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REVIEW

Human Sulfotransferases and Their Role in Chemical Metabolism

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Sulfonation is an important reaction in the metabolism of numerous xenobiotics, drugs, and endogenous compounds. A supergene family of enzymes called sulfotransferases (SULTs) catalyze this reaction. In most cases, the addition of a sulfonate moiety to a compound increases its water solubility and decreases its biological activity. However, many of these enzymes are also capable of bioactivating procarcinogens to reactive electrophiles. In humans three SULT families, SULT1, SULT2, and SULT4, have been identified that contain at least thirteen distinct members. SULTs have a wide tissue distribution and act as a major detoxification enzyme system in adult and the developing human fetus. Nine crystal structures of human cytosolic SULTs have now been determined, and together with site-directed mutagenesis experiments and molecular modeling, we are now beginning to understand the factors that govern distinct but overlapping substrate specificities. These studies have also provided insight into the enzyme kinetics and inhibition characteristics of these enzymes. The regulation of human SULTs remains as one of the least explored areas of research in the field, though there have been some recent advances on the molecular transcription mechanism controlling the individual SULT promoters. Interindividual variation in sulfonation capacity may be important in determining an individual's response to xenobiotics, and recent studies have begun to suggest roles for SULT polymorphism in disease susceptibility. This review aims to provide a summary of our present understanding of the function of human cytosolic sulfotransferases.

Key Words: **human sulfotransferases; SULT1A1 regulation; SULT crystal structures; bioactivation.**

INTRODUCTION

Sulfonate conjugation was first reported by Baumann in 1876 (Baumann, 1876) and has since been shown to be an important pathway in the biotransformation of numerous xeno- and endobiotics such as drugs, chemical carcinogens, hormones, bile acids, neurotransmitters, peptides, and lipids. The universal sulfonate donor for these reactions is 3′-phosphoadenosine 5′-phosphosulfate (PAPS), and the transfer of sulfonate (SO_3^-) to a hydroxyl or amino- group is catalysed by a super gene family of enzymes called sulfotransferases (SULTs). In the case of most xenobiotics and small endogenous substrates, sulfonation has generally been considered a detoxification pathway leading to more water-soluble products and thereby aiding their excretion via the kidneys or bile. For a drug like acetaminophen or a neurotransmitter such as dopamine, this is certainly the case; however, for xenobiotics such as N-hydroxy arylamines, N-hydroxy heterocyclic amines, and hydroxymethyl polycyclic aromatic hydrocarbons, sulfonation is a metabolic activation process leading to highly reactive electrophiles that are both mutagenic and carcinogenic (Falany, 1997; Weinshilboum *et al.*, 1997). Further, for the hair growth stimulant, minoxidil, and the neuroendocrine peptide cholecystokinin (CCK), the sulfonated forms of these molecules elicit their biological effects (Falany, 1997; Vargas *et al.*, 1994).

Two broad classes of SULTs have been identified: (1) membrane-bound SULTs that are located in the Golgi apparatus of the cell and are responsible for the sulfonation of peptides (e.g., CCK), proteins, lipids, and glycosaminoglycans, affecting both their structural and functional characteristics (Falany, 1997; Negishi *et al.*, 2001) and (2) cytosolic SULTs that are responsible for the metabolism of xenobiotics and small endogenous substrates such as steroids, bile acids, and neurotransmitters. The focus of this review is on the human cytosolic SULTs, with particular emphasis on those isoforms that have been shown to metabolize a broad range of drug, xenobiotic, and endobiotic substrates. Their particular role in the metabolic activation of xenobiotics to mutagens and carcinogens will be addressed.

The sulfonation of xenobiotics and small endogenous substrates such as steroids and neurotransmitters is widely distributed in nature and occurs in organisms ranging from microbes to man (Blanchard *et al.*, 2004; Nagata and Yamazoe,

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2000; Rikke and Roy, 1996). The process of sulfonation involves the transfer of a sulfonyl (SO_3^-) group, generally to a hydroxyl on an acceptor molecule, which is catalysed by SULTs. On many occasions, this reaction has been incorrectly termed sulfation in the literature, because it creates a sulfated product. Further, the fact that sulfate is the fourth most abundant anion in human plasma (Markovich, 2001) and is used in the synthesis of the universal sulfonate donor, PAPS, has helped many investigators to incorrectly call the process sulfation. The importance of PAPS in the regulation of sulfonation has been expertly reviewed (Klaassen and Boles, 1997; Schwartz, 2005). In general, the overall enzymatic mechanism requires the sulfonate acceptor (ROH) and donor (PAPS) to bind to a sulfotransferase, which results in the release of the sulfonated product and 3′-phosphoadenosine-5′-phosphate (PAP). SULTs are capable of sulfonating a wide range of substrates including phenols (2-naphthol), primary (ethanol) and secondary alcohols (2-butanol), N-hydroxy arylamines (2-acetylaminofluorene; 2-AAF), N-hydroxy heterocyclic amines (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; PhIP), benzylic alcohols of polycyclic aromatic hydrocarbons (1-hydroxymethylpyrene; 1-HMP), phenolic (17β-estradiol; E2) and alicyclic hydroxysteroids (dehydroepiandrosterone; DHEA), and iodothyronines. SULTs are also capable of sulfonating amino groups of arylamines such as 2-naphthylamine (Jakoby *et al.*, 1980). The broad substrate specificity of SULTs is due to the fact that multiple forms of these enzymes exist and that the binding sites of some isoforms is plastic, allowing the enzyme to adopt varying architectures so that it can interact with small aromatics, L-shaped aromatics, and fused ring compounds (Gamage *et al.*, 2003).

SULFOTRANSFERASES

Rat SULT2A2 was the first SULT cloned and was originally identified as a senescence marker protein (Chatterjee *et al.*, 1987). Since then at least 47 mammalian SULT isoforms, one insect isoform, and eight plant enzymes, which represent nine separate SULT families and 14 subfamilies, have been cloned and characterized (Blanchard *et al.*, 2004). The latter paper provides a significant breakthrough in nomenclature of SULTs, which until then was even confusing to those working full-time in the field. It broadly follows the gene family nomenclature systems developed for other drug-metabolizing enzyme families such as cytochromes P450, UDP-glucuronosyltransferase, glutathione transferases, and N-acetyltransferase. The nomenclature system is based on family members sharing at least 45% amino acid sequence identity and subfamily members being at least 60% identical. To date, five distinct gene families of SULTs have been identified in mammals: SULT1, SULT2, SULT3, SULT4, and SULT5 (Blanchard *et al.*, 2004). The SULT3 family has only been found in mouse and rabbit, and has been shown to primarily sulfonate amino groups (Yoshinari *et al.*, 1998a). Sult5a1 has only been isolated from mice, and limited information is available on this family (Nagata and Yamazoe, 2000).

Table 1 summarizes the human forms of SULTs that have been characterized to date. These can be divided into three families and collectively account for thirteen distinct members: SULT1—A1, A2, A3, A4, B1, C2, C4, E1; SULT2—A1 and B1 (SULT2B1_v1 and SULT2B1_v2); SULT4A1 (SULT4A1_v1 and SULT4A1_v2) (Blanchard *et al.*, 2004; Hildebrandt *et al.*, 2004; Mammalian Gene Collection (MGC) Program Team, 2002). At least two *SULT* genes (human SULT2B1 and SULT4A1) encode two SULT isoforms: SULT2B1—SULT2B1_v1 and SULT2B1_v2 differ in their N-terminal amino acid sequences as a result of either alternate transcription initiation or alternate splicing (Blanchard *et al.*, 2004; Fuda *et al.*, 2002); SULT4A1—isoforms v1 and v2 differ in their C-terminal amino acid sequences (Mammalian Gene Collection (MGC) Program Team, 2002). Figure 1 shows a dendogram of these human SULTs. While SULT1D1 has been isolated from the dog (Tsoi *et al.*, 2001), mouse (Sakakibara *et al.*, 1998a), and rat (Herrmann and Stoffel, unpublished), no equivalent human form of this enzyme has been identified.

SULT1 Family

SULT1A1. Members of the SULT1A1 subfamily have been identified in the largest range of species including the rat, mouse, cow, dog, rabbit (refer Blanchard *et al.*, 2004 for references), monkey (Ogura *et al.* unpublished), pig (Lin *et al.*, 2004), and platypus (Bolton-Grob and McManus, unpublished). A solitary SULT1A1 isoform has been characterized in all the above-mentioned species, but in humans, four SULT1A subfamily members have been identified (SULT1-A1, A2, A3, and A4; Blanchard *et al.*, 2004; Hildebrandt *et al.*, 2004). Their genes are all clustered on the short arm of chromosome 16 and are thought to have arisen following gene duplication or gene duplication plus recombination events (Aksoy *et al.*, 1994; Dooley, 1998a; Rikke and Roy, 1996). The gene sequences of *SULT1A1* and *SULT1A2* are 93% similar, whereas *SULT1A3* shares approximately 60% identity with the other two genes. The differences are most apparent in the 5′ promoter and intron sequences, as the coding exons of all three genes are >90% identical. Based on the available data, the human *SULT1A1* is most probably the ortholog of the equivalent animal isoforms (Blanchard *et al.*, 2004).

Wilborn *et al.* (1993) were the first to isolate a SULT1A1 cDNA from a human liver library. The full-length cDNA was shown to encode a protein of 295 amino acids, which had high activity toward the model substrate *p*-nitrophenol (*p*NP). The same protein also had activity toward minoxidil as a substrate (Meisheri *et al.*, 1993). The general fidelity of the Wilborn *et al.* sequence has now been confirmed by a number of groups (Table 1), and allelic variants of SULT1A1 have been shown to exist in the human population. SULT1A1 is by far the dominant

TABLE 1
cDNAs and Genes Comprising the Human SULT Superfamily

SULT	Chromosomal location		Name given by author	Accession No.	Reference
SULT1A1	16p11.2–12.1	cDNA	P-PST-1	L19999	Wilborn *et al.*, 1993
			HAST1	L10819	Zhu *et al.*, 1993b
			ST1A3	X78283	Ozawa *et al.*, 1995
			P-PST	X84654	Jones *et al.*, 1995
			H-PST	U26309	Hwang *et al.*, 1995
			HAST2	L19955	Zhu *et al.*, 1996
				U09031	
			SULT1A1	NM_001055	Her *et al.*, 1996
			SULT1A1	AJ007418	Raftogianis *et al.*, 1996
		Gene	*STP1*	U71086	Dooley and Huang 1996
			STP	U54701	Bernier *et al.*, 1996
			TS- PST1 (STP1)	U52852	Raftogianis *et al.*, 1996
SULT1A2	16p11.2–12.1	cDNA	ST1A2	X78282	Ozawa *et al.*, 1995
			HAST4v	U28169	Zhu *et al.*, 1996
			HAST4	U28170	Zhu *et al.*, 1996
		Gene	*STP2*	U76619	Dooley and Huang, 1996
				U34804	Her *et al.*, 1996
				U33886	Gaedigk *et al.*, 1997
SULT1A3	16p11.2	cDNA	HAST3	L19956	Zhu *et al.*, 1993a
			HAST3-intron1	L19957	
			TL- PST	U08032	Wood *et al.*, 1994
			hEST	L25275	Bernier *et al.*, 1994a
			m-PST	X84653	Jones *et al.*, 1995
			hm-PST	-	Ganguly *et al.*, 1995
		Gene	*STM,*	U20499	Aksoy *et al.*, 1994,1995
			HAST	U37686	Dooley *et al.*, 1994
				L34160	Bernier *et al.*, 1994b
SULT1A4	16p12.1	cDNA	SULT1A4	MGC5178	Hildebrandt *et al.*, 2004
		Gene	*SULT1A4*		Hildebrandt *et al.*, 2004
SULT1B1	4q11–13	cDNA	ST1B2	D89479	Fujita *et al.*, 1997
				U95726	Wang *et al.*, 1998
		Gene	*SULT1B2*	AF184894	Wang *et al.*, 1998
SULT1C2	2q11.2	cDNA	SULT1C1	U66036	Her *et al.*, 1997
			ST1C2	AB008164	Yoshinari *et al.*, 1998b
			HAST5	AF026303	Hehonah *et al.*, 1999
		Gene	*SULT1C1*	AF186257	Freimuth *et al.*, 2000
SULT1C4	2q11.2	cDNA	hSULT1C	AF055584	Sakakibara *et al.*, 1998b
		Gene	*SULT1C2*	AF186263	Freimuth *et al.*, 2000
SULT1E1	4q13.1	cDNA	hEST (STE)	U08098	Aksoy *et al.*, 1994
			hEST-1	S77383	Falany *et al.*, 1995
			SULT1E1	Y11195	Rubin *et al.*, 1999
		Gene	*STE*	U20514-21	Her *et al.*, 1995
SULT2A1	19q13.3	cDNA	DHEA-ST	U08024	Otterness *et al.*, 1992
				U08025	
			hSTa	S43859	Kong *et al.*, 1992
				L02337	
			DHEA-ST8	X70222	Comer *et al.*, 1993
		Gene	*STD*	U13056-61	Otterness *et al.*, 1995
				L36191-196	Luu –The *et al.*, 1995
SULT2B1_v1	19q13.3	cDNA	hSULT2B1a	U92314	Her *et al.*, 1998
SULT2B1_v2			hSULT2B1b	U92315	Her *et al.*, 1998
		Gene	*SULT21B1*	U92316-22	Her *et al.*, 1998
SULT4A1_v1	22q13.1–13.2	cDNA	hBR-STL	AF188698	Falany *et al.*, 2000
SULT4a1_v2			hSULT4A1	AF251263	Walther *et al.*, 1999
			SULTX3	AF115311	Sakakibara *et al.*, 2002
				AAH28171	MGC Program Team
		Gene	*SULT4A1*	Z97055	Dunham *et al.*, 1999

