













Regulation of Drug Transport Proteins—From Mechanisms to Clinical Impact: A White Paper on Behalf of the International Transporter Consortium

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Membrane transport proteins are involved in the absorption, disposition, efficacy, and/or toxicity of many drugs. Numerous mechanisms (e.g., nuclear receptors, epigenetic gene regulation, microRNAs, alternative splicing, post-translational modifications, and trafficking) regulate transport protein levels, localization, and function. Various factors associated with disease, medications, and dietary constituents, for example, may alter the regulation and activity of transport proteins in the intestine, liver, kidneys, brain, lungs, placenta, and other important sites, such as tumor tissue. This white paper reviews key mechanisms and regulatory factors that alter the function of clinically relevant transport proteins involved in drug disposition. Current considerations with *in vitro* and *in vivo* models that are used to investigate transporter regulation are discussed, including strengths, limitations, and the inherent challenges in predicting the impact of changes due to regulation of one transporter on compensatory pathways and overall drug disposition. In addition, translation and scaling of *in vitro* observations to *in vivo* outcomes are considered. The importance of incorporating altered transporter regulation in modeling and simulation approaches to predict the clinical impact on drug disposition is also discussed. Regulation of transporters is highly complex and, therefore, identification of knowledge gaps will aid in directing future research to expand our understanding of clinically relevant molecular mechanisms of transporter regulation. This information is critical to the development of tools and approaches to improve therapeutic outcomes by predicting more accurately the impact of regulation-mediated changes in transporter function on drug disposition and response.

Transport proteins of the solute carrier (SLC) and ATP-binding cassette (ABC) superfamilies are widely recognized as key determinants of the absorption, distribution, and excretion of many endogenous compounds and xenobiotics, including drugs, bile acids, hormones, and nutrients, which may thereby directly or indirectly impact medication efficacy and/or safety. Not surprisingly, intersubject variability in drug response due to transporters has been attributed to altered transporter function in organs, such as the intestine, liver, and kidneys.^{1,2} Mechanisms of alterations in drug transporter activity have focused primarily on (i) differences in gene expression and/or protein abundance due to genetic polymorphisms; and (ii) drug-drug interactions (DDIs) predominantly involving direct

inhibition of transporters.¹ These factors have been reviewed previously, as detailed in numerous International Transporter Consortium White Papers (<https://www.itc-transporter.org/publications.html>). Whereas incorporation of these factors into drug disposition predictions has been successful to some extent, current knowledge of tissue protein levels and direct inhibitory DDI mechanisms do not always adequately explain clinically observed transporter-mediated DDIs, provide accurate predictions of drug disposition *in vivo*, or explain the extent of intersubject variability in drug exposure and response. Possible reasons for this may be due to alterations in mechanisms beyond genetic polymorphisms that regulate transporter expression, localization, and/or function.

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Transport proteins are synthesized in the endoplasmic reticulum, assembled in the Golgi apparatus, and translocated to the apical or basolateral plasma domain of cells where they may be localized directly in the plasma membrane or reside in intracellular storage vesicles.³ Transporters stored in intracellular compartments may be recruited “on demand” to the membrane, and cycle on and off membranes by exocytic insertion and endocytic retrieval prior to lysosomal degradation.⁴ Recently, more complex drug-transporter interactions and disease-related changes in transporter expression, localization, and function⁵ have emphasized the need for a deeper understanding of the various factors that regulate the structural modifications and requisite localization for transport activity.

For example, reduced systemic exposure of numerous organic anion transporting polypeptide (OATP) 1B1 uptake transporter substrates (e.g., rosuvastatin, pravastatin, pitavastatin, and coproporphyrin I) has been reported following multiple dose administration of the pregnane X receptor (PXR) activators rifampin and/or carbamazepine (see detailed review by Zamek-Gliszczyński *et al.*).⁶ Although no consistent effect on *SLCO1B1*/OATP1B1 mRNA or protein could be demonstrated *in vitro* or *in vivo*, and PXR does not regulate OATP1B,⁷ physiologically based pharmacokinetic (PBPK) modeling suggested that increased OATP1B1 activity could describe the clinical results. These data suggest that mechanisms other than PXR-mediated transcriptional activation of *SLCO1B1* may be involved. Unexpected clinical DDIs with some US Food and Drug Administration (FDA)-approved tyrosine kinase inhibitors (TKIs) may be explained by the recent discovery that OATP1B1 function is regulated by tyrosine kinase-mediated phosphorylation.⁸ Another intriguing example involves unexpected discrepancies between gene expression and protein levels of intestinal MDR1 P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) 2, and breast cancer resistance protein (BCRP) after chronic treatment with the PXR ligands rifampin and carbamazepine, which were associated with different expression patterns of regulatory microRNAs (miRNAs) correlating with differential effects on protein amounts of these transporters in the human intestine.⁹ Thus, in addition to well-established genetic and transcriptional regulation, epigenetic and post-transcriptional factors also may contribute to variability and poor predictability of pharmacokinetics, efficacy, as well as DDIs.¹⁰

Transporter regulation mechanisms also are important from a drug safety standpoint. For example, accurate assessment of drug-induced liver injury liability associated with bile salt export pump (BSEP) inhibition is a widely recognized challenge in drug development. BSEP inhibition is typically assessed in drug discovery using *in vitro* screening tools that lack functional cellular regulatory machinery.¹¹ Increased hepatic bile acids due to direct inhibition of BSEP activate nuclear receptors (NRs), most notably farnesoid X receptor (FXR). FXR regulates compensatory mechanisms that control bile acid homeostasis, including bile acid synthesis, qualitative and quantitative composition, and excretion by other transporters (e.g., organic solute transporter α/β (OST α/β), MRP3, and MRP4). The interplay of these factors may contribute to the low predictive accuracy for assessing drug-induced liver injury liability when only measuring direct BSEP inhibition.¹¹ These

clinically relevant examples emphasize the importance of considering the regulation of transport proteins by intrinsic and/or extrinsic factors in drug development.

The science of understanding relevant underlying mechanisms of transporter regulation is at an early stage and many of the published studies have used *in vitro* and animal models. Lack of *in vitro*-to-*in vivo* correlations and species differences in transporters and regulatory pathways may limit translation of data from these systems.¹² This white paper reviews important mechanisms that can influence the activity of transporters that are relevant in drug development with a focus on new information that may contribute to intersubject variability in transporter expression and/or function. It further highlights experimental approaches to investigate regulation mechanisms of drug transporters, including established tools and emerging techniques, as well as modeling and simulation approaches to predict the clinical impact of transporter regulation.

MECHANISMS OF TRANSPORTER REGULATION

Nuclear receptors

One of the most prominent mechanisms of transporter regulation involves NRs, which act as transcription factors. **Table 1** provides a snapshot of the importance of NRs in transporter regulation. After binding endogenous or exogenous ligands (e.g., drugs and nutrients), NRs typically dimerize with another NR (usually retinoid X receptor) and subsequently bind to specific DNA sequences (receptor-specific DNA-binding domains) to initiate transcription of multiple detoxification genes (**Figure 1a**).¹³ Generally, NR activation results in markedly increased expression and function of drug transporters and metabolizing enzymes. Whereas this mechanism is highly desirable from an evolutionary standpoint to accelerate elimination of potentially harmful xenobiotics from the human body, it causes challenges for pharmacotherapy with certain combinations of drugs. Co-administration of NR ligands (i.e., potent inducers of drug transporter expression) such as rifampin, hyperforin (the ingredient in St. John's wort that activates PXR), and carbamazepine with drug transporter substrates can result in clinically relevant DDIs due to diminished systemic or tissue drug exposure, which may reduce drug efficacy.¹⁴ With respect to transporter regulation, the NRs PXR, constitutive androstane receptor (CAR) and FXR are of high relevance. Although it is known that the activation of different NRs is associated with a receptor-specific induction profile (e.g., PXR regulates P-gp, MRP2, and cytochrome P-450 (CYP) 3A4/2C enzymes), knowledge of the actual impact of NR agonists and antagonists on transporter function in humans remains incomplete because studies have been performed *in vitro* or via indirect inference *in vivo*. Direct regulation of human drug transporters (mRNA and/or protein abundance) was demonstrated in only a few studies for the human intestine and focused on the ABC transporters P-gp and MRP2 after oral administration of PXR ligands.^{7,15} However, the underlying mechanism(s) cannot always be derived directly from such rather descriptive studies. Although there is also functional evidence from many pharmacokinetic DDI studies with NR ligands, data interpretation is complex and does not allow discrimination between transporter regulation in the intestine, liver, kidneys, or other organs.¹⁴

Table 1 Snapshot of mechanisms of regulation for drug transporters^a

Transport proteins	Nuclear receptors/ Transcription factors	Epigenetic	miRNA	Alternative splicing	Post-translational	Trafficking
MATEs						
MATE1		Methylation (CL-K)				
MATE2-K		Methylation (CL-K)				
OATs						
OAT1	HNF1 (NC-K); HNF4 (CL-K)	Methylation (PC-K)			Phosphorylation (CL-K) Glycosylation (CL) Ubiquitination (CL-K)	Glycosylation (CL) Ubiquitination (CL-K)
OAT3	HNF1 (PC-K); HNF4 (NC-K)	Methylation (PC-K)			Phosphorylation (CL-K) Glycosylation (CL) SUMOylation (CL-K)	Glycosylation (CL) SUMOylation (CL-K)
OATPs						
OATP1B1	FXR (PC-H) LXR α (PC-H) HNF4 α (PC-H)	Methylation (C-H)	CL-H	C-H	Phosphorylation (CL-H)	CL-H
OATP1B3	FXR (PC-H) HNF1 α (CL-H)	Methylation (C-H)		CL-H	Phosphorylation (PC-H)	CL-H
OATP2B1	HNF4 α (CL-H) TNF α (C-P)		CL-I CL-B, NC-B C-H	C-H, CL-H C-I		CL-I
OCTs						
OCT1	HNF1 α (CL, C-H) HNF4 α (CL-H)	Methylation (C-H)			Phosphorylation (CL-H)	
OCT2	HNF4 (NC-K)	Methylation (PC-K) Acetylation (PC-K)			Phosphorylation (CL-K) Glycosylation (CL-K)	Glycosylation (CL-K)
BCRP	AhR (CL-I), PPAR α / γ (CL-I) PPAR α (CL-B) TNF α (CL-B, C-P) EGF (PC-P) HNF2 (C-P) CAR (PC-H) PXR (PC, C-H) AhR (PC-H) NRF2 (PC-H, CL-LU)		CL-I C-P		Tyr-Phosphorylation (C-I)	CL-I, CL-LU
BSEP	FXR (PC-H)					Ubiquitination CL-H
P-gp	PXR (C-I) PXR (PC-H) CAR (PC, C-H) VDR (CL-B)	Methylation (CL-B)	CL-I; C-I CL-H, C-P, CL-LU	C-B, CL-B, CL-K	Phosphorylation (CL-K)	CL-K, CL-LU
MRPs						
MRP1	NRF2 (CL-LU)		NC-B, CL-LU		CL-LU	CL-LU
MRP2	PXR (C-I) PXR (PC-H) CAR (PC-H) NRF2 (PC-H)		CL-I CL-H			CL-H, CL-LU

Not included are single nucleotide polymorphisms, disease-related, age, gender, race, or other intrinsic/extrinsic factors. Focus is on clinically relevant findings and transporters highlighted by the ITC.² Nonclinical data are only included if human-relevant data are not available. For references, the reader is referred to [Tables 2–4](#); [Tables S1–S3](#).

