BIOCHEMICAL PHARMACOLOGY 77 (2009) 689–699

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Coordinate regulation of human drug-metabolizing enzymes, and conjugate transporters by the Ah receptor, pregnane X receptor and constitutive androstane receptor

Christoph Köhle, Karl Walter Bock *

Department of Toxicology, Institute of Pharmacology and Toxicology, University of Tübingen, Tübingen, Germany

ARTICLE INFO

Article history:
Received 13 March 2008
Accepted 19 May 2008

Keywords:
Ah receptor
Pregnane X receptor
Drug metabolism
Conjugate transporters
Cytochrome P450s
UDP-glucuronosyltransferases

ABSTRACT

Coordinate regulation of Phase I and II drug-metabolizing enzymes and conjugate transporters by nuclear receptors suggests that these proteins evolved to an integrated biotransformation system. Two major groups of ligand-activated nuclear receptors/xenosensors evolved: the Ah receptor (activated by aryl hydrocarbons and drugs such as omeprazole) and type 2 steroid receptors such as PXR and CAR, activated by drugs such as rifampicin, carbamazepin and phenytoin. It is increasingly recognized that there is considerable cross-talk between these xenosensors. Therefore, an attempt was made to discuss biotransformation by the Ah receptor together with that of PXR and CAR. Due to considerable species differences the emphasis is on human biotransformation. Agonists coordinately induce biotransformation due to common xenosensor-binding response elements in the regulatory region of target genes. However, whereas different groups of xenobiotics appear to more selectively stimulate CYPs (Phase I), their regulatory control largely converged in modulating Phase II metabolism and transport. Biotransformation appears to be tightly controlled to achieve efficient homeostasis of endobiotics and detoxification of dietary phytochemicals, but nuclear receptor agonists may also lead to potentially harmful drug interactions.

1. Introduction

It is increasingly recognized that cascades of Phase I and II enzymes and transporters act together to protect the body against the accumulation of potentially harmful lipid-soluble compounds. In addition to polymorphisms in the coding region, interindividual levels and activities of the involved proteins are largely determined by complex transcriptional control including actions of xenosensors such as the Ah receptor (AHR) [1,2], CAR (NR1I3) and PXR (NR1I2) [2–7]. Notably, these nuclear receptors act as multifunctional switches; for example, the AHR is involved in organ development in addition to adaptive regulation of detoxification. Previously, coordinate regulation of Phase I

* *Corresponding author*. Tel.: +49 7071 2972274; fax: +49 7071 292273.
E-mail address: bock@uni-tuebingen.de (K.W. Bock).

Abbreviations: AHR, aryl hydrocarbon receptor; ARE, antioxidant response element; BaP, benzo[*a*]pyrene; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CAR, constitutive androstane receptor; FXR, farsenoid X receptor; GR, glucocorticoid receptor; LXR, liver X receptor; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; Nrf2, nuclear factor erythroid 2-related factor; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anionic transport proteins; OST, organic anion steroid transporter; PAH, polycyclic aromatic hydrocarbon; PBREM, phenobarbital responsive enhancer module; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; UGT, UDP-glucuronosyltransferase; tBHQ, *tert* butylhydroquinone; XRE, xenobiotic response element.

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.05.020

and II enzymes by AHR and Nrf2 has been discussed, the latter transcription factor controlling a gene battery involved in protection against oxidative stress [8]. However, due to extensive cross-talk between AHR, CAR and PXR, it seemed desirable to discuss their regulation and impact on drug metabolism and transport together; for example, the AHR appears to be a target gene of PXR [4], and CAR may be regulated by the AHR [9]. They belong to different gene superfamilies; the AHR is a member of the basic helix–loop–helix PAS (Per-Arnt-Sim) family [1] whereas PXR and CAR are type 2 members of the steroid receptor family characterized by heterodimerization with the common partner RXR (NR2B1/2/3) [7]. CAR/PXR and the AHR are the key mediators of the classic phenobarbital- and 3-methylcholanthrene-type induction of microsomal drug metabolism, respectively [10–12]. The genetic basis for coordinate expression of enzymes and transporters is represented in part by common DNA binding motifs present in the regulatory region of target genes. Evolution of common binding motifs hints to considerable functional advantages for the mammalian organism by coordinate regulation of Phase I and II metabolism and transport. It is noteworthy that – in addition to up- and downregulation of target genes – these receptors also regulate basal expression of proteins [13]. Furthermore, the receptors are again under the hierarchical control of regulators [14].

Accumulating evidence suggests considerable species differences in the regulation of biotransformation between rodents and humans. Therefore, the present commentary focuses on human drug metabolism and transport. Hepatic and intestinal CYPs and UGTs as major Phase I and II enzymes together with conjugate uptake and efflux transporters are emphasized to eventually be able to compare regulatory mechanisms with pharmacokinetic data on the bioavailability of drugs and their enterohepatic circulation. Phase II metabolism necessitates transport of the resulting polar conjugates out of cells. These export transporters have been subsummized as Phase III. Conjugates can also be taken up into cells, for example, into hepatoctes for biliary excretion; these uptake processes have been termed Phase 0.

Interestingly, xenobiotic metabolizing enzymes, conjugate transporters as well as their xenosensors are also involved in biotransformation of endobiotics including bile acids, bilirubin, and thyroxin [2,5,6,15]. Expectedly, transcriptional regulatory coupling between these enzymes and transporters is tight in homeostatic control of endobiotics. Conceivably, coupling was also shaped by detoxification of dietary phytochemicals and ubiquitous contaminants to which organisms were exposed in evolution for millions of years [16]. Newly discovered drugs are accidentally handled by the same biotransformation system. The perception that drug metabolism uses an evolved biotransformation or detoxification system, may facilitate understanding of multiple interactions between endo- and xenobiotics. In the following, a brief overview of Phase I and II enzymes and of uptake and export transporters (Phases 0 and III, respectively) is given; subsequently, examples for coordinate transcriptional regulation of these proteins by nuclear receptors are discussed (Table 1). Finally, functional aspects are summarized.

Table 1 – Coordinate regulation of prototypical human xenobiotic-metabolizing enzymes and transporters by nuclear receptors

Enzyme/transporter/xenosensor	AHR	PXR/CAR
Phase I		
CYP1A1 [42,43]	+[a]	0[a]
CYP1A2 [43]	+	0
CYP1B1 [17]	+	0
CYP2B6 [7]	0	+
CYP2C9 [24,25]	0	+
CYP3A4 [44–46]	0	+
CYP4A1[47]	0	0
CYP7A1[69]	0	–
Phase II		
NQO1 [48]	+*	–
GSTA1/A2 [49]	+*	+*
UGT1A1 [50–52]	+	+
UGT1A6 [52,53]	+	+
UGT1A9 [52,54]	+	+
Export and uptake transporters		
(Phase III)		
MDR1 (ABCB1) [39,55]	±*	+
MRP2 (ABCC2) [55,56]	0	+*
MRP3 (ABCC3) [57,58]	+	+
BCRP (ABCG2) [40,55]	+*	+*
(Phase 0)		
OATP1B1 (SLCO1B1) [55,90]	–*	+*
OATP1B3 (SLCO1B3) [55,90]	–*	+*
OATP2B1 (SLCO2B1) [55,90]	–*	+
Xenosensor		
AHR [1]	–	+
PXR [4,90]	0	+
CAR [9]	+	0*

[a] Primary target genes are classified as (+) upregulated; (–) downregulated, and (0) unchanged. (±) Upregulation was only found in liver samples from a few individuals. (+*) No functional response elements have been characterized. The same is true for all downregulated genes.

