8 UDP-Glucuronosyltransferases

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Introduction

Glucuronidation represents one of the most important phase-II biotransformation reactions converting thousands of lipophilic endobiotics and xenobiotics (drugs, dietary plant constituents, etc.) and their phase-I metabolites into hydrophilic and excretable conjugates (Dutton 1980; see Chapter 1). For many compounds such as plant constituents which already contain functional groups ($-OH$, $-COOH$, $-SH$, $-NH_2$) glucuronidation represents the primary biotransformation reaction. Glucuronidation is catalysed by a supergene family of UDP-glucuronosyltransferases (UGTs) which are integral proteins of the membranes of the endoplasmic reticulum and the nuclear envelope. UGTs are present in many tissues of vertebrates (mammals, fish and—although no sequences have been published—in amphibia, reptiles and birds; Dutton 1980).

In general, glucuronidation occurs in concert with other biotransformation reactions which have been termed phase-I (functionalisation reactions), phase-II (conjugation) and phase-III (export of conjugates from cells). As shown in Figure 8.1, lipophilic compounds (X) (entering cells by passive diffusion and by uptake carriers such as OATPs (organic anion transporting proteins), a process which has been termed phase 0) are usually converted in phase I, mainly by cytochromes P450 (CYPs), into a number of electrophilic and nucleophilic metabolites. Reactive electrophiles are often conjugated by glutathione *S*-transferases. When reactive metabolites accumulate in cells they may interact with cellular macromolecules such as DNA (a reaction which may initiate carcinogenesis) or with proteins (in some cases initiating autoimmune diseases; see Chapter 1). Nucleophilic metabolites are mainly conjugated by UGTs, sulphotransferases, etc. Some phenolic metabolites such as benzo[a]pyrene diphenols are readily autoxidised to electrophilic quinones which, in turn, may undergo redox cycling leading to oxidative stress. It is noteworthy that some acyl glucuronides are electrophilic and known to react with cellular proteins. Conjugates with glucuronic acid, sulphate or glutathione need to be excreted from cells by ATP-dependent export pumps such as multidrug resistance proteins (MRPs). It is noteworthy that transport by
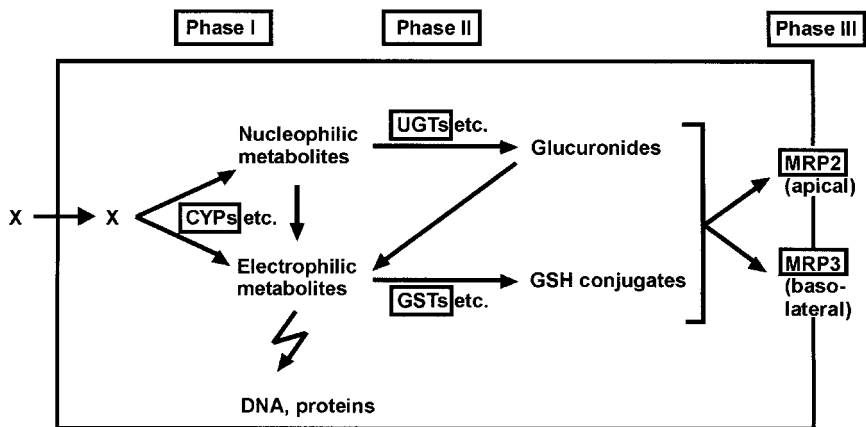
Figure 8.1 Integration of glucuronidation into the biotransformation system of lipophilic endo- and xenobiotics. CYPs, cytochromes P450; UGTs, UDP-glucuronosyltransferases; GSTs, glutathione S-transferases; MRPs, multidrug resistance proteins.

MRPs often determine the disposition of glucuronides: MRP2 has been characterised as an apical export pump which in hepatocytes is exclusively incorporated into canalicular membranes, and therefore secretes conjugates via the bile into the intestine. In contrast, MRP3 secretes glucuronides via the basolateral membrane into the blood. Concerted actions of phase I, II and III enzymes/proteins (the biotransformation system) are supported by their occasional coinduction by xenobiotics; for example, by Ah receptor agonists and by phenobarbital-type inducers. Biotransformation of lipophilic compounds probably represents a detoxification process which is essential for life.

UGTs catalyse the transfer of glucuronic acid from UDP-glucuronic acid to phenols, hydroxylamines, carboxylic acids etc . In this way glucuronides with differing pH stability are formed (Figure 8.2(A)). Whereas ether glucuronides are quite stable (a), *N*-glucuronides of some arylamines such as 2-naphthylamine and 4-aminobiphenyl including *N*-glucuronides of their hydroxylamines are acid-labile at pH < 7 (b) (Beland and Kadlubar 1990). Ester (acyl) glucuronides are unstable at neutral and alkaline *pH >7* (c) (Benet *et al.* 1993).

Based on kinetic data and chemical modification of the enzyme protein, a general acid-base Sn2 mechanism has been proposed for transfer of phenolic aglycones to glucuronic acid (Figure 8.2(B)). A general base (B) of the active site may protonate phenolic compounds facilitating their transfer to glucuronic acid. The base may involve a charge relay system between the catalytic aspartate–glutamate residue and the histidyl residue. The leaving group (UDP) would then be protonated by an acidic amino acid residue of the active site. The catalytic cycle would be completed by a proton exchange by the basic and acidic catalytic residues (Radominska-Pandya *et al.* 1999).

Application of molecular biology techniques to UGTs led to an explosion of our
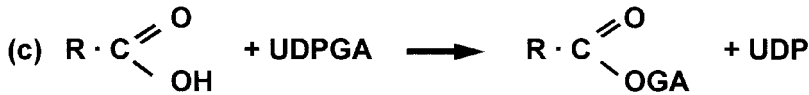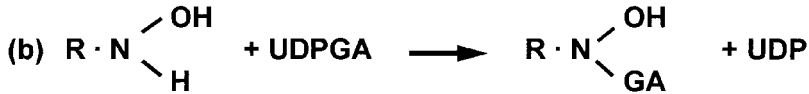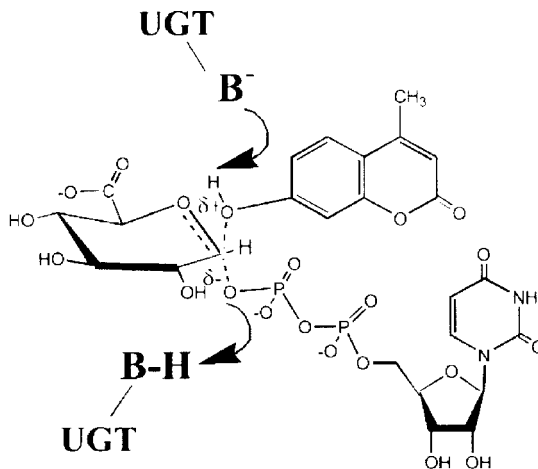
A

(a) R - OH   + UDPGA ⟶ R - OGA   + UDP

(b) R · N（OH, H）   + UDPGA ⟶ R · N（OH, GA）   + UDP

(c) R · C（=O, OH） + UDPGA ⟶ R · C（=O, OGA） + UDP

B

Figure 8.2 UGT reactions. (a) Reactions with differing pH stabilities of resulting glucuronides. UDPGA, UDP-glucuronic acid. (b) Mechanism proposed for the glucuronidation of phenolic compounds (Reproduced from Radominska-Pandya *et al.* 1999 *Drug Metabolism Reviews*, **31**, 817–899. Published by Marcel Dekker, Inc., New York).

knowledge about UGT isoforms and about their functions in the metabolism of endobiotics, drugs, dietary plant constituents and in detoxification of carcinogens. A remarkable tissue-specific expression of UGTs was found. It is becoming increasingly clear that the biotransformation system not only deals with drugs or other xenobiotics but also controls the levels of key endogenous compounds such as hormone receptor

ligands (Nebert 1991). Conjugation of endogenous substrates needs to be explored more extensively.

The present review focuses on human UGT isoforms, their functions and their regulation by xenobiotics. Human UGTs are compared to those characterised in experimental animals. Recently excellent reviews have appeared (Burchell *et al.* 1997; Radominska-Pandya *et al.* 1999; Tukey and Strassburg 2000; King *et al.* 2000). A hypothetical model for the integration of UGTs into endoplasmic reticulum membranes is discussed which may have important implications in UGT activities (Radominska-Pandya *et al.* 1999). For recent additions of UGT sequences, the reader should consult the UDP glucuronosyltransferase homepage (http:»www.unisa.edu.au/ pharm_medsci/gluc_trans/default.htm; e-mail address of the developers of the home-page: Peter.Mackenzie@Flinders.edu.au or Ross.McKinnon@unisa.edu.au. It is hoped that the UGT homepage will increasingly be used as a medium to communicate established UGT isoforms.)

Historical perspectives

Soon after the discovery of UGTs (Dutton and Storey 1953; Dutton 1956, 1997) evidence for their multiplicity was obtained from clinical observations on non-haemolytic familial hyperbilirubinaemias such as Crigler–Najjar syndromes I and II (Axelrod *et al.* 1957; Schmid *et al.* 1957; Arias 1962; Arias *et al.* 1969). In liver microsomes from patients, bilirubin glucuronidation was undetectable whereas glucuronidation of other substrates was unimpaired. Multiplicity was also supported by differential induction of rat UGT activities by treatment of rats with 3-methylcholanthrene or phenobarbital (Bock *et al.* 1973; Wishart 1978a). These studies were substantiated by differential appearance of similar groups of UGT activities in late-foetal and neonatal development (Wishart 1978b). Purification of membrane-bound UGTs proved to be very difficult due to their strong interaction with phospholipids and their existence as strongly interacting oligomers in microsomal membranes. Nevertheless, purification helped to isolate UGT sequences from rats (Jackson *et al.* 1984; Mackenzie *et al.* 1984; Iyanagi *et al.* 1986) and humans (Harding *et al.* 1988). The growing number of sequences allowed a nomenclature system based on their evolutionary divergence (Burchell *et al.* 1991). This system allowed not only the naming of glucuronosyl transferases in vertebrates (fish and mammals) but also finding related glycosyltransferases in invertebrates (*Caenorhabditis elegans, Drosophila melanogaster*), bacteria, yeasts and plants (Mackenzie *et al.* 1997).