B, brain; C, clinical; CL, cell line; H, hepatic; I, intestine; K, kidney; NC, nonclinical *in vivo* organ; P, placenta; PC, primary cell; LU, lung.

^aAhR, aryl hydrocarbon receptor; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CAR, constitutive androstane receptor; EGF, epidermal growth factor; FXR, farnesoid X receptor; HNF, hepatocyte nuclear factor; LXR, liver X receptor; MATE, multidrug and toxin extrusion; miRNA, microRNA; MRP, multidrug resistance-associated protein; NRF2, nuclear factor-erythroid factor 2-related factor 2; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; P-gp, MDR1 P-glycoprotein; PPAR, peroxisome proliferator-activated receptors; PXR, pregnane X receptor; TNF α , tumor necrosis factor alpha; Tyr, tyrosine; VDR, vitamin D receptor.

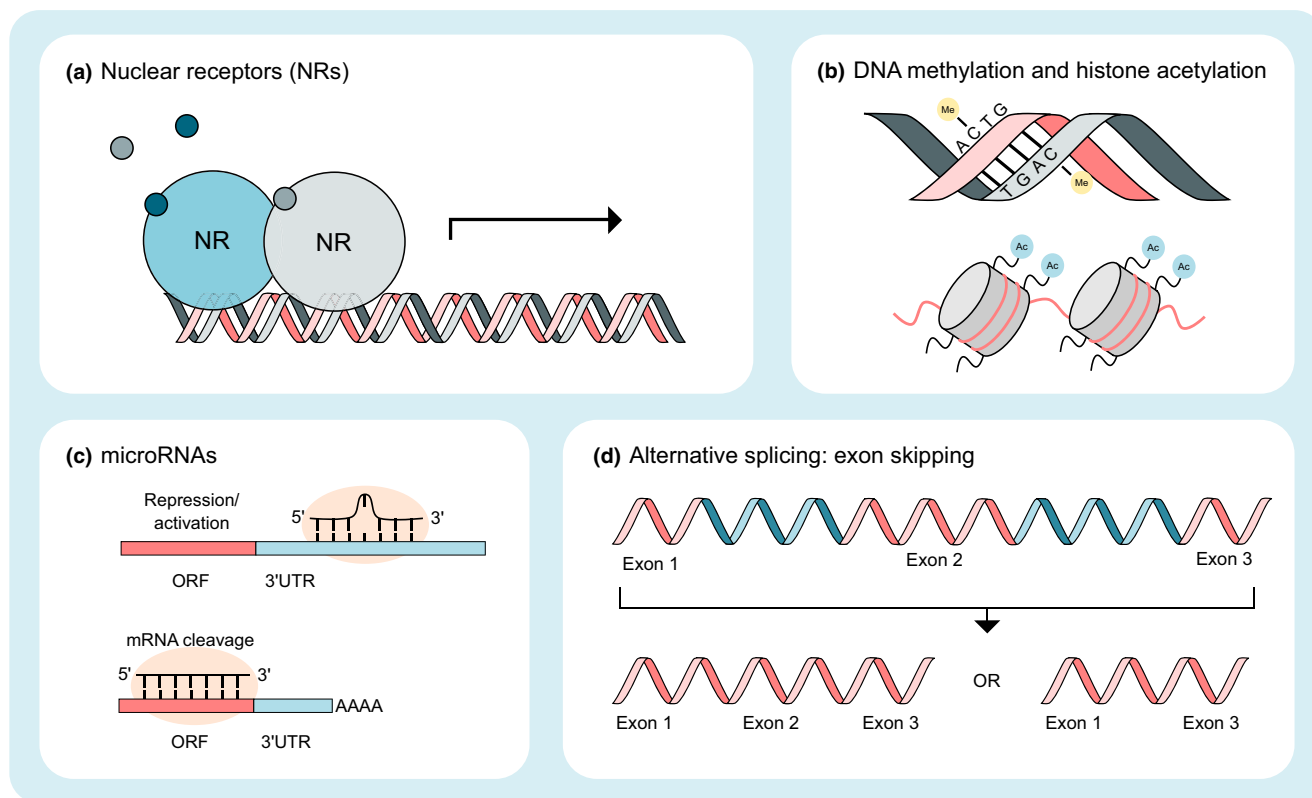


Figure 1 Mechanisms of transporter regulation. **(a)** Nuclear receptors (NRs) bind ligands and attach to specific DNA sequences, often dimerized with another NR, to initiate transcription. **(b)** DNA methylation can decrease gene expression by disturbing the binding of transcription factors or co-activators. Histone acetylation can unfold chromatin leading to a decrease in the binding affinity between histones and DNA, thereby resulting in an increase in gene expression. **(c)** microRNAs (miRNAs) are small non-coding RNAs that can suppress (or potentially activate) translation by binding to 3'-UTR regions of mRNA or initiate mRNA degradation through perfect complementarity with the mRNA. **(d)** With alternative splicing, multiple mRNAs can be produced from one gene, which can then result in different proteins. In humans, exon skipping is the most common form of alternative splicing. ORF, open reading frame; UTR, untranslated region.

Moreover, very little is known about the expression pattern of NRs in different tissues, and the distinct tissue concentrations of NR ligands, which are indispensable prerequisites to conclude that transporter regulation occurs *in vivo*. Tissue-specific NR expression profiles suggest profound differences in the induction potential of NR ligands in different organs depending on their binding affinity to the various NRs and the respective abundance of NRs and transporters.¹⁶ Based on current knowledge, PXR seems to play an important role in the clinically relevant regulation of human intestinal drug transporters, whereas in the liver, CAR and to a lesser extent PXR appear to be more relevant for DDIs involving hepatic transporter regulation.^{9,17} Hence, the route of drug administration (i.e., oral vs. intravenous) can have a profound impact on the extent of DDIs for drugs undergoing significant NR-mediated regulation (e.g., CYP3A4 metabolism and/or P-gp efflux).¹⁵

In addition to DDIs caused by drug-mediated activation of NRs, interindividual differences in the plasma concentration of endogenous NR ligands (e.g., hormones and bile acids) may contribute to clinical variability in transporter expression and function. For example, in cholestatic liver diseases, intrahepatic bile acid accumulation leads to FXR and PXR activation and to significant changes in hepatic transporter expression.⁵ Likewise, lifestyle factors, including diet, alcohol consumption, and smoking, can

affect transporter expression and function.¹⁸ However, for several transport proteins, including BCRP, organic cation transporter (OCT) 1, OCT3, peptide transporter (PEPT) 1, and OATP2B1, although some evidence for transcriptional regulation exists based on results obtained in cell lines (Tables 1–4), the *in vivo* regulation by NRs remains uncertain and requires further research.

Epigenetic gene regulation

Epigenetics is defined as heritable variation of the expression of a gene that is not caused by changes in the DNA sequence. Epigenetic mechanisms, such as DNA methylation and histone acetylation, are also known to regulate transporters (Figure 1b). The process of DNA methylation causes the addition of methyl groups to the DNA strands, and predominately occurs at the dinucleotide CpG sequences in the promoter region of a gene. As a result, transcription of the corresponding gene may be inhibited.¹⁹ For example, the expression of the uptake transporter OCTN1 in acute myeloid leukemia cell lines and potentially in patients is dependent on a DNA methylation-based mechanism that is sensitive to modulation by hypomethylating agents such as cytarabine (see reference 150 in Table S1). Histone acetylation and deacetylation primarily occur at lysine residues at the N-terminus of histones that are enriched around the transcription start site and are

associated with gene activation and inactivation, respectively.²⁰ Histone deacetylation, in addition to DNA hypermethylation, has been found to be relevant for repression of *SLC22A2* (encoding OCT2) in renal cell carcinoma (RCC).²¹ This may be particularly relevant in the case of treatment of patients with platinum compounds, such as the OCT2 substrate oxaliplatin. In RCC cell lines, the DNA methylation inhibitor decitabine and the histone deacetylase inhibitor vorinostat increased OCT2 abundance, which sensitized the cells to oxaliplatin.²¹

Post-transcriptional regulation by miRNAs

In addition to transcriptional regulation by NRs, post-transcriptional regulation by miRNAs—a form of epigenetics—may be another source of interindividual variability in the expression and function of drug transporters (Table 1). The miRNAs are small non-coding RNAs that bind to certain mRNA molecules, thereby initiating their degradation or blocking subsequent translation processes (Figure 1c).¹⁰ One example highlighting the potential relevance of miRNAs and drug transporters relates to the differences in the protein abundance of P-gp, PEPT1, and MRP3 along the length of the human intestine, which correlated with the expression of regulatory miRNAs.²² Therefore, environmental factors that affect the expression pattern of miRNAs, such as smoking, diet, and certain drugs, may result in modified transporter protein abundance.¹⁰ Moreover, pathological conditions, such as cancer or inflammatory diseases, have been associated with changes in the expression pattern of transporter-regulating miRNAs, and, in turn, with changes in the mRNA expression and/or protein abundance of the respective transporters.^{23,24} Furthermore, the highly variable expression of NRs was attributed not only to genetic factors,²⁵ but also to miRNA regulation.⁹ *Vice versa*, NR ligands also demonstrated significant induction of miRNA expression, indicating a complex coordinated network between the different regulatory factors.²⁶ This may also explain, in part, the observed differences in the induction properties between different NR ligands (e.g., rifampin vs. carbamazepine) that cause different induction patterns of regulatory miRNAs.⁹

Alternative splicing

RNA splicing refers to the editing process of precursor mRNA transcripts to form mature mRNAs. Alternative splicing, the process where differential splicing can produce multiple mRNAs from one gene, results in proteins that potentially can have different functions.²⁷ In humans, the most prevalent form of alternative splicing is exon skipping (Figure 1d, Table 1). For *ABCB11* (encoding BSEP), patients with the c.1445A>G variant develop PFIC-2 due to the use of a cryptic splice site in exon 14 resulting in the insertion of 14 new amino acids and a premature stop codon.²⁸ Exon skipping or aberrant splicing in *ABCC2* (encoding MRP2) has been reported to result in Dubin-Johnson syndrome.²⁹ For *ABCB1* (encoding P-gp), alternative splicing in intron 27 resulted in significant 24-hour oscillation in P-gp protein levels in human renal proximal tubular epithelial cells and adenosine deaminase acting on RNA expression correlated with the alternative splicing event.³⁰ Whether this effect will translate to humans is not clear yet.

