

## **Transcriptional Regulation of Solute Carrier (SLC) Drug Transporters**

Shiwei Zhou<sup>1,2</sup> and Yan Shu<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Sciences (S.Z., Y.S.)

School of Pharmacy, University of Maryland at Baltimore, Baltimore, MD, USA

<sup>2</sup>Department of Thyroid Surgery, Hunan Cancer Hospital/the Affiliated Cancer Hospital of

Xiangya School of Medicine, Central South University. (S.Z.)

**Running title: Transcription of SLC drug transporters**

**Correspondence:**

Yan Shu, MD, PhD

Department of Pharmaceutical Sciences

University of Maryland School of Pharmacy

20 N. Pine St., PH Room N519

Baltimore, MD 21201

Tel: 410-706-7358; Fax: 410-706-0886

Email: [yshu@rx.umaryland.edu](mailto:yshu@rx.umaryland.edu)

**Text page:** 29 pages

**Table:** 2 tables

**Figure:** 2 figures

**Reference:** 188 references

**Abstract word count:** 177 words

**Significance Statement word count:** 77 words

**Introduction word count:** 629 words

**Discussion word count:** N/A

**Manuscript total word count:** 7946 words

**Abbreviations:**

SLC, Solute carrier; ABC, ATP-binding cassette; OATPs, Organic anion-transporting polypeptides; OATs, Organic anion transporters; OCTs, Organic cation transporters; OCTNs,

Organic cation/carnitine transporters; PEPTs, Peptide transporters; MATEs, Multidrug and toxin extrusion proteins; HATs, Histone acetylases; HDACs, Histone deacetylases; BBB, Blood-brain barrier; NRs, Nuclear receptors; PXR, Pregnane X receptor; AhR, Aryl hydrocarbon receptor; CAR, Constitutive androgen receptor; FXR, Farnesoid X receptor, LXR, Liver X receptor; HIF-1 $\alpha$ , Hypoxia-inducible factor 1 alpha; HNFs, Hepatocyte nuclear factors; Nrf2, Nuclear factor erythroid 2-related factor 2; RXR, Retinoid X receptor; RAR, Retinoic acid receptor; VDR, Vitamin D receptor; PPAR $\alpha$ , Peroxisome proliferator-activated receptor alpha; PCN, Pregnenolone 16 $\alpha$ -carbonitrile; SHP, Small heterodimer partner 1; CDCA, Chenodeoxycholic acid; TNF- $\alpha$ , Tumor necrosis factor alpha; UDCA, Ursodeoxycholic acid; HCC, Hepatocellular carcinoma; atRA, All-trans retinoic acid; BCL6, B-cell CLL/lymphoma 6; CBF, CCAAT-box-binding transcription factor; HNRNPK, Heterogeneous nuclear ribonucleic protein K; USF, upstream stimulating factor; RCC, Renal cell carcinoma; MLL1, Mixed-lineage-leucemia 1; H3K4me3, Lysine 4 on histone 3; H3K9me3, Lysine-9 on histone H3; H3K27me3, Lysine 27 trimethylation; SRC-1, Steroid receptor coactivator 1; CML, Chronic myeloid leukemia; ERE, Estrogen-responsive element; AREs, Antioxidant response elements; AP-1, Activating protein-1; AP-2rep, Activating protein-2 repressor.

## Abstract

Facilitated transport is necessitated for large size, charged, and/or hydrophilic drugs to move across the membrane. The drug transporters in the solute carrier (SLC) superfamily, mainly including organic anion-transporting polypeptides (OATPs), organic anion transporters (OATs), organic cation transporters (OCTs), organic cation/carnitine transporters (OCTNs), peptide transporters (PEPTs), and multidrug and toxin extrusion proteins (MATEs), are critical facilitators of drug transport and distribution in human body. The expression of these SLC drug transporters is found in tissues throughout the body, with high abundance in the epithelial cells of major organs for drug disposition, such as intestine, liver, and kidney. These SLC drug transporters are clinically important in drug absorption, metabolism, distribution, and excretion. The mechanisms underlying their regulation have been revealing in recent years. Epigenetic and nuclear receptor-mediated transcriptional regulation of SLC drug transporters have particularly attracted much attention. This review focuses on the transcriptional regulation of major SLC drug transporter genes. Revealing the mechanisms underlying the transcription of those critical drug transporters will help us understand pharmacokinetics and pharmacodynamics, ultimately improving drug therapeutic effectiveness while minimizing drug toxicity.

## Significance Statement

It has become increasingly recognized that solute carrier (SLC) drug transporters play a crucial, and sometimes determinative, role in drug disposition and response, which is reflected in decision-making during not only clinical drug therapy but also drug development. Understanding the mechanisms accounting for the transcription of these transporters is critical to interpret their abundance in various tissues under different conditions, which is necessary to clarify the pharmacological response, adverse effects, and drug-drug interactions for clinically used drugs.

## 1. Introduction

Drug transporter is a general term for proteins that undertake the function of transporting drugs across the cell membrane (International Transporter et al., 2010). These proteins usually belong to two super families: solute carrier (SLC) and ATP-binding cassette (ABC) proteins. Although there are exceptions, most SLC transporters are influx transporters, which mostly mediate the uptake of their substrates into cells, and ABC transporters are usually efflux transporters which transport substrates out of cells by hydrolyzing ATP (Hediger et al., 2004). SLCs are widely distributed in various tissues and organs in human body. They usually utilize the ion concentration gradient across the cell membrane as the driving force to transport their substrates (Anderson and Thwaites, 2010). Most SLC transporter proteins contain 7-14 transmembrane helices and have an intracellular and/or extracellular flexible domain, which can assist transmembrane helices to form transmembrane channels (Anderson and Thwaites, 2010). The SLC proteins include over 400 members belonging to 66 families (Perland and Fredriksson, 2017).

In recent years, many SLC members have been characterized as drug transporters because their substrates include a structurally diverse of clinically used drugs. The genes encoding these SLC drug transporters mainly include *SLC21/SLCO* gene family (encoding for organic anion-transporting polypeptides, OATPs), *SLC22A* gene subfamily (for organic anion transporters, OATs; organic cation transporters, OCTs; and organic cation/carnitine transporters, OCTNs), *SLC15A* gene subfamily (for peptide transporters, PEPTs), and *SLC47A* gene subfamily (for multidrug and toxin extrusion proteins, MATEs) (Liu, 2019) (Fig. 1). It is notable that although MATEs belong to the SLC superfamily, they may function

as efflux transporters in polarized epithelia cells such as hepatocytes and renal proximal tubular cells (Loncar et al., 2016). Due to their critical role in drug absorption, distribution, and elimination, alteration in the activities of these SLC drug transporters may result in changes in pharmacokinetics and consequently in drug response, potentially leading to reduced therapeutic effectiveness, developed drug resistance, increased susceptibility to drug-induced tissue injuries, and endogenous toxin-mediated diseases (Niemi, 2010; Huang et al., 2020; Yang and Shu, 2021). The activity of a drug transporter is subject to regulation by various factors such as genetic polymorphisms, co-medication, environmental toxins, food, and disease conditions.

The molecular biology, substrates, inhibitors, and role in drug disposition and response of these SLC drug transporters have been comprehensively reviewed elsewhere (Roth et al., 2012; Cesar-Razquin et al., 2015; Lin et al., 2015; Bhutia et al., 2016; Murray and Zhou, 2017; Rives et al., 2017; Nigam, 2018; Schlessinger et al., 2018; Liu, 2019; Ayka and Sehirli, 2020; Bednarczyk and Sanghvi, 2020; Huang et al., 2020; Koepsell, 2020; Pacha et al., 2021; Pizzagalli et al., 2021; Yang and Shu, 2021). These major SLC drug transporters are also briefly summarized in Table 1. In contrast to their well-recognized clinical importance, the regulatory mechanisms accounting for the activities of SLC drug transporters remain less characterized in general. However, there have been important progresses in recent years on regulation of individual SLC drug transporters *via* the mechanisms at different levels including transcription, post-transcription, translation, and post-translation. Herein we choose to review the transcriptional regulation of those major SLC drug transporters. Currently, the evidence supporting various transcriptional regulation of an individual SLC drug transporter

is predominantly obtained from cellular studies and animal models. Although further clinical studies with a sufficient sample size are needed to validate those preclinical findings, certain transcriptomic analyses with patient tissue samples, pharmacogenetic studies, and characterization of probe drug disposition and effects in human subjects have begun to reveal clinical relevance of such transcriptional regulation. Below we first provide a brief overview of the general mechanisms involved in transcriptional regulation of SLC drug transporters, which is followed by our review on the research progress of transcriptional regulation on those important SLC drug transporters and then our prospective on future research in this area.

## **2. Overview of the mechanisms involved in transcriptional regulation of SLC drug transporters**

Transcriptional regulation refers to alteration in the level of gene expression by changing the rate of transcription. It is a vital process in regulation of gene function in eukaryotes that plays an important role in the accuracy and diversity of genetic information transmission. Transcriptional regulation in eukaryotes includes various processes that are highly related to each other, such as DNA methylation, histone modification, chromatin remodeling, and control of transcription *via* different regulatory factors (Miller and Grant, 2013).

DNA methylation is a form of chemical modification for DNA that alters gene expression without altering the DNA sequence (Fig. 2). The process results in the addition of methyl (CH<sub>3</sub>) groups to the DNA strands, often to the fifth carbon atom of a cytosine ring (Jin et al., 2011). DNA methylation can be found in all vertebrates. The methylation of specific



cytosines can be determined by using bisulfite sequencing. In eukaryotes, DNA methylation predominantly occurs at the dinucleotide CpG. The methylation of CpG dinucleotides in the promoter region may suppress the transcription of the corresponding gene (Sperling, 2007). Sometimes, it may fully silence gene expression, which in turn can render functional deficiency of the corresponding gene.

The chromosomes of eukaryotes are mainly composed of DNA and proteins that include histones and non-histones. Posttranslational modification of the DNA-binding histones has been characterized as a common mechanism in regulation of gene expression (Fig. 2). It refers to chemical modification of histones, such as methylation, acetylation, phosphorylation, adenylation, ubiquitination, and ADP ribosylation, under the action of related enzymes such as histone acetylase (HAT), deacetylase (HDAC), methyltransferase, and demethylase (Wang et al., 2016; Zhang et al., 2021). Histone modification could regulate gene expression by affecting the binding between histones and DNA double strands and altering the conformation of nucleosomes, which may result in chromatin remodeling, or by affecting the binding between transcription factors and the promoter of a gene (Moore et al., 2013).