2. Overview on human nuclear receptor-regulated Phase I and II enzymes and conjugate transporters

2.1. *Phase I enzymes*

CYPs (cytochrome P450 enzymes) are major Phase I enzymes, encoded by a large supergene family [16,17]. Known AHR-controlled CYPs are CYP1A1, CYP1A2 and CYP1B1. Basal expression of CYP1A1 is low, but it is markedly inducible by the AHR in hepatocytes, intestinal epithelium and in vascular endothelium. It is involved in both bioactivation of the carcinogen benzo[*a*]pyrene and in its first-pass detoxification in the intestinal epithelium [18]. CYP1A2 is constitutively expressed in liver but is also AHR-inducible [19]. CYP1B1 is expressed in extrahepatic tissues [17]. Major CYPs involved in drug metabolism are CYP2C9 and CYP3A4. CAR-inducible CYP2C9 ranks second after CYP3A4 among the most expressed CYPs in human liver. It is involved in metabolism of the antiepileptics phenytoin and mephenytoin, the antiulcer drug

omeprazole, tolbutamide, warfarin, propranolol, and diazepam [20,21].

The PXR-inducible CYP3A4 subfamily accounts for approximately 30% of total CYPs in the human liver [22]. CYP3A4 contributes to disposition of 60 frequently prescribed drugs such as immunosuppressant cyclosporine, contraceptive ethinylestradiol, antihypertensives nifedipine and nitrendipine, midazolam and erythromycin ([23,24] and references therein). Together with the closely related CYP3A5, CYP3A4 plays an important role in the metabolism of the dietary mycotoxin aflatoxin B1 [24]. In vivo, CYP3A4 activity displays at least 20-fold variation in different individuals [22]. CYP4A1 is inducible by fibrates and involved in metabolism of lipoids [17]. CYP7A1 is mostly involved in the synthesis of bile acids (discussed in Section 4.1.1).

2.2. *Phase II enzymes*

2.2.1. *UGTs*

UGTs are major Phase II enzymes, encompassing two different gene families, each encoding 9 UGT isoforms: family 1 members are encoded by the UGT1A gene locus on chromosome 2q37, and UGT2 members are encoded as a gene cluster on chromosome 4q13 [25,26]. Substrate specificity of major human hepatic UGTs (UGT1A1, 1A4, 1A6, 1A9 and UGT2B7) have been summarized [26,27]. For example, UGT1A1 is responsible for elimination of bilirubin, estrogens and drugs such as the chemotherapeutics etoposide and SN38, a major metabolite of topoisomerase inhibitor irinotecan [26]; UGT2B7 is involved in conjugation of bile acids and the majority of drugs cleared primarily by glucuronidation.

2.2.2. *Human SULTs*

Human *SULTs* have been divided into several families based on evolutionary diversion [28]. For example, SULT1A1 (encoded on chromosome 16) is involved in conjugation of iodothyronines, estrogens and simple phenols such as paracetamol. SULT2A1 (encoded on chromosome 19) is involved in conjugation of hydroxysteroids and the toxic bile acid lithocholic acid, discussed under Section 4.1.1. SULTs are often competing with UGTs for the same phenolic substrates; sulfation (or sulfonation) being generally considered the higher affinity but lower capacity pathway [28]. In addition, sulfates of steroid hormones often represent storage forms from where the free hormone can be liberated in target tissues.

2.2.3. *GSTs*

GSTs (including cytosolic, microsomal and mitochondrial enzymes) are an integral part of the biotransformation system protecting against cytotoxic electrophilic chemicals [29]. The Janus face of these enzymes is evident by the fact that on the one hand they are responsible for detoxification of electrophiles and on the other hand for resistance of tumor cells against chemotherapeutics. Major human hepatic GSTs include GSTA1 and GSTA2 located as a gene cluster on chromosome 6p12 [30]. Since the two enzymes are difficult to differentiate, they are discussed together. GSTA1/2 are involved in detoxification of electrophilic PAH and aflatoxin B1 metabolites [29] and in the activation of the immunosuppressive prodrug azathioprine [31]. Many other GSTs, including the cytosolic GSTM and P classes, are not discussed here.

2.2.4. *Quinone reductase*

Quinone reductase NQO1 (NADP(H):quinone oxidoreductase) represents a prototypical AHR- and Nrf2-controlled chemoprotective enzyme (see Section 4.2.1) [8, for references]. In detoxification of quinones by 2-electron reduction to quinols, NQO1 bypasses the formation of toxic semiquinones and prevents quinone-semiquinone-quinol redox cycles, the latter generating reactive oxygen species and oxidative stress [32]. Notably, NQO1 is also involved in bioactivation of chemotherapeutics such as mitomycin C.

2.3. *Conjugate transporters*

Transport of bile acid and bilirubin conjugates from blood for biliary excretion is a major function of the liver [33–37]. Substrates of major human hepatic uptake and export transporters are summarized in Table 2.

2.3.1. *Uptake transporters*

Major human hepatocytic transporters OATP1B1, OATP1B3, and OATP2B1 are responsible for uptake of free and conjugated bile acids, bilirubin and drugs from the blood circulation into hepatocytes [33–37]. The uptake of conjugated and unconjugated bile acids from the intestinal lumen is mediated by the bile acid-specific uptake transporter ASBT (apical, sodium-dependent bile salt transporter) which is related to the hepatic NTCP (Na^+ taurocholate cotransporter polypeptide) (Fig. 1) [33]. Recently, OSTα/β (organic anion

Table 2 – Substrates of the discussed human hepatocytic uptake and export transporters

Transporter	Substrate
OATP1B1 [34]	Bilirubin Bilirubin monoglucuronide* Bilirubin diglucuronide 17β-Estradiol glucuronide* Estrone 3-sulfate Dehydroepiandrosterone 3-sulfate Cholyl taurine Cholate
OATP1B3 [34]	Bilirubin monoglucuronide 17β-Estradiol glucuronide
MRP2 [35]	Bilirubin monoglucuronide Bilirubin diglucuronide 17β-Estradiol glucuronide Leukotriene C_4 Methotrexate Etoposide Etoposide glucuronide
MRP3 [35]	Bilirubin monoglucuronide Bilirubin diglucuronide
MRP4 [36]	Chenodeoxycholyltaurine and -glycine Ursodeoxycholyltaurine and -glycine co-transported with GSH

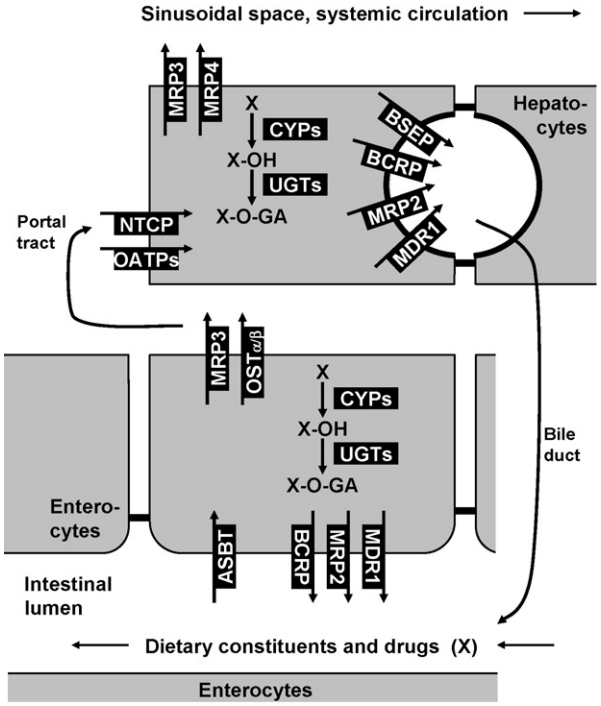

Fig. 1 – Simplified scheme of Phase I and II enzymes and conjugate transporters responsible for enterohepatic circulation of bile acids and xenobiotics/drugs. Apical and basolateral parts of the plasma membrane are separated by tight junctions. Hepatocytic NTCP, enterocytic ASBT and BSEP represent specific bile acid transporters [33].

steroid transporters) have been characterized as key basolateral transporters for reabsorption of bile acids [38].