Alternative splicing in SLC transporters is common. In *SLC22A1* (encoding OCT1), multiple spliced isoforms generated by exon skipping have been identified, several of which result in the loss of transporter function or poor outcomes in patients with cancer treated with imatinib or sorafenib.³¹ A study investigating alternative splicing of the *SLCO1B1* gene (encoding OATP1B1) as a function of age revealed that alternative splicing occurred commonly in pediatric liver tissue. Most of the splice variants, however, were predicted to code for truncated forms that may lack transporter activity or to translate into the same amino acid sequence as the reference isoform of OATP1B1 (Table 3).³² An interesting example of alternative splicing resulting in a new transporter protein is OATP1B3-1B7, which is the combination product of splicing of exons encoding *SLCO1B3* and the pseudo gene *SLCO1B7*.³³ The result is a protein with 12 transmembrane domains mostly localized in the endoplasmic reticulum. Whether OATP1B3-1B7 is of clinical relevance remains to be tested in pharmacogenetic studies.³⁴

Nuclear factor kappa B (NF-κB) and inflammatory responses

Signaling pathways underlying stress-induced regulation of transporters are complex and not well-understood. For instance, in isolated rat blood-brain-barrier (BBB) capillaries, protein levels of efflux transporters were upregulated in response to chronic inflammation, oxidative stress, DNA damage, and seizures.³⁵ Each of these stimuli activates distinct multistep signaling cascades leading to activation of the transcription factor NF-κB resulting in increased P-gp protein levels. Conversely, inhibition of NF-κB activation or nuclear translocation can block increased P-gp activity following exposure to pro-inflammatory cytokines, such as tumor necrosis factor alpha. Interestingly, the effect of NF-κB-mediated stimulation of P-gp expression in the rat brain is different from the mouse liver, where inhibition of NF-κB was associated with an attenuation of the endotoxin-mediated decrease in *Abc* and *Slco* transporter expression.³⁶ Although acute inflammation is known to downregulate *Pxr* in mice, this does not seem to explain the endotoxin-mediated downregulation of transporters.³⁶ Whether the effects of NF-κB are due to a direct or indirect effect on transporter gene transcription is unclear. Of note, it is not known whether the mechanistic studies conducted in rodent models to understand the effect of stress and inflammation on transporters translate to humans.

Post-translational modifications

The most common post-translational modifications (PTMs) involved in transporter regulation include phosphorylation, glycosylation, ubiquitination, SUMOylation, acetylation, and palmitoylation. A comprehensive review on this topic was published recently.³⁷ PTMs can modulate transporter function, expression, efficiency, structure, and trafficking, as described below.

Phosphorylation without changes in membrane expression can result in an on/off switch as has been demonstrated for OCT2.³⁸ Clinically, this could be relevant in patients treated with cisplatin, because TKIs, such as dasatinib, reduced OCT2 function in both cell lines and in kidney tissue.³⁹ As discussed later in this review, TKIs also affect the function of other transporters.

N-glycosylation of transporters occurs on extracellular asparagine residues.³⁷ Although the role of glycosylation is unclear in most cases, evidence suggests that it affects resistance against proteases and, therefore, protein stability. Interestingly, differential glycosylation of MRP4 affected substrate specificity.⁴⁰ In OATP1B1, disruption of all glycosylation sites resulted in lower protein stability with reduced total protein levels. Non-glycosylated OATP1B1 was detected in the endoplasmic reticulum and not in the plasma membrane.⁴¹ *N*-glycosylation plays three roles in the functional expression of SLC26A2 and SLC26A3 proteins: to retain misfolded proteins in the endoplasmic reticulum, to stabilize the protein at the cell surface, and to maintain the transport protein in a functional state.⁴²

Ubiquitination is a common mechanism resulting in degradation of SLC and ABC transporters, and the balance between ubiquitination and de-ubiquitination may regulate transporter activity in the plasma membrane. For instance, ubiquitination has been implicated in P-gp degradation in cancer cell lines and this may lead to reduced resistance to chemotherapeutic drugs.⁴³ Small ubiquitin-like modifier (SUMO) is a small protein that, like ubiquitin, is conjugated to a lysine residue. However, SUMO modification does not cause degradation but rather internalization, and can even result in elevated protein levels as was shown for the glucose transporter (GLUT) 4.⁴⁴ Mrp2 can also undergo SUMO modification, which decreased expression, although it was not clear whether expression at the cell surface was altered.⁴⁵

Palmitoylation can occur at cysteine residues close to the protein-lipid interface and is highly dynamic. By association with lipid rafts, it has been hypothesized that palmitoylation can result in rapid endocytosis or increased transporter efficiency by transporter clustering.³⁷ This mechanism has been described for SLC and ABC transporters involved in the translocation of cholesterol, but it is not clear whether this mechanism is relevant for drug transporters.

Trafficking

Many mechanisms regulate the movement of mature transport proteins from the site of synthesis to the plasma membrane, cycling on and off the membrane, and storage in intracellular compartments, in some cases for ready-response when needed.⁴ Misfolded proteins are retained in the endoplasmic reticulum prior to undergoing degradation. Properly folded proteins are transported from the endoplasmic reticulum to the Golgi apparatus for further processing and covalent modification, followed by packaging into secretory vesicles and transport to the plasma membrane. *N*-linked glycosylation on asparagine residues in the large extracellular loops of some organic anion transporters (OATs) and OATPs,⁴⁶ as well as sodium taurocholate cotransporting polypeptide (NTCP)³⁷ control membrane targeting. However, *N*-linked glycosylation of asparagine 596 was not essential for routing of BCRP to the plasma membrane.⁴⁷ In the case of P-gp, *N*-glycosylation did not directly affect drug transport capacity, but appeared to improve the routing or sorting efficiency of the protein en route to the plasma membrane, or to affect the stability of the protein, either by protection from proteolysis or by facilitating/stabilizing correct protein folding.⁴⁸ In interpreting effects

of glycosylation, it is important to distinguish between effects of *N*-glycosylation on transport protein stability vs. proper routing. Alterations in the Golgi complex by colchicine, a microtubule disruptor, or in the glycosylation status of transporters, can increase intracellular localization of these proteins leading to decreased transporter activity. For example, Western blot analysis revealed that protein amounts of non-glycosylated OATP1B1, OATP1B3, OATP2B1, NTCP, and MRP2, representing nonfunctional proteins, were increased in liver tissue from patients with nonalcoholic steatohepatitis.⁴⁹ Phosphorylation of transporters (either directly or indirectly) catalyzed by kinases, and dephosphorylation by protein phosphatases, can rapidly regulate transporter function by causing internalization or reinsertion of protein into the membrane.³⁷ Protein kinase C (PKC) activation triggered OAT1 and OATP2B1 internalization from the surface of COS-7 cells to intracellular compartments and reduced transporter activity.^{50,51} PKC often initiates cellular signaling that involves other PTMs, as demonstrated for OAT1 where PKC-mediated internalization occurred through ubiquitination.^{37,50} Ubiquitination may also serve as a sorting signal to modulate movement to endosomal compartments, as shown for BSEP, and for lysosomal degradation of internalized MRP2.⁵²

Several molecular partners that interact directly or indirectly with transporters clearly play a role in trafficking of some transporters, which may impact function. For example, p38 mitogen-activated protein kinase, protein kinase A (PKA), PKC, proto-oncogene serine/threonine-protein kinase, and phosphoinositide 3-kinase (PI3K) are important for ABC transporters, and specific inhibitors of these kinases decreased protein levels of some ABC transporters in the canalicular membrane of hepatocytes.⁵³ In addition, small GTPases, class V myosins, and adaptor proteins regulate canalicular ABC transporter endocytosis and recycling. Rab11a and myosin Vb regulate recycling of BSEP from the subapical compartment to the bile canalicular membrane, and disruption of the actin cytoskeleton or microtubules can inhibit BSEP trafficking.⁵³ Ezrin, radixin, and moesin are scaffolding proteins that attach actin filaments to the plasma membrane and serve as anchors for transporters. Cytoskeletal proteins are important regulators of hepatic transporter function, as shown in radixin-knockout mice that developed conjugated hyperbilirubinemia and liver injury due to loss of canalicular Mrp2.⁵⁴ Ezrin, radixin, and moesin proteins have PDZ binding domains that play a role in stabilization, proper membrane localization, and regulation of proteins, including MRP2 and MRP4.⁵⁴

Mechanisms regulating transporter trafficking are, by nature, complex and difficult to precisely evaluate *in vivo*. In part, this is due to lack of specific activators or inhibitors of signaling pathways and transporters. In addition, inhibition of these processes *in vivo* may result in toxicity. Direct inhibition of transporters by modulators, and the compensatory mechanisms that influence overall disposition of transporter substrates, often confound data interpretation. Recognizing potential alterations in transporter trafficking by drugs, disease, and other intrinsic or extrinsic factors that regulate these processes is imperative to understand the potential impact on drug disposition.

TRANSPORTER PROTEIN REGULATION IN ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION ORGANS

Intestine

P-gp, MRP2, MRP3, BCRP, OATP2B1, PEPT1, and apical sodium bile acid transporter (ASBT) are functionally relevant in the human intestine.² Examples of intestinal transporter regulation are provided below with additional details summarized in **Tables 1 and 2**; **Table S1**.

Transcriptional regulation. One of the best understood mechanisms in terms of intestinal transporter regulation from a clinical perspective involves NRs (**Tables 1 and 2**). Human small intestinal tissue shows high expression of PXR followed by FXR, but only traces of CAR.¹⁷ Although transporters are expected to be inducible by respective ligands, PXR-regulated transporters in the intestine (i.e., P-gp and MRP2) may be most likely to cause clinically relevant DDIs after oral drug administration.¹⁴ Similarly, bile acids regulate the expression of ASBT, OST α/β , and MRP3 via FXR activation.

Several studies investigated transporter expression in intestinal biopsies taken from the upper small intestine of healthy volunteers after dosing with prototypical inducers such as rifampin (PXR ligand), carbamazepine (PXR and CAR ligand), or efavirenz (CAR ligand). Potent induction of mRNA and protein was demonstrated for intestinal P-gp and MRP2 after treatment with rifampin for up to 9 days, resulting in a significant decrease in the oral bioavailability of P-gp and MRP2 substrates (**Table 2**).⁵⁵ Interestingly, intestinal BCRP abundance was not affected by rifampin, carbamazepine, or efavirenz treatment, suggesting that it was not regulated by either intestinal PXR or CAR.^{9,56} Evidence from expression and functional *in vitro* studies suggests that aryl hydrocarbon receptor (*Ahr*), peroxisome proliferator-activated receptor (PPAR) α and/or PPAR γ may be involved in the regulation of BCRP,^{57,58} but significance in humans has not been demonstrated.

