette in the 5′-direction are functional

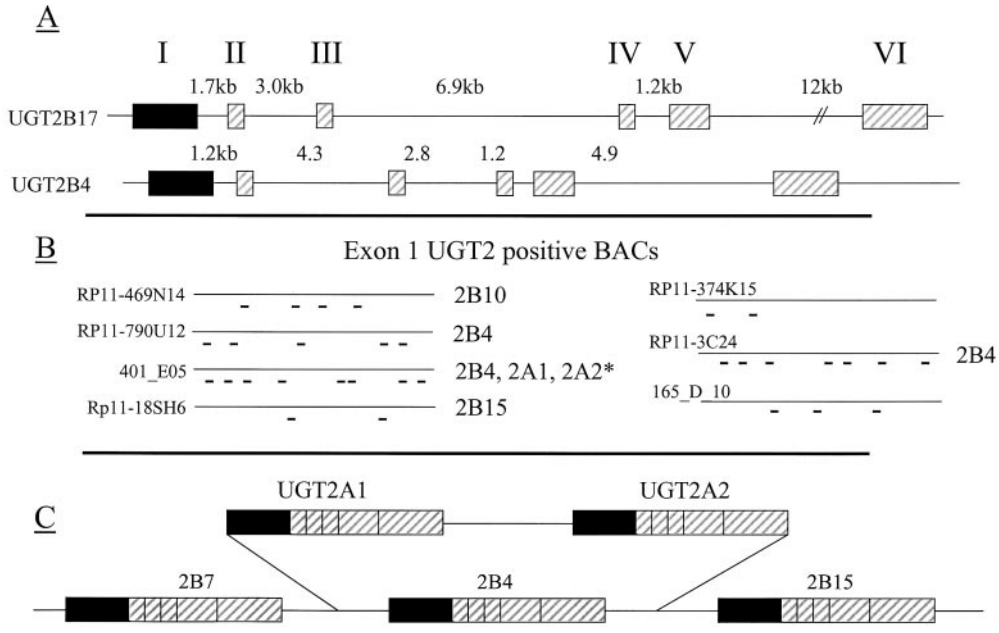

Fig. 3. Organization of the UGT2 gene family. Characterization of *UGT2B4* and *UGT2B17* indicate that the UGT2 genes encode six exons, as displayed in the 3A. Blast N searches of homologous UGT2 sequence have identified a number of BAC clones that contain partial sequence, as displayed in 3B. Each dark underline indicates the predicted position on the BAC clone representing the homologous sequence. The various functional genes are represented as indicated. As of this writing, BAC clones encoding UGT2B7, UGT2B11 and UGT2B17 have not been identified. Figure 3C indicates that based upon the positioning of UGT2B7-UGT2B4-UGT2B15 by Monaghan et al. (1994), it can be concluded that UGT2A1 and UGT2A2 lie in close proximity to these genes.

promotor elements that drive transcription. With the first 3′-spliceosome sequence positioned at the start of exon 2, transcription proceeding at any of the exon 1 cassettes results in RNA synthesis and splicing of the exon 1 RNA sequence to RNA encoding exon 2 (Tukey and Strassburg, 2000). This process would seem to control the tissue-specific expression of these genes.

Regulation of Human UDP-Glucuronosyltransferases in the Hepato-Gastrointestinal Tract

The gastrointestinal tract and the liver are the primary metabolic organs involved in the metabolism of components of our daily diet as well as pharmaceutical drugs administered for therapeutic purposes (Matern et al., 1984; Peters and Jansen, 1988; Iyanagi et al., 1989; McDonnell et al., 1996; Strassburg et al., 1999a). The hepatocyte represents the main metabolic cell type of the liver. Its microanatomical organization is polar, which means that potential candidate compounds for hepatic metabolism reach the hepatocyte at the sinusoidal membrane, where uptake mechanisms (i.e., organic anion transport proteins) are available to channel the agents to subcellular sites of metabolism such as the cytoplasm, endoplasmic reticulum, and cytoplasmic vesicles (Konig et al., 2000; Kullak-Ublick et al., 2000). Export of metabolites as well as other macromolecules such as bile acids proceeds via the apical membrane by specific transport processes (MRP2, MDR3, and bile salt export pump) (Konig et al., 1999; Kullak-Ublick et al., 2000). Exported molecules and conjugates collect in the biliary canaliculi, which eventually lead to the bile ducts and then transport to the duodenum. Thus, this enterohepatic and consequently enterohepatoenteric transit provides the biochemical and anatomical framework of human epithelial detoxification. It is not surprising, then, that such drug-metabolizing enzymes as the UGT family are specifically regulated and distributed in the critical tissues defining the locations of xenobiotic contact (Strassburg et al., 1998a, 2000; Tukey and Strassburg, 2000).

The human digestive tract encompasses one of the largest external transitional surfaces of the body. Food and xenobiotic metabolism begins as soon as contact is established with the squamous cell epithelium of the oral cavity, and continues for a minimum of about 48 h until passage ends in the rectum. The proximal portions of the gastrointestinal tract, such as gastric and small intestinal tissue, participate in resorption and metabolism of therapeutic drugs in addition to the distal colon, which is a common route of nonparenteral drug application. Research into defining the distribution of