2.3.2. *Export transporters*

The *MDR1* gene product P-glycoprotein plays an important role in the transport of unconjugated drugs from the inside to the outside of cells. It was initially discovered in cancer cells as a protein responsible for resistance against several cytotoxic drugs. MDR1 (ABCB1) is expressed in brush border membranes of mature enterocytes and canalicular membranes of hepatocytes. In the gut P-glycoprotein functions as an efflux pump transporting drugs back into the intestinal lumen. Hence, it has an important role in the absorption and presystemic elimination of drugs [39, for references].

MRP2 (ABCC2) is present in apical, *MRP3* and *MRP4* (ABCC3 and ABCC4) in basolateral plasma membranes of hepatocytes. Allelic variants leading to loss of MRP2 in hepatocytes have long been known as the Dubin-Johnson syndrome [35,36]. Recently, *BCRP* (breast cancer resistance protein, ABCG2) was found to contribute to biliary and intestinal conjugate elimination [40].

3. Coordinate regulation of drug biotransformation by AHR, CAR and PXR

Coordinate transcriptional regulation of biotransformation enzymes and transporters appears to enhance the efficiency of homeostatic control of endobiotics and detoxification of dietary xenobiotics.

Table 3 – DNA-binding response elements for AHR, Nrf2, CAR and PXR in the regulatory region of human xenobiotic-metabolizing enzymes

Receptor (typical agonist)/ enzyme	Consensus response element
AHR/XRE (TCDD)	
CYP1A1 [42,43]	TnGCGTG
UGT1A1 [51]	TnGCGTG
UGT1A6 [53]	TnGCGTG
Nrf2/ARE (tBHQ)	
NQO1 [48]	TGACnnnGC
UGT1A1 [62]	GGACnnnGC
CAR (phenobarbital)	
CYP2B6 (distal, NR3) [60]	GGGTGA (n4) ACTCTA
CYP2C9 (distal, NR1) [23]	ACCTGA (n5) ACTGGG
UGT1A1 [51]	ACTCAA (n4) ATTGGA
PXR (rifampicin)	
CYP3A4 (distal, NR1) [61]	ACTTGA (n3) ACTGGG
UGT1A1 [51]	GGTTCA (n3) AGGGTA
UGT1A6 [15]	AGTTCA (n3) AGTTCA
MDR1 [39]	ACTTGA (n4) ACTGGA
PPARα (clofibrate)	
CYP4A1[47]	AGGGTA (n1) AGTTCA
UGT1A1 [63]	AGGGTA (n1) AGTTCA
UGT1A9 [54]	AGGGTA (n1) AGTTCA

3.1. *Phase I and II drug metabolism*

Since more than 20 years it is known that groups of enzyme inducers selectively enhance the expression of the CYP 1 to 4 family members [16,17], and the responsible transcription factors have been identified as AHR, CAR, PXR, and PPARα, respectively (Table 1). Response elements in target genes have been characterized (Table 3). Since PXR and CAR frequently share common ligands and response elements they are listed together. However, despite common agonists, their mode of action is often different. For example, typical CAR activators such as phenobarbital do not bind to CAR but activate nuclear translocation by mechanisms including phosphatases [7] and the protein kinase LKB1 [41], but the true 'receptor' of phenobarbital-type inducers remains to be established [41].

Clusters of functional XREs have been characterized in the enhancer region of human CYP1A1 (Fig. 2) [42,43]. Presence of multiple XREs may allow strong dose-dependent responses upon exposure to AHR agonists. The human CYP1A locus on chromosome 15 includes both CYP1A1 and 1A2 genes in a head to head orientation. Transcription start sites are separated by approximately 20 kb of intervening DNA. This orientation raises the possibility that the genes for CYP1A1 and 1A2 share the 5′-flanking region. CYP1A1 and CYP1A2 appear to be indirectly upregulated by PXR ligands due to induction of the AHR by PXR [3]. Therefore, direct induction by PXR was classified as 0 in Table 1. In contrast to preferential induction of CYPs, conjugating enzymes and transporters appear to be regulated by both AHR and PXR/CAR.

The genetic basis of coordinate induction was identified as the interaction of transcription factors with common response

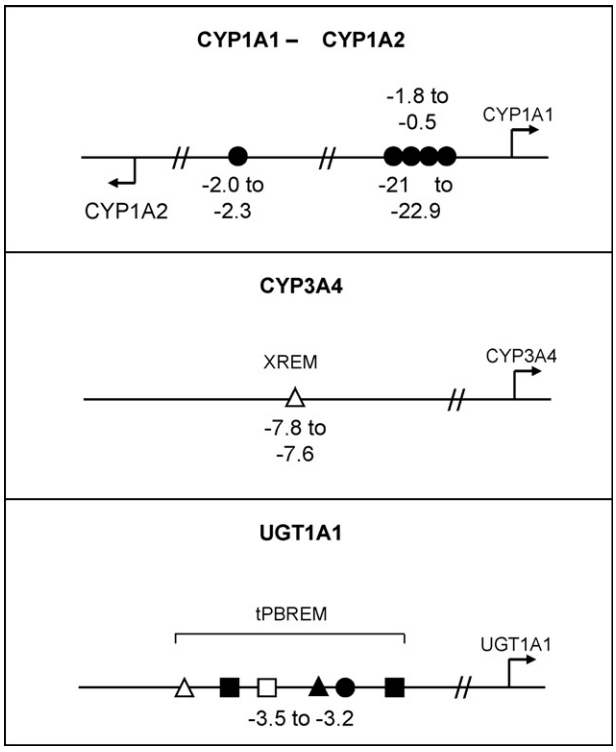

Fig. 2 – Regulation of selected Phase I and II enzymes by nuclear receptors. Illustration of the position of characterized response elements (RE) in the enhancer region of target genes. Closed circles, XRE; closed triangle, ARE; open triangles PXRE; open square, CARE; closed square, GRE. Numbers represent the distance from the transcription start site. CYP1A1 and 1A2 are encoded on chromosome 15 in a head to head orientation. Transcription start sites are separated by approximately 20 kb intervening DNA [43]. Details are described in Section 3.1.

elements in the regulatory region of target genes (Table 3). Similar to binding of AHR/Arnt to XRE domains and of Nrf2/Maf to AREs [8, for references], type 2 steroid receptors in complex with their common partner RXR bind their cognate response elements. The core elements are generally composed of two hexanucleotide half-sites, more or less degenerated with respect to the consensus sequence AGGTCA which are organized either as direct, inverted or everted repeats, separated by a number of nucleotides varying from 1 to 7 [5,6,59]. Major direct repeats (DR) responsible for binding CAR, PXR and PPARα are listed in Table 3. Proximal and distal binding sites have been identified in composite nuclear hormone receptor binding clusters. Only one major portion (e.g. NR1, responsible for CAR/RXR) is listed in Table 3. In the case of CYP2B6 proximal and distal clusters have been termed PBREM (phenobarbital response enhancer module) and XREM (xenobiotic response enhancer module), respectively [60,61]. A PBREM cluster was also detected in the 5′ flanking region of UGT1A1 (termed gtPBREM). Interestingly this 290 bp cluster contains PXR, CAR, GR [51], AHR [50], Nrf2 [62] and PPARα binding motifs [63] (Fig. 2). The cluster appears to be evolutionary conserved since a similar PBREM was characterized in the regulatory region of baboon UGT1A1 [64]. gtPBREM represents a striking example of cross-talk between multiple transcription factors. The interaction between GR and PXR/CAR has been unravelled: while these transcription factors directly bind to their cognate response elements, interaction is achieved by the cofactor GRIP1 (glucocorticoid receptor interacting factor protein-1) which binds to both GR and PXR/CAR, thereby synergistically enhancing the actions of dexamethasone and rifampicin [65]. A role for the interaction between GR and CAR has been proposed: in the neonate the sudden interruption of maternal glucose supply leads to enhanced release of glucocorticoids which could signal enhanced expression of UGT1A1 to suppress icterus neonatorum. However, many possible interactions between other transcription factors within gtPBREM still need to be explored. Notably, allelic variants served to establish functionality of response elements. For example, a polymorphism in the NR3 region of gtPBREM of UGT1A1 (T3263G) has been identified (UGT1A1*60) [66]. Together with the promoter variant $A(TA)_7TAA$ (UGT1A1*28) these allelic variants are responsible for hyperbilirubinemia in Gilbert's syndrome (see UGT website at http://som.flinders.edu.au/FUSA/ClinPharm/UGT). In addition, allelic variants of the transcription factors themselves may influence gene transcription, as shown for UGTs in the case of allelic variants of PXR [67].