Epigenetic/post-transcriptional regulation. Many miRNAs known to regulate drug transporters are expressed in the intestine and thus could affect proteins involved in drug absorption.²⁴ However, there are limited *in vivo* and *in vitro* data characterizing miRNA-mediated transporter regulation in intestine-derived cells or tissue. Differences in miRNA levels in tissue samples from multiple intestinal regions have been linked to regional transporter protein levels.²² For example, miR-27a-3p and miR-409-3p regulate *ABCB1* and their elevated expression in the colon coincides with significantly lower P-gp protein levels compared with the small intestine. Alternative polyadenylation, resulting in different lengths of 3'-UTR regions, as detected for *ABCC1*, *ABCC2*, and *ABCC3* in cell lines and tissues, could affect tissue-dependent regulation: for example, *ABCC2* with the shortest 3'-UTR was not regulated by miR-397, as shown in a luciferase gene reporter assay in HepG2 cells.⁵⁹ Similarly, in colorectal cancer, *ABCG2* 3'-UTR length was shown to affect *ABCG2* regulation mediated by the interplay of miR-519c and RNA binding protein human antigen R.⁶⁰

The role of miRNAs in drug-mediated transporter induction has been studied in human duodenal biopsy samples.⁹ A striking disconnect between the induction of *ABCB1* and *ABCC2* mRNA but no significant increase in P-gp or MRP2 protein abundance was reported after carbamazepine treatment. Levels of several miRNAs were increased by rifampin and carbamazepine treatment and inversely correlated with P-gp, MRP2, and BCRP abundance or protein/mRNA ratios. It is hypothesized that post-transcriptional regulation by miRNAs, which bind to *ABCB1* and *ABCC2* mRNA and block translation of the respective proteins, may help explain this discrepancy and suggests the involvement of NR-independent pathways in transcriptional regulation by some NR ligands. Additional studies are needed to confirm these interesting hypotheses and observations. Furthermore, epigenetics may affect NR expression, in turn influencing NR-mediated regulation of intestinal transporters.⁹

Post-translational regulation. Mutagenesis of asparagine residues increased murine PEPT1 activity, indicating that *N*-glycosylation can affect the function of this intestinal transporter.⁶¹ Mutagenesis of the cysteine residues in OATP2B1 also led to misprocessing and intracellular accumulation in CHO-OATP2B1 cells.⁶² Similarly, *S*-acylated ASBT, the predominant form in ileal brush border membrane vesicles derived from human donor ileum, is important for activity, plasma membrane localization, and protection from degradation.⁶³ Localization of transporters like P-gp and BCRP in plasma membrane "lipid rafts" impacts efflux activity⁶⁴; for example, depletion of cellular cholesterol content by methyl-beta-cyclodextrin caused a 40% decrease in BCRP activity in MDCKII-BCRP cells.⁶⁴ The activation of PKC accelerated OATP2B1 internalization via the clathrin-dependent pathway and subsequent lysosomal degradation in MDCKII-OATP2B1 and Caco-2 cell monolayers.^{51,65}

Taken together, transporter regulation via intestinal NR activation has resulted in clinically relevant DDIs and intersubject variability in the efficacy and safety of drugs. In contrast, whereas exemplar studies show the effects of PTMs and localization on intestinal drug transporter activity, these have been conducted mostly *in vitro* and, therefore, require further investigation to document *in vivo* relevance. Similarly, miRNA-mediated transporter regulation has been demonstrated mostly *in vitro*, but the limited *in vivo* data may provide an explanation for the tissue specific expression of transporters.

Liver

Hepatic transport proteins of clinical relevance include OATP1B1, OATP1B3, BCRP, P-gp, OCT1, OATP2B1, BSEP, and MRP2.² Examples of clinically relevant regulation of hepatic transporters are discussed below with detailed information summarized in **Tables 1 and 3**; **Table S2**.

Transcriptional regulation. The mRNA of hepatic transporters can be induced by NRs, including liver X receptor (LXR) (*SLCO1B1* and *ABCC2*), FXR (*SLCO1B1*, *SLCO1B3*, *ABCC2*, *ABCB11*, *SLC10A1*, and *SLC51A/B*), CAR (*ABCB1*, *ABCG2*, *ABCC2*,

Table 2 Mechanisms of regulation for transporters in the intestine^a

Transport proteins	Mechanism	Model system	Agonist/Causes	mRNA	Protein	Activity	Reference
BCRP	PXR	Duodenal biopsy (N = 12)	Rifampin (600 mg, 6 days)	↔	↔	ND	9
	PXR/CAR	Duodenal biopsy (N = 7)	Carbamazepine (600 mg, 14–18 days)	↑	↔	ND	9
	AhR	Caco-2	Various AhR ligands	↑↑	↑	Apical transport of benzo[a]pyrene-sulfate ↑	57
	DNA methylation, miRNA (indirect)	Colon carcinoma and adjacent “normal” tissue, colon (cancer) cell lines	DNA methyltransferase DNMT3b, siRNA miRNA-203 (indirect)	↑↑	ND	↑↑	119
	miRNA and RNA binding protein	S1 and S1M1-80, Caco-2, HT-29, and SW-620 cells	miRNA-519c	↓	↓↓	↓↓	60
	Localization	HT-29 and Caco-2	Uric acid (6 or 8 mg/dL)	↑	↑	↑	120
	Post-translational	Colon biopsy from patients with chronic low-grade inflammation–associated obesity	Loss of tyrosine phosphorylation (Janus kinase 3)	ND	↓↓	ND	121
MRP2	PXR	Duodenal biopsy (N = 16)	Rifampin (600 mg, 9 days)	↑	↑	ND	122
	PXR/CAR	Duodenal biopsy (N = 7)	Carbamazepine (600 mg, 14–18 days)	↑↑	↔	ND	9
OATP2B1	miRNA	Caco-2 and HEK-OATP2B1	miRNA-24 mimic	Caco-2: ↓↓ HEK-OATP2B1: ↓	Caco-2: ↓↓ HEK-OATP2B1: ↓↓	Caco-2: ↔ HEK-OATP2B1: ↓	123
	Post-translational, internalization	MDCKII-OATP2B1 Caco-2	Phorbol 12-myristate 13-acetate induced PKC activation	ND	↓	↓	51
	Localization	HEK-OATP2B1 Caco-2	Amiodarone Rutin Insulin (via Rab1)	ND	Plasma membrane ↑ Total ↔	↑	124
P-gp	PXR	Duodenal biopsy	Rifampin (600 mg, 10 days)	ND	↑↑	↑	125
	PXR	Duodenal biopsy	Rifampin (600 mg, 9 days)	↑	↑↑	AUC/C _{max} of talinolol reduced by 35%/38%; ↑	126
	PXR/CAR	Duodenal biopsy (N = 7)	Carbamazepine (600 mg, 14–18 days)	↑↑	↔	ND	9
	miRNA	Intestinal tissue, luciferase reporter assay (HepG2)	miRNA-27a-3p and miRNA-409-3p	ND	↓	ND	22

↑, < 2-fold increase; ↑↑, ≥ 2-fold increase; ↓, < 2-fold decrease; ↓↓, ≥ 2-fold decrease; ↔, no change; ND, not determined.

^aAhR, aryl hydrocarbon receptor; AUC, area under the curve; BCRP, breast cancer resistance protein; Caco-2, human colorectal adenocarcinoma cell; CAR, constitutive androstane receptor; C_{max}, maximum concentration; HEK, human embryonic kidney; HT-29, human colonic adenocarcinoma cell line; MDCK, Madin-Darby canine kidney; miRNA, microRNA; MRP, multidrug resistance-associated protein; OATP, organic anion transporting polypeptide; P-gp, P-glycoprotein; PKC, protein kinase C; PXR, pregnane X receptor; siRNA, small interfering RNA; S1, human colon cancer cell line S1; S1M1-80, mitoxantrone resistant S1 cell line derivative; SW-620, human colonic adenocarcinoma cell line.

and *ABCC4*), and PXR (*ABCG2*, *ABCB1*, and *ABCC2*; **Table 3**, **Table S2**). The induction of *ABCB11* and *SLC51A/B*, and increased bile acid transport, have been reported in sandwich-

cultured human hepatocytes by the FXR agonist obeticholic acid, at clinically relevant concentrations, and the endogenous bile acid chenodeoxycholic acid.⁶⁶

Table 3 Mechanisms of regulation for transporters in the liver^a

Transport proteins	Mechanism	Model system	Agonist/Causes	mRNA	Protein	Activity	Reference
OATP1B1	HNF4 α	HH	HNF4 α siRNA	↓ ↓	ND	ND	127
	LXR α	Huh7 HH	T0901317 GW3965	↑ ↑	Huh7, ND HH, Relative Increase	Huh7 (T0901317, GW3965), ↑ ↑ HH (T091317), ↑ ↑	68
	PXR	HH	Rifampin	↔ or ↑ ↑	ND	ND	7 70
	FXR	Huh7 HH	CDCA GW4064 Fexaramine	↑ ↑	HH, CDCA, Relative Increase	Huh7, ↑ HH, CDCA, ↑ ↑	68
	miRNA	Huh7 Human liver tissue Chang liver cells	miRNA-206 miRNA-511	↓ ↓	↓	↓; ND	71, additional references in Table S2
	Epigenetics (DNA methylation)	Human liver tissue	Hypomethylated regions in liver were identified around the transcriptional start site	ND	ND	ND	74
	LYN kinase-mediated tyrosine phosphorylation	HEK-OATP1B1	Nilotinib	ND	ND	↓ ↓	8
	PKC	HEK-OATP1B1	Phorbol 12-myristate 13-acetate	ND	Plasma membrane ↓	↓ ↓	128
	Alternative splicing	Postmortem human livers	Alternative splicing of gene occurs frequently in children	ND	ND	ND	32
	OATP1B3	FXR	HH HG2 Huh7	CDCA	↑ ↑	ND	ND
HNF1 α		HG2	Overexpression HNF1 α	Promoter ↑ ↑	ND	ND	129
PKC		HH	Phorbol 12-myristate 13-acetate	↔	Phosphorylated protein ↑	↓ ↓	76
Epigenetics (methylation)		HG2	5-aza-2'-deoxycytidine (DNA methylation inhibitor)	↑ ↑	ND	ND	130
Alternative splicing		Tumor specific OATP1B3 variant	Lacking N-terminal 28 amino acids	ND	Abundance in plasma membrane ↓; predominantly cytoplasmic expression	↓ ↓	73
OATP2B1	HNF4 α	Huh7	Overexpression of HNF4 α siRNA	Liver-enriched OATP2B1 mRNA variant ↓	ND	ND	131
	Post-translational internalization	MDCKII	Phorbol 12-myristate 13-acetate induced PKC activation	ND	Plasma membrane ↓ ↓	ND	51

(Continued)

Table 3 (Continued)