Nomenclature, UGT domain structure and polymorphisms

UDP-GLUCURONOSYLTRANSFERASE FAMILIES 1 AND 2

Based on evolutionary divergence, mammalian microsomal UDP-glucuronosyltransferases (EC 2.4.1.17) have been grouped into two distinct families: family 1 includes bilirubin and phenol UGTs, and family 2 includes steroid UGTs (Burchell *et al.* 1991). However, it is obvious from Tables 8.1 and 8.2 that both family 1 and 2 members are involved in steroid glucuronidation. For naming each gene, it is recommended that

the root symbol UGT for human (Ugt for mouse), denoting 'UDP-glucuronosyltransfer-ase,' be followed by an Arabic number representing the family, a letter designating the subfamiliy, and an Arabic numeral denoting the individual gene within the family or subfamily, e.g. 'human UGT1A6'.

Interestingly, human family 1 UGT isoforms are formed from a large UGT1 gene locus, spanning over 200 kb, containing more than a dozen promoters/first exons which are joined by exon sharing with their common exons 2 through 5 (Figure 8.3). Hence the different family 1 members have identical *C*-terminal halves of the UGT protein but different *N*-terminal halves. The UGT1 gene locus may have evolved by exon 1 duplication. Each first exon is regarded as a distinct gene and numbered according to the distance from the common exons (e.g. UGT1A1, UGT1A2). The human gene complex is present at chromosome 2 (2q37). The gene locus appears to be conserved between humans and experimental animals. Hence, orthologous genes are found at similar distances from the common exons. For example, the major bilirubin UGT of humans, rats and other species is encoded by exon 1 next to the common exons. The phenol UGT conjugating planar phenols is encoded by the sixth exon.

Family 2 consists of two subfamilies. UGT2 enzymes are encoded by six exons. Despite this difference from UGT1 proteins, both families share a high degree of similarity in the *C*-terminal end. The genes of subfamily 2B may have evolved by gene duplication. They are clustered on human chromosome 4q13. Three isoforms are clustered within a 192 kb region in a provisional order of UGT2B7 - 2B4 - 2B15 (Monaghan *et al.* 1994; Riedy *et al.* 2000). UGT2A1 has been identified as a major protein in bovine and rat olfactory epithelium which conjugates a broad substrate spectrum including odorants (Lazard *et al.* 1991). Recently, the human orthologue has been cloned and also mapped to chromosome 4 (4q13; Jedlitschky *et al.* 1999). It shows an identity of 87% with the rat UGT2A1 and of 43–62% with other human
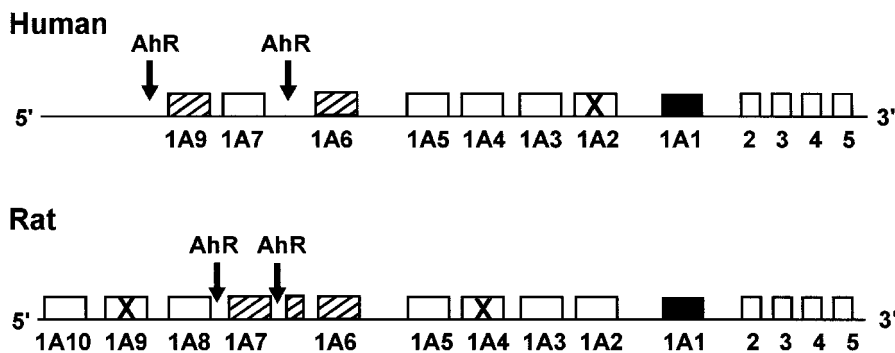

Figure 8.3 Schematic diagram of the human and rat UGT1 gene locus (Ritter *et al.* 1992; Iyanagi 1991, respectively). This gene spanning more that 200 kb consists of at least a dozen promoters/first exons which are linked by exon-sharing with their common exons 2-5. Exons indicated by crosses are pseudogenes. AhR indicates genes controlled by the Ah receptor. The rat UGT1A6 exon 1 is preceeded by a non-coding exon 1a (Emi *et al.* 1996). Recently it was found that UGT1A9 is the next isoform following UGT1A7 in the human UGT1 gene locus which—as illustrated in the figure—is incomplete (Ida S. Owens, National Institutes of Health, Bethesda, USA, personal communication).

UGT isoforms. In addition to odorants, it conjugates some steroids, especially androgens and some drugs. UGT2B isoforms are the most abundant. UGT1B is a minor subfamily that currently contains only one representative, cloned from fish (UGT1B; Mackenzie *et al.* 1997).

UGT DOMAIN STRUCTURE

All human UGTs have a common domain structure. In the variable *N*-terminal domain, a signal peptide has been identified (Blobel and Dobberstein 1975) which determines the transfer of UGTs to the endoplasmic reticulum (ER) followed by a variable *N*-terminal domain. At the conserved *C*-terminal domain, a single transmembrane fragment is found which is followed by a stop transfer signal (Blobel 1980). Evidence has been obtained that the cofactor UDP-glucuronic acid interacts with both the *N*- and *C*-terminal domains (Figure 8.4; Radominska-Pandya *et al.* 1999).

GLUCURONOSYL AND GLYCOSYLTRANSFERASES

It has become evident that UDP-glucuronosyltransferases may be part of a larger family of proteins (both membrane-bound and cytosolic) that preferentially use other
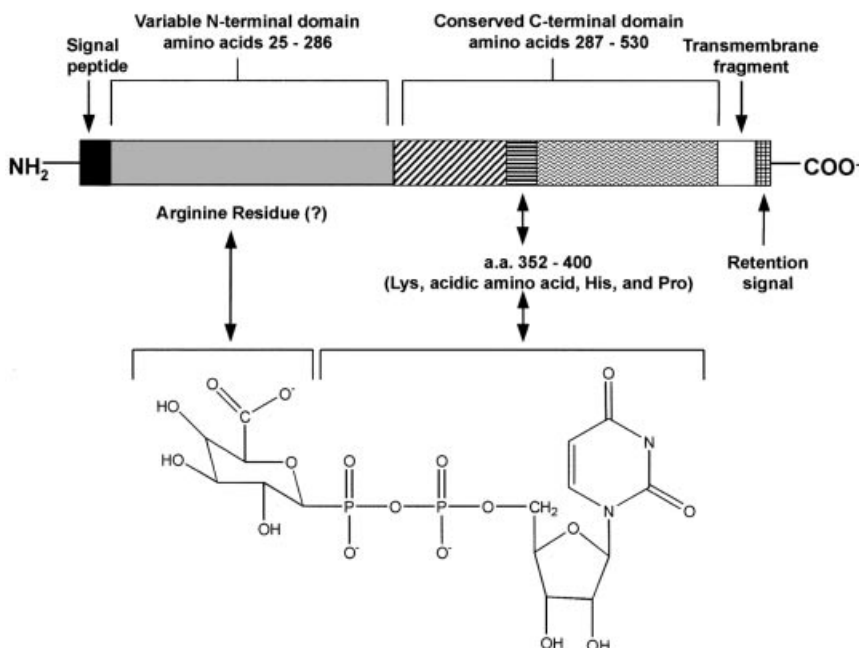

Figure 8.4 Domain structure of UGTs and postulated interactions between specific amino acids of the UGT active site and UDPGA (Reproduced from Radominska-Pandya *et al.* 1999 *Drug Metabolism Reviews*, **31**, 817–899. Published by Marcel Dekker, Inc., New York).

nucleotide sugars, including UDP-glucose and UDP-galactose (Mackenzie *et al.* 1997). Some of these glycosyltransferases reveal a striking homology in their C-terminal halves in which the following bona fide 'signature sequence' of amino acids has been found which determines their membership in the supergene family (h, hydrophobic residue; x, non-specific residue):

$$\text{FhTHGGxxShxExhxxGVPhhxxPhxxDQ}$$
$$\text{S\quad A\qquad\quad G\qquad\qquad\quad T}$$
$$\text{C}$$

This conserved signature sequence probably represents the binding site for UDP. (It is noteworthy that there is a number of UDP-*N*-acetylglucosamine transferases which are not members of this glycosyltransferase supergene family.) Once the preferred nucleotide has been identified, the enzymes should be termed UDP-glucuronosyl-transferases (UGTs), UDP-glucosyltransferases, etc. Inclusion of glucuronosyltrans-ferases in the larger superfamily of glycosyl-transferases (Mackenzie *et al.* 1997) is preliminary because of the limited identification of many invertebrate sequences (Tukey and Strassburg 2000). Nevertheless, the similarity between the mammalian olfactory UGT2A1 and the *Drosophila* olfactory glucosyltransferase, between steroid glucuronosyltransferases and the baculovirus ecdysone glucosyltransferase, etc. appears striking enough to suggest a broader superfamily and to stimulate further studies on the evolutionary relationship between UDP-glucuronosyltransferases and the superfamily of glycosyltransferases.

POLYMORPHISMS AND ALLELIC VARIANTS

An increasing number of allelic variants and polymorphisms of UGT isoforms is being discovered and a nomenclature system for the variants has been proposed (Mackenzie *et al.* 1997).

UGT1A1

Several important inherited hyperbilirubinaemias have been described (Schmid *et al.* 1957; Arias *et al.* 1969). Note that the clinical disorder does not always match the genotype.

