Molecular physiology of bile acid signaling in health, disease, and aging

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RUNNING HEAD: Physiology of bile acid signaling

Abstract

Over the last two decades, bile acids (BAs) have become established as important signaling molecules that enable fine-tuned inter-tissue communication from the liver, their site of production, over the intestine, where they are modified by the gut microbiota, to virtually any organ, where they exert their pleiotropic physiological effects. The chemical variety of BAs, to a large extent determined by the gut microbiome, also allows for a complex fine-tuning of adaptive responses in our body. This review provides an overview of the mechanisms by which BA receptors coordinate several aspects of physiology and highlights new therapeutic strategies for diseases underlying pathological BA signaling.

Call out box for clinicians

Several diseases and conditions have been associated with an uncontrolled rise in BA concentrations. This is often the case when the tight feedback regulation of BA synthesis is compromised to the point that BAs become detrimental. BAs and their cognate receptors, FXR and TGR5, however, exert many beneficial roles as they enable tissues to adapt to environmental, nutritional, and physiological cues. Over the last two decades, BA mimetics targeting FXR, TGR5, or both, have been proven to be efficacious in alleviating chronic metabolic and inflammatory disorders, such as obesity, type 2 diabetes (T2D), atherosclerosis and non-alcoholic steatohepatitis (NASH). While several aspects of BA signaling are still poorly understood, the first therapeutics targeting FXR are making their way into the clinic to treat liver diseases, such as primary biliary cholangitis (PBC) and NASH. Drugs targeting BA signaling may hence have a bright future and the continuing efforts on studying the impact of changing BA signaling pathways in humans will be beneficial to translate our emerging knowledge on BA physiology in model organisms into clinical benefits.

I. Introduction

BAs are a class of structurally diverse molecules with more than 60 species currently identified in mammals. This rich diversity not only suggests the existence of multiple mechanisms driving the synthesis and metabolism of BAs but also indicates that each of these entities may have different bioactive functions. The identification of multiple BA-responsive nuclear and membrane receptors has spurred tremendous interest into the mechanisms by which BAs coordinate signal transduction in various tissues and cell types. The nuclear receptor (NR) farnesoid X receptor (FXR) and the G

protein-coupled receptor (GPCR) Takeda G-protein receptor 5 (TGR5) are the best-studied molecular mediators of BA-dependent adaptive responses and are prospective targets for multiple disorders. Here, we provide an overview of the growing complexity of BA biology, their cross-talk with the microbiome, as well as their role as signaling mediators of cellular and organismal function in health and disease. We also cover a number of novel unanticipated functions of BAs and highlight different modes of intervention in BA signaling as therapeutic options to treat chronic metabolic and inflammatory disorders.

II. BAs in a nutshell

BAs are products of cholesterol catabolism and are composed of a steroid nucleus skeleton and an isopentanoic acid side chain (169). They are synthesized as primary BAs in the liver through well-established enzymatic pathways, which have been extensively reviewed elsewhere (363). Chenodeoxycholic acid (CDCA) and cholic acid (CA) are the main hepatic BA products in humans (Figure 1A-B). There are four main steps that lead to primary BA synthesis: initiation, modifications of the ring structure, oxidation and shortening of the side chain, and conjugation. These sequential processes are carried out in the microsomes, cytosol, mitochondria, and peroxisomes, respectively. At least 7 mono-oxidases of the cytochrome P450 (CYP) family are involved in the incorporation of hydroxyl groups on the ring structures of cholesterol. The bulk of primary BAs is produced by the classic pathway (Figure 1A). This branch is initiated by the rate-limiting enzyme, cholesterol 7α-hydroxylase (CYP7A1). While humans synthesize CDCA, rodents produce MCAs via additional C6 hydroxylation. These BAs are more hydrophilic than CDCA and contribute to the species-specific differences in BA physiology (170). Sterol 12α-hydroxylase (CYP8B1) catalyzes the production of CA and its activity is a major enzymatic determinant of hepatic BA composition. In contrast to the classic pathway, the alternative branch depends on the chain-oxidation action of sterol 27-hydroxylase (CYP27A1), followed by 7α-hydroxylation of its product by oxysterol 7α-hydroxylase (CYP7B1) (363) (Figure 1A). Although in physiological conditions the alternative branch is only marginal, environmental factors, such as cold exposure (452), high fat/high cholesterol (HF/HC) diet feeding (463), or liver disease (235), increase its contribution by enhancing the expression of CYP7B1, suggesting that the pathway mediates adaptive responses to various stresses. Finally, BA biosynthesis terminates with the conjugation of taurine in rodents and glycine or taurine in humans. Additional forms of BA conjugation include sulfation, glucuronidation, and N-acetylglucosamination (169).