3.2. *Conjugate transporters*

Evidence is accumulating that uptake and export conjugate transporters are also co-regulated by the nuclear receptors PXR/CAR and AHR [56–58]. BCRP is upregulated by both AHR and PXR/CAR [40,55]. Expectedly, transporters are also regulated by multiple other mechanisms. For example, MRP3 is upregulated in a compensatory manner when MRP2 is defective, as in the Dubin–Johnson syndrome and in cholestasis [5,6]. Interestingly (with the exception of OATP1B1 which is upregulated by PXR [55]), uptake transporters have been reported to be down-regulated by nuclear receptors [55]. Conceivably, in the case of cellular stress it may be advantageous to limit uptake of conjugates from blood to the hepatocyte.

In conclusion, members of CYP families 1 to 4 appear to be differentially induced by nuclear receptors whereas receptor control appears to have converged in the case of Phase II enzymes, such as UGT1A1 (Fig. 2), and of conjugate transporters such as BCRP (Table 1). Expression of the transcription factors is regulated in turn by multiple factors in a hierarchical order, a fascinating issue discussed under 'regulating the regulators' [14]. Furthermore, nuclear receptors operate in concert with multiple factors, including factors controlling tissue-dependent expression [68] and classic hormone receptors such as GR [52,65]. The complex interaction of all these factors is far from being understood. For example, despite coordinate regulation, the extent of Phase I and II gene induction may be quite different, depending, e.g., on the affinity of the transcription factor to response elements. In addition, there is considerable cross-talk between transcription factors, as described for GR and PXR/CAR in the gtPBREM cluster [65].

4. Functional implications of coordinate regulation

An integrated view of biotransformation is necessary when dealing with in vivo functions. For example, under physiological conditions bile acids are mainly conjugated by sulfation. Under cholestatic conditions, however, bile acid sulfation may be saturated and glucuronidation is taking over. 'Tight coupling' of Phase I and II metabolism and conjugate transport by nuclear receptors can be expected in homeostasis and detoxification of endobiotics such as bile acids and bilirubin. To some extent 'tight coupling' may also have evolved in first-pass detoxification of dietary phytoalexins, compounds to which the animal organism may have been exposed for millions of years. Expectedly, pharmaceutical drugs are also handled by the evolved biotransformation system.

4.1. *Endobiotics*

Bile acids and bilirubin are emphasized since in these cases coordinate regulation of biotransformation in their important homeostatic control has been well studied. It is understood that a number of other examples can be added in the future such as steroid hormone homeostasis and vitamin D receptor pathways.

4.1.1. *Bile acids*

Enterohepatic cycling of bile salts is an impressive example demonstrating tight regulation of metabolism and transport. To facilitate absorption of lipid-soluble compounds, bile salts and their conjugates are essential to emulsify cholesterol, phospholipids, fatty acids and lipid-soluble vitamins. Bile salts are synthesized in liver and secreted via the biliary tract into the intestine. They are efficiently reabsorbed and circulate via the portal tract to the liver. The human bile salt pool (20–40 g) circulates 6–10 times per day. However, only 0.5 g are lost through fecal excretion [33]. As illustrated in Fig. 1, enterocytic ASBT (also termed ileal bile acid transporter IBAT) is the major bile salt uptake transporter in the intestine. After basolateral efflux by the organic steroid transporter OSTα/β [38], bile salts are selectively taken up into hepatocytes by NTCP, OATP1B1, and OATP1B3, and secreted by BSEP (ABCB11) into bile. Other enterocytic transporters (MRP2 and BCRP) are mainly involved in detoxification systems secreting conjugates into the intestinal lumen [40]. In this way, enterocytic metabolism and transport often leads to decreased bioavailability of many orally administered drugs.

Bile salts acting as endogenous detergents are necessarily toxic above certain concentrations. Therefore, their synthesis and catabolism has to be strictly controlled, in particular in pathologic conditions such as cholestasis [33]. In this case PXR is activated by bile salts, such as the hepatotoxic lithocholic acid (LCA). LCA is formed from primary bile acids such as cholic acid and chenodeoxycholic acid by intestinal bacteria. PXR is a master regulator of bile salt synthesis by down-regulating CYP7A1 and by coordinately upregulating OATPs, CYP3A4, SULT2A1, UGTs and MRP2 [69–71]. In addition, monohydroxylated LCA is converted by PXR-controlled CYP3A4 to the less toxic dihydroxylated hyodeoxycholic acid which has been shown to be conjugated by UGT2B4 and UGT2B7 [26]. Hence, PXR may serve as a LCA sensor that protects against liver toxicity of LCA [69–71]. In support of these findings, treatment with rifampicin leads to increased urinary excretion of hyodeoxycholic acid glucuronide [72]. Interestingly, rifampicin has been reported to relieve pruritus, possibly by reducing serum levels of bile acids [73].

PXR acts together with FXR (farnesoid X receptor) and LXR (liver X receptor) which maintain a balanced regulation of bile acid and cholesterol metabolism [5]. The bile acid receptor FXR is mainly activated by primary bile acids such as chenodeoxycholic acid. Similar to PXR, FXR has been shown to down-regulate CYP7A1 and upregulate CYP3A4 expression [5,6]. Interestingly, the AHR may also be involved in cholesterol detoxification. 7-Ketocholesterol, formed from the corresponding oxysterol, has been characterized as an endogenous antagonist of the AHR [74].

Interestingly, bile acids not only regulate bile flow and uptake of cholesterol and fat-soluble vitamins in the intestine but also the size of the adult liver by sensing its functional capacity via FXR [75] and PXR/CAR: inadequate function enhances bile acid levels, the resulting FXR and PXR/CAR activation not only enhances bile acid detoxification by decreasing their synthesis and enhancing their degradation but also senses the functional capacity of the liver and promotes liver growth following partial hepatectomy or liver damage. Of course, the latter function occurs in combination with growth factor and cytokine responses [75].

4.1.2. *Bilirubin*

A significant amount of bilirubin is produced every day (250–400 mg in adult humans) primarily from breakdown of hemoglobin. Bilirubin is removed from the body by selective uptake into hepatocytes, presumably via OATP1B1 (formerly called SLC21A6). It is then conjugated with glucuronic acid by UGT1A1 and excreted into the biliary tract via MRP2 [5,34,35]. It has been established that CAR coordinately stimulates both the expression of UGT1A1 and of the uptake and export transporters, leading to efficient bilirubin clearance [15]. Similar to phenobarbital, bilirubin indirectly activates CAR by stimulating its nuclear translocation [76]. Bilirubin levels have to be strictly controlled since bilirubin is neurotoxic at high serum concentrations, particularly in the newborn. On the other hand, at low levels bilirubin may also act as an antioxidant. These dual actions of bilirubin may be the reason for persistence of allelic variants of UGT1A1 associated with Gilbert's syndrome ([77,78] for references). The AHR-induced CYP1A1 and CYP1A2 may also be involved in catabolism of excessive bilirubin blood levels [78].