Chromatin remodeling refers to the molecular mechanism by which the packaging state of chromatin, histones in nucleosomes, and the corresponding DNA molecules are changed during the replication and recombination (Lorch and Kornberg, 2017). ATP-dependent chromatin remodeling factors play a critical role in this process. They regulate chromatin conformation by altering the assembly, disassembly, and rearrangement of nucleosomes on chromatin, thereby improving the accessibility of transcription factors and other

transcription-related factors to local chromatin DNA (Hota and Bruneau, 2016).

Transcription factors are a set of proteins that bind to specific regions of DNA molecules that control transcription, such as the sequences of promoters and enhancers, promoting or suppressing the transcription of genetic information from DNA into RNA (Lambert et al., 2018). The function of transcription factors can be accomplished alone or by forming complexes with other proteins or molecules. The transcriptional factors are encoded by a variety of genes. About 1600 genes have been predicted in the human genome to encode transcription factors. The nuclear receptors (NRs) are a group of transcription factors in cells. Members of the nuclear receptor superfamily play important roles in cell growth, development, differentiation, metabolism and are particularly known to positively or negatively regulate the transcription of SLC drug transporter genes (Geier et al., 2007; Stieger and Geier, 2011; Svoboda et al., 2011; Stieger and Hagenbuch, 2014).

The transcriptional regulation of a transporter gene could be complicated. Multiple mechanisms described above could be involved in regulation of an individual transporter gene. For example, a single NR may be involved in the regulation of multiple hepatic transporter genes, while a transporter gene can be regulated by multiple NRs in the liver (Staudinger et al., 2013; Amacher, 2016). Those major regulatory mechanisms and/or the related regulatory molecules for transcription of major SLC drug transporter genes are briefly summarized in Table 2.

## 2.1 Transcriptional regulation of OATPs

Studies have shown that the expression of OATPs is subject to the regulation *via* DNA

methylation and histone modification (Imai et al., 2013a). A recent analysis on DNA methylation of *SLCO* genes showed that the CpG dinucleotides around the transcription start site (TSS) of *SLCO1B3* have multiple methylation patterns (Ichihara et al., 2010). These methylation patterns have differential impact on the expression of *SLCO1B3* in various human cancer cell lines (Imai et al., 2013b). Furthermore, it has been reported that the promoter region of *SLCO3A1* was hypermethylated while that of *SLCO4A1* hypomethylated in colorectal cancer tissues and cell lines, which resulted in a low mRNA expression of *SLCO3A1* and high expression of *SLCO4A1*, respectively (Rawluszko-Wieczorek et al., 2015). In addition, in prostate tumor tissues, the promoter region of *SLCO4C1* was found to be significantly hypermethylated, which was negatively correlated with the expression of *SLCO4C1* and the prognosis of patients after radical prostatectomy (Li et al., 2019). Interestingly, the use of demethylating agents or histone deacetylase inhibitor could partially restore the expression of some *SLCOs*, such as *SLCO2A1* in colorectal neoplasia (Holla et al., 2008) and in human head and neck squamous cell carcinoma (Zolk et al., 2013), and *SLCO1B3* in hepatic carcinoma (Ichihara et al., 2010). A genome-wide DNA-methylation profiling has revealed that the expression of *Slco1b2* in mouse liver was regulated by DNA methylation in the promoter and by histone acetylation (Imai et al., 2009). In subsequent analyses, the epigenetic profiles of DNA methylation and histone acetylation around the TSS of mouse and human *SLCOs* (*Slco1a1*, *Slco1a6*, *Slc16a2*, *Slco1a4*, *SLCO1B1* and *SLCO1B3*) were found to be associated with tissue-specific expression of these transporters (Imai et al., 2013a). The DNA methylation may be a determinant of the specific tissue distribution of OATPs.

The role of transcription factors in regulation of OATP expression has been widely characterized. The NRs involved in the transcription of *SLCO* genes include: the typical xenobiotic responsive NRs, such as pregnane X receptor (PXR), aryl hydrocarbon receptor (AhR), and constitutive androgen receptor (CAR), and other NRs of both physiological and pharmacological importance, such as farnesoid X receptor (FXR), liver X receptor (LXR), hypoxia-inducible factor 1 alpha (HIF-1 $\alpha$ ), hepatocyte nuclear factors (HNFs), the nuclear factor erythroid 2-related factor 2 (Nrf2), retinoid X receptor (RXR), retinoic acid receptor (RAR), vitamin D receptor (VDR), and peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ) (Guo et al., 2002a; Cheng et al., 2005; Jigorel et al., 2006; Meyer zu Schwabedissen and Kim, 2009; Klaassen and Aleksunes, 2010; Aleksunes and Klaassen, 2012; Eloranta et al., 2012; Wang et al., 2012b).

PXR is a ligand-dependent transcription factor with a broad spectrum of ligands, including many endogenous and exogenous compounds (Masuyama et al., 2005). After binding with ligand, PXR experiences conformation change and binds to the promoter region of target genes to regulate gene expression (Wang et al., 2014). In animals, it was reported early that treatment with PXR ligand pregnenolone 16 $\alpha$ -carbonitrile (PCN) could induce the protein level of rat *Oatp2* by more than three folds (Guo et al., 2002b). In addition, the mRNA expression of rodent *Slco1a4* and the hepatic uptake of OATP substrates could be up-regulated by several PXR ligands (phenobarbital, PCN and spironolactone) (Hagenbuch et al., 2001; Cheng et al., 2005). In human subjects, carbamazepine (CBZ), which is a PXR agonist, was reported to increase the mRNA expression of *OATP-A* (*SLCO1A2*), *OATP-B* (*SLCO2B1*), *OATP-C* (*SLCO1B1*), and *OATP-8* (*OATP1B3*) in the livers of CBZ-treated

patients (Oscarson et al., 2006). Atorvastatin, a possible PXR agonist, has been shown to increase the mRNA expression of *SLCO2B1* in patient livers as well (Björkhem-Bergman et al., 2013). The *in vivo* findings are supported by abundant *in vitro* evidence. For example, treatment with the PXR ligand rifampicin was found to upregulate the mRNA expression of *SLCO1B1*, *SLCO1B3* and *SLCO2B1* in human primary hepatocytes (Rodrigues et al., 2020). PXR also plays a significant role in regulating the mRNA expression of OATPs in certain cancer cells, such as *SLCO2A1* in head and neck squamous cell carcinoma cells (Zolk et al., 2013), *OATP-A (SLC21A3)* in breast carcinoma (Miki et al., 2006), and *SLCO1A2* in breast cancer cells (Meyer zu Schwabedissen et al., 2008). Notably, as a possible effect of OATP induction on drug disposition, rifampicin administration could alter the pharmacokinetics of statins that are OATP substrates (Rodrigues et al., 2020). However, we should be careful while interpreting the DDIs involved those PXR ligands that may have multiple pharmacological mechanisms or targets in different species. For example, there are controversial reports in which rifampicin had no direct effect on the gene expression of *SLCO* in hepatocytes *in vitro* and in monkey livers (Meyer Zu Schwabedissen et al., 2010; Benson et al., 2016; Niu et al., 2019). The molecular mechanism of PXR signaling and the effects by PXR ligands in regulation of *SLCO* transcription *in vivo* thus remains to be fully characterized.

AhR and CAR are also ligand-dependent transcription factors that regulate the transcription of downstream target genes, and particularly those xenobiotic response genes including *SLCO* genes (Kawamoto et al., 1999; Murray et al., 2014; Kersten and Stienstra, 2017). The effects of AhR and CAR activation on specific *SLCO* gene expression may be

opposite. Studies have shown that AhR ligands (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD; polychlorinated biphenyl 126; beta-naphthoflavone, and 3-methylcholanthrene) could increase the mRNA expression of certain OATPs, such as *Slco2b1* and *Slco3a1* in mouse liver (Cheng et al., 2005). Treatment with statins, which have not been characterized as direct AhR ligands, were reported to induce the mRNA expression of *SLCO4C1* through AhR activation in human kidney proximal cells (Toyohara et al., 2009; Suzuki et al., 2011). Furthermore, treatment with the natural product shikonin has been reported to effectively upregulate the transcription of *SLCO1B1* and *SLCO2B1* through activation of AhR in hepatocytes as well (Huang et al., 2018). On the other hand, it has been reported that TCDD could repress the mRNA expression of *SLCO2B1* (Le Vee et al., 2015), *Slco1a1* (Aleksunes and Klaassen, 2012), and *Slco1a4* (Guo et al., 2002a). Whereas CAR activation has been reported to cause decreased expression of *SLCO1B3*, *SLCO2B1* (Jigorel et al., 2006), and *Slco1a1* (Cheng et al., 2005; Aleksunes and Klaassen, 2012), it results in an enhanced activity of *SLCO1A2* gene promoter (Meyer zu Schwabedissen et al., 2008) and an increased expression of *Slco1a4* (Aleksunes and Klaassen, 2012). In addition, the CAR ligand diallyl sulfide could increase the mRNA expression of mouse *Slco1a4* (Guo et al., 2002a) but decrease that of *SLCO1B3* in human liver slices (Jigorel et al., 2006).

FXR acts as an intrahepatic "bile acid receptor", regulating bile acid synthesis, detoxification and transport, and maintaining the homeostasis balance between bile acid and liposome (Glastras et al., 2015). FXR can also regulate the transcription of *SLCO*, indirectly or directly (Ohtsuka et al., 2006; Godoy et al., 2013). On the one hand, the accumulation of bile acids can activate FXR, which in turn promotes the transcription of the FXR-responsive

gene encoding small heterodimer partner 1 (SHP) protein. The SHP could repress the expression of HNF4 $\alpha$ , eventually reducing HNF4-mediated expression of *SLCO1B1* and *SLCO1B3* (Kullak-Ublick et al., 2004). On the other hand, the activated FXR could directly bind to the promoter of *SLCO* genes and regulate its transcription. This is exemplified by several lines of evidence. The genetic polymorphism of FXR promoter has been associated with a decreased expression of *SLCO1B1* (Marzolini et al., 2007). Treatment with the bile acid chenodeoxycholic acid (CDCA), an FXR ligand, could increase the mRNA expression of *SLCO1B3* in human hepatocellular carcinoma cells (Jung et al., 2002). The synthetic FXR agonists GW4064 and fexaramine could significantly increase the transcription activity of *SLCO1B1* gene (Meyer Zu Schwabedissen et al., 2010). In addition, berberine could induce the nuclear translocation of FXR and LXR $\alpha$ , which in turn increased the expression of *SLCO1B1* (Liu et al., 2020). Both agonists and antagonists of FXR could also regulate the transcription of other *SLCO* such as *SLCO1A2*, *SLCO1B3-1B7* (*LST-3TM12*) (Yang et al., 2014; Malagnino et al., 2019). *SLCO1B3-1B7* is a splice variant of *SLCO1B3* and *SLCO1B7*. For OATP1B3–1B7 protein, the N-terminal part of its mRNA derives from *SLCO1B3*, whereas the remaining part of the transcript originates from the neighboring *SLCO1B7* gene locus. Notably, the regulation of *SLCO* transcription by FXR signaling is often involved with other NRs.