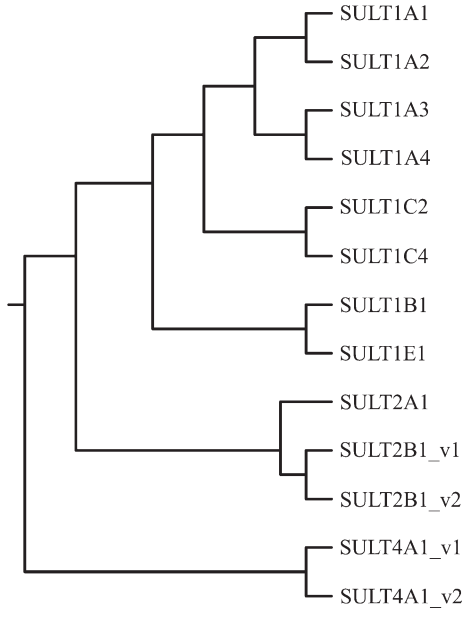

FIG. 1. The phylogenetic tree showing the relationship between human cytosolic sulfotransferases. The tree was generated using Clustalw (http://align.genome.jp/). The Pub Med Accession numbers are as follows: SULT1A1 (U26309), SULT1A2 (U28169), SULT1A3 (L19956), SULT1A4 (BK004132), SULT1B1 (U95726), SULT1C2 (U66036), SULT1C4 (AF055584), SULT1E1 (U08098), SULT2A1 (U08024), SULT2B1_v1 (U92314), SULT2B1_v2 (U92315), SULT4A1_v1 (AF188698) and SULT4A1_v2 (AAH28171).

SULT1A protein expressed in human liver and has an estimated molecular weight on SDS–PAGE of 32 kDa. This form was initially labelled by Weinshilboum's group as the TS or P (thermostable or phenol, TS-PST) form of sulfotransferase, and SULT1A3 as the TL or M (thermolabile or monoamine, TL-PST) sulfonating form, and collectively they were shown to be responsible for the metabolism of most phenolic compounds (Hempel *et al.*, 2005; Reiter *et al.*, 1983). In relation to their thermostabilities, SULT1A3 shows no activity toward dopamine as a substrate after treatment at 45°C for 15 min, but SULT1A1 retains approximately 90% of its activity toward *p*NP following similar treatment. It is also possible to differentiate between SULT1A1 and SULT1A3 activity in tissue fractions using the inhibitor 2,6-dichloro-4-nitrophenol (DCNP), as the sulfonation of *p*NP by the former is highly sensitive to this compound (Veronese *et al.*, 1994). Unlike SULT1A1 or SULT1A3, SULT1A2 exhibits no activity toward dopamine as a substrate and possesses a K_m for *p*NP sulfonation (~70 μM) between that of SULT1A1 and SULT1A3 (Zhu *et al.*, 1996).

SULT1A2. As indicated above, no equivalent form of SULT1A2 has been identified in species other than humans. Ozawa *et al.* (1995) were the first to clone a SULT1A2 cDNA from a human liver library, and it was originally given the name ST1A2. These authors showed that COS [cells derived from the kidney of an adult male African green monkey (*Cercopithecus aethiops*)] cell-expressed SULT1A2 sulfonated *p*NP, minoxidil, and β-naphthol, but at a lower rate than SULT1A1 (ST1A3). In another study, Zhu *et al.* (1996) isolated from a human liver library two forms of this cDNA (HAST4 and HAST4v) that differ by two amino acids (Thr7 to Ile and Thr235 to Asn). In their coding domains HAST4 and HAST4v were 97% and 94% identical to SULT1A1 and SULT1A3, respectively. On expression of these cDNAs in COS cells, the encoded proteins exhibited markedly different K_m values for the sulfonation of *p*NP: K_m values for HAST4 and HAST4v being 74 and 8 μM, respectively. However, unlike SULT1A1 or SULT1A3, SULT1A2 exhibits no activity toward dopamine as a substrate, even though it shared >93% amino acid identity with these proteins. At least 13 different allelic variants of human SULT1A2 have been identified that encode four different amino acid changes resulting in six different SULT1A2 allozymes (Raftogianis *et al.*, 1999).

SULT1A3. To date, a *SULT1A3* gene has only been identified in humans, and it appears that through evolutionary pressures we have acquired a *SULT1A3* gene whose expressed protein fulfils a specific role in sulfonating catecholamines such as dopamine (Coughtrie, 1998; Dooley, 1998b; Hempel *et al.*, 2005). SULT1A3 was initially isolated from a human brain cDNA library and was called HAST3 (Zhu *et al.*, 1993). The cDNA isolated was shown to encode a 34 kDa protein that was 93% similar to SULT1A1 and under the new nomenclature was termed SULT1A3 (Blanchard *et al.*, 2004, Zhu *et al.*, 1993). Based on its substrate preference for dopamine, thermal stability, and sensitivity to DCNP inhibition, it was shown to be the thermolabile or M-form of sulfotransferase initially identified by Reiter *et al.* in 1983 (Veronese *et al.*, 1994). The fidelity of the original sequence was subsequently confirmed (Table 1). An identical cDNA was also isolated by Bernier *et al.* (1994a), who originally reported it as an estrogen sulfotransferase (hEST). A recent study by Hildebrandt *et al.* (2004) showed that two copies of SULT1A3 exist in the human genome (SULT1A3 and SULT1A4), and they appear to be transcriptionally active. At least four nonsynonymous single nucleotide polymorphisms (cSNPs) were reported by the above authors for these genes, which show different enzyme activity.

SULT1B1. The first member of this SULT subfamily was cloned from a rat liver cDNA library and was described as the 3,4-dihydroxyphenylalanine (dopa)/tyrosine sulfotransferase, having activity toward tyrosine and dopa (Sakakibara *et al.*, 1995). The same authors also showed that SULT1B1 had activity toward various thyroid hormone substrates, *p*NP and dopamine. Since then, SULT1B1 enzymes have been isolated from mouse, dog (Blanchard *et al.*, 2004), and brush-tailed possum (Bolton-Grob and McManus, unpublished). The human form of SULT1B1 was isolated and characterized by Fujita *et al.* (1997) and shown to be the major thyroid hormone sulfotransferase, having slightly higher affinity for the triiodothyronine than SULT1A1.

TABLE 2
Endogenous and Xenobiotic Prototypic Substrates of Human Cytosolic SULTs

Subfamily	Endogenous substrates	Xenobiotic substrates
SULT1A1	Iodothyronines: 3,3′diiodothyronine (T2), 3,3′,5-triiodothyronine (T3), (Anderson *et al.*, 1995; Li *et al.*, 2001); Estrogens: β-estradiol (E2) (Falany, 1997)	Simple phenolic compounds: *p*-nitrophenol, *m*-nitrophenol, *p*-ethylphenol, *p*-cresol (Wilborn *et al.*, 1993; Brix *et al.*, 1999b); Drugs: paracetamol (Lewis *et al.*, 1996), minoxidil (Meisheri *et al.*, 1993); Carcinogens: N-Hydroxy -PhIP (Ozawa *et al.*, 1994).
SULT1A2	Not known	Simple phenolic compounds: *p*-nitrophenol (Zhu *et al.*, 1996); Carcinogens: N-Hydroxy −2-AAF (Glatt, 2000)
SULT1A3	Catecholamines: dopamine, (Brix *et al.*, 1999b; Dajani *et al.*, 1999b), norepinephrine (noradrenaline) (Ganguly *et al.*, 1995)	Simple Phenols: *p*-nitrophenol (Brix *et al.*, 1999b); Carcinogens: 1-Hydroxymethylpyrene (Glatt, 2000)
SULT1B1	Iodothyronines: 3,3′diiodothyronine (T2), 3,3′,5-triiodothyronine (T3), 3,3′,5′-reverse triiodothyronine (r-T3), and thyroxine (T4) (Wang *et al.*, 1998)	Simple Phenol: 1-Naphthol (Wang *et al.*, 1998)
SULT1C2	Not known	Simple Phenols: *p*-nitrophenol (Sakakibara, 1998b); Carcinogens: *N*-Hydroxy-2-AAF (Yoshinari *et al.*, 1998b)
SULT1C4	Not known	Simple Phenols: *p*-nitrophenol (Yoshinari *et al.*, 1998a); Carcinogens*: N*-Hydroxy-2-AAF (Sakakibara *et al.*, 1998b)
SULT1E1	Estrogens: E2, estrone (E1) (Falany *et al.*, 1995)	Estrogens: 17-ethinyl-E2, equilenin (Falany *et al.*, 1995); Catechol estrogens: 2-hydroxyestrone, 2-hydroxyestradiol, 4-hydroxyestrone; 4-hydroxyestradiol (Adjei and Weinshilboum, 2002)
SULT2A1	Steroids: DHEA (Comer and Falany, 1992)	Carcinogens: 1- Hydroxymethylpyrene (Meinl *et al.*, 2002), 6 Hydroxymethylbenzo[a]-pyrene, hycanthone (Glatt, 2000)
SULT2B1_v1	DHEA (Her *et al.*, 1998), pregnenolone (Meloche and Falany, 2001)	Not known
SULT2B1_v2	DHEA (Her *et al.*, 1998), pregnenolone (Meloche and Falany, 2001), cholesterol and oxysterols (Geese and Raftogianis, 2001)	Not known
SULT4A1	Not known	Not known

SULT1C. SULT1C subfamily members have been isolated from a variety of species including Sult1c1, 1c2, and 1c3 from the rat, 1c1 from the mouse, and 1c2 from the rabbit (see Blanchard *et al.*, 2004 for references). Weinshilboum's group was the first to isolate a human 1C2 cDNA from a fetal liver-spleen cDNA library (Her *et al.*, 1997) and demonstrate that the gene was located on chromosome 2 at 2q11.2. The identical construct was also cloned and characterized by Yoshinari *et al.* (1998b) and Hehonah *et al.* (1999). Another member of the human 1C subfamily, 1C4, was identified by Sakakibara *et al.* (1998b). To date, no endogenous substrates have been identified for members of the IC subfamily.

SULT1E. The SULT1E enzymes have been widely studied due to their important role in steroid homeostasis. The bovine SULT1E1 was the first cDNA cloned as a known sulfotransferase (Nash *et al.*, 1988). Since this initial study, members of the 1E subfamily have been isolated from a number of species including the guinea pig, rat, mouse, and pig (Blanchard *et al.*, 2004). Aksoy *et al.* (1994) were the first to isolate a SULT1E1 cDNA from a human liver library. The protein encoded by this cDNA was subsequently shown to have high affinity (nM range) for E2 and estrone and a variety of synthetic estrogens, including diethylstilbestrol and tamoxifen (Falany, 1997; Falany *et al.*, 1995). While other SULTs such as SULT1A1 and SULT2A1 exhibit high activity toward E2 and estrone, this only occurs at nonphysiological concentrations (Falany, 1997; Falany *et al.*, 1995). Iodothyronines are also good substrates for SULT1E1 (Kester *et al.*, 1999).

SULT2 Family

The SULT2 family contains the hydroxysteroid sulfotransferases, which have been subdivided into two subfamilies based on their amino acid sequence identities. Generally, all members of the SULT2 family display overlapping substrate specificities toward an array of hydroxysteroids and related compounds (Table 2).

SULT2A. The rat senescence marker protein (SULT2A2) that is predominantly expressed in aging male rats and mentioned above (Chatterjee *et al.*, 1987) was subsequently shown to be a hydroxysteroid sulfotransferase. This became apparent on the cloning of three additional rat SULT2A isoforms, SULT2-A1, A3, A4 (see Blanchard *et al.*, 2004 for references). SULT2A isoforms have also been identified in a variety of species, with SULT2A1 being isolated from the mouse, rabbit (Blanchard *et al.*, 2004), and monkey (Ogura

et al. unpublished). Unlike the rat, which has multiple forms of these 2A enzymes, humans have only a solitary isoform, SULT2A1 (Comer *et al.*, 1993; Forbes *et al.*, 1995; Kong *et al.*, 1992; Otterness *et al.*, 1992). Human SULT2A1 is responsible for the sulfonation of hydroxysteroids including DHEA, androgens, pregnenolone, and bile acids and was initially cloned using liver and fetal adrenal RNA and termed DHEA sulfotransferase for its preferred substrate (Comer *et al.*, 1993; Forbes *et al.*, 1995; Kong *et al.*, 1992; Otterness *et al.*, 1992; Radominska *et al.*, 1990).