Crigler–Najjar syndromes, type I and II

The inheritable and severe defects were analysed with respect to the gene structure of the UGT1 locus. Human UGT1A1 is the primary isoform responsible for bilirubin metabolism and maintenance of normal levels of serum bilirubin. A spectrum of mutations in the common exons 2–5 or of exon 1 is responsible for the phenotypic profiles (UGT1A1*2 - 27, Mackenzie *et al.* 1997). The development of 'chimera-plasty', a form of gene transplantation, is being evaluated as potential therapy (Gura 1999).

Gilbert's syndrome

This is characterised by a mild unconjugated hyperbilirubinaemia. It appears to be present in *ca* 5% of the Caucasian population. The basis for this syndrome is an atypical mutation of the TATA box region of the UGT1A1 promoter. The variant allele consists of seven TA repeats in the A(TA)$_n$TAA motif whereas six TA repeats characterise the common allele (UGT1A1*1). The presence of the A(TA)$_7$TAA allele (UGT1A1*28) was found to decrease UGT1A1 gene expression *in vivo*. Homozygote individuals carrying the A(TA)$_7$TAA allele show significantly higher plasma levels of unconjugated bilirubin caused by a 30% reduction of UGT1A1 gene transcription (Bosma *et al.* 1995; Monaghan *et al.* 1996). Additional UGT1A1 alleles have been reported exclusively in an African–American population who have five or eight repeats (termed UGT1A1*33 and UGT1A1*34, respectively; Beutler *et al.* 1998). In general, there is a correlation between the number of repeats and the bilirubin levels. Evidence has been obtained recently that the (TA)$_8$ allele may be associated with lower oestradiol glucuronidation, higher oestrogen levels and a higher risk in breast cancer (Guillemette *et al.* 2000). Lower glucuronidation in Gilbert patients may also be the reason for more frequent side-effects after treatment with the topoisomerase inhibitor irinotecan due to lower glucuronidation rates of its major metabolite SN-38 (Gupta *et al.* 1994; Iyer *et al.* 1998).

UGT1A6

Two missense mutations were uncovered leading to E181A and R184S mutations of the UGT1A6 gene (UGT1A6*2, Ciotti *et al.* 1997). This genotype is present in about one third of the population and leads to lower glucuronidation of simple phenols than in carriers of the wild-type allele. The functional consequences are not yet clear. Co-occurence of UGT1A6*2 with the mutation of the UGT1A1 promoter (UGT1A1*28) has also been observed (Lampe *et al.* 1999).

UGT2B4

Several polymorphic UGT2B4 isoforms have been described (2B4-D[458], 2B4-E[458], etc.) which differ in the conjugation of the bile acid hyodeoxycholic acid. They are also involved in the conjugation of androgen metabolites, catechol oestrogens and eugenol (Lévesque *et al.* 1999).

UGT2B7

Variants have been described which poorly glucuronidate *S*-oxazepam (Patel *et al.* 1995). Recently, stable expression of both UGT2B7 (H[268]) and UGT2B7 (Y[268]) revealed differences in UGT activities towards several substrates with similar K_m values. However, no difference in oxazepam glucuronidation was found (Coffman *et al.* 1998).

UGT2B15

Variants of UGT2B15 (D^{85}) and UGT2B15 (Y^{85}) have been described (Lévesque *et al.* 1997). No difference in substrate specificity but slightly higher V_{max}-values were found when Y^{85} was compared with D^{85}.

Tissue distribution

LIVER, OESOPHAGUS, GASTROINTESTINAL TRACT AND KIDNEY

The liver is the major site of glucuronidation in the living organism. In this organ, UGTs are differentially expressed in the centrilobular and periportal zones (Ullrich *et al.* 1984; Saarikoski *et al.* 1998). It is becoming increasingly clear that the small intestine plays also a significant role in the first-pass metabolism of orally administered drugs such as morphine (Lin *et al.* 1999). Some UGT isoforms are expressed in a remarkably tissue-specific fashion in the gastrointestinal tract. In fact, hUGT1A7 has been found to be expressed only in gastric tissue and in the oesophagus, the latter also expressing UGT1A8, 1A9 and 1A10 (Strassburg *et al.* 1997a, 1999). UGT1A8 and UGT1A10 are expressed in the colon but not in the liver (Mojarrabi and Mackenzie 1998a; Strassburg *et al.* 1998). Evidence has been obtained that some isoforms (UGT1A1, UGT1A6, UGT2B4 and UGT2B7) are intriguingly expressed in a polymorphic manner in the small intestine (Strassburg *et al.* 2000). UGTs are expressed at other external surfaces such as skin and lung, as well as in kidney. The level of UGT1A9 in the kidney appears to be higher than in human liver (McGurk *et al.* 1998). Furthermore, remarkable species differences have been observed. For example, rat kidney is capable of glucuronidating bilirubin in contrast to the human kidney (McGurk *et al.* 1998).

OLFACTORY EPITHELIUM

An isoform, UGT2A1, has been found selectively in rat and human olfactory epithelium (Lazard *et al.* 1991; Jedlitschky *et al.* 1999). This isoform is involved in the metabolism of a variety of endo- and xenobiotics including steroid hormones and odorants. Inactivation of the latter compounds may facilitate removal of odorant signals thereby terminating their action. The comparative evolutionary approach made possible the identification of a related UDP-glucosyltransferase in the olfactory organ of *Drosophila melanogaster* (Wang *et al.* 1999).

STEROIDOGENIC TISSUES

It is becoming increasingly clear that UGTs play a role not only in overall pharmaco-kinetics but also in local protection of cells against toxicants. For example, the broadly expressed UGT1A6 has been detected in steroidogenic tissues such as testis and ovary (Münzel *et al.* 1994; Becedas *et al.* 1998). In the testis it appears to be expressed in Sertoli cells and in spermatogonia (Brands *et al.* 2000). The blood–testis barrier generated by Sertoli cells does not sufficiently protect spermatogonia against lipophilic compounds. This may explain why UGT1A6 is expressed in the spermatogonia themselves.

In addition to a protective role in steroidogenic tissues, UGTs may control endogenous receptor ligands such as testosterone or dihydrotestosterone, as suggested by Nebert (1991). This has been shown in a prostate carcinoma cell line LNCaP (Bélanger *et al.* 1998). These steroid target cells have been shown to express both UGT2B15 and UGT2B17, the latter being expressed at a lower level but being inducible by antioxidants. For example, treatment of the cells with the soy bean isoflavone biochanin A increased testosterone glucuronide formation and reduced formation of the androgen-dependent prostate-specific antigen, suggesting that UGTs are responsible for control of this endogenous receptor ligand (Sun *et al.* 1998).

BRAIN

Recently, expression of UGTs (UGT1A6 and UGT2B7) has been detected in rat and human brain (Martinasevic *et al.* 1998; King *et al.* 1999), particularly in Purkinje cells of the cerebellum and hippocampal pyramidal cells (King *et al.* 1999; Brands *et al.* 2000). UGT1A6 has been shown to glucuronidate serotonin. However, more work is needed to establish whether UGTs control the levels of catecholamines. UGT activities have also been found in brain microvessel fractions which are derived from the blood-brain barrier (Suleman *et al.* 1998). Expression of UGT2B7 in brain may be interesting since it is the only UGT isoform which forms morphine 6-glucuronide. This glucuronide is biologically active and a strong analgesic (Paul *et al.* 1989; Osborne *et al.* 1992). It has been found to be more potent on μ opioid receptors than morphine itself. Formation of morphine 6-glucuronide in brain tissue from morphine would circumvent the blood-brain barrier which is poorly penetrable by morphine 6-glucuronide from the blood side (Wu *et al.* 1997).

Substrate specificity

UGTs conjugate a remarkable diversity of endobiotics and xenobiotics containing a number of different functional groups (e.g. $-OH$, $-COOH$, $-NH_2$ and $-SH$). Most glucuronides are biologically inactive. However, some are bioactive (morphine 6-glucuronide) or even toxic (oestrogen D-ring glucuronides and acyl glucuronides. Substrate hydrophobicity is essential for glucuronidation by UGTs. For lipophilic compounds the membrane may be involved in the transport of the substrate to the active site of the enzyme (see below).

Heterologous expression of UGT isoforms has been a powerful tool in determining their substrate specificity (Guengerich *et al.* 1997; Townsend *et al.* 1999). The results of these studies are summarised in Tables 8.1 and 8.3 for family 1 members and Tables 8.2 and 8.4 for family 2 members. Quantitative comparison of glucuronidation rates of UGT isoforms is not yet possible since the protein levels of recombinant UGT isoforms are seldom known.

For UGT1A5, 2B10 and 2B11 no substrates have been found so far (July) 2000. The substrates have been grouped here into endobiotics (Tables 8.1 and 8.2) and xenobiotics (Tables 8.3 and 8.4). Endobiotic substrates have been grouped as steroids [C18 steroids (oestradiol), C19 steroids (dihydrotestosterone) and C21 steroids (pregnandiol)] and as a miscellaneous group of other endobiotics such as bilirubin, bile acids

Table 8.1 Endobiotic substrates of human UGT1 isoforms

Isoform	Substrate	
	Steroids	Others
UGT1A subfamily		
UGT1A1	Oestriol	Bilirubin
	β-Oestradiol	atRA[a]*
	2-Hydroxyoestriol	5,6-Epoxy-atRA[a]*
	2-Hydroxyoestrone	
	2-Hydroxyoestradiol	
UGT1A3	2-Hydroxyoestrone	5,6-Epoxy-atRA*
	2-Hydroxyoestradiol	4-OH-atRA
	Oestrone	Lithocholic acid
UGT1A4	Androsterone	
	Epiandrosterone	
	5α-Androstane-3α, 17β-diol	
	5β-Androstane-3α, 11α, 17β-diol	
	5α-Pregnan-3α, 20α-diol	
	5α-Pregnan-3β, 20β-diol	
	5-Pregnene-3β-ol-20-one	
UGT1A6		Serotonin[b]
UGT1A7	not known	
UGT1A8	Oestrone	
	2-Hydroxyoestrone	
	4-Hydroxyoestrone	
	2-Hydroxyoestradiol	
	4-Hydroxyoestradiol	
	Dihydrotestosterone	
	5α-Androstane-3α, 17β-diol	
UGT1A9		Thyroxine
		Reverse triiodothyronine
UGT1A10	2-Hydroxyoestrone	
	4-Hydroxyoestrone	
	Dihydrotestosterone	

For references see Radominska-Pandya *et al*. (1999) unless indicated.
*atRA, all-*trans*-retinoic acid
[a]Radominska-Pandya *et al*. (1997) [b]King *et al*. (1999)

(hyodeoxycholic acid), etc. Xenobiotic substrates have been grouped as marketed drugs, dietary plant constituents (coumarin derivatives, flavonoids etc.) and carcinogens including benzo[*a*]pyrene phenols and arylamines. A list of 350 substrates tested by utilising recombinant UGTs is found in the addendum to the review of Tukey and Strassburg (2000).