LXR is also activated by ligands, which could consequently initiate and regulate the transcriptional expression of its target genes that are important in endogenous metabolism (Wang et al., 2015). It has been reported that treatment with the LXR $\alpha$  agonists TO-901317 and GW3965 could up-regulate the mRNA expression of *SLCO1B1* in Huh-7 cells (Meyer Zu

Schwabedissen et al., 2010). LXR $\alpha$  collaborates with FXR to co-regulate *SLCO1B1* transcription. There is an interaction among LXR $\alpha$ , FXR and the specific DNA binding module of the 5' non-coding region of the *SLCO1B1* gene.

HIFs are transcription factors that respond to oxygen content in cellular environment and are activated in the presence of reduced oxygen levels or hypoxia (Ziello et al., 2007). Several lines of evidence have indicated that a role of HIF1 $\alpha$  in *SLCO* transcriptional regulation. Studies have shown that HIF1 $\alpha$  could bind to the promoter sequences of certain cancer-specific OATP1B3 variants and regulate their mRNA expression (Han et al., 2013). Consistently, HIF1 $\alpha$  was found to regulate the transcription of *SLCO1B3* via two HIF response elements in intron 1 of *SLCO1B3* gene (Ramachandran et al., 2013). In addition, the mRNA expression of *SLCO1B3* and *SLCO2B1* has been found to be induced by HIF-1 $\alpha$  stabilizers but reduced by HIF-1 $\alpha$  knockdown (Shi et al., 2014).

HNFs are a group of phylogenetically unrelated transcription factors that regulate the transcription of a wide range of genes (Lau et al., 2018). Functional HNF1 $\alpha$  response elements have been identified in the proximal promoter of *SLCO1B1* (Jung et al., 2001; Maher et al., 2006). The regulation of *SLCO* transcription by HNFs seems to be related to disease conditions. Lipopolysaccharide (LPS) could down-regulate the mRNA expression of *Slco1b2*, which was attributed to a reduced binding of HNF1 $\alpha$ , HNF3, and RXR:RAR to *Slco1b2* promoter (Li and Klaassen, 2004). The increased expression of the cytokine tumor necrosis factor alpha (TNF- $\alpha$ ) was reported to be a mechanism underlying the reduced expression of *Slco1a1* and *Slco1a4* in the mice with liver injury, which was probably through a HNF1-dependent signaling (Geier et al., 2003). In HNF1 $\alpha$ -null mice, the mRNA expression



of *Slco1a1*, *Slco1a4*, *Slco1a5*, *Slco1b2*, *Slco2b1* in the liver, *Slco1a1*, *Slco1a6*, *Slco2b1*, *Slco3a1*, *Slco4c1* in the kidney, and *Slco2a1* in the duodenum were altered (Maher et al., 2006). There is also *in vivo* evidence in human subjects suggesting the regulation of *SLCO1B1* expression by HNF1 $\alpha$  (He et al., 2008). Ursodeoxycholic acid (UDCA) is an inhibitor of HNF1 $\alpha$ . In healthy subjects, treatment with UDCA could cause significant alteration in the pharmacokinetics of rosuvastatin that is a well characterized OATP1B1 substrate. The regulation of *SLCO* transcription by HNFs could involve other NRs. For example, HNF1 $\alpha$  and HNF3 could partner with RXR:RAR at the *Slco1b2* promoter (Li and Klaassen, 2004). The down-regulation of *SLCO1B3* expression in hepatocellular carcinoma (HCC) has been related to the high expression of HNF3 $\beta$  (Vavricka et al., 2004). Later, it was found that FXR, HNF1 $\alpha$  and HNF3 $\beta$  simultaneously regulated the transcription of *SLCO1B3* (Ohtsuka et al., 2006). In addition, HNF4 $\alpha$  has also been found to promote *SLCO1B3* transcription through the co-activation of  $\beta$ -catenin in HCC (Kitao et al., 2018).

Nrf2 is a nuclear transcription factor that regulates about 250 genes involved in cell homeostasis, including antioxidant proteins, detoxifying enzymes, drug transporters, and many cell protective proteins (Tonelli et al., 2018). In rodents, the activation of Nrf2 could down-regulate the transcriptional expression of *Slco1a1* but up-regulate those of *Slco2b1* and *Slco1b2* (Cheng et al., 2005; Cheng et al., 2011; Aleksunes and Klaassen, 2012; Wu et al., 2012). In the liver of Nrf2 knockout mice, the mRNA expression of *Slco1a6*, *Slco2b1* (Anwar-Mohamed et al., 2011) and *Slco1b2* (Tanaka et al., 2009) were significantly decreased.

There are additional NRs which role in the transcription of *SLCOs* has been explored. In

human hepatocytes, all-trans retinoic acid (atRA) could activate the formation of RXR:RAR heterodimers which could down-regulate the mRNA and protein expression of OATP1B1 and OATP2B1 (Le Vee et al., 2013). The effects of atRA treatment on *SLCO* expression could be counteracted by knockdown of RXR and RAR. VDR, an NR that in complex with hormonally active vitamin D, 1,25(OH)<sub>2</sub>D<sub>3</sub>, regulates the expression of a wide array of physiologically important genes. In small intestinal epithelial cells, VDR has been shown to be involved in the transcriptional activation of *SLCO1A2* (Eloranta et al., 2012). In addition, PPAR $\alpha$  has also been reported to play a vital role in the down-regulation of *Slco1a1*, *Slco1a4*, *Slco1b2* in mouse livers by the environmental toxic chemicals perfluorooctanoic acid and perfluorodecanoic acid (Cheng and Klaassen, 2008). In wild-type mice, treatment with PPAR $\alpha$  ligands, including clofibrate, ciprofibrate, and diethylhexylphthalate, could significantly decrease the mRNA levels of *Slco1a1*, *Slco1b2*, *Slco2a1*, and *Slco2b1* in the liver (Cheng et al., 2005). As recently reviewed elsewhere (Rodrigues et al., 2020; Zamek-Gliszczyński et al., 2021), however, we would like to point out that the direct clinical evidence remains very limited to support an importance of alteration in OATP transcriptional regulation in determining drug disposition and response. Further clinical studies are therefore needed to characterize the clinical relevance for these various regulatory mechanisms of OATP transcription.

## 2.2 Transcriptional regulation of OATs

Epigenetic mechanisms are involved in the transcriptional regulation of *OATs*. Both DNA methylation and histone modification may play a vital role. By identifying the TSS of

*SLC22A8* gene, the Sugiyama group found that the promoter region of *SLC22A8* has multiple CpG dinucleotides as the targets of DNA methylation (Kikuchi et al., 2006). In their follow-up studies, DNA methylation was found to repress the activity of the *SLC22A8* promoter and decrease the expression of *SLC22A8*, whereas the transcription could be resumed when using methyltransferase inhibitors (Kikuchi et al., 2006). DNA methylation is also highly related to the tissue specific expression of OAT1 and OAT3 (Jin et al., 2012). Compared to the renal cortex, most of the CpG dinucleotides around the TSSs of hepatic *Slc22a6* and *Slc22a8* were highly methylated, which consistent with their high expression levels in the kidney but low levels in the liver. Recently, another study showed that histone hypoacetylation rather than DNA hypermethylation was involved in the transcriptional suppression of *SLC22A7* in HCC cell lines (Wang et al., 2021b). When histone hypoacetylation was mitigated with a histone deacetylase inhibitor, the expression of *SLC22A7* was increased.

Earlier findings have revealed the transcriptional regulation of *OATs* by transcriptional factors and particularly various NRs. As early as in 2003, studies have found that the promoter regions of *SLC22A6* and *SLC22A8* have the binding sites for several transcription factors including PAX1, PBX, WT1 and HNF1 (Eraly et al., 2003). There is considerable evidence supporting that transcription factors function in transcription of *OATs*. For example, in reporter gene assays, expression of the transcription factor B-cell CLL/lymphoma 6 (BCL6) led to increased activities of the reporter luciferase that was driven by the promoters of *Slc22a6* and *Slc22a8* (Wegner et al., 2012). However, BCL6 seemed to not directly activate the promoter of *SLC22A6*. It indirectly enhanced the transcription by increasing the protein expression of HNF1 $\alpha$  (Wegner et al., 2014). In addition, ER $\alpha$  can indirectly regulate

the transcription of *SLC22A6* as well (Euteneuer et al., 2019). There was no direct binding of ER $\alpha$  to the *SLC22A6* promoter. Instead, the ligand-activated ER $\alpha$  could activate the CCAAT-box-binding transcription factor (CBF) and heterogeneous nuclear ribonucleic protein K (HNRNPK), both of which could bind to the promoter of *SLC22A6* and enhance its transcription. On the other hand, the ligand-activated LXRs could reduce *SLC22A6* expression in the renal S2 cells, which in turn resulted in a decreased transport of OAT1 substrates in these cells (Kittayaruksakul et al., 2012). However, LXRs may have a cell-specific effect on the expression of different OATs. In the hepatocytes isolated from the rats with hypercholesterolemia, the expression of both LXR $\alpha$  and Oat2 were found to be reduced. In follow-up experiments with HepG2 cells treated with hypercholesterolemic serum, LXR $\alpha$  activation could rescue the reduced expression of *SLC22A7* (Liu et al., 2016a).

