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Review

Molecular regulation of the hepatic bile acid uptake transporter and HBV entry receptor NTCP

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ABSTRACT

Transporters expressed by hepatocytes and enterocytes play a critical role in maintaining the enterohepatic circulation of bile acids. The sodium taurocholate cotransporting polypeptide (NTCP), exclusively expressed at the basolateral side of hepatocytes, mediates the uptake of conjugated bile acids. In conditions where bile flow is impaired (cholestasis), pharmacological inhibition of NTCP-mediated bile acid influx is suggested to reduce hepatocellular damage due to bile acid overload. Furthermore, NTCP has been shown to play an important role in hepatitis B virus (HBV) and hepatitis Delta virus (HDV) infection by functioning as receptor for viral entry into hepatocytes. This review provides a summary of current molecular insight into the regulation of NTCP expression at the plasma membrane, hepatic bile acid transport, and NTCP-mediated viral infection.

1. Introduction

1.1. Bile acids and the enterohepatic circulation

Bile acids are synthesized in the liver and subsequently conjugated to taurine or glycine for secretion into bile. Conjugated bile acids are actively secreted by hepatocytes across the apical membrane into the canalicular space via the bile salt export pump (BSEP) (Fig. 1) [1]. These bile acids are subsequently stored in the gallbladder and, following food consumption, released into the small intestine to facilitate digestion and absorption of nutrients [1]. In the terminal ileum they are taken up by the apical sodium-dependent bile acid transporter (ASBT) into the enterocyte. Here, bile acids are bound by the intestinal bile acid binding protein (IBABP) and shuttled through the basolateral side of the cells where they are then released into the portal circulation via the organic solute transporter complex (OSTαβ) [1]. Subsequently, the bile acids are transported directly back to the liver via the portal vein and taken up at the basolateral side of the hepatocyte mainly by the Na^+-taurocholate *Co*-transporting Polypeptide (NTCP, gene name *SLC10A1*) and to a lesser extent by members of the organic anion transporting polypeptide (OATP) family [1]. The bile acids complete this cycle 6–10 times a day with less than 1 g/day of the total bile acid pool lost via the feces, which is compensated by de novo synthesis of bile acids [1]. This is a tightly regulated process where bile acids themselves provide a feedback signal via the nuclear bile acid receptor Farnesoid X receptor (FXR) [2]. In the enterocyte, activation of FXR instigates the production of the hormone

Abbreviations: ASBT, (apical sodium-dependent bile acid transporter); BSEP, (bile salt export pump); cAMP, (cyclic Adenosine monophosphate; CYP7A1, (cholesterol 7 alpha-hydroxylase); FGF15/19, (fibroblast growth factor 15 or 19); FXR, (farnesoid X receptor); GLP-1, (glucagon-like peptide 1); GR, (glucocorticoid receptor); HBV/HDV, (hepatitis B and delta virus); HNF4α, (hepatocyte nuclear factor 4 α; IBABP, (intestinal bile acid binding protein); IBAT, (ileal bile acid transporter); IL-1β, (interleukin-1β; IL-6, (interleukin-6); KO, (knockout); NAFLD, (non-alcoholic fatty liver disease); NTCP, (Na^+ taurocholate co-transporting polypeptide); OATP, (organic anion transporting polypeptide); OSTαβ, (organic solute transporter alpha-beta complex); PI3K, (phosphoinositide 3-kinase); PKB, (protein kinase B); PKC, (protein kinase C); PP2B, (protein phosphatase 2B); RAR, (retinoic acid receptor); RXR, (retinoic X receptor); S6K, (P70 S6 kinase); SHP, (small heterodimer partner); SULT, (sulfotransferase); TCA, (taurocholate); TDC, (taurodeoxycholate); TCDC, (taurochenodeoxycholate); TGR5 or GPBAR1, (G protein-coupled bile acid receptor); TLC, (taurolithocholate); TNFα, (tumor necrosis factor α); UDCA, (ursodeoxycholic acid); WT, (wild type).

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fibroblast growth factor 19 (FGF19, FGF15 in mice) which travels via the portal circulation to the hepatocyte where it activates FGF4/B-klotho complex resulting in inhibition of bile acid synthesis via CYP7A1 (Fig. 1) [2]. FGF15/19 signaling also represses hepatic bile acid uptake via inhibition of both NTCP and members of the OATP family, further contributing to protection against hepatic bile acid overload [3]. FXR activation in hepatocytes also inhibits CYP7A1, via small heterodimer partner (SHP) activation. Besides FXR, several other bile acid receptors may further contribute to bile acid signaling and homeostasis [2,4]. One of the best described bile acid receptors is TGR5, a G-protein coupled receptor detecting bile acids at the plasma membrane. TGR5's role in bile acid synthesis is not yet demonstrated, although TGR5 deficient mice have an increased expression of Cyp7a1 [2].

In this review, we focus on the molecular function and regulation of the hepatic uptake transporter NTCP, as this protein has a pivotal role in bile acid dynamics, bile acid signaling and in viral infection of the liver.

1.2. Role of NTCP in humans and mice

NTCP is encoded by the gene *SLC10A1* [solute carrier family 10 (sodium/bile acid contransporter) member 1] [5], located at 14q24 in the human genome. The gene is conserved amongst numerous species, and the human protein exhibits 77% amino acid homology with the rat and mouse *Slc10a1* gene [5,6]. Vaz et al. were the first to report an NTCP-deficient individual, firmly demonstrating the pivotal role of NTCP as the main hepatic bile acid transporter [7]. They describe a child with a single homozygous nonsynonymous mutation, leading reduced NTCP expression at the plasma membrane with dramatic loss of taurocholate uptake, resulting in conjugated hypercholanemia and otherwise a relatively mild clinical phenotype. Since then, numerous NTCP deficient individuals, mainly found in South-East Asia, have been identified with an inactive variant polymorphism, perhaps due to its protective role against hepatitis type B and delta virus infection (HBV/HDV) (see below). These individuals are mostly asymptomatic, although transient hyperbilirubinemia and a slight thickening of the gallbladder has been described [1].

In 2015 the first genetic *Slc10A1* knockout mouse model was generated by Slijepcevic et al. [8], which showed that a lack of NTCP resulted in increased plasma bile acid concentrations. Interestingly, only a subset of mice display strongly elevated conjugated bile acids levels, while the majority of NTCP knockout (KO) mice have normal serum bile acids concentration, upon fasting [8]. The mechanisms underlying this bi-stable system were later explained using *Slco1a/1b* cluster KO mice that lack OATP1a/1b proteins. In this model, pharmacological inhibition of NTCP-mediated bile acid transport completely blocked plasma TCA clearance, while plasma conjugated bile acids levels rapidly increased in all mice, proving that NTCP and OATP1A/1B members together govern all hepatic bile acid uptake in mice. Hypercholanemic NTCP deficient mice showed reduced expression of OATP1A1, which was attributed to increased intestinal fibroblast growth factor 15/19 (FGF15/FGF19) signaling (see below) [3]. These findings indicate that NTCP dominates hepatic uptake of conjugated bile acids in humans, whereas the activity of the OATP1A/1B family members can dampen the elevation in bile acid levels in plasma in NTCP-deficient mice. FGF15/19 reduces the capacity of this OATP-pathway leading to hypercholanemia, despite its dampening effect on bile acid synthesis. In humans, loss of