UGT FAMILY 1 MEMBERS (TABLES 8.1 AND 8.3)

UGT1A1

This major isoform is the only human UGT involved in the conjugation of bilirubin. Normally it has to glucuronidate 200–400 mg of bilirubin formed daily from the

Table 8.2 Endobiotic substrates of human UGT2 isoforms

Isoform	Substrate	
	Steroids	Others
UGT2B subfamily		
UGT2B4 alleles	Androsterone	Hyodeoxycholic acid
	5α-Androstane-3α, 17β-diol	
	5β-Pregnan-11α, 17β-diol-20-one	
	5β-Pregnan-11α, 17β-triol-20-one	
UGT2B7 (Y)	4-Hydroxyoestrone	Hyodeoxycholic acid
	4-Hydroxyoestradiol	
	Androsterone	
	Epitestosterone	
	5β-Androstane-3α, 17β-diol	
	5β-Pregnan-3α-ol-11,20-dione	
	5β-Pregnen-11α-l-3,20-dione	
UGT2B7 (H)	Androsterone	Lithocholic acid
	Epitestosterone	Hyodeoxycholic acid
	4-Hydroxyoestrone	atRA[a]*
	2-Hydroxyoestrone	5,6-epoxy-atRA[a]*
	4-Hydroxyoestradiol	4-OH-atRA[a]*
	Oestriol	4-OH-atRA[a]
		Linoleic acid[b]
		Linoleic acid 9,10-diol
		Linoleic acid 12,13-diol
		Arachidonic acid
		Phytanic acid
UGT2B15	Androsterone	
	Dihydrotestosterone	
UGT2B17	Androsterone	
	Dihydrotestosterone	
	Testosterone	

For references see Radominska-Pandya *et al.* (1999) unless indicated.
*atRA, all-*trans*-retinoic acid.
[a]Samokyszyn *et al.* (2000) [b]Jude *et al.* (2000)

catabolism of haem. As already discussed, UGT activity of UGT1A1 is a major factor determining the blood level of bilirubin.

This enzyme is also involved in the conjugation of retinoic acid, oestrogens and ethinyloestradiol at the 3-OH position (Ebner and Burchell 1993). Senafi *et al.* (1994) have shown that UGT1A1 conjugates dietary phenolic plant constituents such as the flavonoid quercetin and anthraquinones. The enzyme also conjugates opioids such as buprenorphine and nalorphine (King *et al.* 1996) and SN-38, a major metabolite of the chemotherapeutic topoisomerase inhibitor irinotecan (Iyer *et al.* 1998).

UGT1A3 and UGT1A4

These isoforms are known for the conjugation of tertiary amines to quarternary ammonium glucuronides, including important drugs such as imipramine, cyprohepta-dine, ketotifene, etc. (Green and Tephly 1998). Rat UGT1A4 is a pseudogene. This

Table 8.3 Xenobiotic substrates of human UGT1A isoforms

Isoform	Drugs	Plant constituents	Carcinogens, others
UGT1A1	Ethinyloestradiol Buprenorphin SN-38	Quercetin Naringenin 4-Methylumbelliferone	1-Naphthol
UGT1A3	Cyproheptadine	Scopoletin Naringenin 4-Methylumbelliferone	
UGT1A4	Amitriptyline Imipramine Clozapine	Diosgenin Tigogenin	Benzidine
UGT1A6	Paracetamol[a]	Methylsalicylate 4-Methylumbelliferone[a]	3-OH-BaP BaP-3,6-diphenol[c] 1-Naphthylamine[d] 2-Naphtlylamine[d] N-OH-2-naphthylamine[d]
UGT1A7	SN-38[b]		7-OH-BaP[e] N-OH-PhIP[e]
UGT1A8		Scopoletin Naringenin Eugenol	N-OH-PhIP[e]
UGT1A9	Propofol Paracetamol[a]	Quercetin Alizarin 4-Methylumbelliferone[a]	3-OH-BaP BaP-3,6-diphenol[c] BaP-7,8-dihydrodiol N-OH-PhIP[e]
UGT1A10	Mycophenolic acid		

For references see Radominska-Pandya *et al.* (1999) unless indicated.
BaP, benzo[a]pyrene; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
[a]Bock *et al.* (1993) [b]Ciotti *et al.* (1999b) [c]Gschaidmeier *et al.* (1995) [d]Orzechowski *et al.* (1994) [e]Strassburg *et al.* (1999).
SN-38: 7-Ethyl-10-hydroxycamptothecin.

Table 8.4 Xenobiotic substrates of human UGT2B isoforms

Isoform	Drugs	Plant constituents	Carcinogens, others
UGT2B4 alleles		Eugenol	
UGT2B7	Morphine Naloxone Codeine Buprenorphine Carboxylic-acid-containing drugs		BaP-7,8-dihydrodiol[a]
UGT2B15		Eugenol Scopoletin Naringenin 4-Methylumbelliferone	
UGT2B17		Eugenol	

For references see Radominska-Pandya *et al.* (1999)
BaP, Benzo[a]pyrene
[a]Jin *et al.* (1993b)

may be the reason why rats cannot form quarternary ammonium glucuronides. Human UGT1A4 is more active than UGT1A3. UGT1A3 is also involved in the conjugation of catechol oestrogens and UGT1A4 in the conjugation of androsterone and pregnane-diol (Table 8.1).

UGT1A6

This broadly expressed isoform is mostly involved in the conjugation of simple planar phenols. Serotonin can be considered as an endogenous substrate of UGT1A6 (King *et al.* 1999). It has been shown to be a major enzyme in the conjugation of paracetamol (acetaminophen; Bock *et al.* 1993). Paracetamol glucuronidation is enhanced in cigarette smokers (Bock *et al.* 1987, 1994). UGT1A6 is also involved in the conjugation of a variety of planar primary amines such as 1- and 2-naphthylamine and the corresponding hydroxylamines (Orzechowski *et al.* 1994). Both the human and rat orthologues are able to form conjugates of benzo[*a*]pyrene (BaP) phenols and diphenols such as BaP-3,6-diphenol to monoglucuronides. Rat UGT1A6, but not human UGT1A6, forms both BaP-3,6-diphenol mono- and diglucuronides (Gschaid-meier *et al.* 1995).

UGT1A7

In humans this isoform is expressed in a remarkably tissue-specific manner. It was found in the oesophagus and gastric tissue but not in liver (Strassburg *et al.* 1999). It conjugates 7-hydroxy-BaP and 4-hydroxy-PhIP (2-amino-1-methyl-6-phenylimida-zo[4,5-b]pyridine). In the rat it is expressed in liver and intestine where it very efficiently conjugates both planar and bulky phenols (Burchell *et al.* 1997). In particular, it is involved in the detoxification of benzo[*a*]pyrene (BaP) metabolites such as 3-OH-BaP- and BaP-7,8-dihydrodiol (Grove *et al.* 1997).

UGT1A9

The human isoform is important in the hepatic conjugation of both planar and bulky phenols (Ebner and Burchell 1993). It conjugates endogenous substrates such as thyroxin and drugs such as the anaesthetic propofol. Paracetamol is also conjugated but with much lower affinity than by UGT1A6 (Bock *et al.* 1993).

UGT1A8 and UGT1A10

Both isoforms are expressed in the intestine but not in the liver. Endogenous substrates of UGT1A8 are catechol oestrogens and androgens, coumarins, flavonoids and anthraquinones (Mojarrabi and Mackenzie 1998a; Cheng *et al.* 1999). Both isoforms have been shown to conjugate metabolites of the potential colon carcinogen PhIP (Nowell *et al.* 1999). UGT1A10 has been demonstrated to conjugate the immuno-suppressive agent mycophenolic acid (Mojarrabi and Mackenzie 1998b).

UGT FAMILY 2 MEMBERS (TABLES 8.2 AND 8.4)

UGT2B4

Several UGT2B4 alleles have been described which are expressed in the liver and extrahepatic tissues. They are involved in the conjugation of hyodeoxycholic acid, androgen metabolites, catechol oestrogens and eugenol (Lévesque *et al*. 1999). More work is needed to characterise the substrate specificity of the different polymorphic variants since alleles have been described which appeared to be selective for hyodeoxycholic acid (Pillot *et al*. 1993).

UGT2B7

This isoform represents a major UGT isoform of family 2 which is expressed in the liver and many other organs. Two allelic variants have been described: UGT2B7 (Y) and UGT2B7 (H). They conjugate a wide variety of drugs such as morphine, (non-steroid antiinflammatory drugs) and zidovudine (Barbier *et al*. 2000), and endogenous compounds such as catechol oestrogens at 4-OH, androsterone, bile acids such as hyodeoxycholic acid, retinoids, fatty acids such as linoleic acid and in particular hydroxy fatty acids (Jin *et al*. 1993a; Coffman *et al*. 1998; Radominska-Pandya *et al*. 1999).