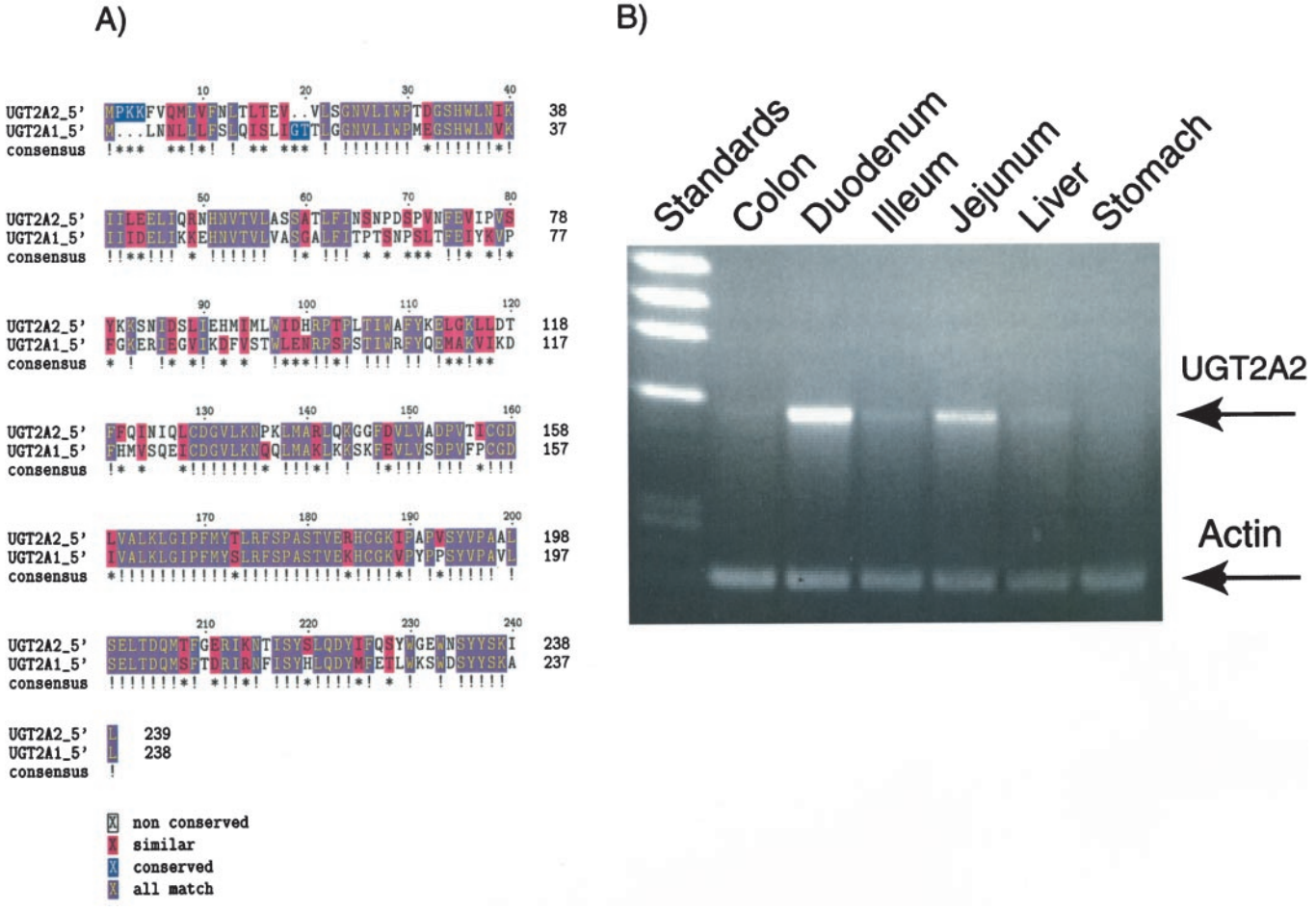

Fig. 4. Amino acid sequence and expression of UGT2A2*. A portion of clone 401_E_05 (accession number 011254) encoding bases 7132–7869 encodes a protein that is approximately 56% similar in amino acid sequence to UGT2A1. The alignment is shown on the left (A). The right side of the figure (B) shows expression of UGT2A2 RNA in several human tissues of the gastrointestinal tranct. In this experiment, oligonucleotide primers to the DNA spanning bases 7132 to 7869 were generated. After reverse transcription, duplex polymerase chain reaction using a combination of the human actin primers and the UGT2A2 primers was performed. The actin RNA is observed in each sample along with differential expression of UGT2A2.

oxidative drug metabolism in humans has established that the hepatocyte and other organs of the gastrointestinal tract, such as the small intestine, are active in cytochrome P-450–directed metabolism (Kolars et al., 1992, 1994). Thus, because most drugs and xenobiotics are eventually targeted for elimination through glucuronidation, it is not surprising that a role for this process in the gastrointestinal tract has been perceived to play an important role.

Glucuronidation of phenolic substrates (bilirubin as well as bile acids in extrahepatic tissue extracts from the small intestine, colon, and kidney) emphasizes the potential importance of this pathway in extrahepatic metabolism (Matern et al., 1984; Pacifici et al., 1986, 1988; Parquet et al., 1988; Peters and Jansen, 1988; Peters et al., 1991; Bock, 1996; McDonnell et al., 1996). Knowledge of the molecular and genetic organization of the UGT supergene family has facilitated the identity of UGT gene expression in extrahepatic tissues. Because many of the individual *UGT* gene products share a high degree of sequence similarity, methods that rely upon sequence hybridization by Northern blot analysis provides limited information. Homology between the UGT1A first exons encoding UGT1A3, UGT1A4, and UGT1A5, as well as the first exons of UGT1A7, UGT1A8, UGT1A9, and UGT1A10 exceeds 93%, which significantly confounds the use of DNA fragments as probes in hybridization techniques such as Northern blot analysis (Strassburg et al., 1997a). In addition, antibodies directed against purified preparations of UGTs share avidity toward epitopes which are conserved between the different proteins (Strassburg et al., 1998a). This makes detection of the individual UGT proteins in specific target tissues a significant challenge. However, antibodies directed against the more diverse isoforms, including UGT1A6, UGT1A1, and UGT2B7 (Radominska-Pandya et al., 1998; Ritter et al., 1999; Walle et al., 2000) have been generated while the immunological discrimination of UGT1A3 and UGT1A4 as well as UGT1A7–10 and UGT2B7-UGT2B15 continues to pose an unresolved obstacle.

The refinement of polymerase chain reaction-based techniques to precisely detect small changes in DNA sequence down to the level of 1- and 2-bp disparities has enabled the identification of highly related *UGT* gene transcripts (Strassburg et al., 1997a,b). This approach has been helpful in the identification and characterization of human tissue-specific glucuronidation patterns. Shown in Fig. 6 is a schematic of the RNAs and the location of the sense and antisense oligonucleotides that have been developed and used in different studies to identify the mRNAs that encode the different UGT1A and UGT2 proteins (Strassburg et al., 1997b). Using cDNA generated from total RNA by reverse transcription, the pairs of oligonucleotides for each UGT cDNA can be used in PCR analysis to identify with remarkable specificity the UGT gene transcripts. Combined with internal markers in the same reaction, quantification of the relative mRNA abundance in the different tissues can also be determined (Strassburg et al., 1997b).