4.1.3. *Thyroid hormones*

The prohormone L-thyroxine (T_4) is the most widely used treatment for hypothyroidism. Interindividual differences in hepatic T_4 metabolism could result in variation in the patient exposure to T_4. Whereas >70% is deiodinated to T3, T4 is also metabolized by conjugation. Under physiological conditions T_4 sulfation initiates irreversible inactivation: T_4 sulfate is very unstable because sulfation accelerates inner ring deiodination by approximately 200-fold. In contrast, T_4 glucuronide is stable and may serve as a mechanism of delivery into intracellular compartments [79]. T_4 is conjugated by several UGTs. UGT1A1

appears to be a major isoform involved, based on strong correlation with probe drugs and allelic variants [79]. T_4 is also conjugated by extrahepatic UGT1A8 and 1A10 which may be involved in first-pass metabolism. Treatment with phenobarbital, phenytoin and carbamazepine has been associated with decreased serum T_4 levels in humans. In addition to anticonvulsant phenobarbital-type inducers, the AHR may also be involved due to its role in UGT1A1 regulation; but this suggestion still needs to be confirmed. Notably, T_4 homeostasis is mainly regulated by negative feedback by the anterior pituitary gland.

4.2. *Xenobiotics*

Dietary phytochemicals to which the mammalian organism was exposed for millions of years may also have shaped the biotransformation system and its regulation by nuclear receptors. Similar considerations may be applicable to dietary PAH contaminants, particularly when animals are foraging close to habitats devastated by natural fires.

4.2.1. *Dietary phytochemicals*

Currently, extensive efforts are undertaken to prevent cancer by altering our diet in favour of fruits and vegetables and by supplementing the diet by chemopreventive phytochemicals. Multiple mechanisms have been identified by which phytochemicals may exert their chemopreventive actions. One frequently studied mechanism is the nuclear receptor-mediated induction of Phase I and II xenobiotic detoxification system. Phytochemicals have been classified as (i) selective AHR agonists such as 3,3′-diindolylmethane (DIM), a major product of the over-the counter food additive indol-3-carbinol, (ii) selective Nrf2 activators such as sulforaphane, generated from broccoli extracts, and (iii) mixed AHR/Nrf2 activators such as the flavonoid chrysin [80, for references]. The bZip transcription factor Nrf2 is normally sequestered in cytoplasm in complex with Keap1 which negatively regulates Nrf2 by facilitating its proteasomal degradation. Under conditions of oxidative/electrophilic stress, Nrf2 and Keap1 dissociate, and Nrf2 is translocated to the nucleus where it binds to AREs of target genes. Expectedly, many potential inducers are detoxified in the intestinal mucosa [81]. An interesting chemoprevention trial with broccoli extracts containing sulforaphane is carried out with residents of Quidong, People's Republic of China, who are at risk to develop hepatocellular carcinoma due to consumption of mycotoxin aflatoxin B1-contaminated foods coupled with endemic hepatitis B virus infection [82]. In addition, the population is exposed to airborne phenanthrene, the most abundant carcinogenic PAH in this area adjacent to and downwind of Shanghai (discussed in the next section). Aflatoxin B1 is mainly bioactivated by CYP1A2 and CYP3A4, and detoxified by GSTs. As part of the above randomized, placebo-controlled trial it was shown that the excretion pattern of the foodborne and airborne toxicants was approximately doubled by consumption of broccoli-extracts presumably by activating the AHR and Nrf2 [82].

4.2.2. *Dietary and airborne PAH contaminants*

Planar PAHs are known to be both ligands of the AHR and substrates of AHR-induced CYP1A1 [83]. PAHs such as benzo[*a*]pyrene (BaP), phenanthrene and pyrene are ubiquitous environmental pollutants to which humans are exposed, for example, in their diet or as components of cigarette smoke. Metabolism and carcinogenicity of the prototypical carcinogen BaP has been intensely studied [84]. As previously discussed, coordinate induction of Phase I and II enzymes by the AHR may efficiently attenuate accumulation of reactive intermediates such as BaP diolepoxides and BaP quinones [8]. Moreover, linkage of the AHR with Nrf2 may reduce oxidative stress generated by quinone–quinol redox cycles. In support of the detoxification role of AHR and Nrf2 activation, an inverse correlation was observed between the urinary excretion of phenanthrene tetraols and dithiocarbamate levels (a measure of glutathione conjugation of PAH metabolites) in broccoli-treated Quidong people [82].

4.3. *Prescription drugs*

4.3.1. *Drug–drug interactions by activation of xenosensors*

Many drug-metabolizing enzymes/transporters are subject to direct translational modification, enabling rapid changes of their activity. However, to achieve intermediate and long-term changes they are regulated by networks of transcription factors including drug-activated xenosensors. Self-medication of St. John's wort extracts as antidepressant led to serious drug interactions, such as heart transplant rejection due to loss of cyclosporin-mediated immunosuppression [85], or to unwanted pregnancy due to loss of ethinylestradiol-mediated

Table 4 – Selected examples of nuclear receptor-mediated drug interactions

Inducing drug (nuclear receptor)	Modulated enzyme/ transporter	Interaction
St. John's wort extract or hyperforin (PXR)	CYP3A4	Heart transplant rejection due to loss of cyclosporin-mediated immunosuppression [85]
Rifampicin (PXR)	CYP3A4	Loss of verapamil effect on arrhythmias [86]
	UGT1A1	Loss of cholesterol-lowering effect of ezetimibe [87]
	MRP2	Loss of morphine-mediated analgesic effect [88]
	OATP1B1	Decreased uptake of bosentan [89]
Carbamazepine (CAR)	CYP3A4	Induced clearance of warfarin and oral contraceptives [90,91]
Brussels sprouts, broccoli (AHR, Nrf2)	CYP1A2, UGTs, GSTs	Increased theophyllin clearance [84], trials on chemoprevention [82]

contraception. St. John's wort extract contains the high-affinity PXR agonist hyperforin. Treatment with the PXR agonist rifampicin led to decreased bioavailability of many drugs such as verapamil [86] or cholesterol-lowering ezetimibe [87] and morphine [88] (Table 4). Interestingly, when rifampicin-treated individuals were taking morphine orally, morphine glucuronides, formed by induced UGTs, were not increased in serum, probably due to efficient secretion of morphine glucuronides into the intestinal lumen via induced MRP2 [88]. Similarly, ezetimibe glucuronide is mostly secreted into the intestinal lumen, and entero-enteric circulation probably determines its duration of action [87]. Notably, in the interpretation of rifampicin studies it has to be kept in mind that final-day dosing may mask induction, possibly due to competitive inhibition of drug uptake by OATPs. For example, rifampicin has been shown to inhibit the uptake of the endothelin antagonist bosentan via OATP1B1 and OATP1B3 [89]. In livers of carbamazepine-treated patients, enhanced CYP3A4-mediated verapamil metabolism was clearly detectable [90]. Coordinate induction of drug-metabolizing enzymes, transporters, and nuclear receptors has been detected in carbamazepine-treated patients, and may be clinically relevant [91]. Recently, metamizole has been shown to induce CYP2B6 and CYP3A4 by a phenobarbital-type mechanism [92]. The influence of AHR activation by components of cigarette smoke has been described in many studies [84]. Ingested smoke components readily induce intestinal CYP1A1, detected in duodenal biopsies from smokers and patients treated with omeprazole, an AHR agonist [93]. AHR activation by omeprazole may be due to cross-talk between PXR/CYP3A4 and AHR [94]. In clinical trials on cancer chemoprevention a number of AhR- and Nrf2-activating phytochemicals are currently tested [80, for references]. However, their clinical significance is still unresolved.