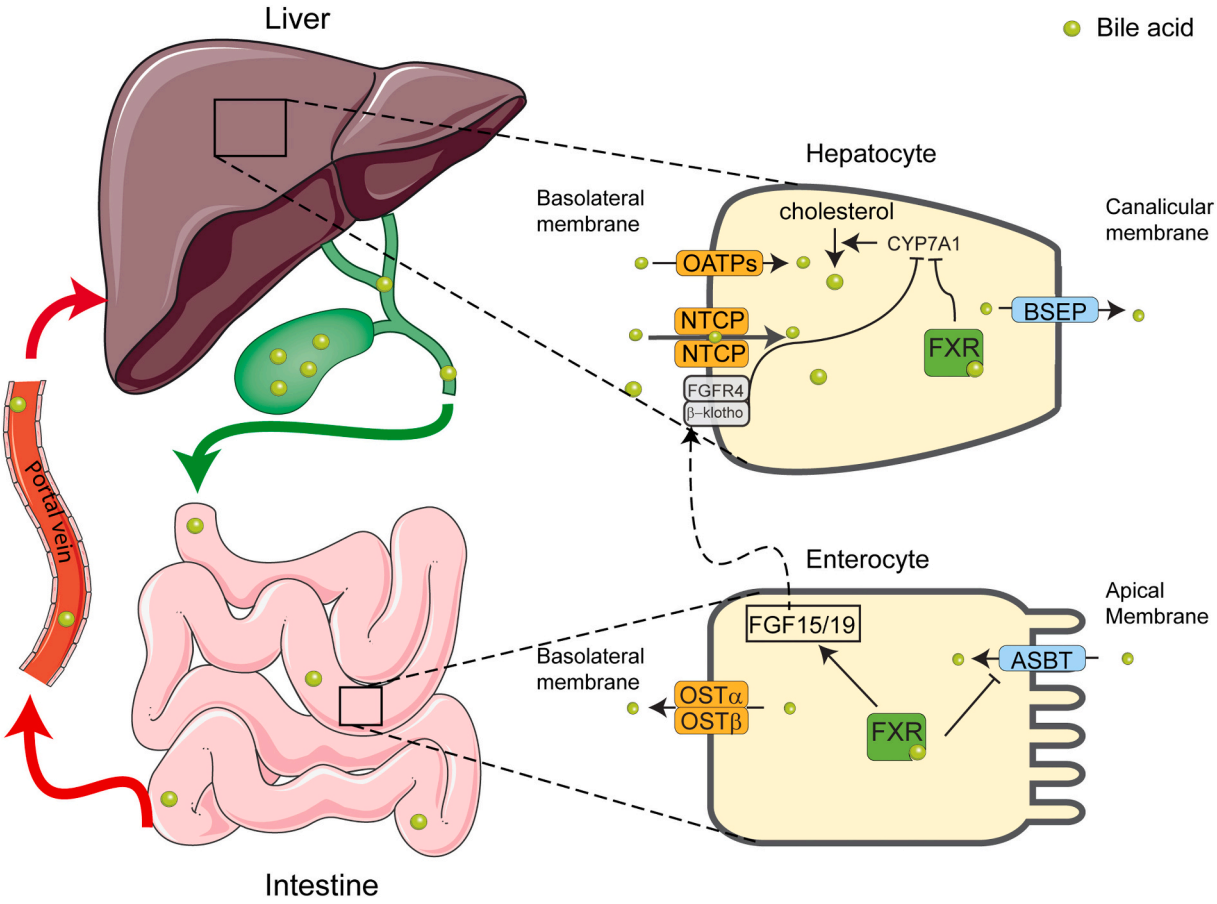

Fig. 1. Schematic overview of the enterohepatic circulation of bile acids. The transport and signaling in the enterocyte and hepatocyte is depicted. The left side of the figure illustrates the enterohepatic circulation, while the right side depicts the bile acid transport within the hepatocyte and enterocyte in which the transport proteins and bile acid receptor FXR are depicted. Activation of FXR induces secretion of FGF15 (mice) or FGF19 (humans). FGF15/19 represses hepatic bile acid synthesis and uptake.

NTCP function results in a gradual decrease in plasma bile acid levels over several years. Whether this decrease is related to altered activity of OATP members and/or bile acid synthesis is yet to be explored. However, an alternative mechanism leading to increased urinary bile acid secretion in SLC10a1$^{-/-}$ mice, was recently suggested. In these mice, an increased expression of members of the sulfotransferase (SULT) family and one of the bile acid sulfates, taurolithocholic acid 3-sulfate was observed [3,8,9]. SULT catalyzes the sulfation of bile acids which increase the solubility of bile acids and decreases their intestinal absorption, elevating their fecal and urinary excretion. The increase in SULT levels was also observed in individuals with a NTCP loss of function variant (Ser267Phe, discussed in the next sections). Therefore, bile acid sulfation could serve as a mechanism for enhanced elimination of bile acids in both NTCP deficient mice and humans [9].

1.3. NTCP as a viral entry receptor

In 2012, NTCP was described to be the functional entry receptor for HBV and its satellite virus, HDV [10]. In humans several synonymous as well as non-synonymous single nucleotide polymorphisms (SNPs) have been reported in the *SLC10A1* locus. The non-synonymous loss of function variant S267F, found in about 9% of the East Asian population, diminishes NTCPs function in the HBV replication cycle [11]. Indeed, recent studies showed that the frequency of this S267F variant was higher in healthy controls than in HBV or HBV/HDV co-infected patients, indicating either a decreased susceptibly or a reduction in the disease progression and chronicity [12,13].

HBV as well as HDV attach with low-affinity to heparan sulfate proteoglycans on the surfaces of hepatocytes that serve as their host cells [14,15]. After this initial attachment, both viruses bind to NTCP through a high-affinity interaction between the PreS1-domains of their large surface protein [16], followed by internalization of the viral particles [15,17,18]. After internalization, the viral membrane likely fuses with the hepatocyte membrane, initiating a productive infection, however the exact role of NTCP during this process and the molecular details of the viral entry process are not completely understood. Nevertheless, the presence of NTCP is essential to render hepatocytes and hepatoma cell lines permissive for HBV [10,19]. In most hepatoma cell lines the expression of NTCP is barely detectable, and primary human hepatocytes downregulate NTCP upon a loss of polarization after cell isolation or during proliferation, resulting in a reduced or even complete loss of HBV infection capacity [20–22]. Therefore, several NTCP-overexpressing hepatoma cell lines have been generated in order to study HBV infection in vitro [21,23–25]. Titration of NTCP expressed in a non-permissive cell line revealed that a specific expression level of NTCP is required to allow HBV infection, while higher expression levels do not confer an additional advantage, indicating that other cofactors are required and limiting (Oswald, Chakraborty & Protzer, unpublished results).