Using UGT1A-specific oligonucleotides, most of the tissues of the gastrointestinal tract have now been studied and *UGT* gene expression has been characterized. The cloning of cDNAs from liver identified UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 (Harding et al., 1988), and their

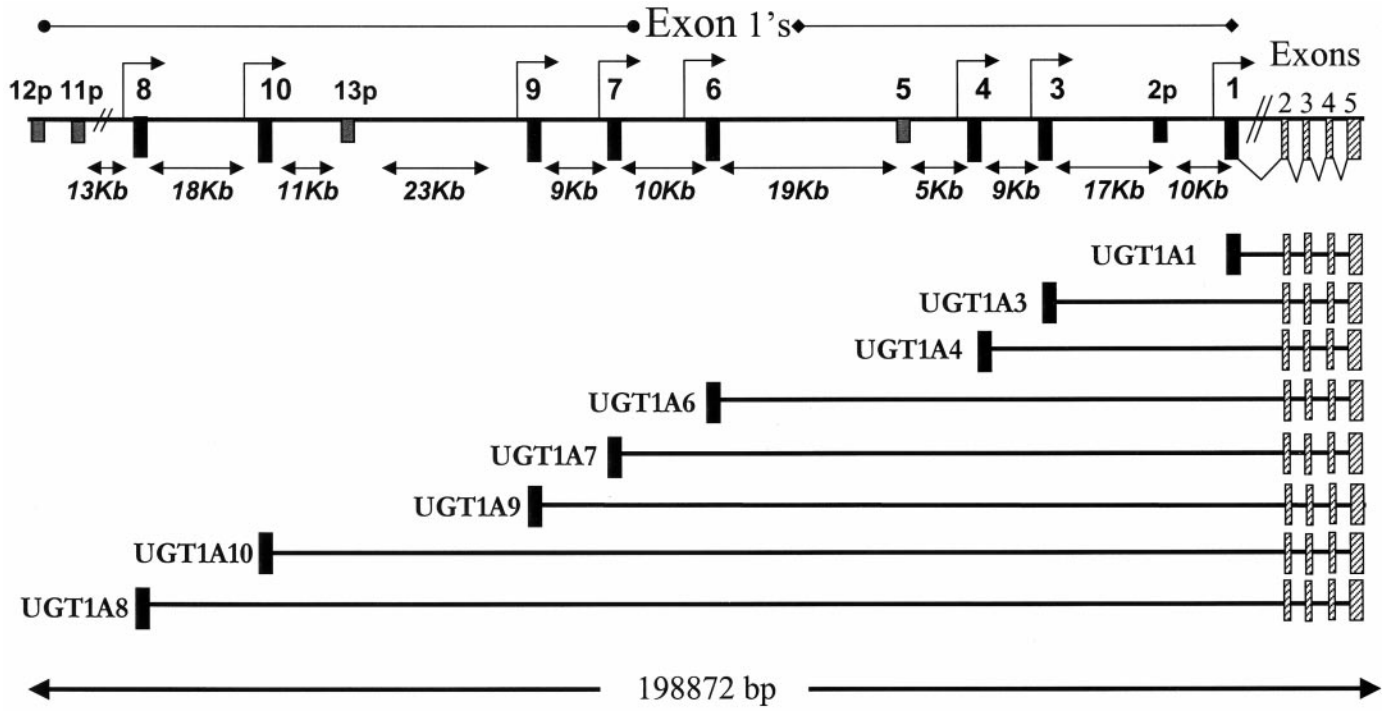

Fig. 5. The organization of the human UGT1A locus. The UGT1A locus has been completely sequenced (Genbank accession number AF297093) and encodes functional genes that can lead to the transcription of RNA encoding UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, and 1A10. The distances between the exon 1 sequences are shown as calculated from the DNA sequence. Nuclear RNA of the predicted sizes are speculated to be synthesized, each containing a different exon 1 element and the same exons 2 to 4. Normal RNA processing of the nuclear transcripts leads to mature mRNA encoding the different UGTs. There are three pseudogenes, 1A11p, 1A12p, and 1A13p, as indicated. Because RNA encoding UGT1A5 has not been identified in any human tissue, we arre designating that exon as a potential pseudogene (indicated by the smaller size). The initial characterized by restriction enzyme mapping and partial DNA sequence leading to the identification of the 3′-exon sequences and the first exons encoding UGT1A1, 1A3, 1A4, 1A5, and 1A6 have been published previously (Ritter et al., 1992b).

expression confirmed by differential RT-PCR analysis (Ritter et al., 1991; Wooster et al., 1991; Mojarrabi et al., 1996; Strassburg et al., 1997b). The availability of divergent exon 1 sequences encoding UGT1A7, UGT1A8, and UGT1A10 made it possible to examine their expression in liver and other tissues. The extension of differential RT-PCR detection to an analysis of tissue samples from the human biliary tree demonstrated that the *UGT1A* locus was differentially regulated between liver and extrahepatic tissues (Strassburg et al., 1997b). Similar to the liver, biliary tissue was found to express UGT1A1, UGT1A3, UGT1A4, and UGT1A6. Two significant exceptions were observed. First, unlike in liver, UGT1A9 was not expressed in biliary epithelium. Second, the expression of UGT1A10 RNA, which was not found in liver, was abundantly expressed in biliary epithelium. This finding demonstrated that UGT1A9 and UGT1A10 are differentially regulated in human tissues that are ontogenetically and functionally linked. In addition, the identification of UGT1A10 seemed to be strictly an extrahepatic UGT. UGT1A10 has since been found to be expressed in multiple extrahepatic organs of the gastrointestinal tract, demonstrating that this UGT may provide an important role in overall homeostasis and based on its anatomical expression pattern, play an important role in both first pass metabolism as well as systemic metabolism (Strassburg et al., 1997b, 2000).

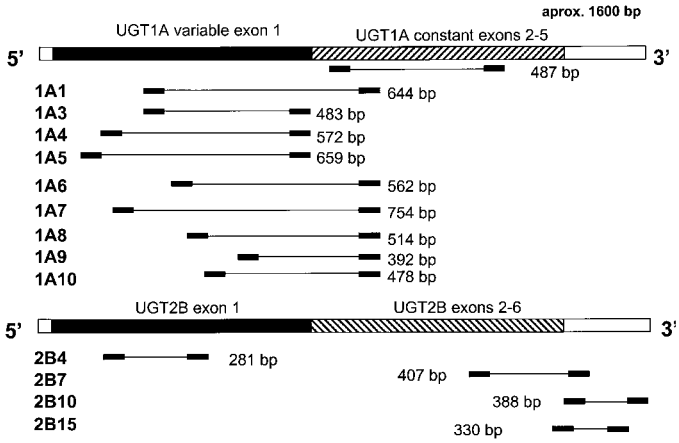

Fig. 6. Tissue-specific identification of human UGT expression through out the hepatic-gastrointestinal tract. Using oligonucleotide primers designed to identify the unique UGT transcripts, RNA isolated from the different human tissues was primed for the synthesis of cDNA by reverse transcriptase and then used for specific PCR reactions, as outlined previously (Strassburg et al., 1998a). Using unpublished structural DNA sequence information deposited into GenBank by Ida Owens from the National Institutes of Health, we were able to use this method to identify the expression of the *UGT1A7*, *UGT1A8* and *UGT1A10* genes in extrahepatic tissues (Strassburg et al., 1997a, 1998a).