Transport proteins	Mechanism	Model system	Agonist/Causes	mRNA	Protein	Activity	Reference
	miRNA	Human liver HG2 HepaRG	miRNA-24	Human liver: expression level of miRNA-24 negatively correlated with OATP2B1 mRNA HG2: promoter activity ↓ ↓ ↓; HG2 and HepaRG: mRNA ↓ ↓ ↓	Human liver: expression level of miRNA- 24 negatively correlated with OATP2B1 protein HG2: ND HepaRG: ↓ ↓ ↓	ND	72
OCT1	HNF4α	HepaRG	HNF4α siRNA	↓ ↓	ND	ND	31 and Table S2
	HNF1α	HG2 Huh7 Human liver samples (correlation study)	In silico assay and expression correlation study	Promoter activity; correlation between HNF1 and OCT1 mRNA expression ↑	ND	ND	132
	Epigenetic (methylation)	Human liver tissue biopsies	Hepatocellular carcinoma	Relative decrease	↓ ↓	ND	133
	YES1 kinase- mediated tyrosine phosphorylation	HEK Mouse liver	Dasatinib	ND	ND	↓ ↓ ↓	82
BCRP	CAR	HH	Phenobarbital	↑	ND	ND	69
	PXR	HH Human liver biopsy	Rifampin Carbamazepine	↑ ↑	ND	ND	7
	NRF2	HH	Oltipraz	In 5/7 donors ↑ ↑	ND	ND	134
	AhR	HH	TCDD	↑ ↑	ND	ND	69
BSEP	FXR	HH HG2	CDCA OCA Oxysterol 22(R)- Hydroxycholesterol GW4064	↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑	Increase	ND	135
P-gp	PXR	HH Human liver slices	Rifampin	↑ ↑	↑ (HH only)	ND	7
	CAR	HH Human liver slices	Phenobarbital	↑ ↑	↑ ↑ (HH only)	ND	7
	NRF2	HG2 Liver (mice)	Bajijiasu herb	↑ ↑ ↑ ↑	↑ ↑ ↑ ↑	↑ ND	136
	NF-κB and AKT dependent MAPK activation	HG2	Deoxynivalenol	↑ ↑	↑ ↑	ND	137
	miR-223	HCC cell lines	miRNA	↓ ↓	Relative down regulation	miRNA-223 over- expression increases HCC cell sensitivity to doxorubicin and paclitaxel	138
MRP2	PXR	HH	Rifampin	↑ ↑	ND	ND	134
	CAR	HH	Phenobarbital	7/7 donors ↑ ↑	ND	ND	134
	NRF2	HH	Oltipraz	5/7 donors ↑ ↑	ND	ND	134

Table 3 (Continued)

Transport proteins	Mechanism	Model system	Agonist/Causes	mRNA	Protein	Activity	Reference
	miRNA and alternative polyadenylation	HG2	miRNA-379	Longer 3'-UTR variants ↓	ND	ND	⁵⁹

↑, < 2-fold increase; ↑↑, ≥ 2-fold increase; ↓, < 2-fold decrease; ↓↓, ≥ 2-fold decrease; ↔, no change; ND, not determined.

^aAhR, aryl hydrocarbon receptor; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CAR, constitutive androstane receptor; CDCA, chenodeoxycholic acid; CHO, chinese hamster ovary cells; FXR, farnesoid X receptor; HCC, hepatocellular carcinoma; HEK, human embryonic kidney cells; HepaRG, hepatic progenitor cell line; HG2, human hepatoma (HepG2) cells; HH, human hepatocytes; HL, HeLa cells; HNF, hepatocyte nuclear factor; Huh7, human hepatocyte carcinoma derived cell line; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MDCK, Madin-Darby canine kidney cells; MRP, multidrug resistance-associated protein; NRF2, nuclear factor-erythroid factor 2-related factor 2; NF-κB, nuclear factor kappa B; OATP, organic anion transporting polypeptide; OCA, obeticholic acid; OCT, organic cation transporter; P-gp, P-glycoprotein; PKC, protein kinase C; PRL, prolactin; PXR, pregnane X receptor; siRNA, small interfering RNA; TCCD, 2,3,7,8-Tetrachlorodibenzo-p-dioxin; UTR, untranslated region.

Among examples of drug-mediated regulation of hepatic transporters, induction of OATP1B by rifampin has received the most attention *in vitro* and in clinical studies using OATP1B probe substrates. As thoroughly reviewed,^{6,7} several clinical studies using multiple-dose rifampin as an inducer and OATP1B1 probe substrates as victim drugs support the potential induction of OATP1B1 activity,⁶ but the induction of *SLCO1B1* mRNA and OATP1B1 protein and transport function has not been demonstrated consistently in human hepatocytes (Table 3). One major challenge with mechanistic clinical studies on NR regulation is the fact that NRs modulate gene networks; hence, it may be difficult to separate the impact on OATP1B regulation from that of efflux transporters. To address this challenge, a PBPK model incorporating transporter induction and suppression was developed,⁶⁷ which has potential to dissect differential regulation and inhibition of various transporters. Induction of hepatic transporters including *SLCO1B1* by LXR and FXR has been well-characterized.⁶⁸ For drugs that are agonists of LXR or FXR (e.g., obeticholic acid) in hepatocytes, it will be important to understand whether they are inducers of transporter activity in humans. Induction of *ABCC2*, *ABCC3*, *ABCC4*, and *ABCG2* by PXR, CAR, and aryl hydrocarbon receptor agonists was reported in cultured human hepatocytes, but based on more recent literature, clinical relevance likely is low.^{69,70}

Epigenetic/post-transcriptional regulation. At the post-transcriptional level, miRNAs were involved in downregulation of expression of *SLCO1B1*,⁷¹ *SLCO2B1*,⁷² and *ABCC2*.⁵⁹ Expression levels of miR-206 and miR-24 were associated with expression of *SLCO1B1*⁷¹ and OATP2B1⁷² in the human liver, respectively. In cancer cell lines and cancer tissues, an alternatively spliced variant of OATP1B3 lacking the N-terminal 28 amino acids present in wild-type OATP1B3 was identified.⁷³ The OATP1B3 variant was localized predominantly intracellularly and had negligible transport activity when overexpressed *in vitro*.⁷³ At the epigenetic level, there is evidence that DNA methylation plays a role in the liver specific expression of *SLCO1B1* and *SLCO1B3*.⁷⁴ Tissue-dependent hypomethylated regions in liver DNA relative to kidney DNA were identified around the transcriptional start site of *SLCO1B1* and *SLCO1B3*.

Post-translational regulation. Altered glycosylation of OATP1B1, OATP1B3, OATP2B1, NTCP, and MRP2 has been implicated

in disease states, such as nonalcoholic steatohepatitis.^{49,75} Phosphorylated OATP1B1, OATP1B3, and OATP2B1 has been detected.^{51,76,77} The OATP1B1 variant c.521T>C (rs4149056; p.I74Val>Ala) had reduced transport activity *in vitro* and this variant showed a modest increase in phosphorylation status compared with wild-type OATP1B1.⁷⁸ The causal relationship between altered phosphorylation of the c.521T>C variant and reduced transport function has yet to be demonstrated.

Short-term pre-incubation with inhibitors may reduce transporter activity *in vitro* to a greater extent than simultaneous incubation with the substrate, even after washing the cells. This “pre-incubation-dependent inhibition” phenomenon has been reported for several SLC transporters, including OATP1B1 and OATP1B3, OATP2B1, and OCT1 in recombinant cell lines,^{38,79} and in sandwich-cultured primary human hepatocytes.⁸⁰ For several inhibitors, including cyclosporin A,⁷⁹ the half-maximal inhibitory concentration values after inhibitor preincubation were close to the estimated *in vivo* inhibition constant values. Many of the reported inhibitors or modulators that show pre-incubation-dependent inhibition of transporters are phosphorylation modulators, including TKIs.⁸¹ Transporter-mediated DDIs involving TKIs have received considerable attention due to the chronic use of these drugs. Although multiple mechanisms have been proposed to explain pre-incubation-dependent inhibition,⁷⁹ inhibition of LYN and YES1 kinase-mediated tyrosine phosphorylation was implicated in reduced transport function of OATP1B1 and OCT1, respectively, following treatment with TKIs.^{8,82} The clinical implication of altered transporter phosphorylation, as shown for TKIs, warrants further investigation.

Kidneys

In the kidneys, transporters that are relevant to drug or endogenous compound disposition include multidrug and toxin extrusion (MATE) 1, MATE2K, OCT2, OAT1, OAT2, OAT3, OAT4, OATP4C1, PEPT1, PEPT2, P-gp, MRP2, and MRP4.² Examples of regulation of clinically relevant kidney transporters are summarized below, and additional details are included in Tables 1 and 4; Table S1.

Transcriptional regulation. Despite the established overall protein abundance profiles of kidney transporters, the regulatory factors

contributing to modulation of transporter function in renal tissue are understudied and, thus far, a significant role for NRs in gene regulation has not been reported. However, various regulatory mechanisms have been characterized in preclinical models. For example, corticosteroids altered canine *Slc22a2*/OCT2 expression and activity⁸³ and murine *Slc22a2* mRNA expression fluctuated based on circadian rhythm.⁸⁴ Collectively, this suggests that hormone and external signaling factors play a role in modulating OCT2 function, although, to date, no human data have been reported to confirm these observations.

Epigenetic/post-transcriptional regulation. The impact of epigenetics on the regulation of OCT2 activity is the most established; promoter methylation and loss of histone acetylation in human tissue samples were associated with a renal-specific reduction in *SLC22A2*/OCT2 mRNA expression and protein abundance in RCC.^{21,85} Epigenetics also has been demonstrated as a mechanism that regulates activity of the urate transporter 1, OAT1 and OAT3 *in vitro* and *in vivo* (Figure 1, Table 4).

Post-translational regulation. Evidence continues to accumulate that OCT2 transport activity is dependent on various forms of PTMs: (i) phosphorylation-dependent signaling has long been reported to modulate OCT2-mediated transport. This was first shown with pharmacological activators or inhibitors of PKA, or phosphoinositide 3-kinase, which revealed that these kinases reduce OCT2-dependent uptake.⁸⁶ However, the thesis that PKA regulates OCT2 remains exclusively dependent on nonspecific multi-kinase inhibitors or activators and has not been verified directly with genetic strategies. Thus, it remains unknown which kinases affect OCT2 function or whether such kinases act directly or indirectly on this transporter. (ii) *In vitro* genetic studies showed the functional importance of a YES1-dependent tyrosine phosphorylation event at tyrosine 362.³⁹ This site is located at the substrate binding domain, according to predictive computational modeling, and the negative charge of tyrosine phosphorylation is expected to play a role in substrate binding. Support for this idea came from a mutagenesis approach and a YES1 TKI that abolished transport activity.³⁹ The dependence of OCT2 activity on tyrosine phosphorylation and the sensitivity of OCT2 activity to TKIs was confirmed in rodent models; and (iii) in addition to phosphorylation, glycosylation status also is reported to be involved in OCT2 activity. To date, data are limited to *in vitro* models, but loss of glycosylation at N96 reduced substrate affinity, and N112 glycosylation played a major role in OCT2 cell surface localization.⁸⁷ Protein kinase signaling has been investigated in OAT1 and OAT3 regulation, where PKA promotes phosphorylation of these transporters (along with SUMOylation of OAT3) and enhanced transport activity.⁸⁸ PKC can also influence OAT1 transport through promotion of ubiquitination, internalization, and degradation.⁸⁹ However, these mechanisms have yet to be confirmed *in vivo* or in human tissues. Furthermore, there is a plethora of data lacking for other renal transporters regarding regulatory factors and their contribution to *in vivo* transport function.

Collectively, whereas the information described above and in Table 4 demonstrate some progress in clarifying factors that can

contribute to transport activity in the kidneys, it is evident that more detailed investigations are needed, particularly in translating findings from *in vitro* and animal models to the clinic to confirm the contribution of each regulatory mechanism to drug disposition and/or patient outcomes. Regarding use of animal models, the species-specific expression profile of some transporters currently considered important in the active tubular transport of drugs must be emphasized. One example involves the expression of *Slc22a1*/*SLC22A1*, which is present in rodent kidneys, but not in monkey and human kidneys.¹² Nonetheless, data gathered particularly for transporters involved in tubular uric acid handling and summarized in the “urate transportosome”⁹⁰ support that novel methods are required to translate mechanistic studies into human phenotypes. Moreover, the studies on uric acid handling emphasize that tubular transporters are part of networks, where regulatory mechanisms coordinately change expression of transporters and work in concert to mediate tubular reabsorption and secretion.