SULT2B. While the SULT2A and SULT2B subfamilies are capable of metabolizing a range of similar substrates, it nonetheless appears that SULT2B subfamily members are predominantly cholesterol sulfotransferases (Javitt *et al.*, 2001). To date, three members of this subfamily have been identified, Sult2b1 from the mouse (Sakakibara *et al.*, 1998a) and two human isoforms (SULT2B1_v1 and v2; Her *et al.*, 1998). Human SULT2B1_v1 is 15 amino acids shorter than SULT2B1_v2 at the amino terminus, which imparts a functional distinction. For example, SULT2B1_v1 preferentially sulfonates pregnenolone, whereas SULT2B1_v2 catalyses the sulfonation of cholesterol (Fuda *et al.*, 2002).

SULT4 Family

Falany's group cloned the first members of the SULT4 family from both human and rat brain cDNA libraries (Falany *et al.*, 2000). These authors termed the cDNAs ''brain sulfotransferase-like'' (BR-STL), because of their structural similarity to published SULTs and their selective expression in brain tissue. Liyou *et al.* (2003) cloned an identical cDNA from a human brain cDNA library, and Sakakibara *et al.* (2002) have identified the equivalent mouse brain isoform. At the amino acid level the human, rat, and mouse isoforms are 97% similar, and based on the Blanchard *et al.* (2004) nomenclature, they have been classified as SULT4A1. These proteins are orphan enzymes, as no substantial activity toward endogenous or xenobiotic substrates has been demonstrated (Sakakibara *et al.*, 2002). The predominant brain localization of SULT4A1 and the high degree of sequence identity across species is suggestive of an important, yet unidentified physiological function.

LOCALIZATION OF HUMAN SULFOTRANSFERASES

SULT1A Subfamily

Much of the early work on the specific cellular localization of human SULT1A members is clouded by the fact that it was not until, 1995 that we definitively knew this subfamily consisted of three closely related members that shared >93% identity at the amino acid level (Blanchard *et al.*, 2004; Hempel *et al.*, 2005). The recent finding of Hildebrandt *et al.* (2004), which shows that SULT1A3 has undergone a gene duplication and both SULT1A3 and 1A4 appear to be transcriptionally, active requires a more cautious interpretation of SULT1A3 data. However, the available data obtained using an array of methods including metabolic probes, immunohistochemistry, hybridization histochemistry, immunoblotting, and RT-PCR analysis have shown that SULT1A members exhibit probably the widest tissue distribution of any cytosolic SULT subfamily (Blanchard *et al.*, 2004; Dooley *et al.*, 2000; Hempel *et al.*, 2005). SULT1A1 is by far the major adult liver SULT1A subfamily member and has also been identified in brain (Richard *et al.*, 2001; Whittemore *et al.*, 1986; Young *et al.*, 1985), breast (Windmill *et al.*, 1998), intestine (Teubner *et al.*, 1998), endometrium (Falany *et al.*, 1998), adrenal gland, platelets, and placenta (Abenhaim *et al.*, 1981; Hart *et al.*, 1979; Heroux *et al.*, 1989), kidney and lung (Vietri *et al.*, 2003), and jejunum (Sundaram *et al.*, 1989). SULT1A3, as indicated above, is barely detectable in the adult human liver (Eisenhofer *et al.*, 1999; Heroux *et al.*, 1989) but is highly expressed in jejunum and intestine (Eisenhofer *et al.*, 1999; Richard *et al.*, 2001; Sundaram *et al.*, 1989; Teubner *et al.*, 1998) and also present in platelets and placenta (Heroux *et al.*, 1989) and brain (Whittemore *et al.*, 1985; Young *et al.*, 1985). Using hybridization histochemistry, employing a general SULT1A ribo-probe, a specific SULT1A3 ribo-probe, and a SULT1A antibody, a positive signal was observed in epithelial cells lining the lumen of the stomach and the gastric pits, and in the epithelial cells lining the lumen surface of the crypts of Lieberkuhn of the small intestine and colon. In human lung cytosol, SULT1A1 and SULT1A3 proteins are detectable, and histological studies have shown these proteins present in epithelial cells of the respiratory bronchioles (Hempel *et al.*, 2005; Windmill *et al.*, 1998). Since both the intestine and lungs are major portals of entry of drugs and xenobiotics into the body, the above localization pattern suggests that both SULT1A1 and SULT1A3 may play a significant role in the extrahepatic detoxification and metabolic activation of these chemicals. From a developmental perspective, both SULT1A1 and SULT1A3 are abundantly expressed in the fetal liver, but SULT1A3 almost disappears in adult liver and kidney (Cappiello *et al.*, 1991; Hempel *et al.*, 2005; Pacifici *et al.*, 1993; Richard *et al.*, 2001). These results are suggestive of a role for SULT1A members in protecting the fetus from exogenous toxins and in the homeostasis of hormones such as dopamine and iodothyronines. Further, immunoblotting of placenta cytosol showed the presence of both SULT1A1 and SULT1A3, indicating they may have a potential role in the metabolism of xenobiotics entering the fetal circulation from the maternal side (Heroux *et al.*, 1989; Stanley *et al.*, 2001). The fact that placental UDP-glucuronosyltransferases are relatively low and variable in humans (Collier *et al.*, 2002; Pacifici *et al.*, 1998) suggests that SULT1A members may play a significant role in phase II metabolism in this tissue.

The localization and physiological function of SULT1A2 is the least understood of the SULT1A subfamily members. cDNAs of SULT1A2 have been isolated from both human liver and colon libraries (Blanchard *et al.*, 2004; Ozawa *et al.*, 1995; Zhu *et al.*, 1996), and lower mRNA levels than other SULT1A members have been found in liver, kidney, brain, lung, ovary, and some sections of the gastrointestinal tract (Dooley *et al*., 2000; Glatt *et al.*, 2001). However, it appears that SULT1A2 mRNA expression does not translate into the formation of protein. For example, Nowell *et al.* (2005), using a specific anti-peptide antibody for SULT1A2, screened more than 200 cytosolic fractions from 10 different human tissues and found no evidence of immunoreactive protein. Dooley *et al.* (2000) have suggested that the SULT1A2 gene is a quasi-effective pseudogene of SULT1A1 that is occasionally expressed at the RNA level. While SULT1A2 has been shown to be more efficient than SULT1A1 in the metabolic activation of several aromatic amines (Glatt and Meinl, 2004; Meinl *et al.*, 2002) and capable of activating 3-nitrobenzanthrone and its metabolites in model *in vitro* systems, the current weight of evidence suggests this enzyme is not expressed *in vivo* in humans. Because of this data, Nowell *et al.* (2005) have cautioned the interpretation of data from SULT1A2 genotype/disease association studies.

SULT1B Subfamily

The major physiological role for human SULT1B1 appears to be in thyroid hormone metabolism (Fujita *et al.*, 1997; Wang *et al.*, 1998). To date, SULT1B1 (hST1B2) mRNA has been detected in liver, small intestine, colon, and blood leukocytes (Wang *et al.*, 1998). In the same study, it was also demonstrated that SULT1B1 protein was clearly detectable on immunoblots in cytosol from liver, small intestine, and colon.

SULT1C Subfamily

The physiological role of SULT1C members is currently unknown. Her *et al.* (1997) performed dot blot analysis and obtained positive signal for SULT1C2 (called SULT1C1 in paper) in the adult human stomach, kidney, and thyroid, as well as fetal liver and kidney. Both Yoshinari *et al.* (1998b) and Hehonah *et al.* (1999) isolated identical human sequences from fetal liver and adult stomach cDNA libraries, respectively. Sakakibara *et al.* (1998b) identified another member of this subfamily from human fetal lung that has been termed SULT1C4. At the RNA level, SULT1C4 was shown to be expressed at higher levels in fetal lung and kidney and at lower levels in fetal heart. Positive signal was also found in the adult kidney, ovary, and spinal cord (Sakakibara *et al.*, 1998b).

SULT1E Subfamily

SULT1E1 is present on immunoblots in both human liver and jejunum cytosol (Forbes-Bamforth and Coughtrie, 1994; Falany *et al.*, 1995). Falany *et al.* (1998) showed that, on immunoblots, SULT1E1 was not detectable in proliferative endometrial cytosol, but was consistently found in the secretory endometrial cytosols. Coughtrie (2002) has also reported significant levels of SULT1E1 in human fetal liver, lung, and kidney, using E2 activity as a diagnostic indicator of this protein.

SULT2A Subfamily

Northern analysis has shown SULT2A1 to be present in the human liver, adrenal, and small intestine (Luu-The *et al.*, 1995; Otterness *et al.*, 1992; Tashiro *et al.*, 2000). In a more extensive investigation using RT-PCR, Javitt *et al.* (2001) showed that SULT2A1 mRNA was highly expressed in steroidogenic organs (adrenal and ovary), androgen-dependent tissue (prostate), and in the liver, stomach, small intestine, and colon. Barker *et al.* (1994) have used immunohistochemistry to demonstrate SULT2A1 (DHEA-ST) expression in embryonic human hepatocytes and that this pattern continues into adulthood, when immunostaining is localized around the central vein. The same authors also reported that SULT2A1 expression was detected in fetal zone of the fetal adrenal, and in the adult, staining was localized in the zona reticularis. Further, kidney SULT2A1 immunostaining was present in the proximal and distal tubules, loops of Henle, collecting ducts, and their progenitors.

SULT2B Subfamily

An initial study using Northern analysis showed SULT2B1 to be localized to the human prostate, placenta, small intestine, and trachea (Tashiro *et al.*, 2000). Javitt *et al.* (2001) extended this study to both isoforms of SULT2B1 using RT-PCR and demonstrated that SUL2B1_v2 was more widely expressed than its counterpart, particularly in a variety of hormone-responsive tissues including the placenta and prostate, which is suggestive of a differential regulatory mechanism of the two transcripts (Geese and Raftogianis, 2001). For example, SULT2B1_v2 mRNA was present in adrenal gland, placenta, ovary, prostate, lung, kidney, colon, stomach, small intestine, spleen, thymus, thyroid, and liver, whereas SULT2B1_v1 mRNA was present in all these tissues except the last six. Interestingly, SULT2A1 mRNA is present in brain and bone marrow but absent from the skin, where the mRNA of both isoforms of SULT2B are expressed.

SULT4A Subfamily

The current data shows an exclusive brain localization of SULT4A1 in rats, mice, and humans (Falany *et al.*, 2000; Liyou *et al.*, 2003; Sakakibara *et al.*, 2002). However, recent microarray data is suggestive of a wider tissue distribution than the brain for the localization of this SULT (Mm.248796 at <http://www.ncbi.nlm.nih.gov/Unigene/>).

TABLE 3
Crystal Structures of Human SULTs as of Mid-2005

SULT Structure	Cofactor bound	Substrate/inhibitor bound	Resolution (Å)	PDB code	Publication
SULT1A1*2	PAP	*p*-nitrophenol	1.9	1LS6	Gamage *et al.*, 2003
SULT1A1*3	PAP	estradiol	2.3	2D06	Gamage *et al.*, 2005
	#	#	#	#	Lu *et al.* unpublished
SULT1A2	#	#	#	#	Lu *et al.* unpublished
SULT1A3	$SO4^{2-}$	—	2.4	1CJM	Bidwell *et al.*, 1999
	PAP	—	2.5	—	Dajani *et al.*, 1999a
	PAP	dopamine	2.6	2A3R	Lu *et al.*, 2005
SULT1B1	PAP	—	2.1	1XV1	Dombrovski *et al.* (unpublished)
SULT1C1	PAP	—	2.22	1ZHE	Dong *et al.* (unpublished)
SULT1E1	PAP	(OH-PCB)[a]	1.7	1G3M	Shevtsov *et al.*, 2003
	PAPS	—	1.8	1HY3	Pedersen *et al.*, 2002
	PAP	vanadate	2.1	1BO6	Kakuta *et al.*, 1998
SULT2A1	PAP	—	2.4	1EFH	Pedersen *et al.*, 2000
SULT2A3	—	DHEA[b]	1.99	1J99	Rehse *et al.*, 2002
DHEA-ST	—	ADT[c]	2.7	1OV4	Chang *et al.*, 2003
SULT2B1_v1	PAP	—	2.91	1Q1Q	Lee *et al.*, 2003
SULT2B1_v2	PAP	—	2.4	1Q1Z	Lee *et al.*, 2003
	PAP	pregnenolone	2.3	1Q20	Lee *et al.*, 2003
	PAP	DHEA[b]	2.5	1Q22	Lee *et al.*, 2003
SULT4A1	—	—	2.24	1ZD1	Dong *et al.* (unpublished)
H3-OST-1[d]	PAP	—	2.1	1ZRH	Dong *et al.* (unpublished)
HSNST[e]	PAP	—	2.3	1NST	Kakuta *et al.*, 1999
3-OST-3[f]	PAP	—	1.85	1T8T	Moon *et al.*, 2004
	PAP	Tetrasaccharide	1.95	1T8U	Moon *et al.*, 2004

Note. # indicates structure deposited with the Protein Data Bank but not yet released; details not available.
[a]3,5,3′,5′-Tetrachloro-Biphenyl-4,4'-Diol.
[b]Dehydroepiandrosterone.
[c]Androsterone.
[d]Heparan sulfate glucosamine 3-O-sulfotransferase-1.
[e]SULT domain of heparin sulfate-N-deacetylase sulfotransferase.
[f]3-O-sulfotransferase.