4.3.2 *Nuclear receptors as factors responsible for interindividual variation of drug disposition*

Interindividual variation in levels and activities of drug-metabolizing enzymes and transporters represents a major challenge in drug therapy. It is due to multiple factors, including polymorphisms in both the coding and regulatory regions of biotransformation-associated genes as well as previous drug treatment, hormonal factors or pathologic conditions such as inflammation, the latter leading to decreased expression of drug-metabolizing enzymes [2]. Interindividual variation of CYP3A4-mediated midazolam metabolism was approximately 20-fold in Caucasians [19]. Interindividual variation of drug glucuronidation may also in part be due to drug treatment. UGT1A isoforms were found to be moderately induced by both AHR and PXR/CAR agonists [95–97]. In line with these observations, studies with transgenic mice expressing the entire human UGT1 locus suggest that expression of all UGT1 members is under the control of both the AHR and PXR [52]. Considerable interindividual variation of export transporter expression has also been observed; 15% of all individuals could be classified as high BSEP, MRP2, or MDR1 expressors and 1–5% as very low expressors [98]. In addition, interindividual variation in levels of nuclear receptors such as PXR and AHR are clearly detectable [4,90]: patients treated with carbamazepine showed significantly higher levels of PXR, and a trend was seen for enhanced AHR expression, possibly explaining increased CYP1A2 expression in these patients. Understanding the influence of the large variety of factors including tissue-specific factors on the expression of drug-metabolizing enzymes and transporters represents a major task for the future.

5. Conclusions

Coordinate regulation of Phase I and II drug-metabolizing enzymes and conjugate transporters by nuclear receptors PXR, CAR and AHR suggests that these proteins evolved to an integrated biotransformation system. The genetic basis for coordinate regulation may be largely due to common nuclear receptor-binding elements in the regulatory region of target genes. Previously, the AHR gene battery and its linkage with the Nrf2 gene battery was discussed, the latter protecting against oxidative stress [8]. However, considerable cross-talk exists between PXR, CAR, and the AHR. Therefore, an attempt was made to integrate PXR and AHR gene batteries. Due to considerable species differences, the discussion was restricted to human drug metabolism. Moreover, we focused on intestinal and hepatic drug metabolism to eventually be able to compare nuclear receptor actions with drug bioavailability, a widely studied in vivo pharmacokinetic parameter. The relative contribution of PXR and CAR in the induction response to different drugs is difficult to assess. Furthermore, activation of CAR by drugs is complicated by the fact that it occurs by an indirect, poorly understood mechanism [2,7]. Therefore, the discussion was mainly focused on two prototypical ligand-activated receptors PXR and AHR.

Differential induction of human CYP1 enzymes via the AHR, of CYP2B6 via CAR, CYP3A4 via PXR, and CYP4A1 via PPARα has been known for a long time. Early suggestions were substantiated by the identification of cognate response elements in their regulatory region. However, responses to AHR and PXR/CAR agonists appear to have converged in the induction of Phase II enzymes and conjugate transporters. Combined discussion of these gene batteries is warranted due to multiple mechanisms of cross-interaction between these chemosensors; for example, AHR may be a target gene of PXR [4]; PXR- and AHR-binding response elements are found in close proximity in clusters such as gtPBREM in the conserved regulatory region of both human and baboon UGT1A1 [51,64].

Tight coupling between Phase I and II enzymes and transporters attenuates the risk posed by accumulation of toxic intermediates of the metabolic pathways. Expectedly, coupling is extensive in detoxification of endobiotics such as bile acids, leading to efficient enterohepatic circulation of bile acids and their conjugates. Coupling may also have evolved in the case of dietary phytochemicals, compounds to which the mammalian organism was exposed for millions of years. Obviously, coupling is loose for newly developed drugs. In the latter case, particular care has to be taken to investigate potential generation of toxic intermediates. Recognition of drug metabolism and transport as a coordinately-regulated biological system may help to understand the accumulation of reactive intermediates depending among other factors upon the degree of Phase I and II coupling.

Acknowledgements

We thank Dietrich Keppler (DKFZ Heidelberg, Germany) for critical discussions, and apologize for often citing reviews instead of original studies to reduce the number of references.

REFERENCES

[1] Gu YZ, Hogenesch JB, Bradfield CA. The PAS superfamily: sensors of environmental and developmental signals. Annu Rev Pharmacol Toxicol 2000;40:519–61.
[2] Pascussi JM, Gerbal-Chaloin S, Duret C, Daujat-Chavanieu M, Vilarem MJ, Maurel P. The tangle of nuclear receptors that controls xenobiotic metabolism and transport: crosstalk and consequences. Annu Rev Pharmacol Toxicol 2008;48:1–32.
[3] Xie W, Evans RM. Orphan nuclear receptors: the exotics of xenobiotics. J Biol Chem 2001;276:37739–42.
[4] Maglich JM, Stolz CM, Goodwin B, Hawkins-Brown D, Moore JT, Kliewer SA. Nuclear pregnane X receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. Mol Pharmacol 2002;62:638–46.
[5] Handschin C, Meyer UA. Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR, and FXR. Arch Biochem Biophys 2005;433:387–96.
[6] Eloranta JJ, Meier PJ, Kullak-Ublick GA. Coordinate transcriptional regulation of transport and metabolism. Methods Enzymol 2005;400:511–30.
[7] Tien ES, Negishi M. Nuclear receptors CAR and PXR in the regulation of hepatic metabolism. Xenobiotica 2006;36:1152–63.
[8] Köhle C, Bock KW. Coordinate regulation of Phase I and II xenobiotic metabolism by the Ah receptor and Nrf2. Biochem Pharmacol 2007;73:1853–62.
[9] Patel RD, Hollingshead BD, Omiecinski CJ, Perdew GH. Aryl hydrocarbon receptor activation regulates constitutive androstane levels in murine and human liver. Hepatology 2007;46:209–18.
[10] Conney A, Miller EC, Miller JA. The metabolism of methylated amino azodyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. Cancer Res 1956;16:450–9.
[11] Remmer H. Die Beschleunigung des Evipanabbaues unter der Wirkung von Barbituraten. Naturwissenschaften 1958;8:189.
[12] Remmer H, Merker HJ. Drug-induced changes in the liver endoplasmic reticulum: association with drug-metabolizing enzymes. Science 1963;142:1657–8.
[13] Tijet N, Boutros PC, Moffat ID, Okey AB, Tuomisto J, Pohjanvirta R. Aryl hydrocarbon receptor regulates distinct dioxin-dependent and dioxin-independent gene batteries. Mol Pharmacol 2006;69:140–53.
[14] Harper PA, Riddick DS, Okey AB. Regulating the regulator: factors that control levels and activity of the aryl hydrocarbon receptor. Biochem Pharmacol 2006;72:267–79.
[15] Xie W, Yeuh MF, Radominska-Pandya A, Saini SPS, Negishi Y, Bottroff BS, et al. Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. Proc Natl Acad Sci USA 2003;100:4150–5.
[16] Nebert DW, Gonzalez FJ. P450 genes: structure, evolution and regulation. Annu Rev Biochem 1987;945–93.
[17] Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, et al. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 1996;6:1–42.
[18] Nebert DW, Dalton TP, Okey AB, Gonzalez FJ. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. J Biol Chem 2004;279:23847–50.
[19] Eaton DL, Gallagher EP, Bammler TK, Kunze KL. Role of cytochrome P4501A2 in chemical carcinogenesis: implications for human variability in expression and enzyme activity. Pharmacogenetics 1995;5:259–74.
[20] Ferguson SS, LeCluyse EL, Negishi M, Goldstein JA. Regulation of human CYP2C9 by the constitutive androstane receptor: discovery of a new distal binding site. Mol Pharmacol 2002;62:737–46.
[21] Gerbal-Chaloin S, Daujat M, Pascussi JM, Pichard-Garcia L, Vilarem MJ, Maurel P. Transcriptional regulation of CYP2C9 gene. J Biol Chem 2002;277:209–17.
[22] Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 1994;270:414–23.
[23] Özdemir V, Kalow W, Tang BK, Paterson AD, Walker SE, Endrenyi L, et al. Evaluation of the genetic component in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics 2000;10:373–88.
[24] Wojnowski L, Turner PC, Pedersen B, Hustert E, Brockmöller J, Mendy M, et al. Increased levels of aflatoxin-albumin adducts are associated with CYP3A5 polymorphisms in The Gambia, West Africa. Pharmacogenetics 2004;14:691–700.
[25] Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, et al. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. Pharmacogenet Genom 2005;15:677–85.
[26] Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. Annu Rev Pharmacol Toxicol 2000;40:551–616.
[27] Burchell B, Lockley DJ, Staines A, Uesawa Y, Coughtrie WH. Substrate specificity of human hepatic UDP-glucuronosyltransferases. Methods Enzymol 2005;400: 46–57.
[28] Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, et al. Human sulfotransferases and their role in chemical metabolism. Toxicol Sci 2006;90:5–22.
[29] Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2004;45:51–88.
[30] Morel F, Rauch C, Coles B, Le Ferrec E, Guillouzo A. The human glutathione transferase alpha locus: genomic organization of the gene cluster and functional characterization of the genetic polymorphism in the hGSTA1 promoter. Pharmacogenetics 2002;12:277–86.
[31] Eklund BI, Moberg M, Bergquist J, Mannervik B. Divergent activities of human glutathione transferases in the bioactivation of azathioprine. Mol Pharmacol 2006;70: 747–54.
[32] Ross D. Quinone reductases multitasking in the metabolic world. Drug Metab Rev 2004;36:639–54.
[33] Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. Physiol Rev 2003;83:633–71.
[34] Cui Y, König J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. J Biol Chem 2001;276:9626–30.
[35] König J, Nies AT, Cui Y, Leier I, Keppler D. Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity and MRP2-mediated drug resistance. Biochim Biophys Acta 1999;1461:377–94.