BAs cycle between the liver and the intestine and this dynamic process guarantees the distribution of adequate BA concentrations at sites of physiological actions (230). After conjugation to taurine or glycine, BAs are secreted from the liver into the bile and stored in the gallbladder along with other

bile constituents. Food intake is the main trigger promoting bile secretion in the intestinal tract. This process is mediated by the gut hormone CCK, which promotes hepatic secretion of BAs and gallbladder contraction. Once released in the duodenum, the amphipathic BAs exert their detergent-like activity by forming micelles with dietary lipids and fat-soluble vitamins to facilitate intestinal lipid absorption. BAs return to the liver via the portal vein through active transport mechanisms and undergo several enterohepatic cycles a day (173). This process, termed enterohepatic circulation, is controlled by several dedicated transporters that limit fecal and urinary loss (reviewed in (80, 147)) (Figure 1B). BAs in the portal circulation are taken up by hepatocytes through sodium-dependent taurocholate co-transporting polypeptide (NTCP) and organic anion-transporting polypeptide 1 (OATP1). Hepatic multidrug resistance protein 3 (MRP3), MRP4 and organic solute transporter α/β (OSTα/β) provide excretion routes for BAs into the systemic circulation while bile acid export pump (BSEP) exports them across the canalicular membrane (Figure 1B and reviewed in (147)). At the terminal ileum, most of the conjugated BAs are reabsorbed by the enterocytes via the apical sodium-dependent BA transporter (ASBT), chaperoned from the apical to the basolateral membrane by the cytosolic ileal bile acid binding protein (I-BABP), and secreted into the portal circulation via basolateral BA transporters OSTα/β, and MRP3. The enterohepatic circulation is efficient and recycles about 95% of the total BA pool. The remaining 5% is lost in the feces but rapidly restored through *de novo* synthesis in the liver, hence maintaining a constant BA pool size (363).

During their intestinal transit, BAs undergo several modifications through the action of bacteria residing in the distal part of the intestine, which deconjugate BAs or produce the secondary BAs deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), hyocholic acid (HCA), murideoxycholic acid (MDCA), ω-muricholic acid (ωMCA) and hyodeoxycholic acid (HDCA) (Figure 1A-B). The details of BA biotransformation by the gut microbiota are described elsewhere (430) and briefly summarized in Table 1.

III. BAs as signaling factors

The identification of dedicated BA receptors has triggered a remarkable rejuvenescence of the field and led to the novel concept that BAs, in addition to their detergent-like properties and their use as substrates for microbial metabolism (described in section IV-F), act as bonafide hormones (Figure 2). Below we describe in detail the main findings related to FXR and TGR5, the most extensively studied BA-responsive receptors, while briefly summarizing the most pertinent studies related to other, less known, BA-responsive receptors (Figure 3A).