UGT2B15 and UGT2B17

UGT2B15 and 2B17 are expressed in the liver and the prostate (Green *et al*. 1994; Beaulieu *et al*. 1996). They conjugate dihydrotestosterone, other androgens and various plant constituents. UGT2B17 has been found to be inducible by antioxidants (Sun *et al*. 1998) and by cytokines (Lévesque *et al*. 1998).

UGTs INVOLVED IN METABOLISM AND DISPOSITION OF CARCINOGENS

Glucuronidation plays a major role in detoxification of carcinogenic compounds, for example of the tobacco-specific nitrosamine NNAL [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol]. Several UGT isoforms are involved in its detoxification such as UGT1A6, 1A7, 1A9 and 1A10 (Nguyen *et al*. 2000). In the rat, NNAL has been shown to be conjugated by UGT2B1 (Ren *et al*. 1999). A number of UGTs are also involved in detoxification of polycyclic aromatic hydrocarbons (PAHs; Bock 1991; Bock and Lilienblum 1994) and of simple and heterocyclic arylamines (Lee Chiu and Huskey 1998).

Benzo(a)pyrene (BaP)

The role of glucuronidation in the metabolism of PAHs is discussed using BaP as example. Selective pathways are listed in Figure 8.5. Extensive studies of the carcinogenicity of BaP metabolites revealed that BaP-7,8-dihydrodiol-8,9-epoxide is one of the ultimate carcinogens leading to DNA adducts (Conney 1982). As an intermediate in this pathway BaP-7,8-dihydrodiol is formed. This compound was found to be a
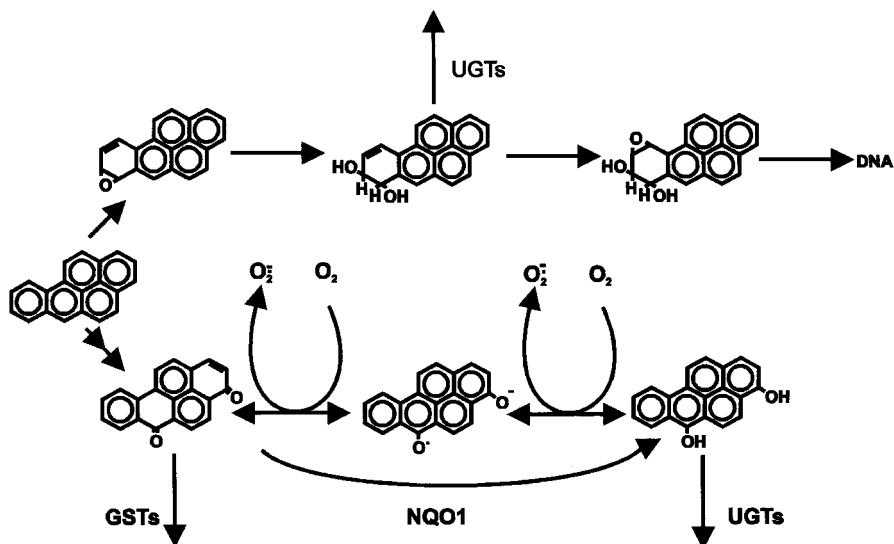
Figure 8.5 Selected pathways of benzo[*a*]pyrene (BaP) metabolism. Upper pathway, formation of a major ultimate carcinogen (Conney 1982), lower pathway, prevention of BaP quinone toxicity by the actions of glutathione S-transferases (GSTs), NADPH quinone oxidoreductase-1 (NQO1) and UGTs, BAP-3,6-quinone as example.

substrate of rat UGT1A7 (Grove *et al.* 1997) and of human UGT1A9 and UGT2B7 (Jin *et al.* 1993b). The mutant Gunn rat strain does not form any functional UGT1 proteins (Hu and Wells 1992). It shows higher BaP DNA adducts than congenic Wistar rats, indicating that glucuronidation by family 1 members is able to reduce the formation of DNA adducts.

A major fraction of BaP metabolites is excreted in rat bile. For example, when BaP was injected intravenously, 68% of the metabolites were excreted during 6 hours, and of this amount 34% and 9% represented glucuronide and sulphate conjugates, respectively. The remaining 25% were probably glutathione conjugates (Boroujerdi *et al.* 1981). BaP-3,6-diphenol diglucuronide was identified as a major glucuronide secreted into rat bile (Bevan and Sadler 1992; Yang *et al.* 1999).

Phenolic metabolites were found to be conjugated more efficiently than BaP-7,8-dihydrodiol. Conjugation of diphenols may efficiently reduce or prevent toxic quinone/quinol redox cycles (Lilienblum *et al.* 1985). Glucuronidation also reduced the mutagenicity of BaP-3,6-quinone in the Ames test (Bock *et al.* 1990a).

Rat UGT1A7 has been shown to prevent the cytotoxicity of BaP-3,6-quinone (Grove *et al.* 2000). BaP-3,6-diphenol is conjugated to mono- and diglucuronides. Several UGTs including UGT1A6 are involved in the formation of monoglucuronides. Diglucuronides are formed by rat UGT1A7 (Bock *et al.* 1999) and human UGT1A9 (Gschaidmeier *et al.* 1995). They are not formed by human UGT1A6. As an exception, rat UGT1A6 forms both mono- and diglucuronides of BaP-3,6-diphenol. This example

may be interesting since the monoglucuronide has to change its position in the UGT binding site for diglucuronide formation (see below).

Arylamines

In studies on the metabolism of 2-naphthylamine and 4-aminobiphenyl, it has been shown that glucuronides determine the target of their carcinogenicity, the urinary bladder. For example, 2-naphthylamine is oxidised to *N*-hydroxy-2-naphthylamine which is efficiently conjugated. The resulting *N*-hydroxy-*N*-glucuronide represents a stable transport form and can be considered as a proximal carcinogen. It decomposes at the slightly acidic pH of urine to the hydroxylamine and is protonated to a nitrenium ion, which readily reacts with DNA, and may potentially initiate bladder cancer (Beland and Kadlubar 1990; see chapter 1). The *N*-hydroxy-*N*-glucuronide of 2-naphthylamine was shown to be conjugated by human UGT1A6 and UGT1A9 facilitating detoxification of this reactive compound within the cell (Orzechowski *et al*. 1994). Benzidine and metabolites have been shown to be efficiently conjugated by UGT1A4 and UGT1A9 (Ciotti *et al*. 1999a).

Recently, mutagenic heterocyclic arylamines such as PhIP have been discovered which are formed at trace levels in food such as meat and fish in typical household cooking practices (Sugimura and Sato 1983). They also have been found to be oxidised to hydroxylamines and conjugated with glucuronic acid to N–OH–N–glucuronides (Alexander *et al*. 1991). The N–OH–N–glucuronide of PhIP has been found to be acid-stable and not hydrolysed by *E. coli* β-glucuronidase (Kaderlik *et al*. 1994a). It is a major metabolite in humans (Malfatti *et al*. 1999), and hence this pathway is a major detoxifying pathway. N-OH-PhIP has been found to be *O*-acetylated by *N*-acetyltransferases. Evidence was obtained that reactive *N*-acetoxy-PhIP is transported via the bloodstream and may lead to DNA adduct formation in the colon via this route (Kaderlik *et al*. 1994b). Several family 1 members (UGT1A3, 1A8, 1A9, 1A10) have been shown to conjugate metabolites of PhIP, and may effectively prevent bioactivation by acetylation or sulphation (Nowell *et al*. 1999).

Reactive acyl glucuronides as chemically reactive intermediates

Glucuronides are generally considered to be biologically inactive. Therefore, it is important to stress that acyl glucuronides are chemically reactive at pH > 7. Acyl glucuronides are formed from a number of carboxyl-containing endobiotics (bilirubin, lithocholic acid, retinoic acid) and drugs such as NSAIDs, clofibrate and the anti-epileptic drug valproic acid. They are known to undergo 'acyl migration' along the hydroxyl groups at C2–C4 of glucuronic acid. These glucuronides are β-glucuronidase-resistant and therefore escape the usual urinary analysis of glucuronides. It has been suggested that these β-glucuronidase-resistant glucuronides of NSAIDs may be transport forms through the small intestine, explaining their action in the colon (Dickinson 1998).

Acyl glucuronides have attracted considerable interest because they form protein adducts and are possibly responsible for immune reactions. It is striking that many

drugs withdrawn from the market (benoxaprofen, zomepirac, etc.) belong to this class (Benet *et al.* 1993). Adducts may be generated by two mechanisms (Figure 8.6):

(1) Nucleophilic displacement. In this mechanism the drug covalently binds to the protein and the glucuronic acid moiety is liberated.
(2) Imine mechanism: After acyl migration, the aldehyde group of the ring-open tautomer condenses with a lysine group on the protein to form an imine. At the completion of the reaction, the adduct contains the drug together with the glucuronic acid moiety.