STRUCTURAL STUDIES AND ENZYMATIC MECHANISM IN CYTOSOLIC SULTS

SULTs can exhibit quite broad, overlapping substrate specificities; however, individual enzymes often demonstrate strict regio-specificity toward a particular substrate (Falany, 1997). Understanding the structural basis of such specificity has been crucial for elucidating the catalytic mechanism and function of these enzymes. It will also aid in predicting the metabolic fate of drugs and chemical carcinogens that are sulfonated and could provide a more rational approach to drug design and chemical risk assessment.

The major catecholamine SULT, SULT1A3, was the first human cytosolic SULT to be structurally characterized (Bidwell *et al.*, 1999; Dajani *et al.*, 1999a). Prior to this, the mouse cytosolic estrogen SULT (SULT1E1) (Kakuta *et al.*, 1997) and the SULT domain of the human Golgi membrane-bound heparan sulfate N-deacetylase/N-sulfotransferase-1 (HSNST; Kakuta *et al.*, 1999) were published. Since then, the number of crystal structures of cytosolic and membrane-bound SULTs have grown rapidly. Despite this progress, only a few crystal structures have been determined with both cofactor and substrate bound (Table 3). This relative lack of information has hindered elucidation of the structural principles underlying the recognition and utilization of a given substrate.

Overall SULT Structure

The SULT crystal structures have shown that the enzymes are generally globular proteins with a single α/β domain that forms characteristic five-stranded parallel β-sheet surrounded on either side with α-helices (Fig. 2). The β-sheets contribute the PAPS-binding site and the core catalytic residues. These catalytic residues have been shown to be conserved across the cytosolic and membrane-bound SULTs. Interestingly, SULTs share similarities in their structural fold with nucleotide kinases such as uridylate kinase, adenylate kinase, and guanylate kinase (Kakuta *et al.*, 1997).

PAP Binding Site

Early sequence analysis suggested that the sequence motif GxxGxxK, present in nearly all cytosolic SULTs, and shown to

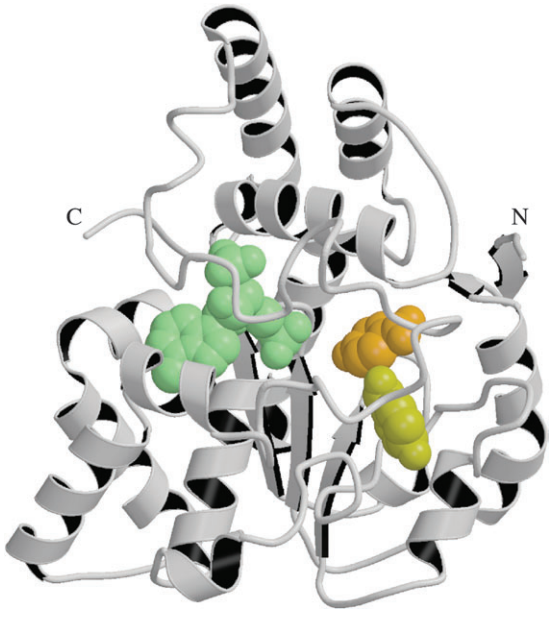

FIG. 2. Overall structure of a cytosolic SULT: structure of human SULT1A1 complex with 3′-phosphoadenosine-5′-phosphate (PAP) and *p*-nitrophenol (*p*NP; Gamage *et al.*, 2003). Secondary structural elements are depicted as coils for helices and arrows for strands. The bound ligands are shown as spherical atomic models; PAP, green; pNP^1, orange; pNP^2, yellow.

be important for PAPS binding, was equivalent to the P-loop found in ATP- and GTP-binding proteins (Chiba *et al.*, 1995; Driscoll *et al.*, 1995). However, structural studies on mouse SULT1E1 revealed that the ''P-loop'' equivalent in SULTs actually corresponds to another loop region, comprising residues 45-TYPKSGT-51 of SULT1E1 (Kakuta *et al.*, 1997). This loop, termed the phosphosulfate binding (PSB) loop, of SULTs provides the major binding site for the 5′-phosphate group of PAP (Kakuta *et al.*, 1997) and is thought to be important for orienting the cofactor for in-line sulfuryl transfer to the acceptor substrate (Dajani *et al.*, 1999a; Kakuta *et al.*, 1997; Pedersen *et al.*, 2002). Please note that the term PAP is used because mouse SULT1E1 was crystallized in the presence of this molecule and not with PAPS.

The 3′-phosphate of PAP interacts with two conserved regions of sequence, residues 257–259 located at the beginning of the GxxGxxK region, and two additional residues, Arg130 and Ser138 in mouse SULT1E1 (Kakuta *et al.*, 1997). The positioning of the adenine ring of PAP is determined by the residues Trp53, Thr227, and Phe229. These general features identified in SULT1E1 for binding PAP are conserved in other SULT structures, suggesting that the cytosolic and membrane-bound SULTs evolved from a common ancestor (Yoshinari *et al.*, 2001).

Substrate Binding Site

Generally, cytosolic SULTs have a covered hydrophobic substrate binding site, whereas the presumed substrate binding pocket of the membrane-bound HSNST is a large open cleft to allow sulfonation of carbohydrates, glucosaminylglycans, and proteins. As mentioned before, SULTs display broad substrate specificity, though a given enzyme can often be characterized by having a preference for a specific substrate. The underlying principles that regulate this specificity most probably reside in the substrate binding sites of these enzymes. Thus, in contrast to the PAPS binding site, which is characterized by conserved residues across all the SULTs, the substrate binding pocket of SULTs shows a great deal of variability. Despite the elucidation of several SULT crystal structures, the structural principles that underpin substrate specificity are still not fully understood. As indicated above, this is due to the fact that only a few crystal structures have been solved with bound substrate and cofactor present.

In the SULT1A family, the crystal structures of SULT1A1*2, SULT1A1*3, SULT1A2, and SULT1A3 (Table 3) have been determined. The Bidwell *et al.* (1999) structure of SULT1A3 was solved with a sulfate ion in the cofactor site, whereas the Dajani *et al.* (1999a) SULT1A3 structure was complexed with PAP. Both structures show large stretches of disordered regions, and this was thought to be a consequence of the lack of a bound substrate. In contrast, the crystal structure of SULT1A1 was crystallized in the presence of both PAP and a model xenobiotic substrate *p*NP that revealed an L-shaped and very hydrophobic substrate-binding pocket (Gamage *et al.*, 2003). Indeed, Gamage *et al.* (2003) has shown that the binding site of SULT1A1 is plastic, allowing this enzyme to adopt varying architectures so that it can interact with small aromatics (*p*NP), L-shaped aromatics (diiodothyronine), and fused ring compounds (E2; Gamage *et al.*, 2005).

Human SULT1A1 and 1A3 share 90% sequence identity, though they exhibit distinct substrate preferences. SULT1A1 prefers uncharged simple phenolic compounds such as *p*NP, *p*-cresol, or *p*-ethylphenol, whereas 1A3 prefers positively charged substrates such as dopamine or tyramine (Brix *et al.*, 1999b). The crystal structure of SULT1A1 revealed the hydrophobic substrate binding pocket mentioned above, which clearly favors binding of uncharged substrates. By contrast, the SULT1A3 substrate binding site includes acidic residues such as Glu146 and Glu89, which favors binding of positively charged substrates. Indeed, site-directed mutagenesis and molecular modeling studies identified Glu146 as a critical residue for the recognition of dopamine by SULT1A3 (Brix *et al.*, 1999a ; Dajani *et al.*, 1998).

Mouse SULT1E1 was the first cytosolic SULT to be structurally characterized (Kakuta *et al.*, 1997); its structure showed the enzyme in a complex with both the substrate E2 and PAP. In the active site, His108 is directly coordinated to the 3-phenolic group of E2 and acts as the catalytic base in the sulfuryl transfer mechanism. This histidine residue is conserved in all cytosolic SULTs, and its mutation has been shown to abolish activity of mouse SULT1E1 (Kakuta *et al.*, 1998). Thus, this structure provided the basis for understanding

β-estradiol binding in the active site and for catalysis of sulfonation by the proposed S_N2 in-line displacement mechanism. The crystal structures of SULT1E1:PAP:vanadate (Kakuta *et al.*, 1998) and human SULT1E1:PAPS (Pedersen *et al.*, 2002) provided further evidence as to the structure of the transition state during sulfuryl transfer and gave supporting evidence for the proposed mechanism.

In the SULT2A subfamily, the crystal structures of the human dehydroepiandrosterone sulfotransferase enzymes (SULT2A1, SULT2A3), which sulfonate steroids such as DHEA, androsterone, E2, and pregnenolone, have been solved in complex with PAP (Pedersen *et al.*, 2000), DHEA (Rehse *et al.*, 2002), and androsterone (Chang *et al.*, 2003). In the DHEA-bound structure, two alternative substrate-binding orientations were identified for DHEA, and the authors suggested that the second orientation may reflect a binding mode associated with substrate inhibition. The work of Chang *et al.* (2003) demonstrated that this enzyme recognizes androsterone as a cognate substrate, with similar kinetics but higher specificity and stronger substrate inhibition than DHEA.

In the SULT2B subfamily, SULT2B1_v1 (SULT2B1a) and 2B1_v2 (2B1b), as outlined above, are splice variants (Her *et al.*, 1998) and have different substrate specificities (Fuda *et al.*, 2002). The crystal structures of SULT2B1_v1 and 2B1_v2 bound with PAP and that of SULT2B1_v2 with its substrate pregnenolone have been determined (Lee *et al.*, 2003). These structures reveal a different catalytic binding orientation for the acceptor substrate, pregnenolone, than that observed for the related steroid DHEA in SULT2A1. It was shown that the amino-terminal helix comprising residues19–26 determines the substrate specificity between the two isoforms. The residues 19-Asp-Ile-Ser-Glu-Ile-23 are responsible for the ability of SULT2b1_v2 to sulfonate cholesterol (Fuda *et al.*, 2002; Lee *et al.*, 2003). Thus, the substrate specificity difference between the two SULT2B1 isoforms appears to lie at the unique amino terminus.

The crystal structures of SULT1B1:PAP, SULT1C1:PAP, and SULT4A1 have also been determined and submitted to the Protein Data bank (PDB, http://www.rcsb.org/pdb), but are not yet described in the literature (Table 3).

Sulfuryl Transfer Mechanism

Duffel and Jakoby (1981) reported that *p*NP sulfonation by rat aryl sulfotransferase IV has a random Bi Bi mechanism in which PAPS and *p*NP bind to the enzyme independently. Kinetic studies on the catalytic mechanism of recombinant mouse SULT1E1 suggested that sulfonation follows a random Bi Bi mechanism with dead-end complexes (Zhang *et al.*, 1998). On the other hand, studies using purified human brain aryl sulfotransferase (Whittemore *et al.*, 1986) and flavonol sulfotransferase (Varin and Ibrahim, 1992) suggested that sulfonation occurs via an ordered Bi Bi mechanism. All these studies agree that the sulfonate transfer reaction occurs without formation of intermediates. When the crystal structure of mouse SULT1E1 was solved in the presence of PAP and E2, it became clear that the core structure resembles that of uridylate kinase, with striking similarities between the PAP and ADP binding sites (Kakuta *et al.*, 1997). These structural features of SULT1E1 suggest that the sulfotransferase reaction takes place via an S_N2 in-line displacement, a mechanism similar to phosphoryl transfer (Kakuta *et al.*, 1997, 1998). The active site and transition state mimicked by SULT1E1:PAP:vanadate (Kakuta *et al.*, 1998) and human SULT1E1:PAPS complexes (Pedersen *et al.*, 2002) provide further supporting evidence for this mechanism.

Substrate Inhibition

Substrate inhibition, observed at high concentrations of their preferred substrates, is a characteristic feature of SULTs (Raftogianis *et al.*, 1999; Reiter *et al.*, 1983). However, several of the published studies have assumed a Michaelis–Menten model to analyse the kinetics of these enzymes using limited substrate concentration ranges below the overtly inhibitory range (Brix *et al.*, 1999b; Lewis *et al.*, 1996). The recent studies on the crystal structure of SULT1A1 from our laboratory gave the first clues to the molecular basis of substrate inhibition that takes place with small planar substrates such as *p*NP (Gamage *et al.*, 2003). In the SULT1A1:PAP:*p*NP structure (Gamage *et al.*, 2003), we observed two *p*NP molecules bound in the L-shaped active site. When the kinetic implications of this observation were investigated using a wide array of *p*NP concentrations, we found that there was slight positive cooperativity at low substrate concentrations and substrate inhibition at higher *p*NP concentrations (above 2 μM; Fig. 3A). From these data, a general kinetic model was constructed (Fig. 3B). The model fits well to the experimental data, and we proposed that impeded catalysis results when both binding sites are occupied, and this gives rise to the observed substrate inhibition with *p*NP in SULT1A1. Furthermore, we have also shown by molecular modeling and site-directed mutagenesis that the SULT1A3 active site could accommodate two molecules of dopamine (Barnett *et al.*, 2004). From these studies, we have been able to conclude that the substrate inhibition at high concentrations of the substrate is due to impeded catalysis when both binding sites are occupied.