A. FXR, a dedicated NR for BAs

FXR (also known as NR1H4) is a NR that earned its name from the identification of farnesol as an activator (113). Later, BAs were demonstrated to be the natural ligands of FXR (274, 320, 434), with CDCA being the most potent (Table 2). Although initially assumed to be limited to the liver, intestine, kidney, and adrenal glands, subsequent studies showed that FXR is more broadly expressed. Upon ligand binding, FXR heterodimerizes with the retinoic acid receptor α (RXRα; NR2B1) to activate the transcription of its target genes (Figure 3B). The transcriptional activity of FXR is fine-tuned by a set of coregulators, including transcriptional coactivators, such as steroid receptor coactivator 1 (SRC-1) (274, 320), peroxisome-proliferator-receptor (PPAR)-γ coactivator-1α (PGC-1α) (475), and methyltransferases represented by coactivator-associated arginine (R) methyltransferase-1 (CARM-1) (13, 25) and protein arginine (R) methyltransferase-1 (PMRT-1) (359). Furthermore, FXR can be post-translationally modified. Elevated concentrations of plasma glucose favor FXR stabilization and function through O-Glc-N-acylation (31), whereas acetylation, methylation and SUMOylation inhibit its transcriptional activity (20, 21, 214). Moreover, AMP-activated protein kinase (AMPK) phosphorylates and inactivates FXR in the context of cholestasis (259).

B. TGR5, a dedicated GPCR for BAs

TGR5, also known as G-protein coupled BA receptor (GPBAR1), is a member of the Rhodopsin-like subfamily of GPCRs and classified as the founder BA receptor of this sub-class (411). TGR5 is encoded by a single exon gene, generating a protein comprised of seven transmembrane domains, three extracellular loops and three intracellular loops (279). Consistent with the signal-amplifying properties of GPCRs, TGR5 is lowly to moderately expressed in almost every tissue or cell type, with the exception of the gallbladder epithelium, where it is abundantly expressed (425). TGR5 is activated by both conjugated and unconjugated BAs with the following order of potency LCA>DCA>CDCA>CA (Table 2). The taurine-conjugated BAs are usually more potent activators than the glycine-conjugated or unconjugated BAs (203). In addition to BAs, some steroid hormone intermediates, such as pregnandiol and 5α-pregnandione, also modulate TGR5 activity (210, 369). Semi-synthetic agonists for TGR5 have been developed and are listed in Table 2. Upon BA stimulation, TGR5 couples to Gαs proteins, and activates adenylate cyclase to initiate a transient cAMP rise (203), which, in turn, induces the activity of various downstream effectors, including PKA (231, 337, 350), or the exchange protein directly activated by cAMP (EPAC) (231, 350). TGR5 stimulation was also reported to activate MAPK signaling, mainly via ERK1/2 (191, 353, 427), proto-oncogene protein-tyrosine kinase (SRC) (175) and the mechanistic target of rapamycin (mTOR) (333, 471) (Figure 3B). The impact of β-arrestins on TGR5 signaling has been studied by several groups. Initial observations reported that TGR5 internalizes after activation (203) and that induction of TGR5 anti-inflammatory signaling is dependent on β-arrestin 2 (442). However, other studies demonstrated that TGR5 signaling does not require β-arrestins (191, 337). Recent evidence reported

that TGR5 only indirectly interacts with β-arrestin through G protein-coupled receptor kinase (GRK) to activate the innate antiviral immune response (175).

C. Other receptors involved in BA signaling

C.1. Nuclear receptors

Other members of the NR family, including the pregnane X receptor/steroid and xenobiotic-sensing receptor (PXR/SXR; NR1I2), constitutive androstane receptor (CAR; NR1I3) and the vitamin D3 receptor (VDR; NR1I1) can respond to BAs (recently reviewed in (383)). Higher concentrations of BAs are often required for their activation, suggesting that their functions become particularly relevant during pathological conditions such as cholestasis. This is exemplified by PXR/SXR, which is a xenobiotic sensor that coordinates cytochrome P450-induced detoxification and inhibition of BA synthesis in conditions requiring protection from LCA overload (398, 456). PXR acts in synergy with CAR to control BA clearance as well as bilirubin detoxification (142, 180, 366, 371, 429) but whether BAs act as ligands for CAR is still a matter of debate. On the contrary, VDR binds LCA at lower concentrations than PXR and mediates the detoxification of its ligand by inducing the transcription of *Cyp3a* (273). While this feature supports a protective role for VDR in gut homeostasis, recent evidence suggests that the LCA-VDR axis regulates biological pathways that go beyond BA detoxification, and coordinate processes as diverse as adaptive and innate immunity (338, 395, 403) and gut microbiota modulation (435).