Analysis of other gastrointestinal tissues confirmed the complexity of *UGT1A* gene expression patterns. In human colon, the extrahepatic expression of UGT1A8 was observed (Cheng et al., 1998; Mojarrabi and Mackenzie, 1998; Strassburg et al., 1998a). Although the expression of UGT1A8 mRNA has been reported in jejunum and ileum (Cheng et al., 1998), a separate study using 18 small intestinal samples was unable to detect the expression of UGT1A8 in small intestine (Strassburg et al., 2000). Human colon shows the most complex patterns of UGT1A expression, with all of the isoforms expressed except UGT1A5 and UGT1A7 (Strassburg et al., 1999a). Interestingly, in esophagus, expression patterns showed the exclusive transcription of the UGT1A7–10 cluster of gene products (Strassburg et al., 1999b). Three of these UGTs—UGT1A7, UGT1A8, and UGT1A10—are expressed only in extrahepatic tissues. In combination, these recent findings demonstrate a tissue specific expression of *UGT1A* gene products and support the hypothesis that glucuronidation requirements of different metabolically active tissues are regulated in a tissue specific fashion.

Not surprisingly, the *UGT2* genes are also under the influence of tissue specific regulation; however, a clear distinction between hepatic and extrahepatic isoforms has not been observed to date. The most significant differential expression pattern seems to be that observed for UGT2A1 (Jedlitschky et al., 1999). It has been demonstrated that UGT2A1 expression is restricted mainly to the sensory tissues, such as the olfactory mucosa, and is not found in the gastrointestinal tract. Like *UGT1A* gene expression, there does not seem to be a form of the UGT2 family that is restricted selectively to the liver. With the exception of UGT2A1, all of the *UGT2B* gene products have been found in liver (Tukey and Strassburg, 2000). Initial observations indicated that UGT2B4 may be

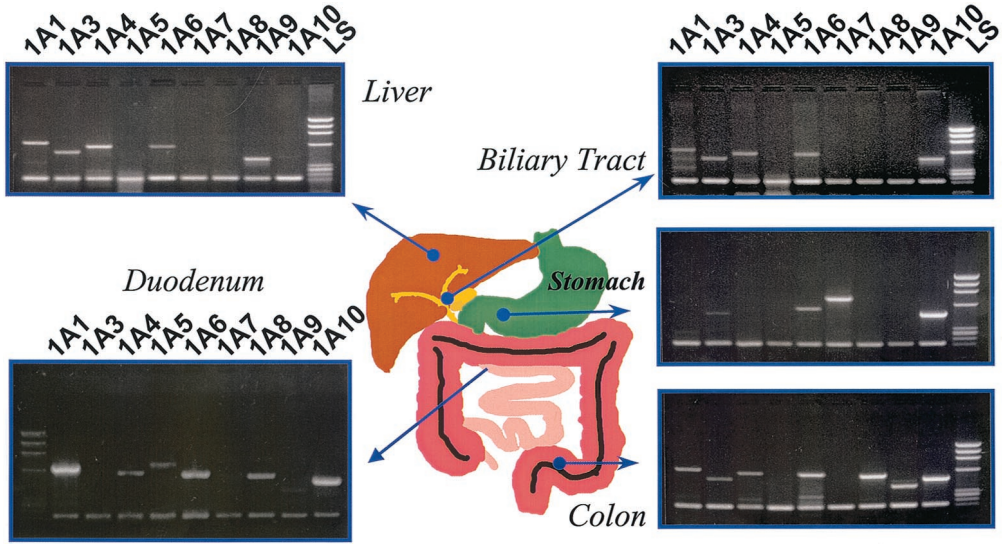

Fig. 7. Schematic diagram showing primers designed for the specific amplification of individual UGT1A and UGT2B transcripts. Descriptions of the primers are as published previously (Strassburg et al., 1997b, 2000).

selective to the liver (Radominska-Pandya et al., 1998), but recent observations using multiple samples of small intestine have shown that UGT2B4 is expressed polymorphically in the small intestine (Strassburg et al., 2000). Careful analysis of the small intestine shows that UGT2B10 is not detectable in the duodenum or jejunum and is expressed in only ~12.5% of samples of ileum (Strassburg et al., 2000). Experiments carried out in our laboratory have demonstrated that UGT2B4 is not expressed in colon, whereas UGT2B7, UGT2B10, and UGT2B15 are abundant in large intestine. UGT2B7, UGT2B10, UGT2B15, and UGT2B17 can be found in many tissues of the gastrointestinal tract, as well as in steroid-sensitive target tissues (Beaulieu et al., 1996, 1997; Levesque et al., 1997).

A detailed picture of tissue-specific regulation of the *UGT* gene families in the human digestive tract are becoming available. This differential regulation can be regarded as the biochemical basis determining tissue specific glucuronidation in man. When the specific UGT activities in the epithelial compartment of the different gastrointestinal organs are compared, a pattern emerges in which glucuronidation activity in the most proximal (esophagus, stomach) (Strassburg et al., 1998b, 1999b) and most distal locations (colon) (Peters and Jansen, 1988; Strassburg et al., 1999a) is clearly reduced (Fig. 8). In the human digestive system, the highest overall glucuronidation activity has been identified in the jejunum and in the liver (Strassburg et al., 1999a, 2000). For some compounds that are glucuronidated through phenolic linkages, such as polycyclic aromatic hydrocarbons and some steroid hormones, small intestinal glucuronidation activity exceeds that of the liver (Strassburg et al., 2000). Given the amount of small intestinal surface, duodenal, jejunal, and ileal glucuronidation is likely to have considerable relevance for human detoxification.

Polymorphic Interindividual Regulation of UGT Genes in the Gastrointestinal Tract.