NTCP has also been reported to be a crucial factor defining the species barrier for HBV in macaques. When macaque hepatocytes are modified to express human NTCP in vitro or in vivo, they become permissive for HBV [19,26]. A single arginine amino acid difference at position 158 was identified, showing the specificity of HBV binding and subsequent infection [27]. However, this polymorphism does not affect NTCPs function as a bile acid transporter, indicating a specific evolution of NTCP preventing macaques of HBV infection [28,29]. Currently, researchers in this area focuses on the development of transgenic macaques that express humanized NTCP in order to generate an urgently needed immunocompetent animal model that allows studying novel antiviral strategies which have the potential to cure HBV [30].

Comparative species analysis of the NTCP sequence has highlighted additional regions involved in HBV binding and entry. For example, the I263V mutation in woodchucks increases HBV infection by 50%, whereas, in mice NTCP region 84–87 prevent both HBV and HDV infection, irrespective of binding capacity. [31–33]. This argues for a functional role of NTCP in membrane fusion additionally to its function in binding and uptake of the viruses. However, substitution of this region with its human counterpart renders mice only permissive for HDV infection, indicating that another, post-entry step is crucial for productive HBV infection of mice [32,33].

1.4. NTCP as an antiviral target

As NTCP plays an essential role in in HBV and HDV infection cycle, it has been exploited to develop new antiviral therapies [34]. After the discovery of NTCP as a functional receptor for HBV and HDV, known NTCP-substrates such as taurodeoxycholate (TDC), taurocholate (TCA) and taurochenodeoxycholate (TCDC) have been identified to inhibit HBV and HDV infection [20], as well as chemically-distinct small molecules such as Ezetimibe [35], Irbesartan [36] and Cyclosporine A including its derivatives [25,37,38]. The bile acid substrates binding to NTCP directly interfere with the HBV and HDV binding to NTCP, dampening viral entry.

HBV binds to its receptor through the PreS1 domain of its large envelop protein with high affinity [16], therefore it was used to generate a synthetic, myristoylated peptide spanning amino acids 2–48 of the PreS1 domain (Myrcludex B/Bulevirtide) [39,40]. This peptide specifically binds human NTCP and inhibits both bile acid uptake as well as HBV infection [41,42]. These results confirmed NTCP as a bona-fide HBV receptor, and peptide binding can be used to distinguish between permissive and non-permissive NTCP variants [10]. Most importantly, the myristoylated PreS1 peptide is able to block HBV infection with high efficacy at an IC50 of 80 pM and was used to develop a therapeutic drug initially referred to as Myrcludex B [40]. This peptide has a longer half-life than NTCP itself, as it can transfer from a pre-existing plasma membrane localized NTCP molecule to a newly synthesized NTCP molecule [43] and a once-daily injection strongly inhibits virus entry [34]. After a series of successful clinical trials, it has recently been approved in the European Union for the treatment of a chronic HDV infection, and is marketed under the name Bulevirtide (Hepcludex®) [44]. Latest clinical trials of patients treated has shown that while the effect on HBV was moderate (NCT02881008), the drug induced a strong decline in HDV RNA (Studies NCT02637999; NCT03546621 and NCT02888106), indicating a promising antiviral therapeutic for HBV/HDV co-infected individuals [38].

1.5. Consequences of NTCP inhibition

The discovery of Bulevirtide as specific and potent inhibitor of NTCP allowed for the functional pre-clinical investigation into three additional potential applications of pharmacological NTCP inhibition [1].

Firstly, pharmacological inhibition of NTCP effectively reduced cholestatic liver injury in several cholestatic mouse models [45]. The phospholipid to bile acid ratio in bile increases upon NTCP inhibition rendering the bile less toxic [45]. NTCP inhibition also reduces bile acid accumulation in hepatocytes and increases extracellular bile acid levels. An overload of intracellular hydrophobic bile acids triggers inflammatory processes [46] by activating the inflammasome amongst others [47]. Conversely, high levels of circulating bile acids may reduce inflammation in peripheral cells expressing the TGR5 receptor, such as macrophages, as TGR5 directly dampens activation of the inflammasome [48].

Secondly, NTCP inhibition or genetic NTCP inactivation leads to reduced weight gain in mice fed a high fat diet, via increased energy expenditure and reduced intestinal fat absorption [49,50], making it an interesting mechanism to study in the context of obesity. The increase in energy expenditure was due to increased uncoupled respiration, a process where the proton gradient in mitochondria is not used to create ATP, but instead to generate heat. The generation of heat is mediated by high levels of Uncoupling Protein 1 (UCP1) present in brown adipose tissue. Bile acids can induce UCP1 activity, amongst others via TGR5 [2].

Thirdly, transient hypercholanemia induced by NTCP-inhibition leads to increased release of glucagon-like peptide 1 (GLP-1) in mice [50]. GLP1-release from entero-endocrine cells is mediated by TGR5 activation by bile acids [2], providing a likely rationale for this effect. A subsequent improved glucose handling was not obvious and whether this effect is also present in humans upon NTCP inhibition is not yet elucidated. Nevertheless, the combined data of pharmacological and genetic NTCP inactivation in mice and humans suggests that NTCP inhibition is safe and has beneficial effects in HDV-infected patients and possibly in certain metabolic/inflammatory disorders. Therefore, a more detailed insight into NTCP regulation is also warranted.

1.6. NTCP topology and associated proteins

Human NTCP mRNA is translated into a 349 amino acid protein with a molecular mass of 37 kDa (core glycosylated) or 50–55 kDa (complex glycosylated) [5]. Although the structure of NTCP has not yet been resolved, crystallographic studies of its close relative ASBT from bacteria suggest that NTCP crosses the plasma membrane nine times and contains very small extracellular loops with a large extracellular N-terminus and an intracellular C-terminus (Fig. 2) [51]. The C-terminal domain of NTCP is essential for its targeting to the plasma membrane [52,53]. In line with this, truncation of the C-terminus of NTCP results in a diminished bile acid uptake due to reduced NTCP plasma membrane localization. Mutating the C-terminal tyrosine-based sorting motifs (Y307-E-K-I and Y321-K-A-A) resulted in a decreased basolateral localization of NTCP without affecting its molecular transport activity [6,52]. The N-terminus is located on the outside of the hepatocyte, containing two glycosylated asparagine residues (N5, N11). Losing the glycosylation of one of the two residues has no effect on NTCP localization at the plasma membrane, NTCP-mediated bile acid uptake or on HBV infection [54,55]. However, the absence of both glycated residues has not yielded identical results between studies. One study showed a drastically reduced plasma membrane abundancy of NTCP, a decrease in NTCP-mediated bile acid uptake and a reduction in HBV infection [54]. Whereas Lee et al., showed that HBV can still enter HepG2 cells expressing the glycosylation-deficient form of NTCP [55]. A third study demonstrated that inhibition of NTCP glycosylation using tunicamycin reduced NTCP-mediated HBV infection [56]. However, tunicamycin is known to induce ER-stress, which also reduces NTCP abundance at the plasma membrane [57]. The latter effect is mediated via ER-stress induced changes in expression of Calnexin, an ER chaperone that binds NTCP [57]. The latter points towards a potentially hepatoprotective, FXR-independent pathway where cholestasis-induced ER-stress leads to a reduced protein expression of NTCP.