Covalent binding of drugs to proteins may generate epitopes which may initiate autoimmune disease. One of the targets of covalent binding has been shown to be the UGT protein itself (Terrier *et al.* 1999). This covalent binding of the substrate leads to inhibition of UGT activity. Reactivity depends on the stability of the acyl glucuronide which varies considerably. For example, telmisartan acyl glucuronide appears to be much more stable than other acyl glucuronides (Ebner *et al.* 1999).
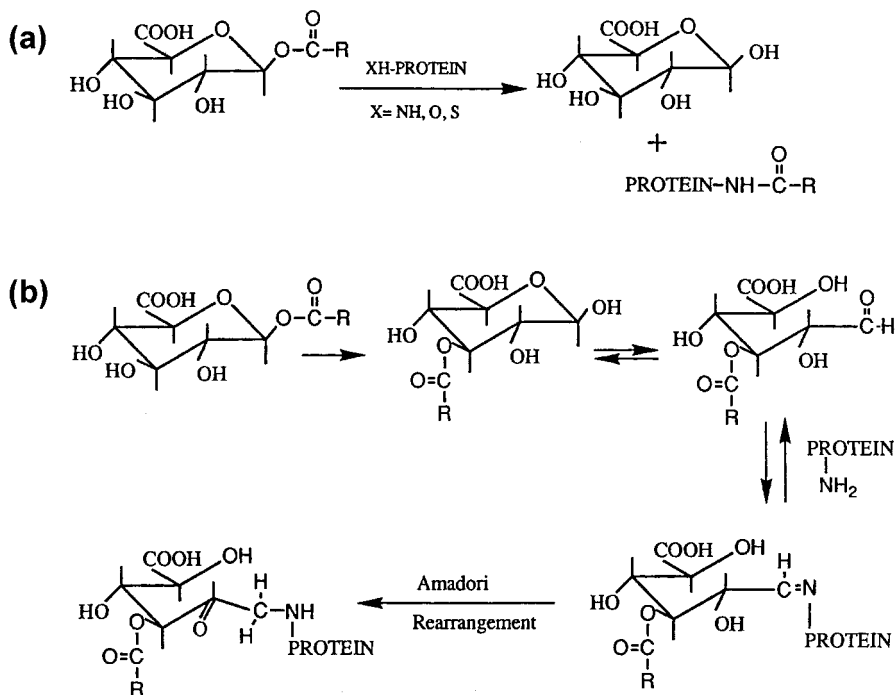

Figure 8.6 Postulated mechanisms for the irreversible binding of carboxylic acids to proteins via their glucuronides. (a) Nucleophilic displacement mechanism, leading to an acylated protein and liberation of D-glucuronic acid. (b) Imine mechanism, by which the glucuronic acid is part of the adduct (Reproduced from Benet *et al.* 1993. *Life Sciences,* **53**, 141–146. Published by Elsevier Science, Oxford).

Factors regulating formation and export of glucuronide from cells

MEMBRANE TOPOLOGY AND COFACTOR SUPPLY

After many years of discussion of the conformation (Berry and Hallinan 1974) and the compartmentation hypothesis (Zakim *et al.* 1988), the advent of UGT sequences provided compelling evidence for the localisation of UGTs behind a lipid barrier in the lumenal part of the endoplasmic reticulum (ER). As already discussed, UGT sequences are preceded by a signal peptide (Blobel and Dobberstein 1975) mediating the integration of the UGT polypeptide with the ER membrane (Figure 8.4). This signal peptide is cleaved and the protein *N*-glycosylated on the lumenal site of the ER. The polypeptide is retained in the ER by a transmembrane domain and stop transfer signal (Blobel 1980) at the *C*-terminus of the protein. However, experiments with truncated proteins revealed that the variable *N*-terminal domain (amino acids, 25–286) containing the aglycone binding site may also be involved in strong interactions with the lipid bilayer. In fact, deletion of the signal peptide and the stop transfer signal did not prevent membrane targeting and insertion of UGT1A6, suggesting the presence of internal topogenic elements able to translocate and retain the isoform in the ER membrane (Ouzzine *et al.* 1999).

Current information about the structure of the active site of UGTs has been discussed recently (Radominska-Pandya *et al.* 1999). It provided the basis for the proposed acid-base catalytic mechanism discussed in Figure 8.2(B) and the UDPGA binding illustrated in Figure 8.4. The proposed model is supported by three-dimensional structure data at 1.8 Å resolution of *E. coli* galactose-1-phosphate uridylyltransferase (Wedekind *et al.* 1995) which belongs to the glycosyltransferase supergene family including UGTs. More work is needed to elucidate the structure of the active site and in particular the hydrophobic pockets of the multiple aglycone-binding sites of UGTs, for example those encoded by the exons 1 of family 1 members.

Localisation of UGTs on the lumenal part of ER membranes raises the question as to how the cofactor UDP-glucuronic acid (UDPGA), synthesized in the cytosol, is transported through the membrane. This transport is the reason for the known 'latency' of UGT activity. The activity is low in native microsomes and can be activated by various procedures: for example, addition of detergents, of pore-forming agents such as alamethicin, repeated freezing and thawing, spontaneous activation by endogenous phospolipases generating detergents such as lysophosphatidylcholines, etc. (Dutton 1980). Transporters may exist both for transport of the cofactor UDPGA and for glucuronides produced intralumenally. These transporters have not been characterised. Recent findings suggest the participation of two asymmetric antiporters: UDPGA influx is coupled to UDP-*N*-acetylglucosamine efflux, and UDP-*N*-acetylglucosamine influx is coupled to UMP efflux (Bossuyt and Blanckaert 1995). The latter nucleotide is a product of UDP. Hence, these antiport pathways may link UDPGA influx with UGT activity. The reason for UDP-*N*-acetylglucosamine being a physiologic activator of UGT activity has not been elucidated. Recently, evidence was provided for an UDPGA/glucuronide antiport (Bánhegyi *et al.* 1996).

Interestingly, accumulating evidence suggests that the active enzyme consists of dimers of 2 UGT polypeptide chains. Homo- and heterodimers have been found by crosslinking and co-immunoprecipitation (Meech and Mackenzie 1997; Ikushiro *et al.*

1995). Oligomer formation between UGT monomers is also supported by radiation-inactivation analysis of liver microsomal UGTs (Peters *et al.* 1984; Vessey and Kempner 1989; Gschaidmeier and Bock 1994). Based on these findings, a dynamic topological model has been proposed in which dimerisation of UGT monomers at the *C* terminus may lead to the formation of a channel that allows access of UDPGA to the enzyme active site, possibly in conjunction with highly specialised transport proteins (Bossuyt and Blanckaert 1995; Hirschberg *et al.* 1998). The produced glucuronides may be expelled into the cytosol via the same channel used for the entry of UDPGA (Figure 8.7). The lipophilic aglycone may diffuse through the bilayer and bind to the active site. This may explain the correlation between UGT activities and substrate lipophilicity (Illing and Benford 1976; Bock and Lilienblum 1994).

Radiation-inactivation analysis revealed the existence of tetramers for the formation of the diglucuronide of BaP-3,6-diphenol (Gschaidmeier and Bock 1994). Cooperation of several functional UGTs may be necessary, in this case since the position of the monoglucuronide at the active site has to be changed for diglucuronide formation. Monoglucuronides may be expelled from one UGT unit, and neighbouring UGTs may then accept the monoglucuronide as substrate for diglucuronide formation.

The cofactor UDPGA is formed by UDP-glucose dehydrogenase from UDP-glucose. The latter is readily formed either from glycogen or from glucose. The level of UDPGA in liver cells was found to be 0.2–0.3 μmoles/g liver tissue, in the range of the K_m values for UGTs (Bock and White 1974). Therefore, any change in the cellular level of UDPGA may affect glucuronidation. For example, addition of ethanol inhibits UDP-glucose dehydrogenase and lowers the level of UDPGA (Moldeus *et al.* 1978). However, in the liver of fed rats high glucuronidation rates did not decrease the intracellular UDPGA concentration, suggesting efficient regeneration of the cofactor (Bock and White 1974).

GLUCURONIDE TRANSPORTERS (MRPs), BILIARY TRANSPORT OF GLUCURONIDES AND ENTEROHEPATIC CYCLING

Multispecific transporters for glucuronides (as well as for sulphate and glutathione conjugates) have been characterised (Jedlitschky *et al.* 1996). Interestingly, they are
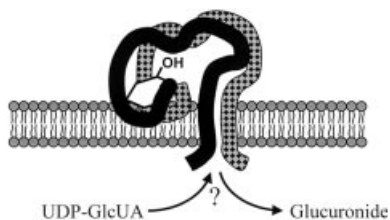

Figure 8.7 Hypothetical model of UGT topology. The catalytic unit is formed by dimerisation of UGT monomers. Lipophilic aglycones diffuse through the bilayer and bind to the active site formed by interactions of two monomers. UDP-GlcUA = UDPGA may gain access to the active site via a proteinaceous channel formed when the monomers dimerise. After conjugation, the glucuronide is expelled into the cytosol via the same channel used for entry of UDPGA (Reproduced from Radominska-Pandya *et al.* 1999 *Drug Metabolism Reviews*, **31**, 817–899. Published by Marcel Dekker, Inc., New York).

expressed in different parts of epithelial cells such as hepatocytes and thus determine the disposition of glucuronides. For example, MRP1 and MRP3 are present in the basolateral membrane whereas MRP2 is present exclusively in the apical membranes of cells (Keppler and König 1997; Kool *et al.* 1997). Their functions have been characterised for etoposide glucuronide, oestradiol-17β glucuronide (Jedlitschky *et al.* 1996), and for bilirubin mono- and diglucuronides (Jedlitschky *et al.* 1997). Absence of MRP2 is the cause of the Dubin–Johnson syndrome, which is characterised by heritable conjugated hyperbilirubinaemia. MRPs are also differentially expressed in other polarised epithelial cells; for example, in intestinal epithelia and Caco-2 cell monolayers (Walle *et al.* 199; Bock *et al.* 2000).

MRPs may determine glucuronide formation in cells since accumulation of glucuronides inhibits the reversible UGT reaction (Bock and White 1974). As discussed in the Introduction section, MRPs also determine the fate of glucuronides. For example, SN-38, a major metabolite of the camptothecin topoisomerase inhibitor irinotecan, has been shown to be rapidly converted to glucuronides which are excreted using MRP2 via the bile into the intestine (Sugiyama *et al.* 1998). After hydrolysis by bacterial β-glucuronidases, the aglycone may be re-absorbed and undergo extensive enterohepatic cycling. This leads to prolongation of the drug's biological half-life and accumulation of the compounds in the organism. However, in intestinal cells glucuronides are released by MRP2 into the intestinal lumen. Subsequent hydrolysis by β-glucuronidases leads to reabsorption of the aglycones and to futile cycles. These futile cycles may explain in part gastrointestinal toxicity (diarrhoea) of the potent chemotherapeutic irinotecan (Gupta *et al.* 1994).