Genetic (structural) polymorphisms of human drug-metabolizing enzymes, including cytochrome P-450, glutathione-*S*-transferases, and *N*-acetyltransferases, have been identified and evaluated as determinants underlying interindividual differences in drug metabolism and potential adverse drug reactions (Daly, 1995; Meyer and Zanger, 1997). For some of the *UGT* genes, such as *UGT1A1*, *UGT1A6*, *UGT2B4*, *UGT2B7*, and *UGT2B15*, polymorphic alleles have also been identified (Ciotti et al., 1997; Levesque et al., 1997; Coffman et al., 1998; Lampe et al., 1999; Levesque et al., 1999). For UGT1A1, which is the only UGT isoform capable of biologically relevant bilirubin glucuronidation (Bosma et al., 1994), as many as 32 allelic variants have been identified (for review, see Tukey and Strassburg, 2000). Many groups have studied these genetic polymorphisms because they form the genetic basis for hereditary unconjugated hyperbilirubinemias that underlie Crigler-Najjar's syndrome (Ritter et al., 1993) and Gilbert-Meulengracht's disease (Bosma et al., 1995; Monaghan et al., 1996). Structural polymorphisms of the *UGT* genes are likely to influence interindividual variations of glucuronidation activity as evidenced by variations of serum unconjugated bilirubin levels in affected persons.

A distinct mechanism separate from allelic polymorphisms is represented by the detection of regulatory polymorphisms resulting in unique interindividual patterns of gene expression in different tissues (Strassburg et al., 1998b; 2000). Examples of this feature have been identified for both *UGT1* and *UGT2* genes in stomach epithelium, duodenal, jejunal, and ileal epithelium. Patterns of altered gene expression were defined by the detection or absence of *UGT* gene products between individual organisms (e.g., by RT-PCR or Western blot in the absence of evidence suggesting structural polymorphisms of the underlying gene sequence). In gastric epithelium, the polymorphic regulation of *UGT1A1*, *UGT1A3,* and *UGT1A6* gene transcripts was observed in 30% of the tissues analyzed, whereas the expression of UGT1A7 and UGT1A10 mRNA were not subject to polymorphic regulation (Strassburg et al., 1998b). Changes in levels of gene activity were found to correlate with interindividual variation in overall microsomal phenolic substrate glucuronidation in gastric mucosa. Interestingly, catalytic activity of

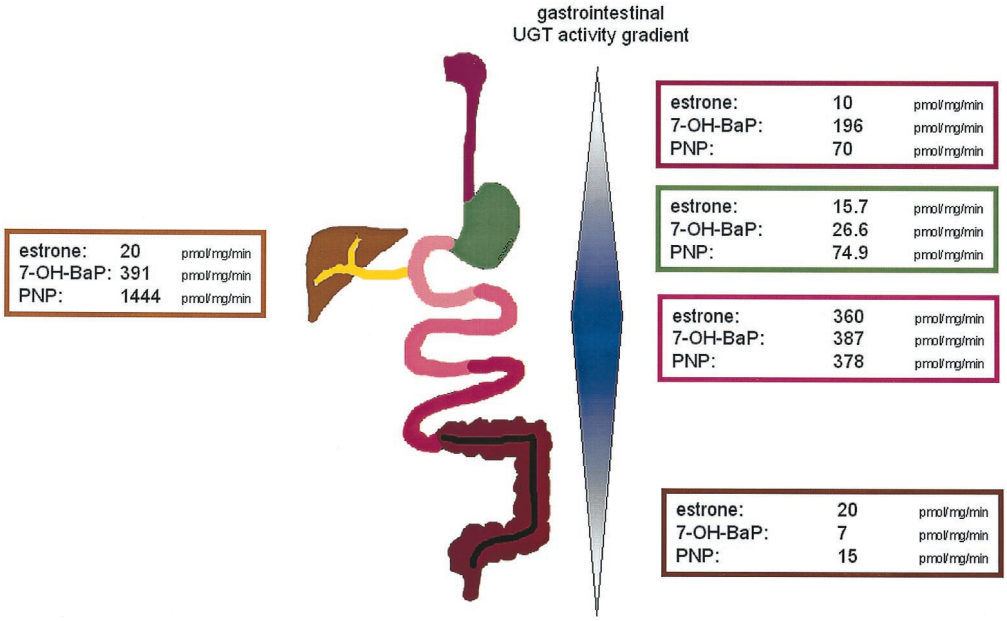

Fig. 8. Schematic diagram of the human digestive system demonstrating a glucuronidation gradient in the course of the intestinal passage. Index activities determined with microsomal protein from the respective tissues in the digestive system are indicated in the boxes corresponding to the colors in the drawing (7-OH-BaP, 7-hydroxybenzo[*a*]pyrene; PNP, para nitrophenol). The lowest glucuronidation activities are found in the esophagus and stomach as well as in the distal colon.

hyodeoxycholic acid, a substrate that is glucuronidated primarily by UGT2B7 and UGT2B4, was evident in one of the four tissue samples analyzed. These data provided evidence for the polymorphic regulation of both the *UGT1A* locus and *UGT2B* genes.

The characterization of UGT expression in hepatic, cholangiocellular, esophageal, and colonic tissue does not share evidence of regulatory polymorphisms, as observed in gastric epithelium (Strassburg et al., 1997b, 1998a, 1999a). However, the analysis of the three levels of the human small intestine—duodenum, jejunum and ileum—has revealed that a second organ of the digestive system is subject to regulatory gene expression (Strassburg et al., 2000). Up to 13 *UGT* gene transcripts were analyzed in small intestine, and only UGT1A7, UGT1A8, and UGT1A9 were not detectable. UGT1A10 was expressed in all of the small intestinal tissues, suggesting that UGT1A10 fulfills a requirement for glucuronidation that is necessary in all extrahepatic gastrointestinal tissues. The other *UGT1A* and *UGT2B* gene products displayed patterns of regulatory polymorphism. A tight correlation between functional activities and *UGT* gene transcript expression is not evident because substrate specificities between the different UGTs are characterized by a high degree of overlap. Hyodeoxycholic acid represents an example of a substrate that is glucuronidated by a limited number of UGT proteins (Ritter et al., 1992a; Levesque et al., 1999). UGT2B7 and UGT2B4 exhibit hyodeoxycholic acid UGT activity; both of these enzymes were found to show a polymorphic pattern of expression in duodenum and jejunum, but not in liver. The characterization of hyodeoxycholic acid UGT activity using small intestinal microsomes and hepatic microsomes confirmed the existence of interindividual variations in catalytic activity only in small intestine. Additionally, the differences found at the functional level were corroborated by immunoblot detecting the absence of small intestinal UGT2B7 protein in persons without UGT2B7 mRNA expression. These results show not only that the qualitative regulation of UGT genes is tissue specific but also that polymorphic regulation is a tissue-specific property.