Besides Calnexin, only few regulatory interaction partners of NTCP have been identified. Two other SLC10A family members proteins (SLC10A4 and SLC10A6) are described to heterodimerize with NTCP, although the functional consequence has not been elucidated yet [53,58]. Furthermore, two additional NTCP-associated proteins are identified, Stomatin and chloride channel CLIC-like 1 (CLCC1) [59]. Stomatin is a single pass membrane protein associated with lipid rafts that can affect NTCP-mediated bile acid uptake, although the precise mechanism remains unclear [59]. CLCC1 is a putative intracellular chloride channel, and a functional consequence of the CLCC1-NTCP interaction has yet to been reported.

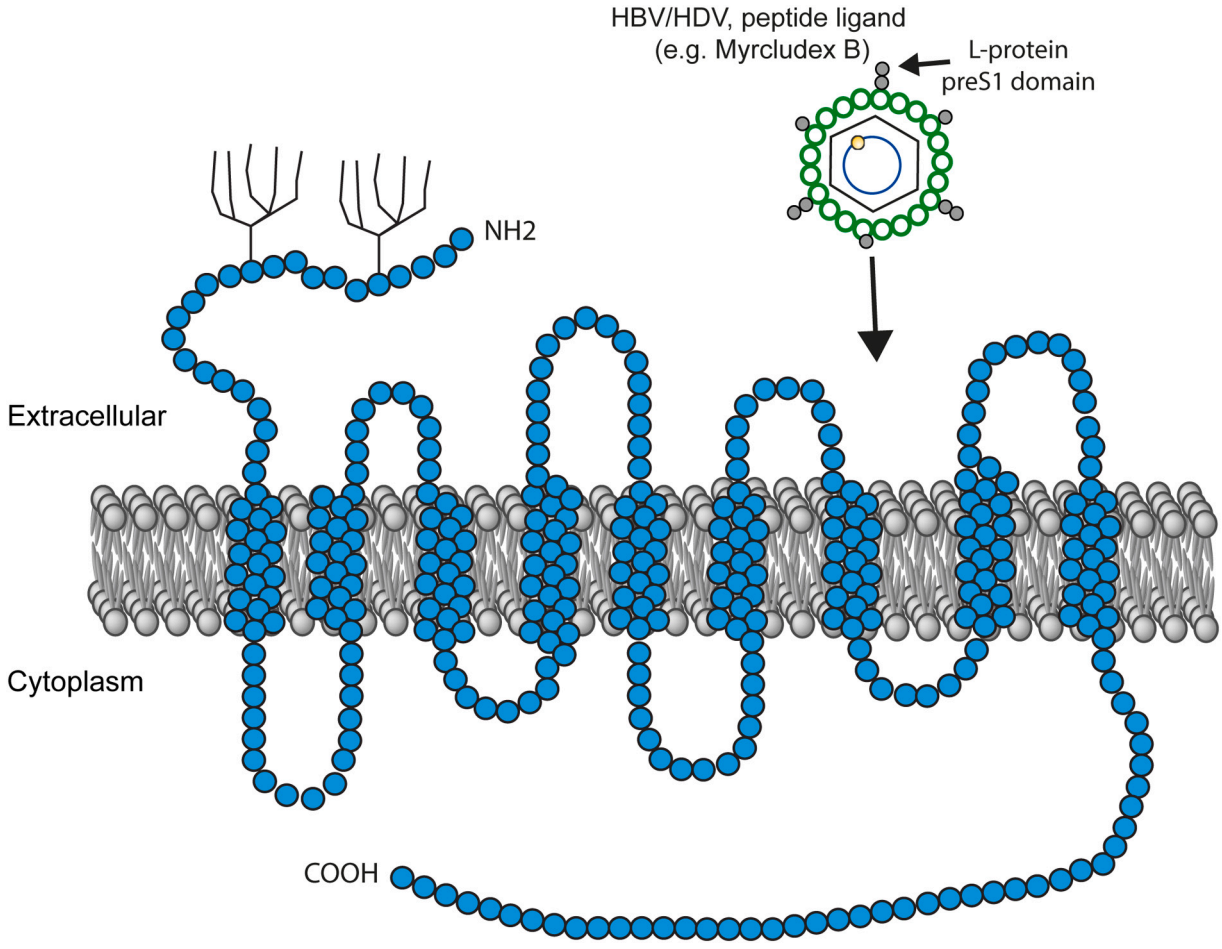

Fig. 2. Topology of NTCP. The transmembrane protein NTCP has its amino-terminus on the extracellular side and its carboxyl-terminus within the cytoplasm. The amino-terminus contains two N-linked glycans. Bile acids and HBV/HDV can interact with NTCP facilitating bile acid uptake and viral entry.

Treatment of NTCP-expressing cells with chemical cross-linking agents yielded an additional band at >250 kDa and ~100 kDa with a strong reduction of the monomeric 50 kDa NTCP signal. This suggests that NTCP is present in a dimeric complex also containing (un)known proteins with the 100 kDa band representing NTCP dimers [53]. Inclusion of a plasma membrane targeted nonfunctional NTCP variant in the dimer resulted in functional bile acid uptake by NTCP, suggesting that each individual NTCP subunit can transport bile acids independently of the other [53]. Conversely, inclusion of an NTCP subunit lacking its C-terminus (Y307stop) had a dominant negative effect on trafficking of the complex and resulted in retention of NTCP-WT in the ER and impaired bile acid uptake [53]. Therefore dimerization occurs early in the secretory pathway in a C-terminus-independent manner and is required for trafficking of NTCP to the plasma membrane [58,60]. In line with the effect of dimerization on NTCP trafficking, interference with NTCP oligomerization significantly impaired the internalization of the NTCP-HBV preS1 complex and infection by HBV [60].

1.7. Regulation of NTCP mRNA expression

Most studies on NTCP transcriptional regulation are based on rodent models, because NTCP shows minimal endogenous expression in hepatocellular cell models and is rapidly downregulated after isolation in primary human hepatocytes [61,62]. Therefore, the regulation of NTCP mRNA expression remains a challenge yet to be unraveled. Most studies demonstrate that NTCP mRNA regulation is mainly linked to bile acid concentration and serves as an adaptive response to reduce bile acid entry into the hepatocyte during pathophysiological conditions. The nuclear receptor FXR as well as inflammation and hormones are shown to regulate NTCP expression levels.

1.8. Regulation of NTCP mRNA expression via FXR-activation

While *SLC10A1* gene expression is regulated via bile acid activation of FXR, FXR does not directly interact with the *SLC10A1* promoter region. Instead it induces expression of small heterodimer partner (SHP), which in turn represses *SLC10A1* gene activation via the retinoid X receptor (RXR) and retinoic acid receptor (RAR) responsive element [63] which exist in the rat SLC10A1 gene but not in the corresponding human and mouse gene. Remarkably, NTCP protein levels were strongly reduced by cholic acid feeding in Vitamin A deficient mice, whereas NTCP mRNA levels were not reduced. This suggests the presence of other RXR-independent (posttranscriptional) NTCP regulatory mechanisms. Furthermore, also in SHP-deficient mice, NTCP mRNA expression is reduced after cholate feeding [64], suggesting that bile acid-induced repression of NTCP expression is at least partly independent of SHP. In the terminal ileum, FXR also stimulates the production of the hormone FGF19 (FGF15 in mice), which travels via the portal circulation to the hepatocyte. Here, FGF19 activates the fibroblast growth factor receptor 4 (FGFR4)/B-klotho complex which triggers intracellular signaling pathways in the liver to maintain the bile acid balance (Fig. 1) [65]. Injections of recombinant hFGF19 down-regulates NTCP mRNA by ~50% in WT mice indicating that FXR further modulates NTCP mRNA expression via FGF15/19 signaling [3].