The mechanism of substrate inhibition that takes place with multi-ring substrates such as E2 is not well understood. SULT1A1 and SULT1E1 both show strong substrate inhibition at high substrate concentrations of E2 (Adjei and Weinshilboum, 2002; Falany and Falany, 1997; Fig. 4A). In our recent crystal structure of SULT1A1, there is one molecule each of PAP and E2 in the active site, but the latter is bound in a nonproductive mode (Gamage *et al.*, 2005). This has led us to propose a model (Fig. 4B) that gives an excellent quantitative explanation of the observed substrate inhibition by E2. In this model, a dead-end complex is formed during

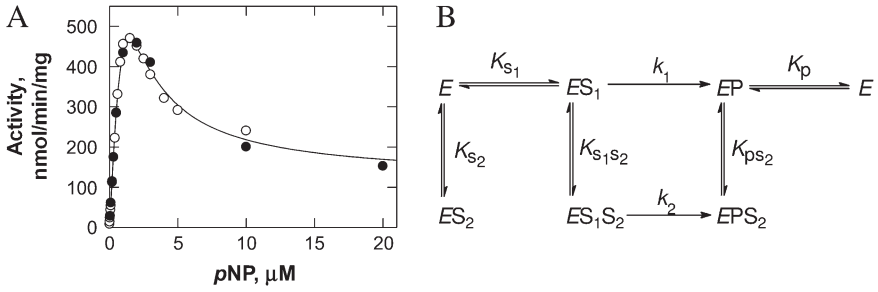

FIG. 3. Kinetic implications of *p*-nitrophenol (*p*NP) sulfonation in SULT1A1. (A) Substrate inhibition is observed above 2 μM of *p*NP. Each data point is a mean of duplicate or triplicate assays. (B) Kinetic model to explain the observed substrate inhibition. The enzyme (E) binds at site 1 to give ES_1 (dissociation constant K_{S1}) or at site 2 to give ES_2 (dissociation constant K_{S2}). If site 2 is occupied, *p*NP will not bind to site 1. The binding of *p*NP at site 1 will not prevent binding of *p*NP at site 2 to give ES_1S_2. ES_1 and ES_1S_2 are catalytically competent species, and they form EP and EPS_2 enzyme product complexes with rate constants k_1 and k_2, respectively. Product is released directly from EP (dissociation constant K_p), and EPS_2 requires prior release of *p*NP from site 2 (dissociation constant K_{pS2}), (Gamage *et al.*, 2003; with permission from *J. Biol.Chem.*).

catalysis, as deduced previously from kinetic studies (Duffel and Jakoby, 1981; Yang *et al.,* 1998). The SULT:PAP:E2 complex that we crystallized is a direct demonstration of this type of dead-end complex, and it provides a ready explanation for the substrate inhibition. A similar type of model may also explain the substrate inhibition that is observed for other SULTs.

Dimerization

Most of the cytosolic SULTs generally exist as dimers in solution, and it appears that they are capable of forming not only homodimers but also heterodimers (Petrotchenko *et al.*, 2001). A conserved dimerization motif was identified by Petrotchenko *et al.* (2001) in human SULT1E1 consisting of 10 residues near the C-terminus and represented by the consensus sequence KXXXTVXXXE (the so-called KTVE motif). The KTVE motif is conserved in nearly all SULTs (Petrotchenko *et al.*, 2001), though the physiological significance of dimerization for the function of SULTs has not yet been identified. However, biophysical studies carried on dimerization and activation of epidermal growth factor receptor (EGFR) by ligand binding may provide future guidance in understanding the role of dimerization in SULT activity (Schlessinger, 2002).

Molecular Modeling of N-Hydroxy Metabolites of 2AAF and PhIP into SULT1A Isoforms

At the time of writing this review, no structural studies have been published that demonstrate the binding of N-hydroxy aromatic and heterocyclic amines or hydroxy methyl polycyclic aromatic hydrocarbons to human SULTs. However, using the published structures of SULT1A1 (Gamage *et al.*, 2003), SULT1A3 (Bidwell *et al.*, 1999), and the computer model of SULT1A2, we have investigated their ability to accommodate these carcinogens.

Specifically, we have modeled the binding of the N-hydroxylated metabolites of the model carcinogen 2-AAF and the major food-derived mutagen PhIP into the active sites of the three enzymes to investigate their binding interactions. The structures of SULT1A2*1 (unpublished data) and 1A3 (Barnett *et al.*, 2004) were modeled based on the crystal

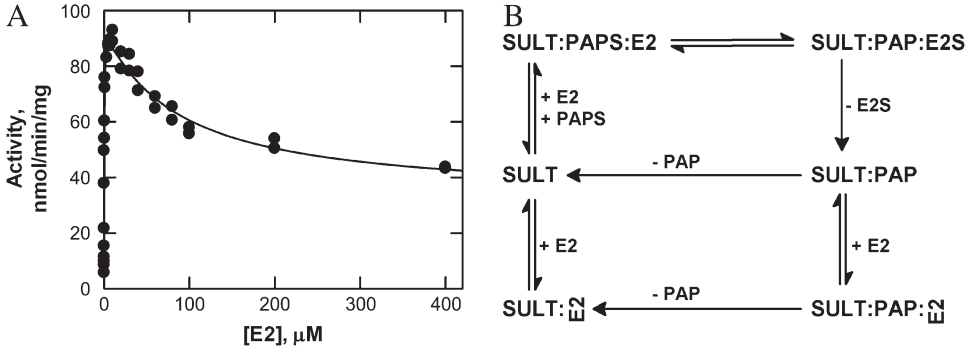

FIG. 4. Kinetic implications of 17β-estradiol (E2) sulfonation in SULT1A1. (A) Substrate inhibition is observed above 1.5 μM of E2. Each data point is an average of duplicate assays. (B) Kinetic model to explain the observed substrate inhibition. The enzyme binds to E2 and 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to give rise to SULT:PAPS:E2, which undergoes catalysis, and E2S and 3′-phosphoadenosine-5′-phosphate (PAP) are released. Alternatively, the SULT:PAP complex can bind E2 in a nonproductive mode (E2 is drawn sideways) to give a dead-end complex. The enzyme can reenter catalysis by releasing PAP followed by E2. The crystal structure of SULT1A1:PAP:E2 demonstrates the dead-end complex mentioned above (Gamage *et al.*, 2005; with permission from *J. Biol.Chem.*).

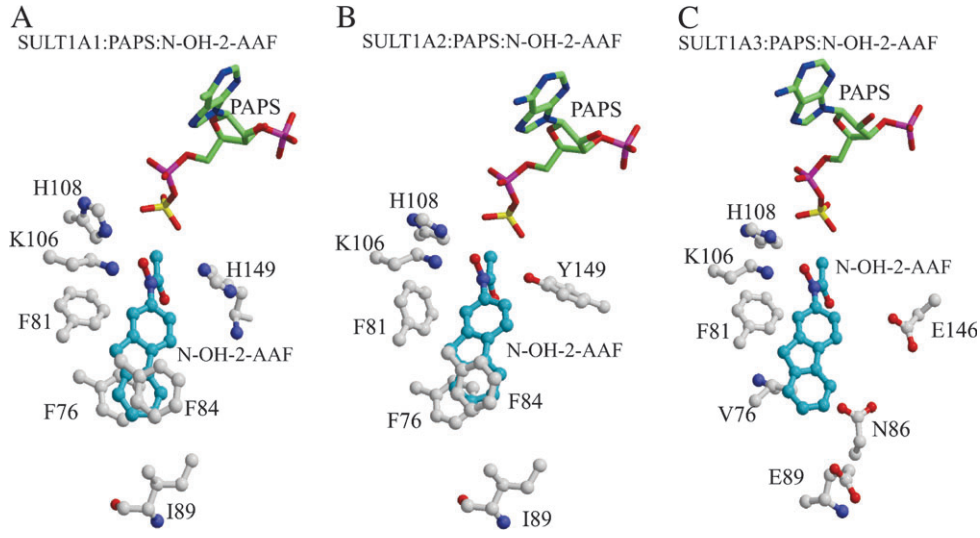

FIG. 5. Models of N-hydroxy 2-acetylaminofluorene (N-OH-2-AAF) bound in the active sites of (A) SULT1A1, (B) 1A2, and (C) 1A3. The cofactor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) is shown as a stick model. N-OH-2-AAF (blue) and enzyme residues (white) are represented as ball-and-stick models. Atom colouring is red for oxygen, blue for nitrogen, yellow for sulfur, and pink for phosphorous.

structure of SULT1A1 (Gamage *et al.*, 2003). PAPS was modeled into each of the three structures, based on the crystal structure of the SULT1E1:PAPS complex (Pedersen *et al.*, 2002). The two ligands were docked into each of the three enzyme structures using the GOLD software (Jones *et al.*, 1997, Fig. 5). The allozyme of SULT1A2 (SULT1A2*1 (HAST4v)) and 1A3 we chose to model with is the wild type. The crystal structure of SULT1A1 is that of SULT1A1*2, which has similar binding characteristics to wildtype SULT1A1*1 (with respect to PAPS and *p*NP) and has slightly lower thermal stability (Rafogianis *et al.*, 1999).

Our results showed that, in all three enzymes, the hydroxyl group of N-OH-2-AAF is within hydrogen bonding distance of the donor sulfonate group of PAPS and of the catalytic residue H108 (Fig. 5), consistent with the possibility of catalysis occurring. In the SULT1A1 and 1A2 models, residues F84 and F76 form stacking interactions with the ligand (Figs. 5A and 5B). However, in the 1A1 model the side chain of F81, the gate residue at the active site, forms an unfavorable interaction with the ligand (2.2 Å). In this case, it seems that a conformational change is necessary in F81 to accommodate the ligand. This is not seen in the SULT1A2 model. This observation could perhaps explain the higher activation of N-OH-2-AAF by SULT1A2 compared with SULT1A1 (Glatt, 2000; Meinl *et al.*, 2002). In our SULT1A3 model (Fig. 5C), the ligand makes unfavorable interactions with both F81 (2.8Å; gate residue) and V84 (2.9 Å) at the enzyme active site. In addition, the active site is relatively acidic as a consequence of residues including E146 and E89; such an environment would be less favorable than the uncharged active sites of SULT1A1 and SULT1A2 for the binding of hydrophobic substrates. Once again, this finding is consistent with the reported lower activation of N-OH-2-AAF by SULT1A3 (Glatt, 2000; Meinl *et al.*, 2002).

The modeling of N-OH-PhIP into the active sites of the enzymes revealed catalytically competent orientations for binding to all three SULT1A structures. In the SULT1A1 and 1A2 models, F76 and F84 form favorable stacking interactions with the benzyl ring of PhIP, as shown in Figures 6A and 6B. Positioning of the residues in the SULT1A1 and 1A2 active sites are similar except for Y149 in SULT1A2. This residue is a histidine in SULT1A1. In the SULT1A2 model, the phenolic hydroxyl of Y149 forms an interaction with the nitrogen of the N-OH group of PhIP. It is not clear whether such an interaction is favorable or unfavorable for binding and catalysis, but given that the interaction is absent in the SULT1A1:N-OH-PhIP model and the two models are otherwise similar, this specific interaction could perhaps explain the lowered metabolic activation of PhIP by SULT1A2 compared with SULT1A1 (Ozawa *et al.*, 1994). By comparison with the very hydrophobic active sites of SULT1A1 and SULT1A2, the SULT1A3 active site is highly charged (Brix *et al.*, 1999a; Dajani *et al.*, 1999a). In our SULT1A3:PAPS:N-OH-PhIP model, we find that the acidic residues (E146 and D86) in the SULT1A3 active site form unfavorable interactions with N-OH-PhIP (Fig. 6C). This could, at least in part, explain the lowered activation of this compound observed with SULT1A3 (Glatt, 2000).