Transcriptional regulation by xenobiotics

In general, differential transcription of different UGT genes largely determines the protein levels and activities of different UGT isoforms. Marked species- and tissue-specific differences exist in constitutive or basal expression, in developmental and xenobiotic-induced expression of UGT isoforms. Factors responsible for tissue-specific expression (such as HNF1 [hepatic nuclear factor 1], C/EBP[CCAAT enhancer binding protein]α and β etc.) are beginning to be unravelled (Hansen *et al.* 1998; Ishii *et al.* 2000; Lee *et al.* 1997). UGTs may also be regulated by interleukins in disease states, as shown for UGT2B17 (Lévesque *et al.* 1998). This chapter deals with induction of UGT isoforms by xenobiotics such as phenobarbital-type inducers, Ah receptor ligands and antioxidants/electrophiles.

PHENOBARBITAL-TYPE AND OTHER INDUCERS

Rat UGT1A1 appears to be induced by phenobarbital (Burchell *et al.* 1997); contradictory findings have been reported and need to be clarified (Ikushiro *et al.* 1995). It has been clearly shown that UGT2B1 is induced by phenobarbital (Mackenzie 1986). This isoform has been shown to conjugate chloramphenicol and morphine and is responsible for the enhanced UGT activities in liver microsomes of phenobarbital-treated rats (Bock *et al.* 1973). The human UGT1A1 has also been shown to be phenobarbital-inducible (Sutherland *et al.* 1993). Preliminary experiments provide

evidence that this isoform is probably controlled by the CAR/RXR [constitutively active receptor/retinoid X receptor] element first identified in the promotor region of phenobarbital-inducible CYP2B genes (Kawamoto *et al*. 1999).

Clofibrate and dexamethasone are known to induce UGTs (Emi *et al*. 1995; Ikushiro *et al*. 1995). However, the induction mechanisms have not been elucidated.

Ah RECEPTOR AGONISTS

Experimental animals

Early genetic studies with 3-methylcholanthrene-responsive and -non-responsive mouse strains suggested induction of liver UGTs via the Ah receptor (Owens 1977). These findings were substantiated by the absence of UGT1A6 expression in Ah receptor knockout mice (Fernandez-Salguero *et al*. 1995). Extensive studies in rat liver showed that basal expression of UGT1A6 in rat liver is low, but it is > tenfold induced by 3-methylcholanthrene treatment (Iyanagi *et al*. 1986; Emi *et al*. 1996). Marked induction by TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) was restricted to the liver whereas high constitutive expression was preponderant in extrahepatic tissues (Münzel *et al*. 1994). Hence, responsiveness to Ah receptor-type inducing agents appears to correlate inversely with the level of constitutive expression. UGT1A7 was also shown to be inducible by 3-methylcholanthrene (Emi *et al*. 1995; Metz and Ritter 1998).

Rabbit UGT1A6 was found to be constitutively expressed in the liver and not further induced by TCDD (Lamb *et al*. 1994), indicating species-dependent differences in UGT1A6 expression. Similarly, microsomal UGT activities towards 1-naphthol and 4-methylumbelliferone, major substrates of UGT1A6, were high in dog liver and only moderately induced by β-naphthoflavone; but induction of UGT activities was observed in dog intestine (Richter von *et al*. 2000). Lack of induction by Ah receptor agonists is probably due to high constitutive expression of liver UGTs in rabbits and dogs.

Humans

UGT1A6 is constitutively expressed in human livers. Induction by TCDD studied in human hepatocyte cultures appears to be moderate with high interindividual variability (Münzel *et al*. 1996). Induction of UGT1A6 by PAH-type inducers is supported by *in vivo* findings that paracetamol glucuronidation (paracetamol being a substrate of UGT1A6, Bock *et al*. 1993) is increased in cigarette smokers (Mucklow *et al*. 1980; Bock *et al*. 1987, 1994). Glucuronidation of propranolol is also increased in smokers (Walle *et al*. 1987). In the Caco-2 cell model, UGT1A6 induction by TCDD is clearly detectable and appears to be Ah receptor-dependent; i.e. the Ah receptor/Arnt (Aryl hydrocarbon nuclear transferase) complex binds to a consensus GTGCG DNA core sequence, the xenobiotic response element (XRE, Münzel *et al*. 1998). Interestingly, a second UGT1 family member, hUGT1A9, also appears to be inducible by TCDD (Münzel *et al*. 1999), similar to at least two rat UGT isoforms (rUGT1A6 and rUGT1A7) which are inducible by polycyclic aromatic hydrocarbons.

ANTIOXIDANTS/ELECTROPHILES

UGTs, for example rat UGT1A6, are known to be inducible by antioxidant-type or phase II enzyme inducers (Buetler *et al.* 1995). Recently, evidence has been obtained in the Caco-2 cell model that three human UGTs (UGT1A6, UGT1A9 and UGT2B7) are induced by the prototype antioxidant t-butylhydroquinone (TBHQ, Münzel *et al.* 1999). Interestingly, antioxidant-inducible proteins also include the apical conjugate export pump MRP2 (multidrug resistance protein 2, Bock *et al.* 2000). Since para-cetamol is a substrate of human UGT1A6 (Bock *et al.* 1993), it is conceivable that increased paracetamol glucuronide excretion found in subjects on a Brussels sprouts and cabbage diet (Pantuck *et al.* 1984) may be due to antioxidant-type induction of UGT1A6 in the intestine and liver.

Antioxidant-type induction may be considered as an adaptive response to oxidative or electrophilic stress which is triggered by a sublethal dose of a variety of antioxidants/prooxidants. Increased glucuronidation has been observed after treatment with TBHQ (a metabolite of the widely used antioxidant BHA [2(3)-tert-butyl-4-hydroxyanisole] and with ethoxyquine (Bock *et al.* 1980). Antioxidant-type inducers also include the phase II enzyme inducer oltipraz (4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione, Kessler and Ritter 1997; Grove *et al.* 1997), a variety of plant flavonoids such as quercetin and polyphenols of green tea (Sohn *et al.* 1994).

For glutathione *S*-transferase Ya (Rushmore and Pickett 1990) and NAD(P)H-quinone oxidoreductase (NQO1, Jaiswal 1994; Venugopal and Jaiswal 1996) it has been shown that antioxidant-type inducers activate a novel redox-sensitive signal transduction pathway. This pathway includes transcription factors such as the zipper proteins Nrf1, Nrf2 and c-Jun, which bind to antioxidant response elements (AREs) or electrophile response elements (EpREs) in the regulatory region of the induced enzymes. Mutational analysis identified TGACNNNGC as the core of the ARE sequences. Activation of this novel antioxidant-responsive pathway is of interest in connection with the efforts of chemoprevention of cancer (Wattenberg 1983; Talalay *et al.* 1995). Prochaska and Talalay (1988) termed the induction of phase II enzymes by antioxidants 'monofunctional induction' in contrast to Ah receptor agonists which induce both phase I (e.g. CYP1A1) and phase II enzymes, the latter termed 'bifunctional induction'. In the case of NQO1, evidence has been presented that its induction by β-naphthoflavone (BNF) is mediated by the 'ARE mechanism' (Venugopal and Jaiswal 1999). As an agonist of the Ah receptor, BNF leads to the induction of CYP1A1. Efficient metabolism of BNF by CYP1A1 may generate antioxidant/electrophile stress which triggers NQO1 induction. Findings with Nrf2- deficient mice suggest that Nrf2 regulates the induction of phase II enzymes, including some UGTs such as UGT1A6 (Itoh *et al.* 1997; Masayuki Yamamoto, Institute for Basic Medical Sciences, University of Tsukuba, Japan, personal communication). It is therefore conceivable that BNF and PAH induction of some UGT isoforms is mediated via an ARE-like mechanism (Figure 8.8). This is supported by recent transient transfection experiments using UGT1A6 reporter gene plasmids containing 3kb of 5'-flanking region (Münzel *et al.* 1996) in which ARE-like domains were found. Treatment of these transfectants with t-butylhydroquinone showed consistent 2-fold induction (Schmohl S, Münzel PA, Bock-Hennig BS and Bock KW, unpublished results). Furthermore, evidence for a linkage between
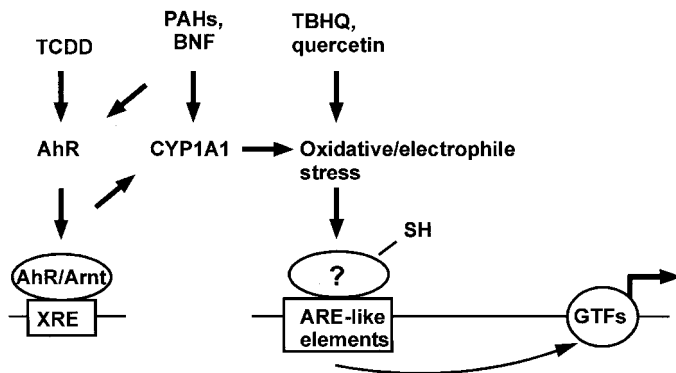
Figure 8.8 Hypothetical mechanism for transcriptional regulation of human UGT1A6 by xenobiotics. XRE, xenobiotic responsive element; ARE antioxidant responsive element; TBHQ, t-butylhydroquinone; PAHs, polycyclic aromatic hydrocarbons; BNF, β-naphthoflavone; GTFs, general transcription factors.