C.2. Cell surface receptors

BAs can trigger acute responses through interaction with other GPCRs including sphingosine 1-phosphate receptor 2 (S1PR2), formyl-peptide receptors (FPRs) and muscarinic acetylcholine receptors (mAChRs) (Figure 3A). While the main ligands for S1PR2 are sphingolipids, TCA and other conjugated BAs can also induce its signaling (402). S1PR2 blockage reduces portal vein pressure and liver injury, suggesting a pathological role for S1PR2 in the setting of cholestasis (198, 440). FPRs are a small group of GPCRs expressed in neutrophils and monocytes (244). High concentrations of CDCA (62) and DCA (61) can interfere with the binding of N-formylmethionyl-leucyl-phenylalanine, an FPR agonist with potent chemoattractant properties in monocytes. Although these findings suggest an anti-inflammatory effect of BAs through FPRs, it is presumed that these receptors are only relevant under pathological conditions. The last group of BA-responsive GPCRs comprises the muscarinic receptors, which, upon exposure to conjugated secondary BAs, promote cancer cell growth in an epidermal growth factor receptor (EGFR)-dependent manner (11, 63). In addition, these receptors are involved in nitric oxide-induced vascular relaxation of the aorta (216), as well as in the pathology of cholestasis-induced cardiac arrhythmia (181, 379). Finally, BAs can

also activate membrane receptors other than GPCRs (Figure 3A). TUDCA, for instance, exerts anti-apoptotic actions in hepatocytes through activation of the β_1 subunit of the $\alpha_5\beta_1$-integrin pathway (132, 392). Interestingly, TUDCA promotes also other processes, such as osteoblast differentiation from mesenchymal stem cells through a similar integrin-mediated pathway (57).

IV. BA signaling in health, disease, and aging

A. Enterohepatic regulation of bile homeostasis

Bile is a physiological fluid composed of water and a mixture of organic and inorganic solutes of diverse complexity, including BAs, cholesterol, phospholipids, and bilirubin (40). Bile is formed in hepatocytes, further processed in the biliary epithelial cells, and stored in the gallbladder, until released postprandially in the gut lumen where the BA fraction facilitates lipid emulsification and absorption. Its main constituents are intrinsically interconnected and are subject to complex regulatory mechanisms in the liver. BA biosynthesis for instance requires hepatic cholesterol as a substrate, yet is tightly controlled by negative feedback regulation involving both liver and gut-driven mechanisms (Figure 4). In addition to its regulatory impact on BA synthesis, BA signaling coordinates the hepatic secretion of biliary cholesterol, BAs, phospholipids, and bilirubin through transporter-mediated mechanisms. As a consequence, BA-responsive receptors play a pivotal role in maintaining cholesterol solubility in bile and in coordinating bile formation and flow (extensively reviewed in (147, 169, 265)). While FXR is considered as an essential regulator of these processes, TGR5 complements these functions by coordinating various aspects of biliary physiology. The emerging roles of liver TGR5 in other non-parenchymal cells is reviewed elsewhere (211).