1.9. Regulation of NTCP mRNA expression via hormones

Besides the increase in bile acid concentration after a meal (or during cholestasis), other hormone concentrations in plasma alter in response to a meal, such as adrenal glucocorticoid, which activates the glucocorticoid receptor (GR). Rose et al., demonstrated that GR can bind to the promoter of the NTCP gene in mice and humans and that both NTCP mRNA and protein levels were downregulated in GR-deficient mice leading to a reduction in NTCP-mediated bile acid transport [66]. In line with this, treatment with glucocorticoid increased NTCP expression in human livers ex vivo and in mice [66]. Other hormones, like estrogen, prolactin, growth hormone and thyroid hormone are suggested to participate in the homeostatic state of NTCP expression as well [67–72]. However, mechanistic insight in the effect of these hormones on NTCP expression is very limited.

1.10. Regulation of NTCP mRNA expression during inflammation

During cholestasis, the prolonged pathological increase in bile acid concentrations triggers the release of cytokines like tumor necrosis factor α (TNFα) interleukin-1β (IL-1β) and interleukin-6 (IL-6), which in turn repress NTCP gene transcription. The effect of IL-6 on NTCP has been investigated in mice in which turpentine injection (induces an acute phase response with IL-6 production) resulted in a downregulation of NTCP mRNA expression, which did not occur in IL-6 KO mice [73]. However reproducibility has been challenging with another study showing no effect [74]. The effect of IL-1β or TNFα has been investigated in rats in which administration of either cytokine resulted in a time-dependent decrease in NTCP mRNA expression. They demonstrated that this effect was possibly mediated via activation of c-Jun N-terminal kinase (JNK), which represses the expression of hepatocyte nuclear factor alpha (HNF4α) [74,75]. This pathway has been further explored in mice in which knockout of HNF4α resulted in a significant reduction of NTCP mRNA expression [76]. Furthermore, promotor analyses revealed that HNF4α strongly activates NTCP expression in mice [77], however, it is unclear whether an HNF4α binding region is present in the human *SLC10A1* gene [77,78]. In summary, NTCP mRNA expression can be regulated by FXR, hormones and cytokines in rodents, but species-specific mechanism and pathways are involved [78].

1.11. Posttranslational regulation of NTCP-mediated bile acid uptake

NTCP is present at the plasma membrane and in endocytic vesicles where it co-localizes with markers of the recycling compartment (Rab11) and early endosomes (Rab4), but not late endosomes [79–81]. This suggests that NTCP might be translocated from an intracellular compartment to the plasma membrane or vice versa, providing a fast and posttranslational response to increased bile acid concentrations after a meal or during acute cholestasis.

After a meal, bile acids from the gall bladder are released into the intestine to facilitate the absorption of fat. During this time period the bile acid concentration in the plasma rises due to re-uptake of the bile acids by enterocytes. About an hour after a meal, plasma bile acid concentration start to rise, peak at 90 min and return to the original level 150 min after a meal [82]. To facilitate this fast decrease in plasma bile acid concentration, hepatic influx of bile acid is stimulated with a fast increase of NTCP expression at the plasma membrane. This rapid phase is regulated via the increase in adenosine 3′,5′- cyclic monophosphate (cAMP) [83,84]. When cAMP levels are induced it increases the maximal transport rate of NTCP within minutes, without affecting NTCP synthesis [83]. Instead, cAMP triggers the translocation of NTCP from intracellular stores towards the plasma membrane [80]. cAMP stimulates Ca^{2+}/calmodulin-dependent protein phosphatase (PP2B) which dephosphorylates NTCP (Fig. 3) [85]. The Phospho-Serine at position 226 is the target site for cAMP-dependent dephosphorylation of NTCP [86].

cAMP activates the phosphoinositide 3-kinase (PI3K) pathway consisting of three groups of downstream targets: protein kinase B (PKB/AKT), P70 S6 kinase (S6K) and protein kinase C (PKC). The mTOR/S6K pathway is not involved in cAMP-induced increase in TCA uptake as rapamycin, an mTOR/S6K inhibitor, failed to inhibit the increase in TCA uptake [87]. Pharmacological activation of PKB resulted in an increased translocation of NTCP towards the plasma membrane, while inhibition of PKB blocked the cAMP-mediated increase in TCA uptake and NTCP translocation [88].

There are multiple isoforms of PKC, only some of which affect NTCP trafficking. Pharmacological activation of PKCζ increases the cAMP-dependent NTCP plasma membrane localization while PKCζ inhibition

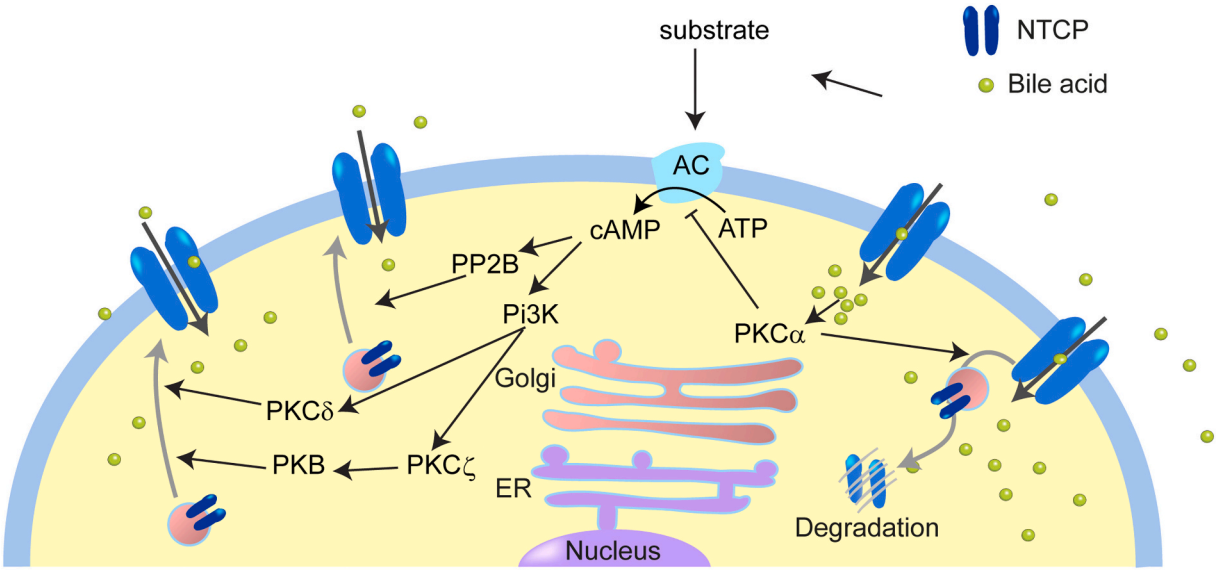

Fig. 3. Schematic representation of endo- and exocytosis of NTCP in a hepatocyte. On the left side, the exocytosis of NTCP is depicted in which several cAMP-driven kinases are involved. On the right side, the endocytosis and subsequently degradation of NTCP is illustrated in which PKC is the main regulator.

blocks this effect, suggesting that PKCζ is required for the cAMP induced intracellular movement of NTCP containing vesicles [79,89]. The isoform PKCδ mediates the NTCP trafficking via activation of RAB4, a small GTPase and an endosomal marker [90]. This activation results in trafficking of PKCδ towards the plasma membrane, thereby stimulating the presence of NTCP at the plasma membrane. For the PKCδ-RAB4 mediated effect on NTCP translocation, the localization of PKCδ at the plasma membrane is required but not the kinase activity of PKCδ [90]. Together the PI3K/PKB/PKC isoforms facilitate the intracellular movement of NTCP towards the plasma membrane after activation of cAMP.