Brain

Clinically relevant transporters for drugs and/or endogenous compounds in the brain are localized in the BBB, the blood-cerebrospinal fluid-barrier, in glial cells, and/or at other brain barriers. Transporters at the BBB, ranked based on currently available abundance data, include glucose transporter 1 with the highest levels, followed by BCRP, OAT2, P-gp, OCT3, OCT1, OATP1A2, OAT7, OAT1, OATP1B3, OATP2B1, OAT3, OATP1C1, and MRP4. However, there is still some controversy regarding the location of brain transporters in polarized brain endothelial cells, especially for the OATPs.⁹¹ Studies on underlying mechanisms in patients with brain diseases (e.g., Alzheimer's, Parkinson's, and epilepsy) have demonstrated that regulatory factors and pathways contribute to modulation of transporter function in the brain leading to changes in transporter expression and abundance⁹² (Table S1). P-gp is the brain transporter that has been characterized best at different regulatory levels (Table 1, Table S1).

Lungs

The influence of transport proteins in the lungs (e.g., OCTs, P-gp) on pulmonary drug disposition and toxicity is an emerging area of research. Human-relevant data documenting transporter regulation in the lungs are limited and are primarily based on cells or lung tissue obtained from patients with pulmonary disease, or *in vitro* cell models (Table S3). The expression, subcellular localization, function, and regulation of OCT transporters in lung tissue and cell lines has been reviewed.⁹³ Pulmonary diseases (e.g., asthma) increased *ABCC2* mRNA.⁹⁴ *ABCB1* mRNA was significantly lower in bronchoalveolar lavage cells from smokers compared with nonsmokers.⁹⁵ Understanding how regulation of pulmonary transporters may impact transporter function is critical in the development of medications for respiratory diseases.

Placenta

The placenta plays an important role in nutrient and waste exchange between mother and fetus and several transporters are expressed in the apical and basolateral membranes of the syncytiotrophoblast. The most abundant and clinically relevant

Table 4 Mechanisms of regulation for transporters in the kidneys^a

Transport proteins	Mechanism	Model system	Agonist/Causes	mRNA	Protein	Activity	Reference
MATE1	Promoter methylation	LS174T and HaCat cells	Demethylation 5-aza-2deoxycytidine	↑↑	ND	ND	139
MATE2K	Histone methylation	769-P and 786-0 cells	H3K4me3 enrichment	↓↓	ND	ND	140
OAT1	PKA activity	OK cells	Forskolin	ND	ND	↑	141
	PKC activity	OK cells	Parathyroid hormone (0.1 μM)	ND	ND	↓↓	141
	MAPK pathway activity	OK cells	PD98059	ND	ND	↑	141
	Ubiquitination and internalization	COS-7 cells	USP8 overexpression	ND	↑	↑	89
	Ubiquitination and degradation	Overexpressing HEK-293 cells	Bortezomib Carfilzomib	ND	↑	↑	142
OAT3	Promoter methylation	HepG2, Caco-2, and HEK293 cells	5-aza-2deoxycytidine	ND	↑↑	ND	141
	PKA activity; SUMOylation ↑ Ubiquitination ↓	Overexpressing COS-7 cells	Bt2-cAMP	ND	Plasma membrane abundance ↑ Total abundance ↔	↑	143 88
OCT2	Unknown	MDCK cells	Dexamethasone Hydrocortisone	↑↑	ND	↑	83
	Promoter methylation	Patient tissue, Renal Cell Carcinoma Cells, HEK-293 cells	Demethylation Decitabine	↑↑	↑↑	↑↑	85
	Histone acetylation	Patient tissue, Renal Cell Carcinoma Cells, HEK-293 cells	Vorinostat	↑↑	↑↑	↑↑	21
	PKA-mediated phosphorylation	Overexpressing HEK-293 cells	Forskolin	ND	ND	↓	86
	PI3K-mediated phosphorylation	Overexpressing HEK-293 cells	Wortmannin	ND	ND	↑	86
	Tyrosine phosphorylation (YES1-mediated)	Overexpressing HEK-293 cells, FVB mice	Dasatinib siRNA Y362F mutant	ND	↔	↓↓	39
	Glycosylation	Overexpressing CHO cells	N96Q	ND	↔	↓↓	87
	P-gp	Post-transcriptional/Alternative splicing	SA7K cells (pseudo-immortalized primary RPTECs)	ADAR1 mouse knockout	↓↓	↓	ND
NRF2 activation		HK-2 shKEAP1 (stable KEAP1 knockdown)	KEAP1 mouse knockout	↑↑	↑↑	↑	144
Transcriptional (decreased Src signalling, JNK activation)		Caki-1	5-aza-20-deoxycytidine	↓	ND	↓	145
Localization (lipid rafts)		MDCK-MDR1 cells	Methyl-β-cyclodextrin	ND	Plasma membrane abundance ↓	↓↓	146
PKC activation		LLC-GA5 Col300 cell (LLC-PK1-MDR1)	Phorbol 12,13-dibutyrate	ND	ND	↑	147

↑, < 2-fold increase; ↑↑, ≥ 2-fold increase; ↓, < 2-fold decrease; ↓↓, ≥ 2-fold decrease; ↔, no change; ND, not determined.

^aADAR, adenosine deaminase; Caki-1, human kidney clear cell carcinoma cell line; COS-2, cercopithecus aethiops kidney cell line; CHO, Chinese hamster ovaries cell line; COS-7, CV-1 in origin with SV-40 genes cells; H3K4me3, trimethylation on histone H3; HaCat, immortalized human keratinocytes; HEPG2, human hepatoma cell line; HK-2, human kidney proximal tubule cells; KEAP, Kelch-like ECH protein; LLC-PK1, Lilly Laboratories culture-porcine kidney cells; MDCK, Madin-Darby canine kidney cells; MAPK, mitogen-activated protein kinase; MATE, multidrug and toxin extrusion; NRF2, nuclear factor-erythroid factor 2-related factor 2; OAT, organic anion transporter; OCT, organic cation transporter; OK, opossum kidney cells; P-gp, MDR1 P-glycoprotein; PI3K, phosphoinositide 3-kinase; PK, protein kinase; RPTECs, renal proximal tubule epithelial cells; SA7K, human proximal tubule epithelial cell line; siRNA, small interfering RNA; USP, ubiquitin specific peptidase 8.

placental drug transporters include BCRP, P-gp, OATP2B1, OCT3, and OAT4.^{96,97} These transporters prevent entry or facilitate efflux of potentially harmful endogenous and exogenous substrates from the fetal compartment. While the placenta develops and grows throughout pregnancy, abundance of the transporters changes: BCRP and P-gp levels decrease while OAT4 and OCT3 levels increase from the first or second trimester to term (Table S1).⁹⁷ Protein levels of both P-gp and BCRP decline almost two-fold near term, leaving the fetus more susceptible to potentially harmful drugs.

Transcriptional regulation. In the placenta, steroid hormones, such as progesterone and estrogen, which physiologically fluctuate during pregnancy or by treatment with glucocorticoids, regulate drug transporter expression.^{98,99} Importantly, pathological conditions, such as preeclampsia, gestational diabetes, growth restriction, or infection, can lead to dysregulation of drug transporters.⁹⁶ Transporter expression changed along with alterations in transcript levels of cytokines and growth factors.⁹⁶ Differences in the extent or direction of mRNA vs. protein changes point to involvement of both transcriptional and translational regulatory pathways. *In vitro* studies in primary cultures of placental cells and tissues have demonstrated that growth factors, hypoxia, and pro-inflammatory cytokines can alter transporter levels and/or activities.^{100,101} However, the regulatory factors contributing to modulation of placental transporters in health and disease are relatively unknown. A better understanding of placental drug transporter regulation in normal and pathological pregnancies will aid in predicting fetal drug exposure and the development of more targeted medication for the mother.

EXPERIMENTAL APPROACHES TO INVESTIGATE REGULATION OF TRANSPORT PROTEINS

Knowledge of transporter protein regulation is relatively limited, and many questions still need to be addressed to better understand and accurately predict the impact of alterations in regulatory pathways on drug efficacy and toxicity. Considerable advances have been made in the methods used to elucidate the mechanisms and factors that regulate transporter activity. Techniques that can be used to study transcriptional regulation, epigenetic alterations, PTMs, and changes in trafficking, transporter expression, and/or abundance are listed in Table 5. Immunohistochemistry/immunocytochemistry and immunofluorescence techniques applied to *in vitro*, nonclinical *in vivo*, or clinical samples can provide insight into tissue levels and, in some cases, more detailed information regarding transporter localization than cell fractionation or biotinylation methods. Protein quantification can be obtained, for instance, by global or targeted proteomics.¹⁰² Global proteomics provides a relative picture of all the proteins in a given sample, but may lack precision relative to targeted proteomics, which measures protein abundance of single or selected proteins with higher reproducibility and sensitivity, particularly for low abundance proteins. Both methods may be able to detect PTMs. However, various forms of protein modifications potentially can be difficult to measure simultaneously. In addition to protein analysis, mRNA expression assessment is

possible in various tissues or cells using current sequencing technology. In fact, this analysis is useful in combination with bisulfite sequencing or chromatin immunoprecipitation to measure epigenetic modifications or transcription factor binding. Each of the procedures outlined are suitable to measure various factors that regulate transporter activity. However, these approaches often cannot be used simultaneously due to sample preparation differences or limited quantities of sample. As such, experiments need to be carefully designed.

Most studies to date have used *in vitro* models, such as cell lines, cells overexpressing transport proteins of interest, or primary cells, to assess transporter regulation. Key considerations in selection of *in vitro* models include: (i) the need for adequate transporter or regulatory protein expression (i.e., NRs); (ii) cell-, organ-, or species-specific differences in transporter expression, membrane localization (i.e., apical vs. basolateral), and/or regulatory pathways; and (iii) laboratory- or culture-specific conditions (e.g., cell source, passage number, media content). It is reasonable to expect that epigenetic- or transcription factor-mediated control of gene promoter activity (if expressed at sufficient levels) would be consistent across *in vitro*, *in vivo*, or clinical conditions. However, the impact of disease or exposure to potential modifiers on these regulatory mechanisms in various human tissues remains to be investigated. This need is similarly essential for RNA and proteomic quantification of transporters in various tissues or disease states. Gene expression and protein abundance using RNA sequencing and proteomics is well-established in cell lines and tissues involved in drug disposition in both animal models and humans. However, these techniques have not been applied robustly to assess changes or variability in mRNA or protein levels throughout development, disease states, gender, or race, which can vary markedly from observations in cell lines or animal models. Additionally, RNA sequencing and proteomics can be applied to clarify changes in splice variant expression, which is understudied, particularly in human tissues where expression may differ significantly from that observed *in vitro*. Likewise, the approaches used to assess changes in PTMs by intrinsic and extrinsic factors, and the impact on transporter localization and activity can be highly dependent on intracellular signaling. These signaling events can differ based on distinct protein abundance among *in vitro* cell models or primary tissues, and also could be changed following drug exposure. Such events, along with regulatory protein expression and activity may differ among species. Thus, care needs to be taken when translating data from animal models to humans, particularly with proteins that are not well-conserved. Therefore, given the lack of characterization in humans, use of primary tissues or clinical samples should be the major focus of future investigations. Method standardization is required to validate cellular models as surrogates for *in vivo* studies, including consistent use of drug and modulator concentrations, exposure times, and culture conditions (e.g., media and additives) for valid comparisons across investigations.