Studies have also shown the regulation of *OAT* transcription by xenobiotic responsive NRs. Ligand-activated CAR and AhR could down-regulate *SLC22A7* expression (Jigorel et al., 2006; Le Vee et al., 2015). The indoxyl sulfate was reported to induce the expression of *SLC22A6* via AhR-EGFR signaling in the kidney, which could be suppressed by AhR antagonism (Jansen et al., 2019). Methotrexate is a well characterized substrate of OATs. Interestingly, treatment with methotrexate could decrease the expression of *Slc22a6* and *Slc22a8* in rat kidneys (Shibayama et al., 2006). As the decrease was accompanied with downregulation of CAR and PXR expression, it is likely that these two NRs was involved in the reduction of *Slc22a6* and *Slc22a8* expression. Moreover, the activation of Nrf2 was reported to cause a significant decrease in *Slc22a7* mRNA expression (Wu et al., 2012). In addition, FXR could regulate the transcription of *SLC22A7* either directly or indirectly

(Popowski et al., 2005). FXR could directly bind to and inhibit the transactivation of *SLC22A7* promoter. FXR activation could also lead to an increased expression of the gene encoding the SHP protein (Lee et al., 2000), which is a corepressor of HNF4 $\alpha$ . The increased SHP would therefore decrease the nuclear binding activity of HNF4 $\alpha$ , ultimately leading to a decrease in the expression of *SLC22A7* (Popowski et al., 2005).

The role of cellular signaling with HNFs in regulation of *OAT* transcription has been widely studied. HNF1 $\alpha$  and HNF4 $\alpha$  may regulate the transcription of many *OAT* genes in developing kidneys because there exist a number of HNF1 $\alpha$ /HNF4 $\alpha$  binding elements at the proximal promoter regions of *OAT* genes (Gallegos et al., 2012; Martovetsky et al., 2013; Nigam et al., 2015). Motif analysis of *cis*-regulatory enhancers in the developing proximal tubules of rodents has suggested that Hnf4a and Hnf1a were the main transcriptional regulators of multiple SLC drug transporters, which include *Slc22a6*, *Slc22a8*, *Slc22a1*, *Slc22a2* and *Slc47a1* (Martovetsky et al., 2013). Human HNF1 $\alpha$ / $\beta$  was also found to bind with the HNF1-motif that was located near the TSS of *SLC22A6*, *SLC22A8*, *SLC22A11*, and *SLC22A12* genes, thereby enhancing the promoter activity and activating the transcription of these transporter genes (Kikuchi et al., 2006; Kikuchi et al., 2007; Saji et al., 2008; Jin et al., 2012). Of note, mutations in the HNF1-motif could fully abolish the enhanced promoter activity that was associated with HNF1 $\alpha$ / $\beta$  binding. Besides HNF1 $\alpha$ / $\beta$ , HNF4 $\alpha$  also regulates the transcription of *OAT* genes in a similar manner (Ogasawara et al., 2007; Thiagarajan et al., 2011). The involvement of HNF1 $\alpha$  in *OAT* gene transcription has also been evidenced in genetic mouse models (Maher et al., 2006; Kikuchi et al., 2007). In HNF1 $\alpha$ -null mice, the mRNA expression of *Slc22a6*, *Slc22a8* and *Slc22a12* was significantly decreased in the

kidney and that of *Slc22a7* was reduced in both liver and kidney. However, the expression of *Slc22a8* was increased in the duodenum. OAT2, OAT5, and OAT7 are three OATs in human liver. Whereas *SLC22A7* gene is subject to the transactivation by HNF4 $\alpha$  (Popowski et al., 2005), the experimental evidence from genetic manipulation and reporter gene assays, together with expression analysis in surgical liver tissues, has indicated that HNF1 $\alpha$  is a critical transcriptional factor in regulation of *SLC22A10* and *SLC22A9* expression (Klein et al., 2010).

### 2.3 Transcriptional regulation of OCTs

The impact of genetic variants and epigenetic regulation of OCTs in general has been briefly summarized recently (Kolz et al., 2021). Herein we select to review the findings related to transcriptional regulation. Among epigenetic mechanisms, DNA methylation is likely the most extensively investigated for the transcriptional regulation of *OCT* genes. OCT1 is highly expressed in human livers. Based on microarray analyses with patient liver tissues, Schaeffeler *et al* found that *SLC22A1* expression was lower in hepatocellular carcinoma as compared with normal liver tissues, which was accompanied with DNA hypermethylation at the promoter of *SLC22A1* (Schaeffeler et al., 2011). In addition, the level of *SLC22A1* mRNA in cisplatin-resistant human esophageal cancer cells was found to be markedly reduced as compared to that in the sensitive cells (Lin et al., 2013). Consistently, the promoter of *SLC22A1* was highly methylated in the cisplatin-resistant cells. As cisplatin is a moderate substrate of OCT1, DNA methylation could be a mechanism accounting for cisplatin chemo-resistance in some cancer patients.

Human OCT2 is a rather kidney-specific transporter. DNA methylation was found to be associated with the tissue specificity of human OCT2. The CpG sites in the proximal promoter of *SLC22A2* gene were reported to be hypermethylated in the liver while hypomethylated in the kidney, which is consistent with a relatively low hepatic expression but a much high renal expression of *SLC22A2* (Aoki et al., 2008). The hypomethylation of *SLC22A2* promoter occurred particularly at the CpG site in the E-box that is the binding site of the basal transcription factor called upstream stimulating factor (USF) 1. Increased methylation of *SLC22A2* proximal promoter could dramatically reduce the binding of USF1 to the E-box, leading to a reduced *SLC22A2* transcription. Renal cell carcinoma (RCC) is characterized by multi-drug resistance. The Zeng group has identified the repressed expression of *SLC22A2* as a mechanism for oxaliplatin resistance in RCC (Liu et al., 2016b). They revealed that the repressed *SLC22A2* expression was characterized by hypermethylation in *SLC22A2* promoter which blocked the interaction between the transcription factor MYC and the E-Box motif and prevented MYC from recruiting the histone methylase mixed-lineage-leucemia 1 (MLL1) to catalyze the trimethylation of lysine 4 on histone 3 (H3K4me3), eventually leading to the repressed *SLC22A2* transcription. Importantly, the epigenetic activation of *SLC22A2* by the DNA methylation inhibitor decitabine could sensitize RCC cells to oxaliplatin treatment in the animal model. They further found that histone acetylation also regulates *SLC22A2* expression in RCC (Zhu et al., 2019). In normal kidney tissues, a dynamic balance of histone acetylation is maintained by histone acetyltransferases (HATs) and histone deacetylases (HDACs). In RCC cells, the interaction between HDAC7 and MYC was disrupted, leading to high abundance of HDAC7 and low

levels of histone acetylation at H3K18ac and H3K27ac around *SLC22A2* promoter that contributed to the repression of *SLC22A2* transcription. In addition, Saito *et al* reported that the placental expression of *SLC22A2* was likely subject to epigenetic regulation as well (Saito *et al.*, 2011). The mRNA expression of *SLC22A2* was higher in the biallelic placental samples than that in monoallelic samples, which was associated with an increased H3 acetylation but decreased trimethylation of lysine-9 on histone H3 (H3K9me3) around *SLC22A2* promoter.

For the widely expressed human OCT3, the proximal promoter has been reported to be located within a large CpG island extending into exon one of which hypermethylation might cause a reduced expression of *SLC22A3* gene (Chen *et al.*, 2013). Compared with normal tissues, the hypermethylation of *SLC22A3* promoter was associated with reduced *SLC22A3* transcript levels in prostate tumors. However, the hypermethylation of *SLC22A3* promoter appeared to be prostate tumor-specific as it was not detected in other tumor tissues (Chen *et al.*, 2013). In addition, metformin, which is a first-line therapy for type 2 diabetes, has been reported to reduce DNA methylation at the genes encoding metformin transporters including *SLC22A3* (Garcia-Calzon *et al.*, 2017). While the exact mechanism has not been revealed, these epigenetic changes seem to be associated with metformin anti-diabetic efficacy.

The role of NRs in regulation of *OCT* gene transcription has also been explored. At present, the regulatory role of PXR in *SLC22A1* expression remains controversial. It has been suggested that doxorubicin treatment could suppress *SLC22A1* expression by increasing the expression of PXR in renal tubular epithelial cells (Nagai *et al.*, 2019). The transcriptional activity of *SLC22A1* promoter and consequently the mRNA expression of *SLC22A1* were reported to be subject to the suppression by ligand-activated PXR in human hepatocytes



(Hyrsova et al., 2016). The effect by a PXR agonist has been ascribed to its competitively sequestering the coactivator steroid receptor coactivator 1 (SRC-1) from the HNF4 $\alpha$  response element and E-box in the *SLC22A1* promoter, reducing the transactivation of *SLC22A1* gene by HNF4 $\alpha$  and USF transcription factors. However, in chronic myeloid leukemia (CML) cell lines and primary CML cells, the expression of *SLC22A1* was upregulated by the agonists of PXR, RAR and RXR (Austin et al., 2015). The PXR agonist rifampin was reported to increase the mRNA expression of *SLC22A1* in peripheral blood cells and enhance the glucose-lowering effect of metformin in healthy human subjects (Cho et al., 2011). In addition, overexpression of PXR was found to increase the mRNA expression of *Slc22a1* in rat hepatocytes, while treatment with the PXR agonist PCN induced the expression of *Slc22a1* in the liver and that of *Slc22a2* in the kidney in rats (Maeda et al., 2007). Further studies are needed to clarify the role of PXR in *OCT* gene transcription in different cells and tissues and the *in vivo* effects.

It has been reported that an increased level of CDCA, which could be induced by cholestasis, down-regulates the mRNA expression of *SLC22A1* and *SLC22A3* in the liver (Saborowski et al., 2006; Nies et al., 2009). As CDCA is a ligand of FXR, the down-regulation has been attributed to the inhibition of HNF4 $\alpha$ -mediated transactivation that results from CDCA-induced expression of the transcriptional repressor SHP *via* FXR signaling. In agreement with this, the expression of *Slc22a1* was significantly downregulated in rats with obstructive cholestasis (Denk et al., 2004). In addition, by treating human renal proximal tubular cells (RPTEC/TERT1 cells) and OCT2-CHO-K1 cells with CDCA and an FXR antagonist, Z-guggulsterone, a recent study have suggested that FXR also plays a role in

regulation of renal *SLC22A2* expression (Wongwan et al., 2020).