[36] Keppler D. Uptake and efflux transporters for conjugates in human hepatocytes. Methods Enzymol 2005;400:531–42.
[37] Kopplow K, Letschert K, König J, Walter B, Keppler D. Human hepatobiliary transport of organic anions analyzed by quadruple-transfected cells. Mol Pharmacol 2005;68:1031–8.
[38] Ballatori N, Christian WV, Lee JY, Dawson PA, Soroka CJ, Boyer JL, et al. Ostα-Ostβ: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. Hepatology 2005;42:1270–9.
[39] Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampicin. J Biol Chem 2001;276:14581–7.
[40] Ebert B, Seidel A, Lampen A. Identification of BCRP as transporter of benzo[*a*]pyrene conjugates metabolically formed in Caco-2 cells and its induction by Ah receptor agonists. Carcinogenesis 2005;26:1754–63.
[41] Blättler SM, Rencurel F, Kaufmann MR, Meyer UA. In the regulation of cytochrome P450 genes, phenobarbital targets LKB1 for necessary activation of AMP-activated protein kinase. Proc Natl Acad Sci USA 2007;104:1045–50.
[42] Kress S, Reichert J, Schwarz M. Functional analysis of the human cytochrome P450 (CYP1A1) gene enhancer. Eur J Biochem 1998;258:803–12.
[43] Ueda R, Iketaki H, Nagata K, Kimura S, Gonzalez FJ, Kusano K, et al. A common regulatory region functions bidirectionally in transcriptional activation of the human CYP1A1 and CYP1A2 genes. Mol Pharmacol 2006;69: 1924–30.
[44] Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, et al. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. Proc Natl Acad Sci USA 1998;95:12208–13.
[45] Blumberg B, Sabbagh W, Juguilon H, Bolado J, van Meter CM, Ong ES, et al. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. Genes Dev 1998;12:3195–205.
[46] Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, et al. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 1998;92:73–82.
[47] Aldridge TC, Tugwood JD, Green S. Identification and characterization of DNA elements implicated in the regulation of CYP4A1 transcription. Biochem J 1995;306:473–9.
[48] Radjendirane V, Jaiswal AK. Antioxidant response element-mediated 2,3,7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) induction of human NADP(H):quinone oxidoreductase 1 gene expression. Biochem Pharmacol 1999;58:1649–55.
[49] Morel F, Fardel O, Meyer DJ, Lanouet S, Gilmore KS, Meunier B, et al. Preferential increase of glutathione *S*-transferase class alpha transcripts in cultured human hepatocytes by phenobarbital, 3-methylcholanthrene, and dithiolthiones. Cancer Res 1993;53:2231–4.
[50] Yueh MF, Huang YH, Hiller A, Chen S, Nguyen N, Tukey RH. Involvement of the xenobiotic response element (XRE) in Ah receptor-mediated induction of human UDP-glucuronosyltransferase 1A1. J Biol Chem 2003;276:15001–6.
[51] Sugatani J, Sueyoshi T, Negishi M, Miva M. Regulation of the human UGT1A1 gene by nuclear receptors constitutive active/androstane receptor, pregnane X receptor, and glucocorticoid receptor. Methods Enzymol 2005;400:92–104.
[52] Chen S, Beaton D, Nguyen N, Senekeo-Effenberger K, Brace-Sinnokrak E, Argikar U, et al. Tissue-specific, inducible, and hormonal control of the human UDP-glucuronosyltransferase-1 (UGT1) locus. J Biol Chem 2005;280:3747–57.
[53] Münzel PA, Schmohl S, Buckler F, Jaehrling J, Raschko FT, Köhle C, et al. Contribution of the Ah receptor to phenolic antioxidant-mediated expression of human and rat UDP-glucuronosyltransferase UGT1A6 in Caco-2 and rat hepatoma 5L cells. Biochem Pharmacol 2003;66:841–7.
[54] Barbier O, Villeneuve L, Bocher V, Fontaine C, Torra PI, Duhem C, et al. The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor α and γ target gene. J Biol Chem 2003;278:13975–83.
[55] Jigorel E, Le Vee M, Boursier-Neyret C, Parmentier Y, Fardel O. Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes. Drug Metab Disp 2006;34:1756–63.
[56] Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stolz CM, et al. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J Biol Chem 2002;277:2908–15.
[57] Hitzl M, Klein K, Zanger UM, Nüssler AK, Neuhaus P, et al. Influence of omeprazole on multidrug resistance protein 3 expression in human liver. J Pharmacol Exp Ther 2003;304:524–30.
[58] Takada T, Suzuki H, Sugiyama Y. Characterization of the 5′-flanking region of human MRP3. Biochem Biophys Res Commun 2000;270:728–32.
[59] Savas Ü, Griffin KJ, Johnson EF. Molecular mechanisms of cytochrome P-450 induction by xenobiotics: an expanded role for nuclear hormone receptors. Mol Pharmacol 1999;56:851–7.
[60] Wang H, Faucette S, Sueyoshi T, Morre R, Ferguson S, Negishi M, et al. A novel distal enhancer module regulated by pregnane X receptor/constitutive androstane receptor is essential for the maximal induction of CYP2B6 gene expression. J Biol Chem 2003;278:14146–52.
[61] Goodwin B, Hodgson E, Liddle C. The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. Mol Pharmacol 1999;56:1329–39.
[62] Yueh MF, Tukey RH. Nrf2-Keap1 signaling pathway regulates human UGT1A1 expression in vitro and in transgenic UGT1 mice. J Biol Chem 2007;282:8749–58.
[63] Senekeo-Effenberger K, Chen S, Brace-Sinnokrak E, Bonzo JA, Yueh MF, Argikar U, et al. Expression of the human UGT1 locus in transgenic mice by 4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (WY-14643) and implications on drug metabolism through peroxisome proliferator-activated receptor α activation. Drug Metab Disp 2007;35:419–27.
[64] Caspersen CS, Reznik B, Weldy PL, Abildskov KM, Stark RI, Garland M. Molecular cloning of the baboon UDP-glucuronosyltransferase 1A gene family Evolution of the primate UGT1 locus and relevance for models of human drug metabolism. Pharmacogenet Genom 2007;17:11–24.
[65] Sugatani J, Nishitani S, Yamakawa K, Yoshinari K, Sueoshi T, Negishi M, et al. Transcriptional regulation of human UGT1A1 gene expression: activated glucocorticois receptor enhances constitutive androstane receptor/pregnane X receptor-mediated UDP-glucuronosyltransferase 1A1 regulation with glucocorticoid receptor interacting protein 1. Mol Pharmacol 2005;67:845–55.
[66] Sugatani J, Yamakawa K, Yoshinari K, Machida T, Takagi H, Mori M, et al. Identification of a defect in the UGT1A1 promoter and its association with hyperbilirubinemia. Biochem Biophys Res Commun 2002;292:492–7.
[67] Gardner-Stephen D, Heydel JM, Goyal A, Lu Y, Xie W, Lindblom T, et al. Human PXR variants and their differential effects on the regulation of human UDP-glucuronosyltransferase gene expresssion. Drug Metab Disp 2004;32:340–7.
[68] Gregory PA, Lewinsky RH, Gardner-Stephen, Mackenzie PI. Regulation of UDP glucuronosyltransferases in the