Given the diversity of glucuronidation enzymes in the small intestine and the complex regulatory mechanisms defining their catalytic contribution to metabolism, it seems that prehepatic metabolism may be significantly underestimated and may play a role in the disposition toward drugs that are taken orally. UGTs catalyze the glucuronidation of many structurally different drugs (Tukey and Strassburg, 2000), and the glucuronidation of these pharmacologically active drugs can be demonstrated with microsomes prepared from epithelial cells of the digestive tract (Matern et al., 1984; Parquet et al., 1988; Peters et al., 1991; Strassburg et al., 1998b, 1999a, 2000). Thus, glucuronidation at the site of resorption would lead directly to an inactivation of the pharmacological properties of the drug and limit the concentration of the active drug that can reach the systemic circulation. In contrast to this mechanism, digestive tract glucuronidation enzymes play a critical role in the enterohepatic circulation of compounds, which include digoxin and bile acids. Intestinal deconjugation would result in the inactivation of biological activity of these compounds and reconjugation in the epithelial cells of the digestive tract would allow a recycling of deconjugated compounds for additional rounds of enterohepatic cycling. The delineated studies and data have provided the first steps aimed at elucidating the biochemical and physiological basis of human digestive tract glucuronidation and its overall significance for metabolism and homeostasis.

References

Barbier O, Belanger A and Hum DW (1999a) Cloning and characterization of a simian UDP-glucuronosyltransferase enzyme UGT2B20, a novel C19 steroid-conjugating protein. *Biochem J* **337:**pp567–574.

Barbier O, Levesque E, Belanger A and Hum DW (1999b) UGT2B23, a novel uridine diphosphate-glucuronosyltransferase enzyme expressed in steroid target tissues that conjugates androgen and estrogen metabolites. *Endocrinology* **140:**5538–5548.

Beaulieu M, Levesque E, Barbier O, Turgeon D, Belanger G, Hum DW and Belanger A (1998a) Isolation and characterization of a simian UDP-glucuronosyltransferase UGT2B18 active on 3-hydroxyandrogens. *J Mol Biol* **275:**785–794.

Beaulieu M, Levesque E, Hum DW and Belanger A (1996) Isolation and characterization of a novel CDNA encoding a human UDP-glucuronosyltransferase active on C19 steroids. *J Biol Chem* **271:**22855–22862.

Beaulieu M, Levesque E, Hum DW and Belanger A (1998b) Isolation and characterization of a human orphan UDP-glucuronosyltransferase, UGT2B11. *Biochem Biophys Res Commun* **248:**44–50.

Beaulieu M, Levesque E, Tchernof A, Beatty BG, Belanger A and Hum DW (1997) Chromosomal localization, structure, and regulation of the *UGT2B17* gene, encoding a C19 steroid metabolizing enzyme. *DNA Cell Biol* **16:**1143–1154.

Belanger A, Hum DW, Beaulieu M, Levesque E, Guillemette C, Tchernof A, Belanger G, Turgeon D and Dubois S (1998) Characterization and regulation of UDP-glucuronosyltransferases in steroid target tissues. *J Steroid Biochem Mol Biol* **65:**301–310.

Belanger G, Beaulieu M, Levesque E, Hum DW and Belanger A (1997) Expression and characterization of a novel UDP-glucuronosyltransferase, UGT2B9, from cynomolgus monkey. *DNA Cell Biol* **16:**1195–1205.

Bock KW (1996) Duodenal biopsies for xenobiotic metabolism studies in human individuals. Identification of bilirubin UDP-GTs in the human alimentary tract in accordance with the gut as a putative metabolic organ. *Hum Exp Toxicol* **15:**860–862.

Bosma PJ, Chowdhury JR, Bakker C, Gantla S, De Boer A, Oostra BA, Lindhout D, Tytgat GNJ, Jansen PLM, Elferink RPJO, et al (1995) The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* **333:**1171–1175.

Bosma PJ, Seppen J, Goldhoorn B, Bakker C, Oude ER, Chowdhury JR, Chowdhury NR and Jansen PL (1994) Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. *J Biol Chem* **269:**17960–17964.

Carrier JS, Turgeon D, Journault K, Hum DW and Belanger A (2000) Isolation and characterization of the human UGT2B7 gene. *Biochem Biophys Res Commun* **272:**616–621.

Chen F, Ritter JK, Wang MG, McBride OW, Lubet RA and Owens IS (1993) Characterization of a cloned human dihydrotestosterone/androstanediol UDP-glucuronosyltransferase and its comparison to other steroid isoforms. *Biochemistry* **32:**10648–10657.

Cheng Z, Radominska-Pandya A and Tephly TR (1998) Cloning and expression of human UDP-glucuronosyltransferase (UGT) 1A8. *Arch Biochem Biophys* **356:**301–305.

Ciotti M, Marrone A, Potter B and Owens IS (1997) Genetic polymorphism in the human UGT1A6 (planar phenol) UDP-glucuronosyltransferase: Pharmacological implications. *Pharmacogenetics* **7:**485–495.

Coffman BL, King CD, Rios GR and Tephly TR (1998) The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). *Drug Metab Dispos* **26:**73–77.

Coffman BL, Tephly TR, Irshaid YM, Green MD, Smith C, Jackson MR, Wooster R and Burchell B (1990) Characterization and primary sequence of a human hepatic microsomal estriol UDP-glucuronosyltransferase. *Arch Biochem Biophys* **281:**170–175.

Daly AK (1995) Molecular basis of polymorphic drug metabolism. *J Mol Med* **73:**539–553.

Dutton GJ (1975) Commentary: Control of UDP-glucuronyltransferase activity. *Biochem Pharmacol* **24:**1835–1841.

Dutton GJ (1978) Developmental aspects of drug conjugation, with special reference to glucuronidation. *Annu Rev Pharmacol Toxicol* **18:**17–35.

Dutton GJ (1980) *Glucuronidation of Drugs and Other Compounds*. CRC Press, Inc., Boca Raton.

Dutton GJ and Storey IDE (1953) The isolation of a compound of uridine diphosphate and glucuronic acid from liver. *Proc Biochem Soc* **53:**xxxvii–xxxviii.