Various clinical approaches may be used to investigate transporter regulation, including use of exogenous transporter substrate probes, endogenous biomarkers, and tissue and liquid biopsies. Exogenous transporter substrate probe cocktails, with multiple transporter and metabolic enzyme substrates, have

Table 5 Experimental methods to study regulation of transporters

Level	Methods	Strengths	Limitations	Application (+)							
				Nuclear receptors	Epigenetics	Post-transcriptional	Alternative splicing	Post-translational	Trafficking	Expression or abundance	Activity
Preclinical	Cell lines	<ul style="list-style-type: none"> Capable of studies at all levels of regulation High growth rate and higher throughput Can be manipulated through genetics Cost effective 	<ul style="list-style-type: none"> May not represent primary tissues and organs Transporter expression and localization may differ from primary tissue Trafficking/regulation mechanisms may be cell-type specific Transporter localization may differ from primary tissues NR expression/activity may differ, even in cell lines of same tissue origin Signaling pathways and proteins expressed may vary Cell source, passage #, culture time/conditions may impact transporters 	+	+	+	+	+	+	+	
	Stem cells	<ul style="list-style-type: none"> Suitable for studies at all levels of regulation Observations are commonly clinically relevant Can be manipulated through genetics 	<ul style="list-style-type: none"> May not represent primary tissues and organs High cost Long time commitment for use 	+	+	+	+	+	+	+	+
	Primary cells	<ul style="list-style-type: none"> Suitable for studies at all levels of regulation Observations are clinically relevant Expression and localization of transporters may match human tissues 	<ul style="list-style-type: none"> Limited availability Relatively high cost Genetic manipulation not possible due to lack of proliferation Changes during culturing can limit results Inter-donor variability due to disease or drugs may impact results Validation is expensive and may be time consuming 	+	+	+	+	+	+	+	+
	Animals	<ul style="list-style-type: none"> Controlled environment Tissues can be used to study all levels of regulation Can be manipulated through genetics 	<ul style="list-style-type: none"> Species/strain differences in transport proteins may limit clinical relevance Transporter regulation mechanisms may be species/strain dependent High cost Low throughput 	+	+	+	+	+	+	+	+
Tissue	Bisulfite Sequencing	<ul style="list-style-type: none"> Highly accurate to measure methylation Can provide whole genome sequencing information 		+	+						
	ChIP	<ul style="list-style-type: none"> Capable of measuring histone modifications Capable of measuring transcription factor binding to gene promoter 		+	+						
	RNA Seq	<ul style="list-style-type: none"> Provides RNA sequence and quantity Can report mRNA stability Can provide single cell expression miRNA 	<ul style="list-style-type: none"> Often does not correlate with transporter abundance 	+	+						+
	Western Blot	<ul style="list-style-type: none"> Provides semi-quantitative protein abundance Can report changes following drug exposure Capable of measuring differences in variant abundance Can be used to measure some PTMs 	<ul style="list-style-type: none"> Antibody dependent Labor intensive 			+	+	+	+		+

(Continued)

Table 5 (Continued)

Level	Methods	Strengths	Limitations	Application (+)							
				Nuclear receptors	Epigenetics	Post-transcriptional	Alternative splicing	Post-translational	Trafficking	Expression or abundance	Activity
	LC/MS global proteomics	<ul style="list-style-type: none"> Provides abundance of most proteins simultaneously Can measure changes following drug exposure Capable of measuring variant expression differences Can measure PTMs (e.g., phosphorylation) 	<ul style="list-style-type: none"> Low protein abundance can be detected Post-translational modifications are not measured Computationally intensive High cost 								
	Targeted LC/MS proteomics	<ul style="list-style-type: none"> Provides protein quantification Can measure PTMs (e.g., phosphorylation) Capable of measuring changes in splice variant expression 	<ul style="list-style-type: none"> Sensitive analysis required Computationally intensive High cost 								
	IHC/IF	<ul style="list-style-type: none"> Provides cellular localization of proteins 	<ul style="list-style-type: none"> Labor intensive Antibody dependent 								
	Surface biotinylation	<ul style="list-style-type: none"> Provides detection of plasma membrane localized proteins 	<ul style="list-style-type: none"> Must quench reaction to prevent binding to free biotin Requires assay optimization to ensure saturation of biotinylation 								
	Cell fractionation	<ul style="list-style-type: none"> Provides cellular localization of proteins Provides post-translational modifications at specific compartments 	<ul style="list-style-type: none"> Some cellular compartments are fragile Some compartments are difficult to separate Requires measurement of enrichment with marker enzymes 								
	Endogenous biomarkers	<ul style="list-style-type: none"> Does not require administration of a probe drug Can assess enzyme-transporter interplay Non-invasive High patient safety Can be measured in first-in-human trials 	<ul style="list-style-type: none"> Variable patient baseline Requires sensitive and validated analytical methods Requires validation and clinical data Requires knowledge of endogenous marker regulation 								
Clinical (non-invasive)	Drug substrates (e.g., cocktail tails)	<ul style="list-style-type: none"> Known and well characterized pharmacokinetics Non-invasive High patient safety 	<ul style="list-style-type: none"> Variable patient baseline/genetics Requires sensitive analytical methods Preparation of dosing solutions requires high level of expertise/training Multiple probes and their transporter profiles required 								
	Liquid biopsy	<ul style="list-style-type: none"> Measure transporter abundance without need for organ biopsy 	<ul style="list-style-type: none"> Purification of exosomes not standardized High volume of sample needed Detection of protein in exosomes requires sensitive analytical methods Unique organ markers needed (e.g., asialoglycoprotein receptor for liver) 								

ChIP, chromatin immuno-precipitation; IHC/IF, immunohistochemistry/immunofluorescence; LC/MS, liquid chromatography/mass spectrometry; miRNA, microRNA; PTMs, post-translational modifications.

been used successfully in numerous clinical studies to characterize DDIs or the effect of disease on transporter activity.^{103,104} Although this approach is informative for identification of DDIs, experimental execution is complex and requires administration of multiple drugs. In contrast, endogenous transporter substrate biomarkers¹⁰⁵ overcome some of these challenges. More specifically, transporter biomarkers do not require additional compound administration, can easily assess baseline characteristics, can be measured over longer periods of time, and require a low volume of blood. However, use of biomarkers to assess transporter regulation has some limitations. For example, synthesis of these endogenous compounds needs to be stable during assessment, and the profile of transporters and/or metabolic enzymes that recognize these molecules must be well-defined.¹⁰⁶ Similar to exogenous probes, changes in systemic biomarker concentrations do not provide mechanistic insight into regulatory alterations caused by DDIs. Liquid biopsies are an emerging technique that may overcome the lack of such mechanistic insights into transporter regulation. Liquid biopsies allow for the collection of circulating exosomes isolated from human plasma that originate from various cells.¹⁰⁷ Following isolation, protein levels can be measured. Theoretically, PTMs can be measured as well, although this strategy has not been reported to date. Current methods require large volumes of plasma to isolate sufficient exosomes,¹⁰⁷ and it remains unknown whether abundance or modifications reflect transport activity at the moment of extraction.

PHARMACOKINETIC MODELING FOR TRANSPORTER INDUCTION AND SUPPRESSION

With the growing understanding that drugs and other xenobiotics can cause clinical DDIs via transporter induction^{6,108,109} and suppression,¹¹⁰ it is imperative to incorporate such complexity into physiologically based mathematical models to accurately predict DDI risks (Table 6). To date, the dynamics of NR-mediated transporter regulation has not been systematically studied, and a widely accepted predictive mathematical and/or biology-based model evaluating the dynamics of transporter induction after NR activation and the effects of post-transcriptional or post-translational modifications is not available. Hence, it is challenging to build suitable mechanistic models for DDIs involving transporter regulation due to induction/suppression. Besides an empirical model that uses a fixed relative scalar,¹¹¹⁻¹¹³ the turnover model that was established for induction/suppression modeling of CYPs and UGTs,¹¹⁴ can be applied to transporters,⁶⁷ because transporters are regulated through mechanisms likely similar to CYP enzymes (Figure 1). *In vivo* transporter levels are governed by the rates of *de novo* protein synthesis and degradation, defined as the protein turnover half-life. The current lack of *in vivo* turnover half-life values for human transporters places a significant limitation on the accurate prediction of changes in drug concentration-time profiles associated with interactions involving transporter induction. Protein turnover is the only system data (i.e., population information) required for the turnover model, besides the baseline protein level (Figure 2), but only sparse data are available for transporter turnover. P-gp is inducible and relevant for clinical DDIs, whereas the induction

of OATP1B is more controversial as discussed above. The turnover number for P-gp is ~ 0.054 /hour based on a meta-analysis of 13 independent published studies from seven laboratories, and the turnover number for OATP1B1 is ~ 0.031 /hour based on two independent studies (author S.N., personal communication). However, robust turnover numbers are still lacking for most transporters. In addition, clinical data for validation of turnover numbers are sparse. In most clinical studies, the concentration of the perpetrator is not reported. For example, only a few concentration-time profiles with low doses of rifampin are available to validate the maximum fold induction/suppression over vehicle control (IndC_{50}). Although the turnover model is semi-mechanistic, it does not link the underlying induction process to the change in the protein level. This requires more detailed *in vitro* experimental design and mathematical models, such as quantitative systems pharmacology models.¹¹⁵

CONCLUSIONS AND FUTURE PERSPECTIVE

The preclinical and clinical evidence presented in this white paper clearly demonstrates that regulation of transporters is highly orchestrated (Table 1), and that the net effect of various regulation mechanisms contributes to variability in drug response in healthy individuals and in patients with various stages of disease. In contrast to the well-established role of NRs, the importance of regulation involving epigenetic, post-transcriptional and post-translational mechanisms, and effects on transporter expression, membrane localization, and function is still fragmented and requires more systematic research to establish to what extent these various mechanisms affect drug response in the clinic. In addition, the effect of food and the intestinal microbiome on transporter regulation is an area where further studies are needed. Currently, the number of drug transporters for which clinically significant regulatory mechanisms have been demonstrated is relatively small (Tables 2–4). Studying transporter regulation in human subjects in the various organs, tissues, and epithelial barriers where membrane transporters play a critical role (including organs not covered in this white paper such as heart, skeletal muscle, and testes) is challenging and, in most cases, based on current experimental methods with limitations, requires invasive procedures to obtain tissue. Thus, there is an urgent need for biorelevant *in vitro* models, such as primary cells and microphysiological systems that possess similar regulatory plasticity to that *in vivo*. Studying regulation in immortalized cell lines can be hypothesis generating, but results obtained in such cells should be interpreted with caution and confirmed in physiologically relevant models. In addition, continued efforts to understand the translatability of endogenous biomarkers and endpoints that can be quantified in liquid biopsies will be valuable. This is also true for analytical advances enabling the highly sensitive and tissue specific analysis of gene and miRNA expression as well as protein abundance of transporters and NRs from liquid biopsies.¹¹⁶