gastrointestinal tract. Toxicol Appl Pharmacol 2004;199:354–63.
[69] Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, Latour A, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci USA 2001;98:3369–74.
[70] Xie W, Radominska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, et al. An essential role for nuclear receptors SXR/PXR in detoxification of bile acids. Proc Natl Acad Sci USA 2001;98:3375–80.
[71] Saini SPS, Sonoda J, Xu L, Toma D, Uppal H, Mu Y, et al. A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. Mol Pharmacol 2004;65:292–300.
[72] Wietholtz H, Marschall HU, Sjövall J, Matern S. Stimulation of bile acid 6-alpha-hydroxylation by rifampin. J Hepatol 1996;24:713–8.
[73] Hoensch HP, Balzer K, Dylewicz P, Kirch W, Goebell H, Ohnhaus E. Effect of rifampicin treatment on hepatic drug metabolism and serum bile acids in patients with primary biliary cirrhosis. Eur J Clin Pharmacol 1985;28:475–7.
[74] Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Ann Rev Pharmacol Toxicol 2003;43:309–34.
[75] Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science 2006;312:233–6.
[76] Huang W, Zhang J, Chua SS, Qatanani M, Han Y, Granata R, et al. Induction of bilirubin clearance by the constitutive androstane receptor (CAR). Proc Natl Acad Sci USA 2003;100:4156–61.
[77] Köhle C, Möhrle B, Münzel PA, Schwab M, Wernet D, Badary OA, et al. Frequent co-occurrence of the TATA box mutation associated with Gilbert's syndrome (UGT1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (UGT1A6*2 and UGT1A7*3) in Caucasians and Egyptians. Biochem Pharmacol 2003;65:1521–7.
[78] Kapitulnik J. Bilirubin: an endogenous product of heme degradation with both cytotoxic and cytoprotective properties. Mol Pharmacol 2004;66:773–9.
[79] Yoder Graber AL, Ramirez J, Innocenti F, Ratain MJ. UGT1A1*28 genotype affects the in-vitro glucuronidation of thyroxine in human livers. Pharmacogenetics Genomics 2007;17:619–27.
[80] Köhle C, Bock KW. Activation of coupled Ah receptor and Nrf2 gene batteries by dietary phytochemicals in relation to chemoprevention. Biochem Pharmacol 2006;72: 795–805.
[81] Smith CM, Graham RA, Krol Wl, Silver IS, Negishi M, Wang H, et al. Differential UGT1A1 induction by chrysin in primary human hepatocytes and HepG2 cells. J Pharmacol Exp Ther 2005;315:1256–64.
[82] Kensler TW, Chen JG, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Quidong, People's Republic of China. Cancer Epidemiol Biomarkers Prev 2005;14:2605–13.
[83] Nebert D, Gelboin HV. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. J Biol Chem 1968;243:6242–9.
[84] Conney AH. Interaction of drug-metabolizing enzymes: a path to the discovery of multiple cytochromes P450. Annu Rev Pharmacol Toxicol 2003;43:1–30.
[85] Ruschitzka F, Meier PJ, Turina M, Luscher TF, Noll G. Acute heart transplant rejection due to Saint John's wort. Lancet 2000;355:548–9.
[86] Fromm MF, Dilger K, Busse D, Kroemer HK, Eichelbaum M, Klotz U. Gut wall metabolism of verapamil in older people: effects of rifampicin-mediated enzyme induction. Br J Clin Pharmacol 1998;45:247–55.
[87] Oswald S, Haenisch S, Fricke C, Sudhop T, Remmler C, Giessmann T, et al. Intestinal expression of P-glycoprotein (ABCB1), multidrug resistance associated protein 2 (ABCC2), and uridine diphosphate-glucuronosyltransferase1A1 predicts the disposition and modulates the effects of the cholesterol absorption inhibitor ezetimibe in humans. Clin Pharm Ther 2006;79:206–17.
[88] Fromm MF, Eckhardt K, Li S, Schänzle G, Hofmann U, Mikus G, et al. Loss of analgesic effect of morphine due to coadministration of rifampicin. Pain 1997;72:261–7.
[89] Treiber A, Schneiter R, Häusler S, Stieger B. Bosentan is a substrate of human OATP1B1 and OATP1B3: inhibition of hepatic uptake as a common mechanism of its interactions with cyclosporine A, rifampicin, and sildenafil. Drug Metab Disp 2007;35:1400–7.
[90] Oscarson M, Zanger UM, Rifki OK, Klein K, Eichelbaum M, Meyer UA. Transcriptional profiling of genes induced in livers of patients treated with carbamazepine. Clin Pharmacol Ther 2006;80:440–56.
[91] Wolbold R, Klein K, Burk O, Nüssler AK, Neuhaus P, Eichelbaum M, et al. Sex as a major factor of CYP3A4 expression in human liver. Hepatology 2003;38:978–88.
[92] Saussele T, Burk O, Blievernicht JK, Nussler AQ, Nussler N, et al. Selective induction of human hepatic cytochromes P450 2B6 and 3A4 by metamizole. Clin Pharmacol Ther 2007;82:265–74.
[93] Buchthal J, Grund KE, Buchmann A, Schrenk D, Beaune P, Bock KW. Induction of cytochrome P450A by smoking and omeprazole in comparison with UDP-glucuronosyltransferase in biopsies of human duodenal mucosa. Eur J Clin Pharmacol 1995;47:431–5.
[94] Gerbal-Chaloin S, Pichard-Garcia L, Fabre JM, Sa-Cunha A, Poellinger L, Maurel P, et al. Role of CYP3A4 in the regulation of the aryl hydrocarbon receptor by omeprazole sulphide. Cell Signal 2006;18:740–50.
[95] Bock KW, Wiltfang J, Blume R, Ullrich D, Bircher J. Paracetamol as a test drug to determine glucuronide formation in man Effects of inducers and of smoking. Eur J Clin Pharmacol 1987;31:677–83.
[96] Bock KW, Schrenk D, Forster A, Griese EU, Mörike K, Brockmeier D, et al. The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. Pharmacogenetics 1994;4:209–18.
[97] Soars MG, Petullo DM, Eckstein JA, Kasper SC, Wrighton SA. An assessment of UDP-glucuronosyltransferase induction using primary human hepatocytes. Drug Metab Disp 2004;32:140–8.
[98] Meier Y, Pauli-Magnus C, Zanger UM, Klein K, Schaeffeler E, Nussler AK, et al. Interindividual variability of canalicular ATP-binding cassette (ABC)-transporter expression in human liver. Hepatology 2006;44:62–74.