Human SULT1E1 and Hydroxylated Polychlorinated Biphenyls (OH-PCBs)

It is reported that certain environmentally relevant PCBs (polychlorinated biphenyls) such as 4,4′-OH-3,5,3′,5′-tetraCB inhibit human SULT1E1 at subnanomolar concentrations (Kester *et al.*, 2000). PCBs are man-made pollutants that persist in the environment and exert a variety of toxic effects on experimental animals by inducing estrogenic activities through

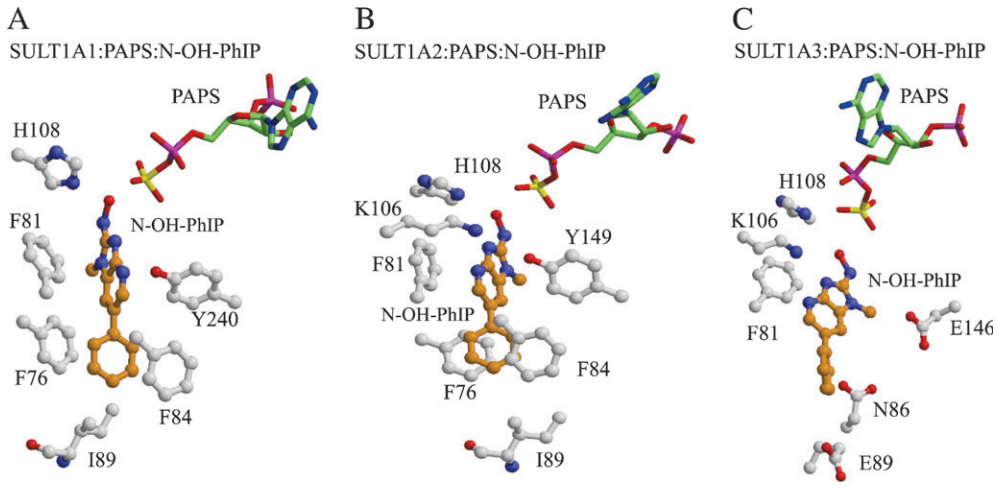

FIG. 6. Docking of N-OH-PhIP (N-hydroxy-2-amino-1-methyl-6-phenylimidazo (4,5b-bipyridine)) into (A) SULT1A1, (B) 1A2, and (C) 1A3 structures. The cofactor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) is shown as a stick model. N-OH-PhIP (orange) and enzyme residues (white) are represented as ball-and-stick models. Atom colouring is oxygen (red), nitrogen (blue), sulfur (yellow), and phosphorous (pink).

increased availability of estradiol (Kester *et al.*, 2000). These compounds and their metabolites have been shown to cause endocrine-disrupting effects such as disturbance of sexual development and reproductive function in animals and humans (Cheek *et al.*, 1998). To address the structural basis of inhibition, the crystal structure of human SULT1E1 was determined with the above environmental pollutant and the cofactor product PAP (Shevtsov *et al.*, 2003). The structure reveals that this compound binds in the active site of SULT1E1 in a very similar way to estradiol (E2) but with a 30° twist between the phenyl rings. This suggests that certain OH-PCBs can act as competitive inhibitors by mimicking E2 binding to SULT1E1 (Shevtsov *et al.*, 2003).

BIOACTIVATION

The early work of the Millers (Millers, 1970, 1978) on the model carcinogen 2-AAF plus other aromatic amines led to the hypothesis that most chemicals require metabolism before being mutagenic or carcinogenic. The first step in the activation of AAF is CYP1A2-mediated N-hydroxylation to N-hydroxy-2-acetylaminofluorene, which is then a substrate for sulfonation, N,O-acyltransfer or deacetylation (McManus *et al.*, 1984; Thorgeirsson *et al.*, 1983). The influential studies by King and Phillips (1968) and the Millers (DeBaun *et al.*, 1968, 1970) were the first to demonstrate the importance of sulfonation as one of the pathways involved in the activation of 2-AAF. These studies followed the seminal finding of Cramer *et al.* (1960), which showed that AAF undergoes N-hydroxylation to N-hydroxy-AAF. This metabolite was found to be more carcinogenic than the parent amide, was often active locally, and was active in the guinea pig that had been shown to be resistant to AAF-induced carcinogenicity (Miller, 1970; Miller, 1978). Sulfonation has now been shown to be important in the activation of a range of compounds such as aminoazo dyes, benzidines, heterocyclic amines, hydroxymethyl polycyclic aromatic hydrocarbons, terpenes, β-aminoethyl alcohols, and 2-nitropropane (Michejda *et al.*, 1994).

Recently, this area has been the subject of a range of reviews (Banoglu, 2000; Glatt, 1997, 2000, 2005; Glatt *et al.*, 1998, 2001; Kauffman, 2004; Surh, 1998), and much of the focus has been on determining the specificity of individual sulfotransferase isoforms for the above substrates using model *in vitro* systems. These studies have shown that certain sulfotransferases are very selective in their activation of promutagens (Glatt, 1997, 2000, 2005), and since they also display tissue-specific expression, it is highly likely that these factors play a role in the organ-selective toxicity of their substrates (Glatt, 2001; Thorgeirsson *et al.*, 1983).

Polymorphisms have been shown in a number of sulfotransferases, and this area has been expertly reviewed by a number of investigators (Glatt, 2000; Glatt and Meinl, 2004; Meinl *et al.*, 2002; Weinshilboum and Adjei, 2005). It was shown that the human SULT1A*Arg (*1) alloenzyme expressed in *Salmonella typhimurium* cells was 12–350 times more active in metabolizing 2-nitropropane, 2,4-dinitrobenzylalcohol, 1-HMP, (−)-1-(α-hydroxyethyl) pyrene, and 2-acetylamino-4-hydroxylaminotoluene to mutagens compared to cells expressing SULT1A1*His (Glatt, 2000). Enantioselectivity has also been demonstrated, with human SULT1E1 and SULT2A1 exhibiting 160-fold preference for the (−)-enantiomer and a 15-fold preference for the (+) enantiomer of 1-(α-hydroxyethyl)pyrene, respectively. Our understanding of such differences in the ability of different sulfotransferases to metabolize chemicals has recently been enhanced with the resolution of a number of the crystal structures of these enzymes (Table 3).

REGULATION OF SULFOTRANSFERASES

Many xenobiotics are effective inducers of transcription and exert their effects via activation of nuclear receptors. These include receptors that have endogenous hormone ligands and orphan nuclear receptors, for which no endogenous ligand have been identified. The role of xenobiotics, and their corresponding nuclear receptors, in the regulation of the Phase I CYP gene family members has been extensively explored, and investigators have now focused on the role of these in the transcriptional regulation of cytosolic SULTs (Runge-Morris, 1998). Sex-specific regulation of rodent isoforms has further broadened our understanding of the transcriptional regulation of cytosolic SULTs. However, it appears that significant interspecies differences in the regulation of SULTs exist. Although there have been some advances in our knowledge of regulation of the human SULT families in recent years, it remains one of the least-explored areas of research in the field.

Xenobiotics such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the model carcinogen 2-AAF are able to regulate transcription via interaction with the nuclear arylhydrocarbon receptor (AhR). Metabolites of these compounds are known substrates for several SULTs, in particular the SULT1 family. From studies in rodents, it is believed that AhR agonists have negative effects on sulfotransferase regulation. β-Napthoflavone, TCDD, 2-AAF, and 3- methylcholanthrene (3-MC) have all been shown to markedly reduce phenol (SULT1A1) and hydroxysteroid (SULT2A) sulfotransferase activities and mRNA levels in rat livers (Runge-Morris, 1998). A recent study showed no effects on expression of SULT1A1, SULT2A1, or SULT1E1 in the human colon carcinoma cell line Caco-2 after exposure to a variety of polycyclic aromatic hydrocarbons (Lampen *et al.*, 2004). Similarly, we have seen no change in SULT1A1 and SULT1A3 mRNA levels after treatment of primary human hepatocytes with 3-MC (Hempel *et al.*, 2004). The consequences of transcriptional regulation by these chemicals are not clear, as sulfonation can lead to both the detoxification and bioactivation of some of these xenobiotic compounds.

Orphan nuclear receptors represent a common mechanism by which xenobiotics elicit their transcriptional activation of metabolic enzymes and drug transporters. Phenobarbital and phenobarbital-like compounds, such as polychlorinated biphenyls, regulate transcription by activating the nuclear constitutive androstane receptor (CAR). A functional nuclear response element responsive to CAR ligands has recently been identified on the mouse SULT2A2 promoter (Saini *et al.*, 2004). There appears to be some conflicting evidence on whether CAR ligands influence the levels of human SULTs. One study describes 11-fold induction of SULT1A1 mRNA after treatment of primary human hepatocytes with the human CAR ligand 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl) oxime [Citco; (Maglich *et al.*, 2003)]. On the contrary, we were unable to reproduce this effect on SULT1A1 or SULT1A3 message levels in human primary hepatocytes (Hempel *et al.*, 2004).

Several SULT isoforms exhibit high affinity for the sulfonation of steroids, and these hormones are involved in transcriptional regulation of some SULTs. The estrogen sulfotransferase (SULT1E1) is one human sulfotransferase expressed under hormonal regulation. Progesterone was shown to control the cyclical expression of SULT1E1 in the menstrual cycle (Falany and Falany, 1996). Transcriptional regulation of SULT1E1 may have therapeutic consequences. For example, it is believed that the progestin-derived anti-breast cancer agents medrogestone and tibolone elicit their effects by inducing SULT1E1 in estrogen-responsive breast cancers, which normally express low levels of this sulfotransferase (Chetrite and Pasqualini, 2001). Sulfonation of estrogens inhibits their action at the estrogen receptor, and SULT1E1 has been implicated as an important enzyme in estrogen homeostasis that may be disrupted in breast cancer. For example, reexpression of recombinant SULT1E1 in the MCF-7 estrogen-responsive breast cancer cell line has been shown to inhibit cell growth (Falany *et al.*, 2002).

Although several sulfotransferases show high affinity for estrogen substrates, studies investigating the role of estrogens in the gene regulation of sulfotransferases require further attention. Evidence suggests that the rat liver hydroxysteroid sulfotransferases (SULT2) may be inducible by estrogens and the anti-breast cancer drug tamoxifen (Hellriegel *et al.*, 1996). It is currently unclear whether the estrogen sulfotransferase is regulated by its own ligand. Treatment of human endometrial Ishikawa cells with β-estradiol did not significantly change SULT1E1 activity (Falany and Falany, 1996), and some studies suggest a potential inhibitory effect of estrogens on SULT1E1 expression, such as the observation of significantly reduced SULT1E1 levels in estrogen-receptor-positive breast cancer biopsy samples and cell lines (Deng *et al.*, 2003; Falany and Falany, 1996). SULT1A1, another sulfotransferase displaying high affinity for estrogens, appears to be positively regulated by tamoxifen, which was shown to significantly increase rat liver and intestinal SULT1A1 mRNA levels (Hellriegel *et al.*, 1996). Interestingly, using serial analysis of gene expression, one study found that human SULT1A enzymes represented the only transcripts up-regulated following treatment of the human breast cancer cell line ZR75-1 with tamoxifen (Seth *et al.*, 2000). Further studies are required to investigate the regulation of human cytosolic sulfotransferases by estrogens.

Glucocorticoids act on two nuclear receptors, the glucocorticoid (GR) and the orphan pregnane X receptor (PXR). Induction by glucocorticoids was confirmed for the human and murine SULT2A1 enzymes (Duanmu *et al.*, 2002a,b), and it has been demonstrated that both PXR and GR directly act on the human *SULT2A1* promoter (Duanmu *et al.*, 2002a). GR was shown to regulate the rat *SULT1A1* promoter at a glucocorticoid response element, which is also shared by other steroid receptors, such as AR and the retinoic acid receptor (RAR).

RAR potentially competes with GR for binding at the response element, and retinoic acid was recently shown to positively affect the expression of human SULT1A1, SULT1E1, and SULT2A1 levels (Maiti *et al.*, 2005). Interestingly, there appears to be interspecies variation of SULT1A1 induction in response to glucocorticoids. Contrary to its rat ortholog, human SULT1A1 enzyme levels are unaffected by treatment with these compounds. For example, human SULT1A1 mRNA levels did not change after treatment of primary human hepatocytes with dexamethasone (Duanmu *et al.*, 2002b; Hempel *et al.*, 2004).

Bile acids are known substrates of the hydroxysteroid sulfotransferases (SULT2) and have been shown to regulate their transcription via the nuclear receptor Farnesoid X Receptor (FXR) (Francis *et al.*, 2003). Recently human SULT2A1 expression was shown to be regulated by the peroxisome proliferator-activated receptor (PPAR), another orphan nuclear receptor, involved in hepatic lipid metabolism (Fang *et al.*, 2005). This effect appears to be species specific, as ligands of this receptor failed to induce SULT2A1 levels in rat primary hepatocytes (Fang *et al.*, 2005).

Slowly studies are focusing on the molecular transcriptional mechanisms controlling the individual sulfotransferase promoters, to reveal a more complex picture of sulfotransferase regulation outside the nuclear receptor field. For example, adrenal-specific expression of human SULT2A1 was recently linked to the ability of another orphan nuclear receptor, steroidogenic factor 1 (SF1), to interact with the transcription factor GATA-6 (Saner *et al.*, 2005). We recently identified the mechanisms regulating the ubiquitous expression of the human SULT1A1 enzyme, which does not appear to be regulated by nuclear receptor activators, unlike its rodent homolog. The human *SULT1A1* promoter was highly activated by the synergistic interaction between the ubiquitous Ets factor GA binding protein (GABP) and Sp1 (Hempel *et al.*, 2004). On the contrary, the highly homologous *SULT1A3* promoter lacks an Ets factor binding repeat, preventing this synergistic interaction (Hempel *et al.*, 2004). This may explain the differences in expression observed between these two human SULT1A members in the adult liver.