Gonzalez FJ (1989) The molecular biology of cytochrome P450s. *Pharmacol Rev* **40:**243–288.

Harding D, Fournel-Gigleux S, Jackson MR and Burchell B (1988) Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells. *Proc Natl Acad Sci USA* **85:**8381–8385.

Harding D, Jeremiah SJ, Povey S and Burchell B (1990) Chromosomal mapping of a human phenol UDP-glucuronosyltransferase, *GNT1*. *Ann Hum Genet* **54:**17–21.

Iyanagi T (1991) Molecular basis of multiple UDP-glucuronosyltransferase isoenzyme deficiencies in the hyperbilirubinemic rat (Gunn rat). *J Biol Chem* **266:**24028–24052.

Iyanagi T, Haniu M, Sogawa K, Fujii-Kuriyama Y, Watanabe S, Shively JE and Anan KF (1986) Cloning and characterization of CDNA encoding 3-methylcholanthrene inducible rat MRNA for UDP-glucuronosylatransferase. *J Biol Chem* **261:**15607–15614.

Iyanagi T, Watanabe T and Uchiyama Y (1989) The 3-methylcholanthrene-inducible

UDP-glucuronosyltransferase deficiency in the hyperbilirubinemic rat (Gunn rat) is caused by a −1 frameshift mutation. *J Biol Chem* **264:**21302–21307.
Jackson MR, McCarthy LR, Harding D, Wilson S, Coughtrie MWH and Burchell B (1987) Cloning of a human liver microsomal UDP-glucuronosyltransferase cDNA. *Biochem J* **242:**581–588.
Jedlitschky G, Cassidy AJ, Sales M, Pratt N and Burchell B (1999) Cloning and characterization of a novel human olfactory UDP-glucuronosyltransferase. *Biochem J* **340:**837–843.
Jin C-J, Miners JO, Lillywhite KJ and Mackenzie PI (1993a) cDNA Cloning and expression of two new members of the human liver UDP-glucuronosyltransferase 2B subfamily. *Biochem Biophys Res Commun* **194:**496–503.
Jin C, Miners JO, Lillywhite KJ and Mackenzie PI (1993b) Complementary deoxyribonucleic acid cloning and expression of a human liver uridine diphosphate-glucuronosyltransferase glucuronidating carboxylic acid-containing drugs. *J Pharmacol Exp Ther* **264:**475–479.
Kapitonov D and Yu RK (1999) Conserved domains of glycosyltransferases. *Glycobiology* **9:**961–978.
Kolars JC, Lown KS, Schmiedlin-Ren P, Ghosh M, Fang C, Wrighton SA, Merion RM and Watkins PB (1994) CYP3A Gene expression in human gut epithelium. *Pharmacogenetics* **4:**247–259.
Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C and Watkins PB (1992) Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J Clin Invest* **90:**1871–1878.
Konig J, Cui Y, Nies AT and Keppler D (2000) Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J Biol Chem* **275:**23161–23168.
Konig J, Nies AT, Cui Y, Leier I and Keppler D (1999) Conjugate export pumps of the multidrug resistance protein (MRP) family: Localization, substrate specificity, and MRP2-mediated drug resistance. *Biochim Biophys Acta* **1461:**377–394.
Kullak-Ublick GA, Beuers U and Paumgartner G (2000) Hepatobiliary transport. *J Hepatol* **32:**3–18.
Lampe JW, Bigler J, Horner NK and Potter JD (1999) UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: Relationships to serum bilirubin concentrations. *Pharmacogenetics* **9:**341–349.
Levesque E, Beaulieu M, Green MD, Tephly TR, Belanger A and Hum DW (1997) Isolation and characterization of UGT2B15(Y85): A UDP- glucuronosyltransferase encoded by a polymorphic gene. *Pharmacogenetics* **7:**317–325.
Levesque E, Beaulieu M, Hum DW and Belanger A (1999) Characterization and substrate specificity of UGT2B4 (E458): A UDP-glucuronosyltransferase encoded by a polymorphic gene. *Pharmacogenetics* **9:**207–216.
Li Q, Lamb G and Tukey RH (2000) Characterization of the UDP-glucuronosyltransferase 1A locus in lagomorphs: Evidence for duplication of the UGT1A6 gene. *Mol Pharmacol* **58:**89–97.
Li Q, Lou X, Peyronneau M-A, Straub PO and Tukey RH (1997) Expression and functional domains of rabbit liver UDP-glucuronosyltranferase 2B16 and 2B13. *J Biol Chem* **272:**3272–3279.
Mackenzie PI (1990) Expression of chimeric cDNAs in cell culture defines a region of UDP glucuronosyltransferase involved in substrate selection. *J Biol Chem* **265:**3432–3435.
Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Belanger A, Fournel-Gigleux S, Green M, Hum DW, Iyanagi T, et al (1997) The UDP glycosyltransferase gene superfamily: Recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* **7:**255–269.
Matern S, Matern H, Farthmann EH and Gerok W (1984) Hepatic and extrahepatic glucuronidation of bile acids in man. Characterization of bile acid uridine 5′-diphosphate-glucuronosyltransferase in hepatic, renal, and intestinal microsomes. *J Clin Invest* **74:**402–410.
McDonnell WM, Hitomi E and Askari FK (1996) Identification of bilirubin UDP-GTs in the human alimentary tract in accordance with the gut as a putative metabolic organ. *Biochem Pharmacol* **51:**483–488.
Meech R and Mackenzie PI (1997) Structure and function of uridine diphosphate glucuronosyltransferases. *Clin Exp Pharmacol Physiol* **24:**907–915.
Meech R and Mackenzie PI (1998) Determinants of UDP glucuronosyltransferase membrane association and residency in the endoplasmic reticulum. *Arch Biochem Biophys* **356:**77–85.
Meyer UA and Zanger UM (1997) Molecular mechanisms of genetic polymorphisms of drug metabolism. *Annu Rev Pharmacol Toxicol* **37:**269–296.
Mojarrabi B, Butler R and Mackenzie PI (1996) cDNA Cloning and characterization of the human UDP glucuronosyltransferase, UGT1A3. *Biochem Biophys Res Commun* **225:**785–790.
Mojarrabi B and Mackenzie PI (1998) Characterization of two UDP-glucuronosyltransferases that are predominantly expressed in human colon. *Biochem Biophys Res Commun* **247:**704–709.
Monaghan G, Burchell B and Boxer M (1997) Structure of the human *UGT2B4* gene encoding a bile acid UDP-glucuronosyltransferase. *Mammalian Genome* **9:**692–694.