Although the role of NRs in the induction of intestinal P-gp and MRP2 is well-established, there are still gaps in understanding which organs are most significantly impacted by transporter induction. Current data indicate that PXR-regulated transporters

Table 6 Physiologically based mathematical models to assess transporter induction or suppression^a

Application (transporter/location)	Substrate	Inducer/suppressor	Advantages	Limitations	Reference
Empirical model					
Intestinal P-gp Hepatic OATP	Dabigatran Pravastatin Rosuvastatin Midazolam Tolbutamide Caffeine	Rifampin	<ul style="list-style-type: none"> Matrix of PXR compounds Simple (no additional model and physiology data are needed) 	Each additional compound to the matrix brings its own uncertainties, however, this limitation will change to an advantage the more compounds can be successfully estimated and therefore included in the matrix	108
Intestinal P-gp Hepatic OATP	Sofosbuvir	Rifabutin Carbamazepine	<ul style="list-style-type: none"> Matrix of PXR compounds Simple (no additional model and physiology data are needed) The application of an established matrix. Any compound that is correctly predicted can be added to the initial matrix 	Same as above	109
REF or RAF scaling within an IVIVE-PBPK framework					
Intestinal (and hepatic) P-gp	Digoxin	Rifampin	<ul style="list-style-type: none"> Simple Use of apparent K_m/J_{max} from Caco-2 	<ul style="list-style-type: none"> An expected maximum fold-change <i>in vivo</i> needs to be generated; in this case, the value was from <i>in vivo</i> biopsies. This is not a generally available parameter. However, there are some <i>in vitro</i> systems that reported comparable fold-change (e.g., hepatocytes, LS180, and LS174T cells) The concentration-dependent induction/suppression course is not included in the model 	111
Intestinal P-gp	Dabigatran etexilate Digoxin Quinidine TalinoIol	Rifampin	<ul style="list-style-type: none"> Simple Used <i>in vitro</i> modeled transporter kinetic data (K_m, J_{max} and/or CL) values 	Same as above	112
Intestinal P-gp	Abemaciclib Acalabrutinib Bosutinib Crizotinib Dabigatran etexilate Digoxin Naldemedine Naloxegol Olaparib Quinidine TalinoIol Verapamil	Rifampin	<ul style="list-style-type: none"> Simple Used <i>in vitro</i> modeled transporter kinetic data (K_m, J_{max} and/or CL) values 	<p>Same as above</p> <p>The more compounds that can be correctly estimated with the same REF, the higher the confidence in this system parameter</p>	113

(Continued)

Table 6 (Continued)

Application (transporter/location)	Substrate	Inducer/suppressor	Advantages	Limitations	Reference
Hepatic OATP1B1	CP-I	Rifampin; OATP1B1 521CC polymorphism	<ul style="list-style-type: none"> Use of sensitive biomarker levels in plasma and urine data Main route of elimination via OATP1B transport (> 85%) Circadian rhythm or food intake are not likely to cause interindividual variability in plasma CP-I baseline 	<ul style="list-style-type: none"> Endogenous factors like MRP2 mutations, hemogenesis triggered by anemia or hemolysis, may alter CP-I baseline Reduced CP-I synthesis in women has been reported Limited information on synthesis and turnover of CP-I, factors influencing baseline 	148, 149
Turnover model within an IVIVE-PBPK model					
Hepatic OATP1B, MRP2	Glibenclamide Repaglinide CP-I	Rifampin	<ul style="list-style-type: none"> This PBPK model incorporated induction of OATP1B and CYP2C8 and inhibition of MRP2 CP-I DDI used to obtain rifampin inhibition parameter data 	Model not accessible to everyone due to program used - low practicality	67
Hepatic OATP1B1	Repaglinide	Rifampin	Using the transporter induction turnover model, DDIs could be recovered reasonably well when an OATP1B1 induction Ind_{max} value (2.3) and a k_{deg} for OATP1B1 of 0.0311/h was included for rifampin in simulations across a range of dosing regimens	Further investigations and data are required to assess the validity of the derived rifampin <i>in vitro</i> OATP1B1 Ind_{max} value (i.e., using different rifampin doses and OATP1B1 substrates) and whether inclusion of an $IndC_{50}$ value would reduce the overpredictions OATP1B1 is a polymorphic transporter and no genotype information was available from the clinical studies. Thus, understanding the effect of polymorphisms was not assessed in this investigation and may contribute to some of the variability observed due to the small sample size of the clinical studies	(SN, Personal communication)
Intestinal P-gp	Digoxin Dabigatran etexilate	Rifampin	<ul style="list-style-type: none"> Measured values of P-gp turnover were incorporated into a semi-mechanistic physiological model to simulate the clinical impact of intestinal P-gp induction by rifampin in humans The DDIs were best recovered across a range of rifampin doses using an $IndC_{50} = 0.25 \mu M$ and $Ind_{max} = 5.6$ 	Although these preliminary results are encouraging, the model needs to be verified against other clinical studies with different rifampin doses to confirm the utility of the model	(SN, Personal communication)
QSP					
Hepatic OATP1B1 and P-gp	Diclofenac Celecoxib	Rifampin	Changes in hepatic OATP1B1 and P-gp can be included in the model	Transporter alterations have not been evaluated in the models for diclofenac and celecoxib	150

^aCaco-2, human colorectal adenocarcinoma cell; CL, clearance; CP-1, coproporphyrin I; CYP, cytochrome P450; DDI, drug-drug interaction; $IndC_{50}$, test compound concentration that supports half-maximal induction/suppression; Ind_{max} , maximum fold induction/suppression over vehicle control; IVIVE-PBPK, *in vitro*-to-*in vivo* extrapolation linked physiologically based pharmacokinetics; J_{max} , maximum rate of transport; k_{deg} , rate of degradation, defined by a first-order rate constant; Km, Michaelis constant; MRP, multidrug resistance-associated protein; OATP, organic anion transporting polypeptide; PBPK, physiologically based pharmacokinetics; P-gp, MDR1 P-glycoprotein; PXR, pregnane X receptor; QSP, quantitative systems pharmacology; RAF, relative activity factor; REF, relative expression factor.

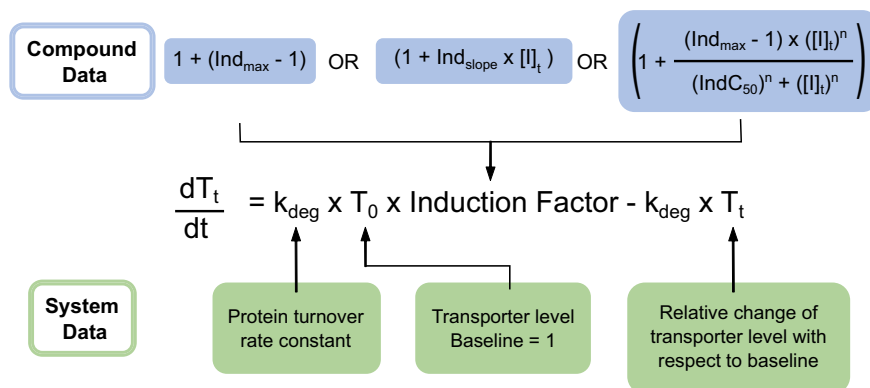


Figure 2 The turnover model as applied to drug-drug interactions. The dynamics of transporter regulation via induction/suppression is represented by a turnover model¹¹⁴ in which the amount of transporter at equilibrium reflects the balance between its rate of synthesis and its rate of degradation, defined by a first-order rate constant (k_{deg}). The required perpetrator data are either: (1) maximum fold induction/suppression over vehicle control (Ind_{max} ; $\text{Ind}_{\text{max}} < 1$ indicates suppression, $\text{Ind}_{\text{max}} > 1$ indicates induction); (2) The slope of the fold induction/suppression versus transporter inhibitor concentration ($[I]_t$) plot when induction/suppression is linear within the range of perpetrator concentrations ($\text{Ind}_{\text{slope}}$ in μM^{-1}); or (3) perpetrator concentration that supports half-maximal induction/suppression (IndC_{50} in μM) together with $\text{Ind}_{\text{slope}}$. This option will link the perpetrator concentration over a range of concentrations directly to induction/suppression and hence will best translate to changes in protein levels. For the IndC_{50} determination, the fraction of unbound drug in the *in vitro* incubation ($f_{u_{\text{inc}}}$) also should be considered. Required system data (population information) are: baseline protein level (T_0) and k_{deg} . T_t is the state variable describing the relative change in the transporter level with respect to baseline because of induction/suppression. Without induction/suppression $T_t = 1$.

(i.e., P-gp and MRP2) are inducible by respective ligands and may cause clinically relevant DDIs after oral drug administration,¹⁴ whereas ligands of CAR do not induce intestinal P-gp or other CAR-regulated enzymes or transporters.^{9,56} To what extent this translates to other PXR activators and NRs is less clear. To increase knowledge in this area, the route of administration (intravenous vs. oral) of a perpetrator drug should be considered carefully to allow a mechanistic interpretation of DDI results.

The role of miRNAs in transporter regulation also is emerging. The correlation of miRNAs with downregulation of transport activity is implied based on data obtained in the intestine and liver. Currently, no systematic data are available to identify the functional significance of miRNAs, and methods to identify miRNA binding sites are indirect and time consuming. To address this gap, application of the chimeric-eCLIP assay recently described to map the hepatocyte miRNA-mRNA interactome¹¹⁷ should be explored to identify miRNAs binding to transporter mRNAs in hepatocytes and cells in other organs. Another class of regulatory RNAs that is understudied are the lncRNAs. Evidence suggests that lncRNAs may be involved in transporter regulation in multidrug resistant tumor cells by either interacting with NRs or with mRNAs encoding ABC transporters,¹¹⁸ but their role in healthy tissue is unclear.

The impact of PTMs and altered protein trafficking on transporter localization and function remains poorly understood and understudied. More focus on the use of human-based *in vitro* and *ex vivo* models with intact regulatory machinery is needed. Proteomic methods used to quantify transporters must distinguish functional from inactive proteins, for example, due to PTMs that affect activity. Transporter localization in the relevant membrane, and appropriate corrections for loss during sample preparation, must be considered when using quantitative cell or tissue abundance data to predict *in vivo* drug disposition.

In summary, based on the evidence presented in this white paper, regulation of transport proteins is highly complex and can occur at multiple levels to impact transporter function and drug disposition. Preclinical and clinical research advances are needed to close knowledge gaps in this field and support the development of improved PBPK and quantitative systems pharmacology models to predict drug response and interpatient variability. As such, these investigations are anticipated among the next frontiers in transporter research.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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