CONCLUSION

Until recently, information on SULTs at the molecular, functional, and structural levels has lagged behind other xenobiotic-metabolizing enzyme systems. However, the advances over the last decade have clearly shown this enzyme system to be comprised of a multi-gene family of proteins that differ markedly in their localization, regulation, and metabolic profiles. A recent book edited by Pacifici and Coughtrie has reviewed each of these fields in depth (Pacifici and Coughtrie, 2005). To date, at least 13 different human SULTs have been characterized. Many of the substrates metabolized by sulfotransferases are also substrates for UDP-glururonosyltransferases, and in this context, the former system has generally been considered a high-affinity, low-capacity pathway, whereas the latter system is considered a low-affinity, high-capacity pathway (Burchell and Coughtrie, 1997). This suggests that SULTs may be more important than UDP-glucuronosyltransferases in the metabolism of chemicals via low-level exposure from the environment or through food consumption.

Recent studies have provided new insights into the transcriptional regulation of both the human *SULT1A1* (Hempel *et al.*, 2004) and *SULT2A1* (Fang *et al.*, 2005) genes. However, this area remains one of the least explored in the human sulfotransferase field. While some of the marked interindividual variation in sulfotransferase activity in the human population (5- to 36-fold) can be explained by polymorphisms in the coding regions of *SULT* genes (Pacifici and Coughtrie, 2005; Pacifici and De'Santi, 1995), variations in regulatory regions have not been fully explored.

The physiological significance of SULT1C2, 1C4, 4A1_v1, and 4A1_v2 are poorly understood, and at the present time, they are orphan enzymes (Blanchard *et al.*, 2004). We have shown that SULT4A1 is localized in discrete regions of the human brain such as cerebral cortex, cerebellum, pituitary, and brainstem (Liyou *et al.*, 2003). In addition, SULT 1A1, 1A3, 2A1, and 1E1 are also expressed in the human brain (Liyou *et al.*, 2003). Recently, Richard *et al.* (2001) have shown that both SULT1A1 and SULT1A3 are widely distributed within the developing human fetal brain. Further, the neurotransmitter systems such as GABA, cholinergic, glutaminergic and σ-opioid receptors are modulated by sulfonated neuro-steroids (Hempel *et al.*, 2005; Kauffman, 2004). These data signal the beginning of a brain mosaic in relation to SULTs, and it should help unravel the tentative associations that have previously been made between SULTs and neurodegenerative disorders (Hempel *et al.*, 2005).

To determine the physiological role and examine the consequences of inactivation of SULTs *in vivo*, the gene knockout approach has already been applied (Qian *et al.*, 2001, Tong *et al.*, 2005). These investigators have generated mice deficient in *Sult1e1* and have shown that these mice had spontaneous fetal loss caused by placental thrombosis. This study provides clear evidence as to the importance of estrogen sulfotransferase in reprouction, and further studies in this area should help evaluate the physiological relevance of other sulfotransferases.

Probably the most confronting difficulty facing toxicologists is the species differences that exist in SULTs, which complicate the extrapolation of animal data to humans. For example, humans have four members of the SULT1A subfamily, where rodents have a single member. In contrast, rats have four members of the SULT2A subfamily, while humans have a single gene. Further, equivalent forms of the mouse Sult3a1 or Sult5a1 have not been identified in humans (Blanchard *et al.*, 2004). In addition, the expression of SULTs in humans has been shown to exhibit a pronounced extra hepatic pattern,

whereas in rodents it appears to be predominantly hepatic (Eisenhofer *et al.*, 1999, Hempel *et al.*, 2005). Finally, marked differences in the way in which human and rodent SULT genes are regulated have also been reported; many of the rodent forms exhibit dramatic sexual dimorphisms (Coughtrie and Johnson, 2001). Such data highlight the importance of recent advances in the structural biology of SULTs. Indeed, *in silico* toxicology modeling of substrates utilizing the crystal structure of human SULTs may be a much more predictive tool in chemical risk assessment than data obtained in animal systems. The brief modeling of N-hydroxy aromatic and heterocyclic amines to human SULT1A subfamily members in this review supports this approach. This advance, together with a bank of model cell and bacterial systems expressing human SULTs, where their toxicity can be determined, may provide the most suitable approach in estimating the importance of sulfonation in chemical risk assessment.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

REFERENCES

Adjei, A. A., and Weinshilboum, R. M. (2002). Catecholestrogen sulfation: Possible role in carcinogenesis. *Biochem. Biophy. Res.Commun.* **292,** 402–408.

Aksoy, I. A., Wood, T. C., and Weinshilboum, R. (1994). Human liver estrogen sulfotransferase: Identification by cDNA cloning and expression. *Biochem. Biophys. Res. Commun.* **200,** 1621–1629.

Barnett, A. C., Tsvetanov, S., Gamage, N., Martin, J. L., Duggleby, R. G., and McManus, M. E. (2004). Active site mutations and substrate inhibition in human sulfotransferase 1A1 and 1A3. *J. Biol. Chem.* **279,** 18799–18805.

Baumann, E. (1876). Ueber sulfosauren im harn. *Dtsch. Chem. Ges.* 54–58.

Bernier, F., Lopez Solache, I., Labrie, F., and Luu-The, V. (1994a). Cloning and expression of cDNA encoding human placental estrogen sulfotransferase. *Mol. Cell Endocrinol.* **99**, 11–15.

Bidwell, L. M., McManus, M. E., Gaedigk, A., Kakuta, Y., Negishi, M., Pedersen, L., and Martin, J. L. (1999). Crystal structure of human catecholamine sulfotransferase. *J. Mol. Biol.* **293,** 521–530.

Blanchard, R. L., Freimuth, R. R., Buck, J., Weinshilboum, R. M., and Coughtrie, M. W. H. (2004). A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics* **14,** 199–211.

Brix, L. A., Barnett, A. C., Duggleby, R. G., Leggett, B., and McManus, M. E. (1999a). Analysis of the substrate specificity of human sulfotransferases SULT1A1 and SULT1A3: Site-directed mutagenesis and kinetic studies. *Biochemistry* **38,** 10474–10479.

Brix, L. A., Duggleby, R. G., Gaedigk, A., and McManus, M. E. (1999b). Structural characterization of human aryl sulphotransferases. *Biochem. J.* **337,** 337–343.

Chatterjee, B., Majumdar, D., Ozbilen, O., Murty, C. V. R., and Roy, A. K. D. (1987). Molecular cloning and characterization of cDNA for androgen-repressible rat liver protein, SMP-2. *J. Biol. Chem.* **262,** 822–825.

Comer, K. A., Falany, J. L., and Falany, C. N (1993). Cloning and expression of human liver dehydroepiandrosterone sulfotransferase. *Biochem. J.* **289,** 233–240.

Coughtrie, M. W. H. (1998). Catecholamine sulfation in health and disease. *Advances Pharmacol.* **42**, 339–342.

Dajani, R., Cleasby, A., Neu, M., Wonacott, A. J., Jhoti, H., Hood, A. M., Modi, S., Hersey, Taskinen, A. J., Cooke, R. M., *et al.* (1999a). X-ray crystal structure of human dopamine sulfotransferase, SULT1A3. Molecular modeling and quantitative structure-activity relationship analysis demonstrate a molecular basis for sulfotransferase substrate specificity. *J. Biol. Chem.* **274,** 37862–37868.

Dooley, T. P. (1998a). Molecular biology of the human phenol sulfotransferase gene family. *J. Exp. Zoology* **282,** 223–230.

Dooley T. P. (1998b). Cloning of the human phenol sulfotransferase gene family: Three genes implicated in the metabolism of catecholamines, thyroid hormones and drugs. *Chemico-Biological Interact.* **109,** 29–41.

Dooley, T. P., Haldeman-Cahill, R., Joiner, J., and Wilborn, T. W. (2000). Expression profiling of human sulfotransferase and sulfatase gene superfamilies in epithelial tissues and cultured cells. *Biochem. Biophys. Res. Commun.* **277,** 236–245.

Duanmu, Z., Locke, D., Kocarek, T. A., and Runge-Morris, M. (2002a). Regulation of human hydroxysteroid sulfotransferase (SULT2A1) gene transcription by dexamethasone. *FASEB J.* **16,** A562–A562.

Duffel, M. W., and W. B. Jakoby (1981). On the mechanism of aryl sulfotransferase. *J. Biol. Chem.* **256,** 11123–11127.

Falany, C. N., Krasnykh, V., and Falany, J. L. (1995). Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. *J. Steroid Biochem. Molec. Biol.* **52**, 529–539.

Falany, J. L., and Falany, C. N. (1997). Regulation of estrogen activity by sulfation in human MCF-7 breast cancer cells. *Oncol. Res.* **9,** 589–596.

Falany, C. N. (1997). Enzymology of human cytosolic SULTs. *FASEB J.* **11**, 206–216.

Falany, J. L., Macrina, N., and Falany, C. N. (2002). Regulation of MCF-7 breast cancer cell growth by beta-estradiol sulfation. *Breast Cancer Res. Treat.* **74,** 167–176.

Falany, C. N., Xie, X., Wang, J., Ferrer, J., and Falany, J. L. (2000). Molecular cloning and expression of novel sulphotransferase-like cDNAs from human and rat brain. *Biochem. J.* **346,** 857–864.

Fang, H. L., Abdolalipour, M., Duanmu, Z., Smigelski, J. R., Weckle, A., Kocarek, T. A., and Runge-Morris, M. (2005). Regulation of glucocorticoid-inducible hydroxysteroid sulfotransferase (SULT2A-40/41) gene transcription in primary cultured rat hepatocytes: Role of CCAAT/enhancer-binding protein liver-enriched transcription factors. *Drug Metab. Dispos.* **33,** 147–156.

Forbes, K. J., Hagen, M., Glatt, H., Hume, R., and Coughtrie, M. W. H. (1995). Human fetal adrenal hydroxysteroid sulphotransferase: cDNA cloning, stable expression in V79 cells and functional characterisation of the expressed enzyme. *Mol. Cell. Endocrinol.* **112**, 53–60.

Fuda, H., Lee, Y. C., Shimizu, C., Javitt, N. B., and Strott, C. A. (2002). Mutational analysis of human hydroxysteroid sulfotransferase SULT2B1 isoforms reveals that Exon 1B of the SULT2B1 gene produces cholesterol sulfotransferase, whereas Exon 1A yields pregnenolone sulfotransferase. *J. Biol. Chem.* **277,** 36161–36166.

Fujita, K., Nagata, K., Ozawa, S., Sasano, H., and Yamazoe, Y. (1997). Molecular cloning and characterization of rat ST1B1 and human ST1B2 cDNAs, encoding thyroid hormone sulfotransferases. *J. Biochem.* (Tokyo) **122**, 1052–1061.

Gamage, N. U., Duggleby, R. G., Barnett, A. C., Tresillian, M., Latham, C. F., Liyou, N. E., McManus, M. E., and Martin, J. L. (2003). Structure of a human carcinogen converting enzyme, SULT1A1: Structural and kinetic implications of substrate inhibition. *J. Biol. Chem.* **278,** 7655–7662.

Gamage, N. U., Tsvetanov, S., Duggleby, R. G., McManus, M. E., and Martin, J. L. (2005). The structure of human SULT1A1 crystallized with estradiol: An insight into active site plasticity and substrate inhibition with multi-ring substrates. *J. Biol. Chem.* **280,** 41482–41486.

Glatt, H. (2000). Sulfotransferases in the bioactivation of xenobiotics. *Chem. Biol. Interact.* **129,** 141–170.

Hehonah, N., Zhu, X., Brix, L., Bolton-Grob, R., Barnett, A., Windmill, K., and McManus, M. (1999). Molecular cloning, expression, localisation and functional characterisation of a rabbit SULT1C2 sulfotransferase. *Int. J. Biochem. Cell Biol.* **31,** 869–882.

Hellriegel, E. T., Matwyshyn, G. A., Fei, P., Dragnev, K. H., Nims, R. W., Lubet, R. A., and Kong, A.-N. T. (1996). Regulation of gene expression of various phase I and phase II drug-metabolizing enzymes by tamoxifen in rat liver. *Biochem. Pharmacol.* **52,** 1561–1568.

Hempel, N., Barnett, A., Gamage, N., Duggleby, R. G., Windmill, K. F., Martin, J. M., and McManus, M. E. (2005). Human SULT1A SULTs. In *Human Cytosolic Sulfotransferases* (G. M. Pacifici and M. W. Coughtrie, Eds.), pp.43–61. CRC Press, Boca Raton, FL.

Hempel, N., Wang, H., LeCluyse, E. L., McManus, M. E., and Negishi, M. (2004). The human sulfotransferase SULT1A1 gene is regulated in a synergistic manner by Sp1 and GA binding protein. *Mol. Pharmacol.* **66,** 1690–1701.