XRE- and ARE-mediated UGT induction has been obtained (Bock *et al.* 1998). Experiments were conducted with rat 5L hepatoma cells in which induction of UGT activity was detected after treatment with the selective Ah receptor agonist TCDD or the prototypical antioxidant t-butylhydroquinone. In the 5L hepatoma cell mutant lacking the Ah receptor (Weiβ *et al.* 1996), UGT induction by both types of inducers were abolished, whereas after stable integration of the Ah receptor both types of induction reappeared.

Alteration of UGTs in disease

It has been known for quite some time that glucuronidation of some drugs (e.g. oxazepam) is little affected in liver cirrhosis whereas the glucuronidation of others (e.g. zidovudine) is decreased, similar to cytochrome P450-dependent drug metabolism (Hoyumpa and Schenker 1991; Kroemer and Klotz 1992; Furlan *et al.* 1999). Only a few studies on the effect of disease states on particular UGT isoforms have been carried out. Since cytokines have been shown to affect gene expression of UGTs, it is likely that inflammation and infections affect glucuronidation (Lévesque *et al.* 1998).

UGT GENE EXPRESSION IN CARCINOGENESIS AND ITS ROLE IN TOXIN RESISTANCE

Early studies showed increased UGT activities in rat liver foci and nodules (Bock *et al.* 1982; Yin *et al.* 1982) associated with a toxin-resistance phenotype consisting of decreased phase I and increased phase II activities. Northern Blot analysis using a selective UGT1A6 probe indicated that UGT1A6 expression was persistently enhanced (Bock *et al.* 1990b). In mouse liver, both UGT-positive and UGT-negative

foci were detected (Bock *et al.* 1989). In humans mostly down-regulation of UGTs has been detected in liver adenomas and carcinomas but not in focal nodular hyperplasia (Strassburg *et al.* 1997b). UGTs were differentially affected. For example, the UGT1A6 isoform was not affected whereas a recent study showed increased UGT2B4 in liver tumours (Kondoh *et al.* 1999). The mechanisms responsible for altered enzyme expression in hepatocarcinogenesis are still unknown.

AUTOANTIBODIES IN VIRAL DISEASE

Patients with chronic hepatitis D often show serum liver–kidney microsomal antibodies type 3 (LKM-3). Surprisingly, UGTs were found to be candidate antigens. Anti-UGT1 antibodies were detected in all LKM-3-positive sera from patients with hepatitis D. Sera from patients with hepatitis B did not react with UGT protein (Philipp *et al.* 1994).

UGTs in experimental animals

This section highlights differences in experimental animals compared to humans. For information on UGT sequences in experimental animals the reader should consult the UGT homepage (see the Introduction).

RAT

Rat UGTs have been extensively studied. The first UGT sequences were characterised from this species. Substrate specificity of rat liver UGTs has been listed in a recent review (Burchell *et al.* 1997). Basal expression of rUGT1A6 is low in rat liver but is increased > 10-fold by Ah receptor agonists (Iyanagi *et al.* 1986). However, there is high constitutive expression in extrahepatic tissues (Münzel *et al.* 1994). Differences in tissue- and species-specific expression of UGT1A6 have already been discussed. Major UGT isoforms in rat liver appear to be the bilirubin-conjugating UGT1A1 and UGT2B1 (Emi *et al.* 1995). UGT2B1 has a broad substrate specificity including morphine, which is conjugated to the 3-glucuronide but not to the 6-glucuronide (Coffman *et al.* 1998), chloramphenicol and the pulmonary carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol = NNAL (Ren *et al.* 1999).

MOUSE

Mouse UGTs of family 1 (UGT1A1 and UGT1A6) and of family 2 (UGT2B5) have been cloned. In Ah receptor knock-out mice both basal expression of UGT1A6 and induction by Ah receptor agonists were absent (Fernandez-Salguero *et al.* 1995).

CAT

The domestic cat is exquisitely sensitive to adverse effects of many drugs such as paracetamol (acetaminophen) and phenolic toxins that are normally glucuronidated before elimination (Gregus *et al.* 1983; Savides *et al.* 1984). Deficient glucuronidation

of planar phenolic compounds has also been identified in other carnivora such as the lion, African civet, forest genet and spotted hyaena (Capel *et al.* 1972; Caldwell *et al.* 1975). To investigate the evolutionary basis for deficient glucuronidation, UGTs of family 1 have been cloned. Whereas cat UGT1A1 was found to be expressed in the liver as a major isoform, a number of stop codons and deletions have been found in the cat UGT1A6 structural gene which is unlikely to produce functional protein (Court and Greenblatt 2000). A similar defect was found in the margay (*Leopardus wiedii*). This gene defect may have arisen via disruption of the UGT1A6 gene in a common ancestor of these species. From an evolutionary standpoint, these species differences may reflect the highly carnivorous nature of the feline diet, and resultant low degree of selection pressure from phytoalexins.

DOG

No dog UGT sequence has been so far published (July 2000). However, one pheno-barbital-inducible morphine UGT has been purified (Oguri *et al.* 1996). Dog liver UGT activities towards morphine, 4-methylumbelliferone and 1-naphthol were found to be 10-fold higher than in human liver, suggesting extensive first-pass metabolism of phenolic compounds in the dog (Richter von *et al.* 2000). Metabolism of retigabin was dominated by *N*-glucuronidation in the dog but not in rats (Hiller *et al.* 1999).

GUINEA PIG

Two guinea pig UGT isoforms (UGT2B21 and UGT2B22) have recently been cloned; they are involved in the conjugation of morphine to both morphine 3- and 6-glucuronides (Miyoshi *et al.* 1998). Interestingly, while UGT2B21 formed the 3-glucuronide but not the 6-glucuronide, UGT2B22 did not have catalytic activity. However, coexpression of both UGT2B21 and 2B22 led to the formation of both morphine 3- and 6-glucuronides, suggesting that hetero-oligomers of the two UGTs were necessary to form morphine 6-glucuronide.

MONKEY

UGT2B9 has been cloned and characterised; it shares a 89% similarity in amino acid sequence to human UGT2B7. UGT2B9 has been shown to conjugate endogenous substrates such as 3-hydroxyandrogens and many drugs such as profene-NSAIDs, fibrates, straight chain fatty acids at the carboxylic acid moiety, and morphine to the morphine 3- and 6-glucuronides (Green *et al.* 1997; Beaulieu *et al.* 1998). Recently, another isoform (UGT2B23) has been characterised which is active on androgens and oestrogens (Barbier *et al.* 1999).

PIG

A liver UGT has been purified and its phospholipid-dependence was studied (Magda-lou *et al.* 1982).

RABBIT

UGTs of families 1 and 2 have been cloned (Tukey *et al.* 1993; Lamb *et al.* 1994). In contrast to rodents, the rabbit UGT1A4 and UGT1A7 are able to form quarternary *N*-glucuronides of imipramine (Bruck *et al.* 1997). Imipramine quarternary *N*-glucuronidation was found to occur at twice the rate of humans (Coughtrie and Sharp 1991).

Conclusions

Molecular biology technology provides a solid foundation to establish individual isoforms of a supergene family of UDP-glucuronosyltransferases expressed in most tissues of vertebrates such as mammals and fish. It also enables the inclusion of glucuronosyltransferases in a much larger supergene family of glycosyltransferases expressed in invertebrates, yeast, bacteria and plants. Interestingly, it was found that all human UGT family 1 members are derived by exon-sharing from one large gene locus located at chromosome 2. Individual isoforms are expressed in a remarkably tissue-specific manner. More work is needed to identify expressed UGTs in particular cell types, for example in the brain or steroidogenic tissues. Furthermore, the evolutionary relationship between glucuronosyl- and related glycosyltransferases has to be substantiated.

Recombinant technology enabled the characterisation of the function of individual UGT isoforms. Most of them show a broad specificity for a variety of xenobiotics (drugs, dietary plant constituents and toxicants). In addition, endobiotics are increasingly recognised as substrates for individual UGT isoforms. UGTs not only inactivate these xeno- and endobiotics but also play a critical role in generating bioactive or even toxic compounds.

Recent studies provide evidence for a dynamic topological model in which UGTs are acting as oligomers in membranes. The model may have implications for the transport of lipophilic aglycones and the cofactor UDP-glucuronic acid to the active site and the subsequent release of glucuronides. More work is needed to substantiate this model, to characterise glucuronide transporters present in the apical and basolateral plasma membrane of epithelial cells, and to investigate the disposition of glucuronides in the organism.

The factors responsible for transcriptional regulation of UGTs are beginning to be characterised, including xenobiotic-activated transcription factors such as the aryl hydrocarbon receptor as well as general enhancers such as HNF1, C/EBPα and β.

Further work along these lines will improve our knowledge of similarities and differences between glucuronidation in experimental animals and humans. Further studies of this major metabolic reaction may facilitate the exrapolation of endo- and xenobiotic biotransformation from experimental models to individual human beings in health and disease.

(1) Studies on the human UGT1 locus (Fig. 8.3) have been completed (Gong QH *et al.*, Pharmacogenetics 11, 357–368, 2001).
(2) A novel hUGT2A2 has been described which is expressed in the gastrointestinal tract (Tukey RH and Strassburg CP, Mol. Pharmacol. 59, 405–414, 2001).

(3) Evidence for transporters of UDP-glucuronic acid to the active site of UGTs in the lumen of endoplasmic reticulum membranes (Fig. 8.7) has been obtained (Kawakita M *et al.*, Abstracts, 10. Int. Workshop on Glucuronidation and the UDP-glucuronosyltransferases, Hemeji, Institute of Technology, Hyogo, Japan, 2001).

(4) Evidence for contribution of Nrf-2 to antioxidant-type induction of mouse UGT1A6 (Fig. 8.8) has been reported by Chan K and Kan YU (Proc. Natl. Acad. Sci. 96, 1231–1236, 1999) and by Enomoto A *et al.* (Tox. Sci. 59, 169–177, 2001).

Acknowledgments

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