When the intrahepatic bile acid concentration acutely increases, the influx of bile acid needs to be rapidly reduced to protect the liver against bile acid overload. Specific bile acids like taurolithocholic acid (TLC) may lead to rapid and prolonged inhibition of bile acid uptake via trans-inhibition [91]. Furthermore, increased bile acid concentration in hepatocytes triggers the rapid retrieval of NTCP from the basolateral membrane. This process involves PKC [83,92], reactive oxygen species and activation of the Src family kinase Yes and Fyn, but not c-Src [93].

2. Conclusion and outlook

NTCP plays a pivotal role in hepatic bile salt uptake and as the HBV/HDV viral entry receptor. Pharmacological inhibition of NTCP activity provides protection in certain cholestatic and metabolic conditions in mice and has recently been approved as anti-HDV therapy. This review provides an overview of the complex regulation of NTCP plasma membrane abundance and activity, which is mediated at multiple levels. At present, the individual contribution of each of these mechanisms under various conditions is largely unclear. One could imagine that these regulatory systems complement each other to ensure effective hepatic bile salt clearance, and simultaneously safeguard hepatocellular protection against acute and chronic bile acid overload, but this hypothesis requires experimental testing. Several additional blind-spots exist in our knowledge on NTCP. The crystal structure of ASBT in various conformations has been elucidated. Despite high sequence similarity, this has not yet been achieved for NTCP, but insight into the 3D structure and conformational changes would be highly valuable to understand the mechanism of bile acid translocation, HBV docking and to develop NTCP-targeting small molecules. Also insight into the quaternary structure of NTCP, involving subunit interaction and interaction with other intrinsic membrane proteins, or adaptor proteins would be valuable in this regard. Finally, the machinery involved in NTCP endocytosis, recycling and degradation is largely unknown, but highly relevant to HBV/HDV viral entry. Undoubtedly, the field will rapidly evolve in the near future as identification of NTCP as the HBV/HDV receptor and novel insight into NTCP as a pharmacological target has created a large inceptive to tackle these scientific challenges.

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Declaration of competing interest

There are no conflicts of interests.

References

[1] R.F. Kunst, et al., Targeting the four pillars of enterohepatic bile salt cycling; lessons from genetics and pharmacology, Hepatology (2020 Nov 22), https://doi.org/10.1002/hep.31651 (in press).
[2] A. Perino, et al., Molecular physiology of bile acid signaling in health, disease and aging, Physiol. Rev. 101 (2) (2020) 683–731.
[3] D. Slijepcevic, et al., Hepatic uptake of conjugated bile acids is mediated by both sodium taurocholate cotransporting polypeptide and organic anion transporting polypeptides and modulated by intestinal sensing of plasma bile acid levels in mice, Hepatology 66 (5) (2017) 1631–1643.
[4] J.M. Donkers, R.L.P. Roscam Abbing, S.F.J. van de Graaf, Developments in bile salt based therapies: a critical overview, Biochem. Pharmacol. 161 (2019) 1–13.
[5] B. Hagenbuch, P.J. Meier, Molecular cloning, chromosomal localization, and functional characterization of a human liver Na+/bile acid cotransporter, J. Clin. Invest. 93 (3) (1994) 1326–1331.
[6] B. Hagenbuch, et al., Functional expression cloning and characterization of the hepatocyte Na+/bile acid cotransport system, Proc. Natl. Acad. Sci. U. S. A. 88 (23) (1991) 10629–10633.
[7] Vaz, F.M., et al., Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholanemia without a clear clinical phenotype. Hepatology, 2015. 61(1): p. 260–7.
[8] D. Slijepcevic, et al., Impaired uptake of conjugated bile acids and hepatitis b virus pres1-binding in na^{+} -taurocholate cotransporting polypeptide knockout mice, Hepatology 62 (1) (2015) 207–219.