Monaghan G, Clarke DJ, Povey S, See CG, Boxer M and Burchell B (1994) Isolation of a human YAC contig encompassing a cluster of *UGT2* genes and its regional localization to chromosome 4q13. *Genomics* **23:**496–499.
Monaghan G, Ryan M, Seddon R, Hume R and Burchell B (1996) Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet* **347:**578–581.
Pacifici GM, Franchi M, Bencini C, Repetti F, Di Lascio N and Muraro GB (1988) Tissue distribution of drug-metabolizing enzymes in humans. *Xenobiotica* **18:**849–856.
Pacifici GM, Giuliani L and Calcaprina R (1986) Glucuronodation of 1-napthol in nuclear and microsomal fractions of the human intestine. *Pharmacology* **33:**103–109.
Parquet M, Pessah M, Sacquet E, Salvat C and Raizman A (1988) Effective glucuronidation of 6 alpha-hydroxylated bile acids by human hepatic and renal microsomes. *Eur J Biochem* **171:**329–334.
Peters WH and Jansen PL (1988) Immunocharacterization of UDP-glucuronyltransferase isoenzymes in human liver, intestine and kidney. *Biochem Pharmacol* **37:**564–567.
Peters WH, Kock L, Nagengast FM and Kremers PG (1991) Biotransformation enzymes in human intestine: Critical low levels in the colon? *Gut* **32:**408–412.
Radominska-Pandya A, Little JM, Pandya JT, Tephly TR, King CD, Barone GW and Raufman JP (1998) UDP-glucuronosyltransferases in human intestinal mucosa. *Biochim Biophys Acta* **1394:**199–208.
Ritter JK, Chen F, Sheen YY, Lubet RA and Owens IS (1992a) Two human liver cDNAs encode UDP-glucuronosyltransferases with 2 log differences in activity toward parallel substrates including hyodeoxycholic acid and certain estrogen derivatives. *Biochemistry* **31:**3409–3414.
Ritter JK, Chen F, Sheen YY, Tran HM, Kimura S, Yeatman MT and Owens IS (1992b) A novel complex locus UGT1 encodes human bilirubin, phenol and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini. *J Biol Chem* **267:**3257–3261.
Ritter JK, Crawford JM and Owens IS (1991) Cloning of two human liver bilirubin UDP-glucuronosyltransferase cDNAs with expression in COS-1 cells. *J Biol Chem* **266:**1043–1047.
Ritter JK, Kessler FK, Thompson MT, Grove AD, Auyeung DJ and Fisher RA (1999) Expression and inducibility of the human bilirubin UDP-glucuronosyltransferase UGT1A1 in liver and cultured primary hepatocytes: Evidence for both genetic and environmental influences. *Hepatology* **30:**476–484.
Ritter JK, Sheen YY and Owens IS (1990) Cloning and expression of human liver UDP-glucuronosyltransferase in COS-1 cells. 3,4-Catechol estrogens and estriol as primary substrates. *J Biol Chem* **265:**7900–7906.
Ritter JK, Yeatman MT, Kaiser C, Gridelli B and Owens IS (1993) A phenylalanine codon deletion at the UGT1 gene complex locus of a Crigler-Najjar type i patient generates a pH-sensitive bilirubin UDP-glucuronosyltransferase. *J Biol Chem* **268:**23573–23579.
Strassburg CP, Kneip S, Topp J, Obermayer-Straub P, Barut A, Tukey RH and Manns MP (2000) Polymorphic gene expression and interindividual variation of udp-glucuronosyltransferase activity in human small intestine. *J Biol Chem* **275:**36164–36171.
Strassburg CP, Manns MP and Tukey RH (1997a) Differential down regulation of the UDP-glucuronosyltransferase 1A locus is an early event in human liver and biliary cancer. *Cancer Res* **57:**2979–2985.
Strassburg CP, Manns MP and Tukey RH (1998a) Expression of the *UDP*-glucuronosyltransferase 1A locus in human colon. Identification and characterization of the novel extrahepatic UGT1A8. *J Biol Chem* **273:**8719–8726.
Strassburg CP, Nguyen N, Manns MP and Tukey RH (1998b) Polymorphic expression of the UDP-glucuronosyltransferase *UGT1A* gene locus in human gastric epithelium. *Mol Pharmacol* **54:**647–654.
Strassburg CP, Nguyen N, Manns MP and Tukey RH (1999a) UDP-glucuronosyltransferase activity in human liver and colon. *Gastroenterology* **116:**149–160.
Strassburg CP, Oldhafer K, Manns MP and Tukey RH (1997b) Differential expression of the *UGT1A* locus in human liver, biliary and gastric tissue. Identification of UGT1A7 and UGT1A10 transcripts in extrahepatic tissue. *Mol Pharmacol* **52:**212–220.
Strassburg CP, Strassburg A, Nguyen N, Li Q, Manns MP and Tukey RH (1999b) Regulation and function of family 1 and family 2 UDP- glucuronosyltransferase genes (*UGT1A*, *UGT2B*) in human oesophagus. *Biochem J* **338:**489–498.
Sutherland L, Bin Senafi S, Ebner T, Clarke DJ and Burchell B (1992) Characterization of a human bilirubin UDP-glucuronosyltransferase stably expressed in hamster lung fibroblast cell cultures. *FEBS Lett* **308:**161–164.
Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40:**581–616.
Turgeon D, Carrier JS, Levesque E, Beatty BG, Belanger A and Hum DW (2000) Isolation and characterization of the human UGT2B15 gene, localized within a cluster of UGT2B genes and pseudogenes on chromosome 4. *J Mol Biol* **295:**489–504.
Von Heijne G (1986) A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res* **14:**4683–4690.
Walle T, Otake Y, Galijatovic A, Ritter JK and Walle UK (2000) Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in the human hepatoma cell line Hep G2. *Drug Metab Dispos* **28:**1077–1082.
Wooster R, Sutherland L, Ebner T, Clarke D, Da Cruz e Silva O and Burchell B (1991) Cloning and stable expression of a new member of the human liver phenol/bilirubin: UDP-glucuronosyltransferase cDNA family. *Biochem J* **278:**465–469.

Send reprint requests to: Robert H. Tukey, Ph.D., Departments of Pharmacology, Chemistry & Biochemistry, University of California, San Diego, La Jolla, CA 92093-0635. E-mail: rtukey@ucsd.edu