Other NRs are also involved in transcriptional regulation of *OCT* genes. By measuring the promoter activity in rat renal epithelial cell line LLC-PK1, Asaka *et al* have demonstrated that testosterone treatment could stimulate the promoter activity of rat *Slc22a2* via the androgen receptor-mediated signaling pathway (Asaka et al., 2006). In the kidney of PPAR $\alpha$ -null mice, the expression of *Slc22a2* was decreased, suggesting that PPAR $\alpha$ , which is highly expressed in the liver and kidney, may regulate the expression of *Slc22a2* (Freitas-Lima et al., 2020). As the uptake of cisplatin into the proximal tubules is significantly mediated by OCTs, deletion of PPAR $\alpha$  was protective of cisplatin-induced nephrotoxicity in mice (Freitas-Lima et al., 2020). PPAR $\alpha$  was also found to mediate the circadian expression of *Slc22a2* in mice (Oda et al., 2014). There are two HNF4 $\alpha$  response elements in human *SLC22A1* promoter which were involved in HNF4 $\alpha$ -mediated transactivation of *SLC22A1* transcription (Saborowski et al., 2006). HNF4 $\alpha$  could enhance *SLC22A1* promoter activity by synergistic interaction with the transcription factor USF1 or USF2 (Kajiwara et al., 2008). Lastly but not least, the activation of glucocorticoid receptor could indirectly increase the expression of *SLC22A1* by upregulating HNF4 $\alpha$  expression in human primary hepatocytes (Rulcova et al., 2013).

## 2.4 Transcriptional regulation of OCTNs

There is evidence supporting a role of DNA methylation in regulation of *OCTN* gene transcription. Qu *et al* identified a region (from -354 to +85) in *SLC22A5* promoter that was critical to the transcription of *SLC22A5* gene (Qu et al., 2013). The methylation of CpG sites

in this region was found to be negatively correlated with the expression of *SLC22A5* in cancer cells. Treatment with the demethylating agent decitabine could significantly reduce the hypermethylation at the *SLC22A5* promoter, which enhanced the expression of *SLC22A5* and consequently the transporter activities in HepG2 and LS174T cancer cells. Scalise *et al* have also indicated that DNA methylation is an important mechanism underlying the down-regulation of *SLC22A5* expression in epithelial cancer cells (Scalise et al., 2012). After treatment with 5-aza-cytidine, which is a demethylating agent, the mRNA expression of *SLC22A5* could increase for 10 times in these cancer cells. Future studies are needed to determine the details of epigenetic regulation on *OCTN* transcription.

PPARs are among the major transcriptional factors characterized in the transcriptional regulation of *OCTNs*. The regulation of *OCTNs* by PPAR has been found to widely exist in humans, rodents, cattle, pigs and other animals. With multiple approaches to activation of PPAR $\alpha$ , including treatment with PPAR $\alpha$  agonists such as clofibrate and WY-14,643, oxidized fat diet feeding, and food restriction, the Eder group have demonstrated that PPAR $\alpha$  activation can lead to the upregulation of *Slc22a5* in mice, rats, and pigs (Luci et al., 2006; Koch et al., 2007; Luci et al., 2007; Ringseis et al., 2007a; Ringseis et al., 2007b; Ringseis et al., 2007c; Koch et al., 2008; Luci et al., 2008; Ringseis et al., 2008a; Ringseis et al., 2008b; Ringseis et al., 2009). In line with this, the expression of *Slc22a5* is significantly reduced in PPAR $\alpha$ -null mice (van Vlies et al., 2007; Koch et al., 2008). Moreover, Maeda *et al* have analyzed the 5'-flanking promoter region of rat *Slc22a5* and identified several putative peroxisome proliferator response elements (PPREs) (Maeda et al., 2008). After treatment with the PPAR $\alpha$  agonist fenofibrate, there was an enhanced binding of PPAR $\alpha$  to the

proximal PPREs of *Slc22a5*. However, the increase of *Slc22a5* promoter activity after PPAR $\alpha$  activation was less than what was expected from that of *Slc22a5* mRNA levels, suggesting existence of additional PPREs which might be located outside the proximal promoter region of *Slc22a5* gene. In fact, a subsequent study by Eder group revealed that there were functional PPREs in the first intron of *Slc22a5*, which were highly responsive to both exogenous PPAR $\alpha$ /RXR expression and agonists (Wen et al., 2010). The sequence of intron 1 containing the PPREs showed a high degree of similarity between mice and rats. Functional PPREs have also been identified in the intron 1 of porcine, bovine, and human *SLC22A5* genes (Luo et al., 2014), suggesting that the regulation of *SLC22A5* by PPAR $\alpha$  is highly conserved across species. Besides *OCTN2*, the transcription of *OCTN3* seems to be also subject to PPAR $\alpha$  regulation. The expression of *Octn3* gene was lower in the kidney and small intestine of PPAR $\alpha$ -null mice as compared to wild-type mice (van Vlies et al., 2007; Koch et al., 2008). PPAR $\alpha$  activation increased the expression of *Octn3* and its transport function in rat astrocytes as well (Januszewicz et al., 2009).

By using different PPAR-null models and specific PPAR inhibitors, D'Argenio *et al* have demonstrated that PPAR $\gamma$  could also regulate the expression of *Slc22a5* gene in colon *via* binding to the PPRE at the first intron as a heterodimer with RXR $\alpha$  (D'Argenio et al., 2010). The expression of *SLC22A5* and the corresponding transport function could be increased by the PPAR $\gamma$  agonists thiazolidinediones and luteolin in mice and colon cells (D'Argenio et al., 2010; Qu et al., 2014). Notably, the colon *Slc22a5* expression in PPAR $\alpha$ -null mice was significantly upregulated by overexpression of a constitutively active PPAR $\gamma$  mutant, indicating an independent regulation by PPAR $\gamma$  (D'Argenio et al., 2010). Additional

transcriptional factors may be involved in regulation of *OCTN* expression. For example, *SLC22A5* has been reported to be an estrogen-dependent gene *via* an intronic estrogen-responsive element (ERE) in breast cancer cells (Wang et al., 2012a). In addition, as a line of clinical evidence, the mRNA level of *SLC22A5* has been reported to be decreased in liver biopsies of those patients received the PXR agonist carbamazepine (Oscarson et al., 2006). However, the number of transcriptional factors in understanding of *OCTN* transcription are still limited.

## 2.5 Transcriptional regulation of PEPTs

The evidence on the transcriptional regulation of PEPT transporters is not as much as that for the SLC drug transporters described above. Most related studies have been focused on PETPT1. There is little understanding of epigenetic regulation of PEPTs until very recently. Wang *et al* reported a suppressed expression of *SLC15A1* (*PEPT1*) in colon cancer due to DNA methylation and histone deacetylation (Wang et al., 2021a). DNA methyltransferase 1 was characterized as the primary determinant of the hypermethylation of *SLC15A1* proximal promoter. In addition, the absence of CBP/P300-mediated H3K18/27Ac combined with the histone deacetylases-mediated histone hypoacetylation around the *SLC15A1* promoter also accounted for the repressed expression of *SLC15A1*.

The transcriptional regulation of *PEPTs* by NRs has not been well investigated either. Among a few studies, cholate-induced FXR/PPAR $\alpha$  activation/inhibition has been reported to play a role in *SLC15A1* transcription (Okamura et al., 2014; Liang et al., 2020). In the intestine of diabetic rats, the level of CDCA was significantly increased. As a natural ligand

of FXR, CDCA could down-regulate the expression of rat *Slc15a1*, which could be reversed by treatment with the FXR inhibitor glycine- $\beta$ -muricholic acid or by FXR knockdown (Liang et al., 2020). On the other hand, treatment with the PPAR $\alpha$  agonist WY-14643 increased the mRNA level of *SLC15A1* and its transport function in rat intestine and human intestinal Caco-2 cells (Shimakura et al., 2006a). The increase in free fatty acids, which are endogenous ligands for PPAR $\alpha$ , could also significantly increase the expression of *Slc15a1* mRNA in rodents (Shimakura et al., 2006a). Two nucleotide sequences homologous to PPRE have been identified within 2.0 kb upstream to the TSS of the mouse and human *SLC15A1* gene (Okamura et al., 2014). The bile acids accumulated in intestinal epithelial cells after eating could interfere with the recruitment of the co-transcriptional activator CBP/p300 to the promoter region of *SLC15A1* gene, thereby inhibiting PPAR $\alpha$ -mediated transactivation of *SLC15A1*. However, the regulatory effects of PPAR $\alpha$  on *SLC15A1* expression has not been fully confirmed in other studies (Hirai et al., 2007; Saito et al., 2008).

A few other transcription factors, including Sp1, Cdx2 and Nrf2, have also been shown to regulate the transcriptional activity of *SLC15A1* promoter (Shimakura et al., 2005; Shimakura et al., 2006b; Geillinger et al., 2014). The -172 ~ -35 bp region of *SLC15A1* promoter lacks TATA-box; however, it contains GC-rich sites which can bind with the transcription factor Sp1 to recruit the TATA-binding protein and initiate transcription (Shimakura et al., 2005). The mutation of the Sp1 sites resulted in a reduced transcriptional activity of human *SLC15A1* promoter. Moreover, while co-expression of Cdx2 and Sp1 has been found to synergistically activate the *SLC15A1* promoter, the mutation of Sp1 sites reduced the effect of Cdx2 (Shimakura et al., 2006b). In addition, an increased Nrf2 activity

was found to upregulate the expression of *SLC15A1* via binding with the antioxidant response elements (AREs) which are close to the start codon in human *SLC15A1* gene (Geillinger et al., 2014).

## 2.6 Transcriptional regulation of MATEs

Only limited findings are available regarding transcriptional regulation on MATE transporters. A CpG island located in the 27 kb upstream of *SLC47A1* gene has been identified as an enhancer (Tanaka et al., 2018). The methylation levels of this CpG island were negatively correlated with the mRNA expression of *SLC47A1* in the liver (Tanaka et al., 2018). However, the detail regulation of *SLC47A1* expression through DNA methylation is currently unclear. As for OCT2 described above, the expression of another MATE transporter, MATE2, has been reported to be suppressed in RCC (Yu et al., 2017). The suppression was related to histone modification. Specifically, the protein scaffold adjacent to the *SLC47A2* gene was found to be enriched with histone H3K4me3 and lysine 27 trimethylation (H3K27me3) in normal renal tissues. In RCC, the binding between the histone methylase MLL1 and the promoter region was lost, reducing H3K4me3 enrichment and the transcriptional activity of *SLC47A2* promoter. In addition, there was deacetylation of H3K27 in RCC which prevented the enrichment of H3K4me3 as well.