A.1. FXR as a regulator of bile and cholesterol homeostasis

Control of BA pool size and composition: Already in 1958, it was demonstrated that the amount of bile supplied to the liver via the portal circulation influences the synthesis rate of BAs in rats (29). Several laboratories confirmed the transcriptional nature of this regulation, with *Cyp7a1* being the main target. Among the transcription factors that control BA synthesis (37, 361, 363), FXR is now recognized as being the master regulator of the BA pool size. The first studies in *Fxr*$^{-/-}$ mice established that hepatic FXR is a cell-autonomous rheostat that regulates BA concentrations by repressing their synthesis and hepatic uptake, while concomitantly stimulating their export (386). In the liver, hepatic FXR contributes to the regulation of *Cyp7a1* via the induction of small heterodimer partner (SHP; NR0B2) (137, 266) (Figure 4, right upper quadrant). SHP is an atypical NR with corepressor activity that potently inhibits its dimerizing NR partners, including the liver receptor homolog-1 (LRH-1; NR5A2) (137, 266), hepatocyte nuclear factor-4α (HNF-4α; NR2A1) (222) and

liver X receptor alpha (LXRα; NR1H3) (41). This molecular network of NRs contributes to the cell-autonomous regulation of *Cyp7a1*. Studies in *Shp*$^{-/-}$ mice have corroborated the importance of SHP in this negative regulatory cascade, however, they also suggested that other mechanisms contribute to this process (215, 436). In fact, additonal studies revealed that feedback regulation of hepatic BA production is also mediated by a gut-driven mechanism, involving the induction of FGF15/19 (FGF15 in mice (185); FGF19 in human (472)). This enterokine is expressed in the enterocytes of the terminal ileum, and was originally discovered as an enterohepatic signaling factor able to blunt hepatic BA synthesis (reviewed in (390)). Tissue-specific *Fxr*$^{-/-}$ mouse models furthermore established that eliminating FXR in the intestine profoundly disrupts BA homeostasis (218). Mechanistically, FGF15/19 reaches the liver via the portal circulation and inhibits *Cyp7a1* expression through hepatic fibroblast growth factor receptor 4 (FGFR4) and ERK/MAPK signaling (172, 185, 253, 393, 467), as well as through SRC-dependent FXR activation (253) (Figure 4, right upper quadrant). FGF15/19 binds to the FGFR4-β-Klotho complex (234, 261) and activates the non-receptor Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2) (253). Accordingly, *Fgfr4*$^{-/-}$, *Klb*$^{-/-}$ and hepatocyte-specific *Shp2*$^{-/-}$ mice are unable to suppress BA synthesis upon FGF15/19 stimulation (188, 253, 468). Of note, intestinal BA-mediated secretion of FGF15/19 also signals, in part, through SHP by increasing its stability in the liver through ERK-dependent phosphorylation that inhibits its proteasomal degradation (291). A more recent study established that FXR signaling also controls BA synthesis independently of SHP and FGF15/19 by regulating *Cyp7a1* mRNA stability (407). Further studies will be needed to establish the relative contribution of these FXR-mediated post-transcriptional responses on BA production.

Activation of FXR signaling also significantly blunts *Cyp8b1* expression, which controls the production of CA. Studies conducted in tissue-specific *Fxr*$^{-/-}$ mouse models showed that unlike *Cyp7a1*, which is more sensitive to inhibition via the intestinal FXR-FGF15 pathway, *Cyp8b1* repression requires FXR activation in the liver, indicating differential regulation of the enzymes controlling the synthesis of the two main BA products (218). Although the molecular basis for this tissue-specific control is still poorly understood, it likely involves complex cross-talk with metabolite- and hormone-sensing transcription factors.

Regulation of BA and bile homeostasis: Secretion of bile constituents, such as cholesterol, BAs, phospholipids, glutathione and bilirubin, is tightly controlled by FXR signaling, which coordinates the expression of hepatic transporters involved in bile formation and flow as extensively reviewed in (80, 147) (Figure 5). Moreover, FXR activation induces the expression of transporters that provide spillover routes for BA efflux to avoid toxic BA overload (reviewed in (414)). Briefly, FXR upregulates the expression of canalicular transporters, including BSEP (12, 336, 386) and MDR3 (Mdr2 in rodents) (263) to increase the biliary concentration of BAs and phospholipids and prevent cholesterol crystallization (302). FXR-dependent protection from hepatic BA overload also occurs via modulation of OSTα/β which acts as an alternative export system to BSEP at the hepatic sinusoidal membrane