Her, C., Kaur, G. K., Athwal, R. S., and Weinshilboum, R. M. (1997). Human sulfotransferase SULT 1C1: cDNA cloning, tissue-specific expression, and chromosomal localization. *Genomics* **41,** 467–470.

Hildebrandt, M. A., Salavaggione, O. E., Martin, Y. N., Flynn, H. C., Jalal, S., Wieben, E. D., and Weinshilboum, R. M. (2004). Human SULT1A3 pharmacogenetics: Gene duplication and functional genomic studies. *Biochem. Biophys. Res. Commun.* **321,** 870–878.

Jakoby, W. B., Sekura, R. D., Lyon, E. S., Marcus, C. J., and Wang, J.-L. (1980). Sulfotransferases. In *Enzymatic Basis of Detoxification* (W. B. Jakoby, Ed.), Vol. 2, pp. 199–228. Academic Press, New York.

Kakuta, Y., Pedersen, L. G., Carter, C. W., Negishi, M., and Pedersen, L. C. (1997). Crystal structure of estrogen sulphotransferase. *Nat. Struct. Biol.* **11,** 904–908.

Kakuta, Y., Petrotchenko, E. V., Pedersen, L. C., and Negishi, M. (1998). The sulfuryl transfer mechanism; crystal structure of a vanadate complex of estrogen sulfotransferase and mutational analysis. *J. Biol. Chem.* **273,** 27325–27330.

Kester, M. H., Kaptein, E., Roest, T. J., van Dijk, C. H., Tibboel, D., Meinl, W., Glatt, H., Coughtrie, M. W., and Visser, T. J. (1999). Characterization of human iodothyronine sulfotransferases. *J. Clin. Endocrinol. Metab.* **84**, 1357–1364.

Klaassen, C. D., and Boles, J. W. (1997). Sulfation and sulfotransferases; the importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J.* **11,** 404–418.

Kong, A.-N. T., Yang, L., Ma, M., Tao, D., and Bjornsson, T. D. (1992). Molecular cloning of the alcohol/hydroxysteroid form (hST_a) of sulfotransferase from human liver. *Biochem.l Biophy.l Res. Commun.* **187**, 448–454.

Lee, K. A., Fuda, H., Lee, Y. C., Negishi, M., Strott, C. A., and Pedersen, L. C. (2003). Crystal structure of human cholesterol sulfotransferase (SULT2B1b) in the presence of pregnenolone and PAP: Rationale for specificity differences between prototypical SULT2A1 and the SULT2B1 isoforms. *J. Biol. Chem.* **278,** 44593–44599.

Lin, Z., Lou, Y., and Squires, J. E. (2004). Molecular cloning and functional analysis of porcine SULT1A1 gene and its variant: A single mutation SULT1A1 causes a significant decrease in sulfation activity. *J. Mamm. Genome.* **15**, 218–226.

Maglich, J. M., Parks, D. J., Moore, L. B., Collins, J. L., Goodwin, B., Billin, A. N., Stoltz, C. A., Kliewer, S. A., Lambert, M. H., Willson, T. M., *et al.* (2003). Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes. *J. Biol. Chem.* **278,** 17277–17283.

Mammalian Gene Collection (MGC) Team. (2002). Generation and initial analysis of more than 15, 000 full-lengths human and mouse cDNA sequences. *Proc. Natl. Acad. Scien. USA.* **99**, 16899–16903.

Markovich, D. (2001). Physiological roles and regulation of mammalian sulfate transporters. *Physiol. Rev.* **81**, 1499–1533.

McManus, M. E., Minchin, R. F., Schwartz, D., Wirth, P. J., Johnson, E. F., and Thorgeirsson, S. S. (1984). Kinetics of 2-acetylaminofluorene metabolism by six highly purified forms of rabbit liver cytochrome P450. *Carcinogenesis* **5,** 1717–1724.

Meinl, W., Meerman, J. H., and Glatt, H. (2002). Differential activation of promutagens by alloenzymes of human sulfotransferase 1A2 expressed Salmonella typhimurium. *Pharmacogenetics* **12,** 677–689.

Meisheri, K. D., Johnson, G. A., and Puddington, L. (1993). Enzymatic and non-enzymatic sulfation mechanisms in the biological actions of minoxidil. *Biochem. Pharmacol.* **45**, 271–279.

Miller, J. A. (1970). Carcinogenesis by chemicals: An overview, G. H. A. Clowes memorial lecture. *Cancer Res.* **30,** 559–576.

Nagata, K., and Yamazoe, Y. (2000). Pharmacogenetics of Sulfotransferase. *Annu. Rev. Pharmacol. Toxicol.* **40,** 159–176.

Nash, A. R., Glenn, W. K., Moore, S.S., Kerr, J., Thompson, A. R., and Thompson, E. O. (1988). Oestrogen sulfotransferase: Molecular cloning and sequencing of cDNA for the bovine placental enzyme. *Australian Journal of Biological Science* **41**, 507–516.

Negishi, M., Pedersen, L. G., Petrotchenko, E., Shevtsov, S., Gorokhov, A., Kakuta, Y., and Pedersen, L. C. (2001). Structure and function of sulfotransferases. *Arch. Biochem. Biophys.* **390,** 149–157.

Otterness, D. M., Wieben, E. D., Wood, T. C., Watson, W. G., Madden, B. J., McCormick, D. J., and Weinshilboum, R. M. (1992). Human liver dehydroepiandrosterone sulfotransferase: Molecular cloning and expression of cDNA. *Mol. Pharmacol.* **41**, 865–872.

Ozawa, S., Nagata, K., Shimada, M., Ueda, M., Tsuzuki, T., Yamazoe, Y., and Kato, R. (1995). Primary structures and properties of two related forms of aryl sulfotransferases in human liver. *Pharmacogenetics* **5,** 135–140.

Pacifici, G. M., and Coughtrie, M. W. H. (Eds.) (2005). *Human Cytosolic Sulfotransferases.* CRC Press. Boca Raton, FL.

Pedersen, L. C., Petrotchenko, E., Shevtsov, S., and Negishi, M. (2002). Crystal structure of the human estrogen sulfotransferase-PAPS complex: Evidence for catalytic role of Ser137 in the sulfuryl transfer reaction. *J. Biol. Chem.* **277,** 17928–17932.

Petrotchenko, E. V., Pedersen, L. C., Borchers, C. H.,Tomer, K. B., Negishi, M., and Pedersen L. C. (2001). The dimerization motif of cytosolic sulfotransferases. *FEBS Lett*. **490,** 39–43.

Radominska, A., Comer, K. A., Zimniak, P., Falany, J., Iscan, M., and Falany, C. N. (1990). Human liver steroid sulphotransferase sulphates bile acids. *Biochem. J.* **272**, 597–604.

Raftogianis, R. B., Wood, T. C., and Weinshilboum, R. M. (1999). Human phenol sulfotransferases SULT1A2 and SULT1A1: Genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. *Biochem. Pharmacol.* **58,** 605–616.

Reiter, C., Mwaluko, G., Dunnette, J., Van Loon, J., and Weinshilboum, R. (1983). Thermolabile and thermostable human platelet phenol sulfotransferase. Substrate specificity and physical separation. *Naunyn Schmiedebergs Arch.Pharmacol.* **324**, 140–147.

Rikke, B. A., and Roy, A. K. (1996). Structural relationships among members of the mammalian sulfotransferase gene family. *Biochim. Biophys. Acta* **130,** 331–338.

Runge-Morris, M. (1998). Regulation of sulfotransferase gene expression by glucocorticoid hormones and xenobiotics in primary rat hepatocyte culture. *Chem. Biol. Interact.* **109,** 315–327.

Sakakibara, Y., Takami, Y., Zwieb, C., Naakayama, T., Suiko, M., Nakajima, H., and Liu, M.-C. (1995). Purification, characterization, and molecular cloning of a novel rat liver dopa/tyrosine sulfotransferase. *J. Biol. Chem.* **270,** 30470–30478.

Sakakibara, Y., Yanagisawa, K., Takami, Y., Nakayama, T., Suiko, M., and Liu, M. C. (1998a). Molecular cloning, expression, and functional characterization

of novel mouse sulfotransferases. *Biochem. Biophys. Res. Commun.* **247**, 681–686.

Sakakibara, Y., Yanagisawa, K., Katafuchi, J., Ringer, D. P., Takami, Y., Nakayama, T., Suiko, M., and Liu, M.-C. (1998b). Molecular cloning, expression, and characterization of novel human SULT1C sulfotransferases that catalyze the sulfonation of *N*-hydroxy-2-acetylaminofluorene. *J. Biol. Chem.* **273**, 33929–33935.

Saner, K. J., Suzuki, T., Sasano, H., Pizzey, J., Ho, C., Strauss, J. F., 3rd, Carr, B. R., and Rainey, W. E. (2005). Steroid sulfotransferase 2A1 gene transcription is regulated by steroidogenic factor 1 and GATA-6 in the human adrenal. *Mol. Endocrinol.* **19,** 184–197.

Schwartz, N. B. (2005). PAPS and sulfoconjugation. In Human cytosolic sulfotransferases (G. M. Pacifici and M. W. Coughtrie, Eds.), pp. 43-61. CRC Press, USA.

Shevtsov, S., Petrotchenko, E. V., Pedersen, L. C., and Negishi, M. (2003). Crystallographic analysis of a Hydroxylated Polychlorinated Biphenyl (OH-PCB) bound to the catalytic estrogen binding site of human sulfotransferase. *Environ. Health Perspect.* **111,** 884–888.

Tashiro, A., Sasano, H., Nishikawa, T., Yabuki, N., Muramatsu, Y., Coughtrie, M. W., Nagura, H., and Hongo, M. (2000). Expression and activity of dehydroepiandrosterone sulfotransferase in human gastric mucosa. *J. Steroid Biochem. Mol. Biol.* **72,** 149–154.

Tsoi, C., Falany, C. N., Morgenstern, R. and Swedmark, S. (2001). Identification of a new subfamily of sulphotransferases: Cloning and characterization of canine SULT1D1. *Biochem. J.* **356**, 891–897.

Vargas, F., Frerot, O., Brion, F., Trung Tuong, M.D., Lafitte, A., and Gulat-Marnay, C. (1994). 3′-Phosphoadenosine 5′-phosphosulfate biosynthesis and the sulfation of cholecystokinin by the tyrosylprotein-sulfotransferase in rat brain tissue. *Chem. Biol. Interact.* **92,** 281–291.

Veronese, M. E., Burgess, W., Zhu, X., and McManus, M. E. (1994). Functional characterization of two human sulphotransferase cDNAs that encode monoamine- and phenol-sulphating forms of phenol sulphotransferase: Substrate kinetics, thermal-stability and inhibitor- sensitivity studies. *Biochem. J.* **302,** 497–502.

Wang, J., Falany, J. L., and Falany, C. N. (1998). Expression and characterization of a novel thyroid hormone-sulfating form of cytosolic sulfotransferase from human liver. *Mol. Pharmacol.* **53,** 274–282.

Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C., and Raftogianis, R. B. (1997). Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. *FASEB J.* **11,** 3–14.

Wilborn, T. W., Comer, K. A., Dooley, T. P., Reardon, I. M., Heinrikson, R. L., and Falany, C. N. (1993). Sequence analysis and expression of the cDNA for the phenol-sulfating form of human liver phenol sulfotransferase. *Mol. Pharm.* **43**, 70–77.

Windmill, K. F., Christiansen, A., Teusner, J. T., Bhasker, C. R., Birkett, D. J., Zhu, X., and McManus, M. E. (1998). Localisation of aryl sulfotransferase expression in human tissues using hybridisation histochemistry and immunohistochemistry. *Chem. Biol. Interact.* **109,** 341–346.

Yoshinari, K., Nagata, K., Ogino, M., Fujita, K., Shiraga, T., Iwasaki, K., Hata, T., Yamazoe, Y. (1998a). Molecular cloning and expression of an amine sulfotransferse cDNA: A new gene family of cytosolic sulfotransferases in mammals. *J. Biochem.* **123**, 479–486.

Yoshinari, K., Nagata, K., Shimada, M., and Yamazoe, Y. (1998b). Molecular characterization of ST1C1-related human sulfotransferase. *Carcinogenesis* **19**, 951–953.

Yoshinari, K., Petrotchenko, E. V., Pedersen, L. C., and Negishi, M. (2001). Crystal structure-based studies of cytosolic sulfotransferase. *J. Biochem. Mol. Toxicol.* **15,** 67–75.

Zhu, X., Veronese, M. E., Bernard, C. C., Sansom, L. N., and McManus, M. E. (1993). Identification of two human brain aryl sulfotransferase cDNAs. *Biochem. Biophys. Res. Commun.* **195,** 120–127.

Zhu, X., Veronese, M. E., Iocco, P., and McManus, M. E. (1996). cDNA cloning and expression of a new form of human aryl sulfotransferase. *Int. J. Biochem. Cell Biol.* **28,** 565–571.