Several studies have explored the role of transcriptional factors in transcription of *MATEs*. The Giacomini group has characterized the transcriptional activity of the basal promoter of human *SLC47A1* gene. They identified polymorphic sequences in the promoter region which could bind to two transcriptional factors, activating protein-1 (AP-1) and

activating protein-2 repressor (AP-2rep), in regulation of the transcription of *SLC47A1* gene (Ha Choi et al., 2009). By using different NR pharmacological activators, a study by Lickteig *et al* have suggested that AhR, CAR, PXR, Nrf2 and PPAR $\alpha$  do not play a critical role in the transcription of *Slc47a1* and *Slc47a2* in mouse livers (Lickteig et al., 2008). However, the mRNA expression of *Slc47a1* in the kidney of PPAR $\alpha$ -null mice was increased (Freitas-Lima et al., 2020) and dramatically decreased in the liver of HNF4 $\alpha$ -null mice (Lu et al., 2010), suggesting a role of these two NRs in tissue-specific MATE transcription. In addition, the mRNA expression of *Slc47a1* was significantly increased in the liver of NADPH-cytochrome P450 oxidoreductase (Cpr)-null mice where Nrf2 was activated (Cheng et al., 2014). Moreover, treatment with the Nrf2 activator bardoxolone methyl was found to significantly increase the mRNA expression of *SLC47A1* in human renal proximal tubular epithelial cells (Atilano-Roque et al., 2016). Interestingly, by using microfluidic culture of human proximal tubules, Fukuda et al have demonstrated that fluid shear stress stimulates MATE2-K expression *via* Nrf2 pathway activation (Fukuda et al., 2017). Lastly but not least, FXR may be involved in the transcriptional regulation of human *MATEs* because treatment with its ligand CDCA could significantly increase the mRNA expression of *SLC47A1* and *SLC47A2* in human renal proximal tubular cells (Wongwan et al., 2020). Further mechanistic studies are needed to clarify the regulatory role of these NRs in transcription of *MATE* genes.

### 3. Closing remarks

SLC drug transporters are present in tissues with varying abundance. It would be a prerequisite to clarify the accurate localization and abundance of these transporters, before



elucidating their role in the absorption, distribution, metabolism, excretion, and consequently the pharmacological and toxicological effects of clinical drugs. The transcriptional regulation is the major determinant of tissue distribution and abundance for these drug transporters. We have provided an overview and collected information on transcriptional regulation of major SLC drug transporters in this review. In consideration of space limit, however, we are unable to discuss the molecular mechanisms in detail for the transcriptional regulation of these transporters. Neither did we include all SLC families whose members sometimes facilitate the movement of clinical used drugs across the membrane. In addition, there are progresses in terms of preclinical evidence and clinical implication which deserve a specific review under individual topics. These limitations have inevitably compromised the comprehensiveness of this review.

Whereas the importance of SLC transporters in drug disposition and response is widely appreciated, understanding the role of epigenetic and NR-mediated transcriptional regulation has just begun. There are progresses suggesting clinical implication of such regulation for SLC drug transporter genes. For example, by targeting the epigenetic mechanism of OCT2 transcription, a sequential combination therapy using the DNA methylation inhibitor decitabine has been proposed to overcome the resistance of RCC to oxaliplatin chemotherapy (Liu et al., 2016b). However, overall, the clinical evidence remains very limited. It is challenging to ascertain the effects by an alteration in transporter transcription on drug disposition and response in animal models and particularly in human subjects *in vivo*. While one mechanism or one NR may govern the transcription of multiple SLC drug transporter genes, an individual transporter can be regulated *via* multiple mechanisms as well. The lack

of selective probe substrates, inhibitors, and regulatory molecules for specific drug transporters has been bringing another layer of complexity in interpretation of *in vivo* data. Current effort in characterizing drug-drug interactions (DDIs) for an increasing number of compounds will bring us much-needed probe drugs to assess the function of SLC drug transporters and their regulation. Moreover, investigating the impact of SLC transcriptional changes on drug disposition and response with appropriate probe compounds in genetic animal models and genetically polymorphic human populations may yield invaluable preclinical and clinical evidence soon. Of note, there are also different levels of regulation on transporter function. For example, post-translational modification on transporter proteins have been widely reported to affect SLC drug transporter function (Murray and Zhou, 2017; Xu and You, 2017; Czuba et al., 2018; Lee et al., 2020). A novel kinase-dependent posttranslational regulation of OATP1B1 activity has been recently characterized as a mechanism underlying DDIs between broad OATP1B1 substrates and the commonly prescribed tyrosine kinase inhibitors (TKI) (Hayden et al., 2021). We have also identified a mechanism regulating the membrane translocation of OCT2 and consequently the nephrotoxicity of cisplatin, a widely prescribed anticancer drug (Yang et al., 2020). Our understanding of a full range of regulatory mechanisms for drug transporters will eventually have an impact on optimization of pharmacotherapy regimens in order to improve drug efficacy and avoid unnecessary toxicity.

## **AUTHORSHIP CONTRIBUTIONS**

Wrote the manuscript: Zhou & Shu



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## FOOTNOTES

This work was supported by the National Institutes of Health [Grant R01GM099742]. No author has an actual or perceived conflict of interest with the contents of this article.



## Figure Legends

**Fig 1. Location of major SLC drug transporters in human liver, intestine, blood-brain barrier (BBB), and kidney.** OATPs: Organic anion-transporting polypeptides; OATs: Organic anion transporters; OCTs: Organic cation transporters; OCTNs: Organic cation/carnitine transporters; PEPTs: Peptide transporters; MATEs: Multidrug and toxin extrusion proteins.

**Fig 2. Epigenetic regulation of gene expression by DNA methylation and histone acetylation.** As shown, the degree of methylated CpG islands in a promoter is usually negatively correlated with the level of gene expression. Histone acetylation is also an important epigenetic mechanism for gene expression regulation. The histone acetylases (HATs) may catalyze the transfer of acetyl groups to the lysine residues on the histone tail and activate gene transcription, whereas histone deacetylases (HDACs) can deacetylate histone and inhibit gene transcription instead.



**Table 1.** Major solute carrier (SLC) drug transporters.<sup>a</sup>

SLC Subfamily <sup>b</sup>	Species	Transporter Gene Name (Protein Name)	Organ with highest expression <sup>c</sup>	Common substrates <sup>d</sup>
OATPs	Humans	<i>SLCO1A2</i> or <i>SLCO1A3</i> (OATP-A or OATP1A2)	Brain	Bromsulphthalein (BSP), Methotrexate, Enalapril, Levofloxacin
		<i>SLCO1B1</i> (OATP1B1)	Liver	Cobimetinib, Lovastatin, Clotrimazole, Valsartan, Cyclosporine, Rifampicin
		<i>SLCO1B3</i> (OATP1B3)	Liver	Rifampicin, Olmesartan, Methotrexate, Digoxin, Pioglitazone
		<i>SLCO1B3-SLCO1B7</i> or <i>LST-3TM12</i> (OATP1B3-1B7)	Liver and Breast	Taurocholic acid, Lithocholic acid, Ezetimibe
		<i>SLCO2A1</i> (OATP2A1)	Lung	Alprostadil, Latanoprost, Iloprost
		<i>SLCO2B1</i> (OATP2B1)	Liver	Azilsartan, Balsalzone, Gavestinel, Valsartan
		<i>SLCO3A1</i> (OATP3A1)	Brain	Benzylpenicillin, Safinamide, Iloprost
		<i>SLCO4A1</i> (OATP4A1)	Lung	Liothyronine, Levothyroxine, Benzylpenicillin,
		<i>SLCO4C1</i> (OATP4C1)	Kidney	Liothyronine, Digoxin, Saxagliptin, Ouabain
	Rodents	<i>Slco1a1</i> (Oatp1a1 or Oatp1)	Liver and Kidney	Estradiol 17beta-d-glucuronide
		<i>Slco1a4</i> (Oatp1a4 or Oatp2)	Brain, Liver and Kidney	Sulforhodamine-101 (SR-101)
		<i>Slco1a5</i> (Oatp1a5)	Kidney	Fexofenadine, Celiprolol
		<i>Slco1a6</i> (Oatp1a6)	Kidney	Taurocholic acid (TCA)
		<i>Slco1b2</i> (Oatp1b2)	Liver	Pitavastatin
		<i>Slc16a2</i> (Oatp1c1)	Liver and Kidney	Sulforhodamine-101 (SR-101), Thyroxine (T4)
		<i>Slco2a1</i> (Oatp2a1)	Lung and Liver	Phenolsulfonphthalein
		<i>Slco2b1</i> (Oatp2b1)	Ubiquitous	Fexofenadine, Rosuvastatin

		<i>Slco3a1</i> (Oatp3a1)	Brain	Sodium fluorescein
		<i>Slco4c1</i> (Oatp4c1)	Kidney and Lung	Digoxin
OATs	Humans	<i>SLC22A6</i> (OAT1)	Kidney	Cimetidine, Methotrexate, Indomethacin, Probenecid, Oxytetracycline, Hydrochlorothiazide
		<i>SLC22A7</i> (OAT2)	Liver	Oxytetracycline, Cimetidine, Methotrexate, Salicylic acid
		<i>SLC22A8</i> (OAT3)	Kidney	Taurocholic acid, Benzylpenicillin, Cimetidine, Tetracycline
		<i>SLC22A11</i> (OAT4)	Kidney	Oxytetracycline, Aminohippuric acid, Methotrexate, Relebactam
		<i>SLC22A10</i> (OAT5)	Liver	None reported
		<i>SLC22A9</i> (OAT7)	Liver	Estrone sulfate, Dehydroepiandrosterone, Butyrate
		<i>SLC22A12</i> (URAT1)	Kidney	Urate
	Rodents	<i>Slc22a6</i> (Oat1)	Kidney	same as SLC22A6
		<i>Slc22a7</i> (Oat2)	Liver and Kidney	same as SLC22A7
		<i>Slc22a8</i> (Oat3)	Kidney	same as SLC22A8
		<i>Slc22a12</i> (Urat1)	Kidney	Urate
	OCTs	Humans	<i>SLC22A1</i> (OCT1)	Liver
<i>SLC22A2</i> (OCT2)			Kidney	Metformin, Cimetidine, Cisplatin, Quinidine,
<i>SLC22A3</i> (OCT3)			Skeletal muscle	Metformin, Cimetidine, Oxaliplatin
Rodents		<i>Slc22a1</i> (Oct1)	Liver, Kidney and Intestine	same as SLC22A1
		<i>Slc22a2</i> (Oct2)	Kidney	same as SLC22A2
OCTNs	Humans	<i>SLC22A5</i> (OCTN2)	Skeletal muscle, Kidney, Heart and Placenta	Amphetamine, Nicotine, Formoterol, Formoterol, Colistin, Oxaliplatin
	Rodents	<i>Slc22a5</i> (Ocn2)	Kidney, Liver and Testis	same as SLC22A5
		<i>LOC303140</i> (Ocn3)	Testes	Acetylcarnitine
PEPTs	Humans	<i>SLC15A1</i>	Intestine	Cephalexin, Enalapril,