[9] F. Mao, et al., Increased sulfation of bile acids in mice and human subjects with sodium taurocholate cotransporting polypeptide deficiency, J. Biol. Chem. 294 (31) (2019) 11853–11862.
[10] H. Yan, et al., Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus, elife 1 (2012), e00049.
[11] H. Yan, et al., Viral entry of hepatitis B and D viruses and bile salts transportation share common molecular determinants on sodium taurocholate cotransporting polypeptide, J. Virol. 88 (6) (2014) 3273–3284.
[12] M.T. Binh, et al., NTCP S267F variant associates with decreased susceptibility to HBV and HDV infection and decelerated progression of related liver diseases, Int. J. Infect. Dis. 80 (2019) 147–152.
[13] P. An, Z. Zeng, C.A. Winkler, The loss-of-function S267F variant in HBV receptor NTCP reduces human risk for HBV infection and disease progression, J. Infect. Dis. 218 (9) (2018) 1404–1410.
[14] A. Schulze, P. Gripon, S. Urban, Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans, Hepatology 46 (6) (2007) 1759–1768.
[15] E.R. Verrier, et al., A targeted functional RNA interference screen uncovers glypican 5 as an entry factor for hepatitis B and D viruses, Hepatology 63 (1) (2016) 35–48.
[16] A. Meier, et al., Myristoylated PreS1-domain of the hepatitis B virus L-protein mediates specific binding to differentiated hepatocytes, Hepatology 58 (1) (2013) 31–42.
[17] A. Chakraborty, et al., Synchronized infection identifies early rate-limiting steps in the hepatitis B virus life cycle, bioRxiv 22 (12) (2020 Dec), e13250.
[18] A. Barrera, et al., Mapping of the hepatitis B virus pre-S1 domain involved in receptor recognition, J. Virol. 79 (15) (2005) 9786–9798.
[19] F.A. Lempp, et al., Sodium taurocholate cotransporting polypeptide is the limiting host factor of hepatitis B virus infection in macaque and pig hepatocytes, Hepatology 66 (3) (2017) 703–716.
[20] A. König, et al., Kinetics of the bile acid transporter and hepatitis B virus receptor Na+/taurocholate cotransporting polypeptide (NTCP) in hepatocytes, J. Hepatol. 61 (4) (2014) 867–875.
[21] R. Yan, et al., Spinoculation enhances HBV infection in NTCP-reconstituted hepatocytes, PLoS One 10 (6) (2015), e0129889.
[22] Y. Yan, et al., Down-regulation of cell membrane localized NTCP expression in proliferating hepatocytes prevents hepatitis B virus infection, Emerg. Microbes Infect. 8 (1) (2019) 879–894.
[23] C. Ko, et al., Hepatitis B virus genome recycling and de novo secondary infection events maintain stable cccDNA levels, J. Hepatol. 69 (6) (2018) 1231–1241.
[24] Sun, Y., et al., *NTCP-Reconstituted In Vitro HBV Infection System*, in *Hepatitis B Virus: Methods and Protocols*, H. Guo and A. Cuconati, Editors. 2017, Springer New York: New York, NY. p. 1–14.
[25] M. Iwamoto, et al., Evaluation and identification of hepatitis B virus entry inhibitors using HepG2 cells overexpressing a membrane transporter NTCP, Biochem. Biophys. Res. Commun. 443 (3) (2014) 808–813.
[26] B.J. Burwitz, et al., Hepatocytic expression of human sodium-taurocholate cotransporting polypeptide enables hepatitis B virus infection of macaques, Nat. Commun. 8 (1) (2017) 2146.
[27] S.F. Muller, et al., Characterisation of the hepatitis B virus cross-species transmission pattern via Na+/taurocholate co-transporting polypeptides from 11 New World and Old World primate species, PLoS One 13 (6) (2018), e0199200.
[28] S. Jacquet, et al., Evolution of hepatitis B virus receptor NTCP reveals differential pathogenicities and species specificities of hepadnaviruses in primates, rodents, and bats, J. Virol. 93 (5) (2019).
[29] J.S. Takeuchi, et al., A single adaptive mutation in sodium taurocholate cotransporting polypeptide induced by hepadnaviruses determines virus species specificity, J. Virol. 93 (5) (2019).
[30] J.M. Wettengel, B.J. Burwitz, Innovative HBV animal models based on the entry receptor NTCP, Viruses 12 (8) (2020) 828.
[31] L. Fu, et al., Woodchuck sodium taurocholate cotransporting polypeptide supports low-level hepatitis B and D virus entry, Virology 505 (2017) 1–11.
[32] W. He, et al., Modification of three amino acids in sodium taurocholate cotransporting polypeptide renders mice susceptible to infection with hepatitis D virus in vivo, J. Virol. 90 (19) (2016) 8866–8874.
[33] H. Li, et al., HBV life cycle is restricted in mouse hepatocytes expressing human NTCP, Cell. Mol. Immunol. 11 (2) (2014) 175–183.
[34] T. Tu, S. Urban, Virus entry and its inhibition to prevent and treat hepatitis B and hepatitis D virus infections, Curr. Opin. Virol. 30 (2018) 68–79.
[35] J. Lucifora, K. Esser, U. Protzer, Ezetimibe blocks hepatitis B virus infection after virus uptake into hepatocytes, Antivir. Res. 97 (2) (2013) 195–197.
[36] C. Ko, et al., The FDA-approved drug irbesartan inhibits HBV-infection in HepG2 cells stably expressing sodium taurocholate co-transporting polypeptide, Antivir. Ther. 20 (8) (2015) 835–842.
[37] S. Nkongolo, et al., Cyclosporin a inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor, J. Hepatol. 60 (4) (2014) 723–731.
[38] S. Shimura, et al., Cyclosporin derivatives inhibit hepatitis B virus entry without interfering with NTCP transporter activity, J. Hepatol. 66 (4) (2017) 685–692.
[39] D. Glebe, et al., Mapping of the hepatitis B virus attachment site by use of infection-inhibiting preS1 lipopeptides and tupaia hepatocytes, Gastroenterology 129 (1) (2005) 234–245.
[40] A. Schulze, et al., Fine mapping of pre-S sequence requirements for hepatitis B virus large envelope protein-mediated receptor interaction, J. Virol. 84 (4) (2010) 1989–2000.
[41] Petersen, J., et al., Prevention of hepatitis B virus infection in vivo by entry inhibitors derived from the large envelope protein. Nat. Biotechnol., 2008. 26(3): p. 335–41.
[42] Y. Ni, et al., Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes, Gastroenterology 146 (4) (2014) 1070–1083.
[43] J.M. Donkers, M.D. Appelman, S.F.J. van de Graaf, Mechanistic insights into the inhibition of NTCP by myrcludex B, JHEP Rep. 1 (4) (2019) 278–285.
[44] European-Medicines-Agency. *Hepcludex* 2020; Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/hepcludex.
[45] D. Slijepcevic, et al., Na(+) -taurocholate cotransporting polypeptide inhibition has hepatoprotective effects in cholestasis in mice, Hepatology 68 (3) (2018) 1057–1069.
[46] S.Y. Cai, et al., Bile acids initiate cholestatic liver injury by triggering a hepatocyte-specific inflammatory response, JCI Insight 2 (5) (2017), e90780.
[47] Z. Gong, et al., Chenodeoxycholic acid activates NLRP3 inflammasome and contributes to cholestatic liver fibrosis, Oncotarget 7 (51) (2016) 83951–83963.
[48] C. Guo, et al., Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome, Immunity 45 (4) (2016) 944.
[49] J.M. Donkers, et al., NTCP deficiency in mice protects against obesity and hepatosteatosis, JCI Insight (2019) 5.
[50] J.M. Donkers, et al., Inhibition of hepatic bile acid uptake by Myrcludex B promotes glucagon-like peptide-1 release and reduces obesity, Cell Mol. Gastroenterol. Hepatol. 10 (3) (2020) 451–466.
[51] N.J. Hu, et al., Crystal structure of a bacterial homologue of the bile acid sodium symporter ASBT, Nature 478 (7369) (2011) 408–411.
[52] A.Q. Sun, et al., The rat liver Na(+)/bile acid cotransporter. Importance of the cytoplasmic tail to function and plasma membrane targeting, J. Biol. Chem. 276 (9) (2001) 6825–6833.
[53] I.T. Bijsmans, et al., Homo- and hetero-dimeric architecture of the human liver Na^{+}-dependent taurocholate co-transporting protein, Biochem. J. 441 (3) (2012) 1007–1015.
[54] M.D. Appelman, et al., N-glycosylation of the Na+-taurocholate cotransporting polypeptide (NTCP) determines its trafficking and stability and is required for hepatitis B virus infection, PLoS One 12 (1) (2017), e0170419.
[55] J. Lee, et al., N-linked glycosylation is not essential for sodium taurocholate cotransporting polypeptide to mediate hepatitis B virus infection in vitro, J. Virol. 92 (15) (2018).
[56] C. Le, et al., In vitro infection with hepatitis B virus using differentiated human serum culture of Huh7.5-NTCP cells without requiring dimethyl sulfoxide, Viruses 13 (1) (2021).
[57] M.J.D. Robin, et al., Calnexin depletion by endoplasmic reticulum stress during cholestasis inhibits the Na(+)-taurocholate cotransporting polypeptide, Hepatol. Commun. 2 (12) (2018) 1550–1566.
[58] S. Noppes, et al., Homo- and heterodimerization is a common feature of the solute carrier family SLC10 members, Biol. Chem. 400 (10) (2019) 1371–1384.
[59] M.D. Appelman, et al., The lipid raft component stomatin interacts with the Na(+) taurocholate cotransporting polypeptide (NTCP) and modulates bile salt uptake, Cells 9 (4) (2020).
[60] K. Fukano, et al., Troglitazone impedes the oligomerization of sodium taurocholate cotransporting polypeptide and entry of hepatitis B virus into hepatocytes, Front. Microbiol. 9 (2018) 3257.
[61] D. Liang, et al., Parallel decrease of Na(+)-taurocholate cotransport and its encoding mRNA in primary cultures of rat hepatocytes, Hepatology 18 (5) (1993) 1162–1166.
[62] S.J. Rippin, et al., Cholestatic expression pattern of sinusoidal and canalicular organic anion transport systems in primary cultured rat hepatocytes, Hepatology 33 (4) (2001) 776–782.
[63] L.A. Denson, et al., The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp, Gastroenterology 121 (1) (2001) 140–147.
[64] L. Wang, et al., Resistance of SHP-null mice to bile acid-induced liver damage, J. Biol. Chem. 278 (45) (2003) 44475–44481.
[65] T. Inagaki, et al., Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis, Cell Metab. 2 (4) (2005) 217–225.
[66] A.J. Rose, et al., Molecular control of systemic bile acid homeostasis by the liver glucocorticoid receptor, Cell Metab. 14 (1) (2011) 123–130.
[67] T.C. Ganguly, et al., Regulation of the rat liver sodium-dependent bile acid cotransporter gene by prolactin. Mediation of transcriptional activation by Stat5, J. Clin. Invest. 99 (12) (1997) 2906–2914.
[68] J. Cao, et al., Differential regulation of hepatic bile salt and organic anion transporters in pregnant and postpartum rats and the role of prolactin, Hepatology 33 (1) (2001) 140–147.
[69] J. Cao, et al., Estradiol represses prolactin-induced expression of Na+/taurocholate cotransporting polypeptide in liver cells through estrogen receptor-alpha and signal transducers and activators of transcription 5a, Endocrinology 145 (4) (2004) 1739–1749.
[70] Simon, F.R., et al., Multihormonal regulation of hepatic sinusoidal Ntcp gene expression. Am. J. Physiol. Gastrointest. Liver Physiol., 2004. 287(4): p. G782–94.
[71] Simon, F.R., et al., Ethinyl estradiol cholestasis involves alterations in expression of liver sinusoidal transporters. Am. J. Phys., 1996. **271**(6 Pt 1): p. G1043–52.
[72] A. Geier, et al., Regulation of basolateral organic anion transporters in ethinylestradiol-induced cholestasis in the rat, Biochim. Biophys. Acta 1609 (1) (2003) 87–94.
[73] E. Siewert, et al., Interleukin-6 regulates hepatic transporters during acute-phase response, Biochem. Biophys. Res. Commun. 322 (1) (2004) 232–238.