		(PEPT1)		Benzylpenicillin,
	Rodents	<i>Slc15a1</i> (Pept1)	Intestine	same as SLC15A1
MATEs	Humans	<i>SLC47A1</i> (MATE1)	Adrenal gland	Metformin, Levofloxacin, Cimetidine, Cisplatin
		<i>SLC47A2</i> (MATE2 or MATE2-K)	Kidney	Metformin, Cimetidine, Cisplatin, Quinidine,
	Rodents	<i>Slc47a1</i> (Mate1)	Kidney and Liver	same as SLC47A1
		<i>Slc47a2</i> (Mate2)	Testis	same as SLC47A2

Note: <sup>a</sup>Only those SLC drug transporters reviewed in the text are presented. <sup>b</sup>OATPs: organic anion-transporting polypeptides; OATs: organic anion transporters; OCTs: organic cation transporters; OCTNs: organic cation/carnitine transporters; PEPTs: peptide transporters; MATEs: multidrug and toxin extrusion proteins. <sup>c</sup>The information is extracted from The Human Protein Atlas ([www.proteinatlas.org](http://www.proteinatlas.org)) and UniProt ([www.uniprot.org](http://www.uniprot.org)). <sup>d</sup>Only selected compounds are presented; Please refer to the literatures for a more comprehensive list of the substrates (Roth et al., 2012; Cesar-Razquin et al., 2015; Lin et al., 2015; Bhutia et al., 2016; Murray and Zhou, 2017; Rives et al., 2017; Nigam, 2018; Schlessinger et al., 2018; Liu, 2019; Ayka and Sehirli, 2020; Bednarczyk and Sanghvi, 2020; Huang et al., 2020; Koepsell, 2020; Pacha et al., 2021; Pizzagalli et al., 2021; Yang and Shu, 2021).

**Table 2.** Regulatory mechanisms and protein factors involved in transcription of solute carrier (SLC) drug transporter genes.<sup>a</sup>

Regulatory mechanisms/ protein factors	Transporters <sup>b</sup>	Representative Agonist/Activator	Representative Antagonist/Inhibitor
DNA methylation	OATP1B1, OATP1B3, OATP3A1, OATP4A1, OATP3A1, OATP4C1, OATP2A1, Oatp1b2, Oatp1a1, Oatp1a6, Oatp1c1, Oatp1a4, OAT1, OAT3, Oat1, Oat3, OCT1, OCT2, OCT3, OCTN2, PETPT1, MATE1		5-Aza-2'-deoxy Cytidine (5azadC), Decitabine, Metformin
Histone modification	OATP2A1, OATP2A1, OATP1B1, OATP1B3, Oatp1b2, Oatp1a1, Oatp1a6, Oatp1c1, Oatp1a4, OAT2, OCT2, PETPT1, MATE2		Histone deacetylase inhibitor: Trichostatin, Vorinostat (SAHA)
Pregnane X receptor (PXR)	Oatp2, OATP1B1, OATP1B3, OATP2B1, OATP2A1, OATP-A, OATP1A2, Oat1, Oat3, OCT1	Pregnenolone 16 $\alpha$ -carbonitrile (PCN), Rifampicin, Phenobarbital, Doxorubicin, Spironolactone	Methotrexate
Aryl hydrocarbon receptor (AhR)	Oatp2b1, Oatp3a1, OATP4C1, OATP1B1, OATP2B1, Oatp1a1, Oatp2, OAT1, OAT2	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), Polychlorinated biphenyl 126, Beta-naphthoflavone, Diesel exhaust particles (DEPs), Indoxyl sulfate, 3-methylcholanthrene	
Constitutive androgen receptor (CAR)	OATP1A2, OATP1B3, OATP2B1, OATP1B3, Oatp1a1, Oatp1a4, Oatp2, OAT2, Oat1, Oat3	Diallyl sulfide, Phenobarbital, 6-(4-chlorophenyl)-imidazo [2,1-b] thiazole-5-Carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO)	Methotrexate
Farnesoid X receptor (FXR)	OATP1B1, OATP1B3, OAT2, PETPT1, Pept1, MATE1, MATE2-K	Vitamin A, Bile acids, Chenodeoxycholic	DY268, Z-guggulsterone, Glycine- $\beta$ -

		acid (CDCA), GW4064, Fexaramine, Berberine	muricholic acid, Lipopolysaccharide
Liver X receptor (LXR)	OATP1B1, Oatp1b2, OAT1, OAT2	Oxysterols, Berberine, TO-901317, GW3965, 22(R)-hydroxy- cholesterol, 22(S)-hydroxy- cholesterol, 9-cis retinoic acid (9cRA), 25-hydroxy- cholesterol (HC)	
Hypoxia- inducible factor 1 alpha (HIF- 1 $\alpha$ )	OATP1B3, OATP1B3, OATP2B1		
Hepatocyte nuclear factors (HNFs)	OATP1B1, OATP1B3, Oatp1b2, Oatp1, Oatp2, Oatp1a1, Oatp1a4, Oatp1a5, Oatp1b2, Oatp2b1, Oatp1a1, Oatp1a6, Oatp2b1, Oatp3a1, Oatp4c1, Oatp2a1, OAT1, OAT2, OAT3, Oat1, Oat3, OCT1, Mate1		Lipopolysaccharide, Ursodeoxycholic acid (UDCA)
Nuclear factor erythroid 2- related factor 2 (Nrf2)	Oatp1a1, Oatp2b1, Oatp1b2, Oatp1a6, OAT2, Oat2, PEPT1, MATE1, MATE2-K	Sulforaphane	Retinoic acid, Oltipraz
Retinoid X receptor (RXR)	OATP1B1, OATP2B1, OCT1, OCTN2	all-trans retinoic acid (atRA), 9-cis retinoic acid (CRA)	
Retinoic acid receptor (RAR)	OATP1B1, OATP2B1, OCT1,	all-trans retinoic acid (atRA), 9-cis retinoic acid (CRA)	
Vitamin D receptor (VDR)	OATP1A2,	1 $\alpha$ 25-dihydroxy- vitamin D3 (D3)	
Peroxisome proliferator- activated receptor alpha (PPAR $\alpha$ )	Oatp1a1, Oatp1a4, Oatp1b2, Oatp2a1, Oatp2b1, OCT1, OCT2, Oct2, OCTN2, Octn2, OCTN3, PETPT1, PepT1, Mate1	Perfluorooctanoic acid, Perfluorodecanoic acid, Clofibrate, Ciprofibrate, Diethylhexylphthalate, Fenofibrate	WY-14,643

PPAR $\gamma$	OCTN2	Thiazolidinediones (troglitazone and rosiglitazone), Luteolin,	Bisphenol diglycidyl ether (BADGE), GW9662
Androgen receptor (AR)	Oct2	Testosterone	
Estrogen receptor (ER)	OAT1, OCTN2	Estrogen	

Note: <sup>a</sup>Only those regulatory mechanisms/protein factors reviewed in the text are presented.

<sup>b</sup>OATPs: organic anion-transporting polypeptides; OATs: organic anion transporters; OCTs: organic cation transporters; OCTNs: organic cation/carnitine transporters; PEPTs: peptide transporters; MATEs: multidrug and toxin extrusion proteins. The upper case letters represent human proteins, while the others are rodent proteins.



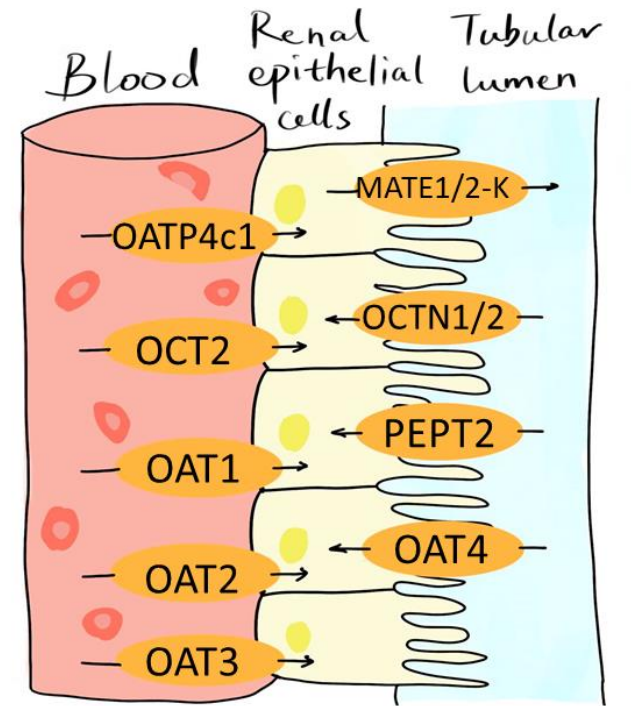
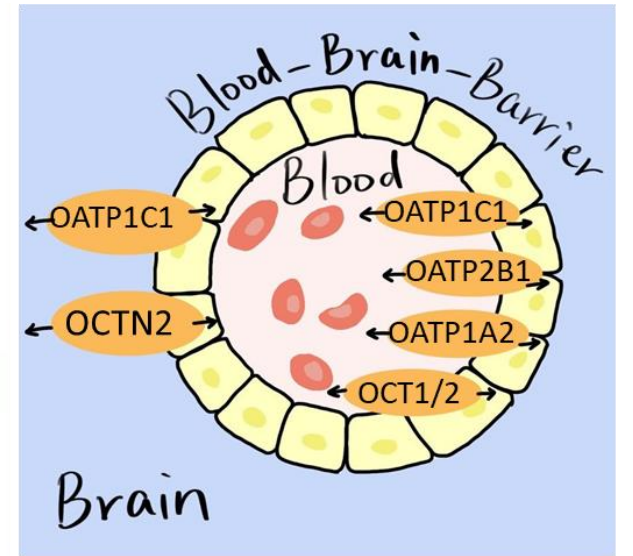
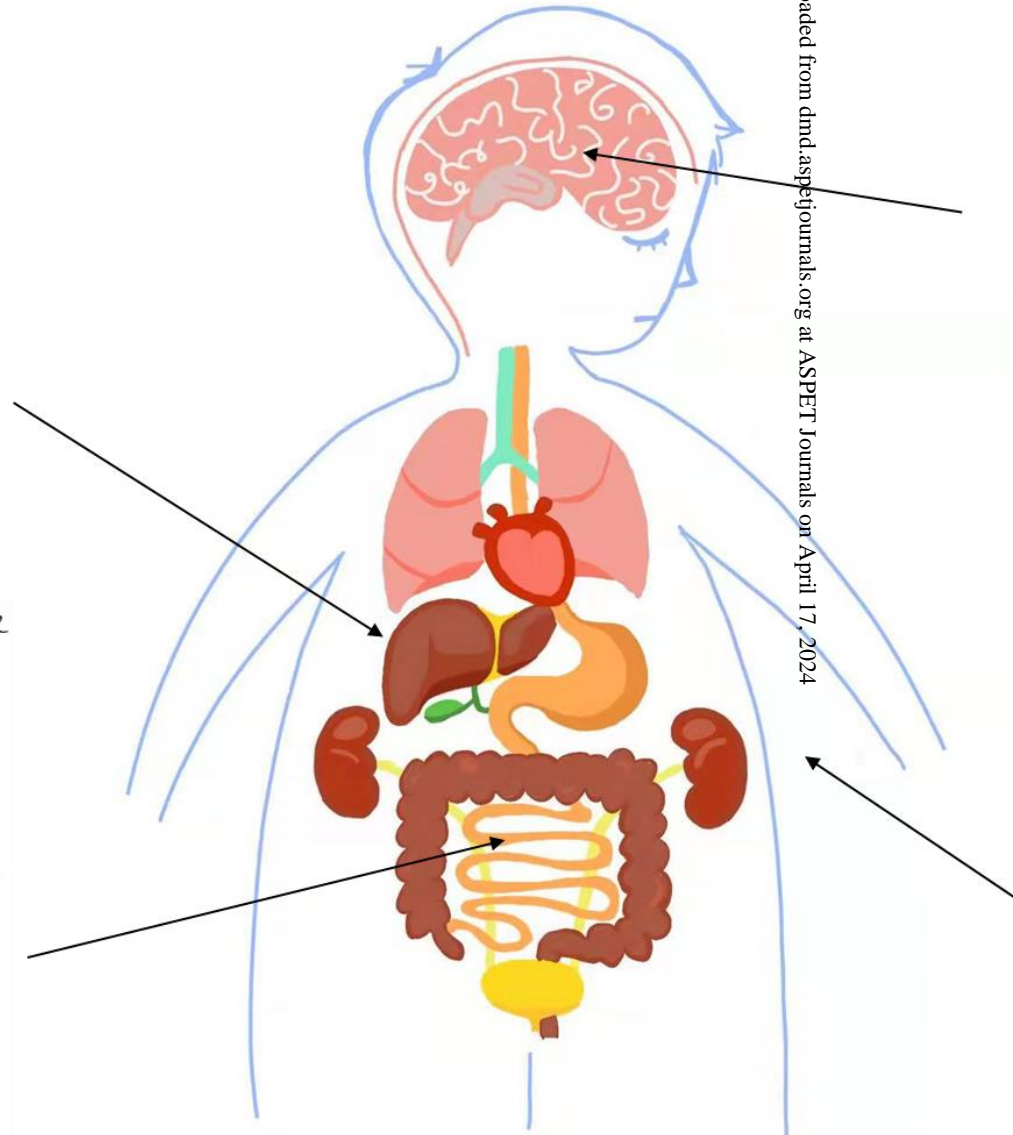
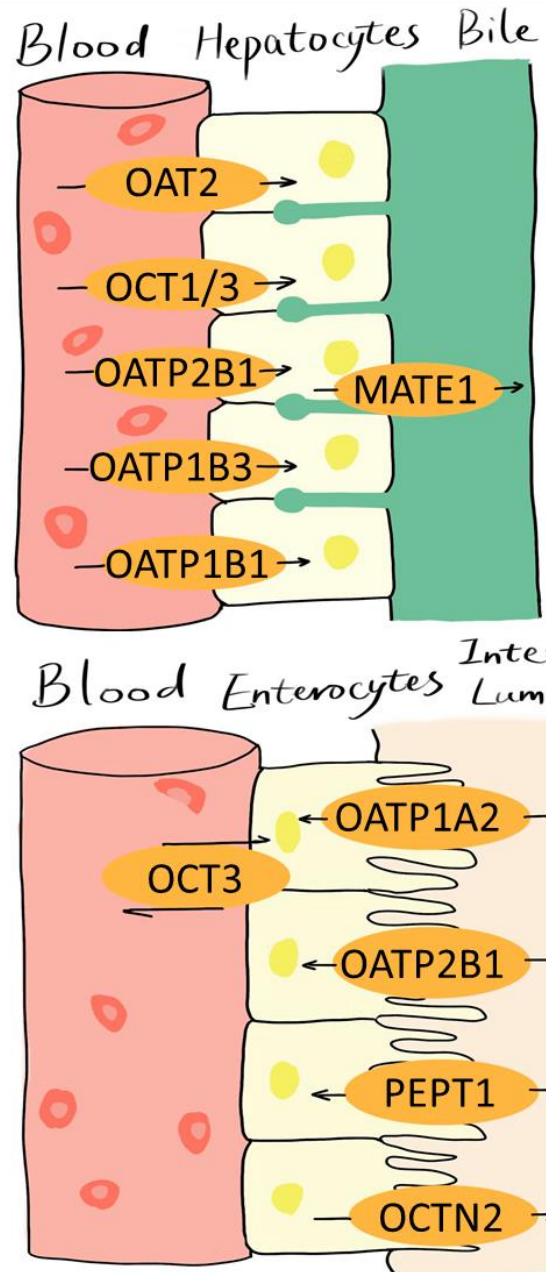


Fig 1

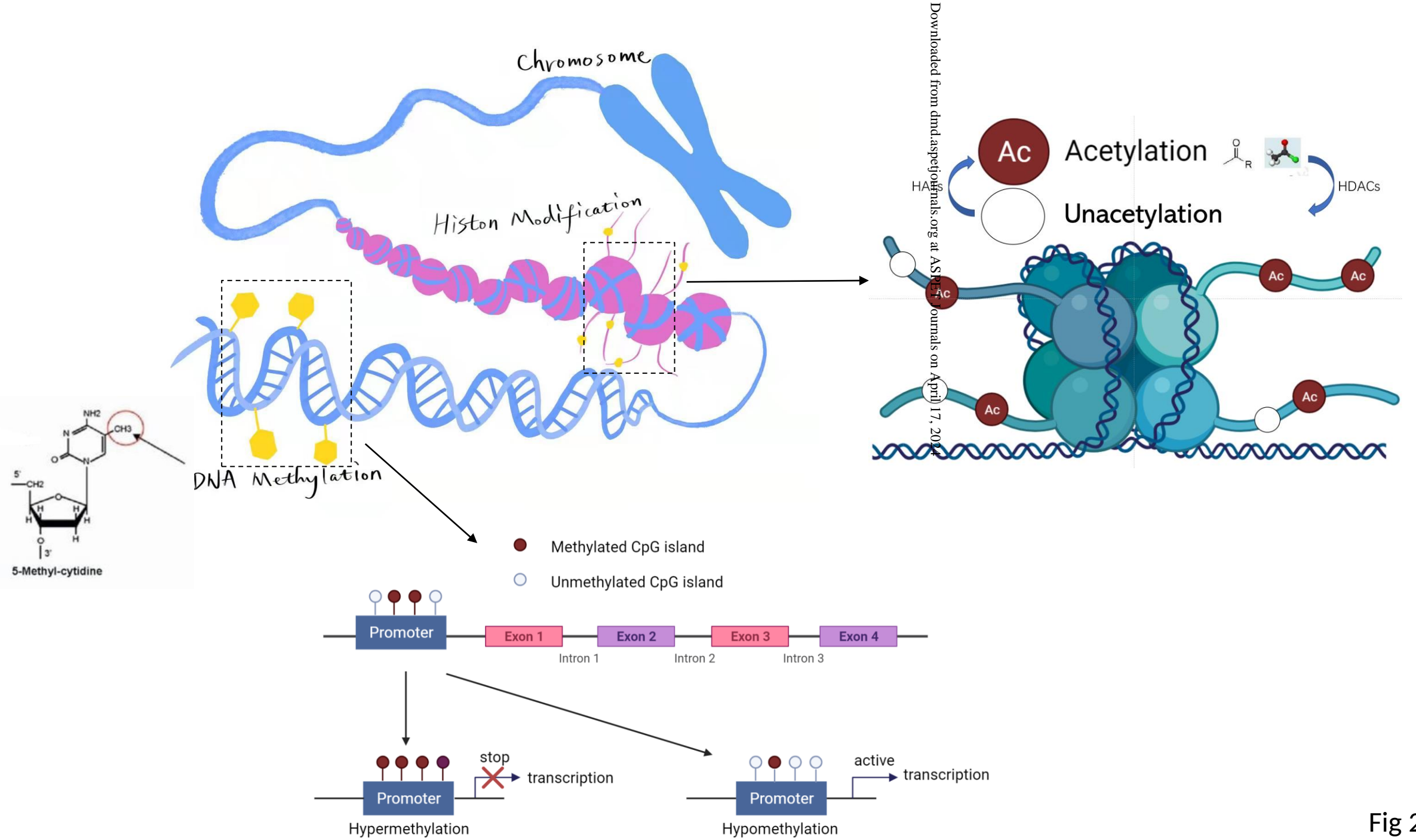


Fig 2