[74] R.M. Green, D. Beier, J.L. Gollan, Regulation of hepatocyte bile salt transporters by endotoxin and inflammatory cytokines in rodents, Gastroenterology 111 (1) (1996) 193–198.
[75] D. Li, et al., Interleukin-1 beta-mediated suppression of RXR:RAR transactivation of the Ntcp promoter is JNK-dependent, J. Biol. Chem. 277 (35) (2002) 31416–31422.
[76] G.P. Hayhurst, et al., Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis, Mol. Cell. Biol. 21 (4) (2001) 1393–1403.
[77] Geier, A., et al., Hepatocyte nuclear factor-4alpha is a central transactivator of the mouse Ntcp gene. Am. J. Physiol. Gastrointest. Liver Physiol., 2008. 295(2): p. G226–33.
[78] Jung, D., et al., Role of liver-enriched transcription factors and nuclear receptors in regulating the human, mouse, and rat NTCP gene. Am. J. Physiol. Gastrointest. Liver Physiol., 2004. 286(5): p. G752–61.
[79] S. Sarkar, et al., PKCzeta is required for microtubule-based motility of vesicles containing the ntcp transporter, Traffic 7 (8) (2006) 1078–1091.
[80] Mukhopadhayay, S., et al., cAMP increases liver Na+-taurocholate cotransport by translocating transporter to plasma membranes. Am. J. Phys., 1997. **273**(4 Pt 1): p. G842–8.
[81] J.A. Dranoff, et al., Short-term regulation of bile acid uptake by microfilament-dependent translocation of rat ntcp to the plasma membrane, Hepatology 30 (1) (1999) 223–229.
[82] B. Angelin, I. Bjorkhem, Postprandial serum bile acids in healthy man. Evidence for differences in absorptive pattern between individual bile acids, Gut 18 (8) (1977) 606–609.
[83] S. Grune, L.R. Engelking, M.S. Anwer, Role of intracellular calcium and protein kinases in the activation of hepatic Na+/taurocholate cotransport by cyclic AMP, J. Biol. Chem. 268 (24) (1993) 17734–17741.
[84] A. Divald, et al., Vasopressin and phorbol-12,13-dibutyrate inhibit glucagon- or cyclic AMP-stimulated taurocholate uptake in isolated rat hepatocytes, Hepatology 20(1 Pt 1 (1994) 159–165.
[85] Webster, C.R., C. Blanch, and M.S. Anwer, Role of PP2B in cAMP-induced dephosphorylation and translocation of NTCP. Am. J. Physiol. Gastrointest. Liver Physiol., 2002. 283(1): p. G44–50.
[86] M.S. Anwer, et al., Dephosphorylation of Ser-226 facilitates plasma membrane retention of Ntcp, J. Biol. Chem. 280 (39) (2005) 33687–33692.
[87] C.R. Webster, et al., Cell swelling-induced translocation of rat liver $Na^{(+)}$/taurocholate cotransport polypeptide is mediated via the phosphoinositide 3-kinase signaling pathway, J. Biol. Chem. 275 (38) (2000) 29754–29760.
[88] C.R. Webster, et al., Protein kinase B/Akt mediates cAMP- and cell swelling-stimulated Na+/taurocholate cotransport and Ntcp translocation, J. Biol. Chem. 277 (32) (2002) 28578–28583.
[89] M. McConkey, et al., Cross-talk between protein kinases Czeta and B in cyclic AMP-mediated sodium taurocholate co-transporting polypeptide translocation in hepatocytes, J. Biol. Chem. 279 (20) (2004) 20882–20888.
[90] Won Park, S., et al., Protein kinase Cδ differentially regulates cAMP-dependent translocation of NTCP and MRP2 to the plasma membrane. Am. J. Physiol. Gastrointest. Liver Physiol., 2012. 303(5): p. G657–65.
[91] K. Lowjaga, et al., Long-term trans-inhibition of the hepatitis B and D virus receptor NTCP by taurolithocholic acid, Am. J. Physiol. Gastrointest. Liver Physiol. 320 (1) (2021) G66–G80.
[92] Stross, C., et al., Protein kinase C induces endocytosis of the sodium taurocholate cotransporting polypeptide. Am. J. Physiol. Gastrointest. Liver Physiol., 2010. 299 (2): p. G320–8.
[93] P.G.K. Mayer, et al., Regulation of plasma membrane localization of the Na(+)-taurocholate co-transporting polypeptide by Glycochenodeoxycholate and Tauroursodeoxycholate, Cell. Physiol. Biochem. 52 (6) (2019) 1427–1445.