(239, 486). In addition to the BA transporters, BAs upregulate MRP2 (200) to excrete bilirubin and gluthatione conjugates, as well as ABCG5/8 (469, 479) in charge of cholesterol efflux. FXR activation by obeticholic acid (OCA) (Table 2) however is not associated with increased biliary cholesterol secretion, suggesting that the effects on hepatic ABCG5/8 induction do not necessarily result in increased transport activity (458). Of note, most of the bile constituent transporters are also upregulated in human liver slices when exposed to OCA (184), which is of relevance for cholestatic disease, as described in section IV-A.3. After participating in the digestion and absorption of dietary lipids in the gut, intestinal BAs are reabsorbed through different mechanisms (81). While only a fraction can diffuse passively in the duodenum (79), most of the BAs are taken up by the terminal ileum through active transport (Figure 5). Intestinal FXR activation downregulates the expression of ASBT (252) while it increases I-BABP and OSTα/β (114, 218, 247, 486) to efficiently export BAs from the enterocytes to the portal blood.

Control of intestinal cholesterol homeostasis: In mice, activation of FXR signaling by obeticholic acid (OCA) (458) and PX20606 (81) (Table 2) changes the BA pool composition leading to inhibition of intestinal cholesterol absorption (458) and increase of intestinal cholesterol excretion (81). Similarly, the FXR agonist GW4064 also stimulates plasma cholesterol clearance by enhancing its fecal elimination and reducing its intestinal absorption (479). Both the hepatic and intestinal pathway of FXR signaling seem to contribute to this process. As described above, hepatic FXR controls *Abcg5/8*. However, it also represses *Cyp8b1* expression, thereby reducing CA levels (218). These changes render the BA pool more hydrophylic and less efficient in emulsifying lipids. In addition to this effect, intestinal FXR stimulates transintestinal cholesterol elimination (TICE), a transport mechanism in the intestine that controls cholesterol homeostasis by increasing its intestinal elimination (190) (Figure 6). Studies revealed that this mechanism largely involves intestinal ABCG5/8 transporter activity and that enhanced excretion rather than decreased absorption of cholesterol accounts for FXR-mediated fecal sterol loss (81, 190). The coordinated activation of FXR in the enterohepatic organs thus not only controls BA production, bile formation and flow; it also ensures the tight control of cholesterol levels in our body.

A.2. TGR5 and biliary physiology

TGR5 influences BA homeostasis in a different, but complementary, manner compared to FXR in part explained by the distinct expression profile of both receptors along the enterohepatic axis (Figure 6). While *Fxr* is abundant in hepatocytes and enterocytes, only marginal levels of *Tgr5* mRNA are found in these cell types. In contrast, *Tgr5* is robustly expressed in cholangiocytes and gallbladder epithelium where TGR5 activation promotes chloride (Cl^-) secretion through cAMP-regulated induction of CFTR. The generated Cl^- gradient is subsequently used by the anion exchanger 2 (AE2) to secrete bicarbonate (HCO_3^-) across the apical membrane (207). In cystic

fibrosis, defective CFTR is responsible for impaired biliary secretion of Cl^- and HCO_3^- promoting ductal cholestasis, which can evolve into sclerosing cholangitis and cirrhosis (110). TGR5 is furthermore localized on cholangiocyte cilia, where its activation modulates bile flow and composition by regulating resorptive and secretory mediators (212). These findings underscore TGR5 as a pivotal regulator of biliary secretion, which is in line with the reduced bile flow in *Tgr5*$^{-/-}$ mice (254) (Figure 6). *Tgr5*$^{-/-}$ mice are furthermore susceptible to BA overload-induced liver injury potentially linked to a more hydrophobic BA pool (92, 327), and a compromised biliary epithelium barrier function (290). These findings underscore TGR5 not only as a regulator of biliary secretion and flow, but also as a cytoprotective protein involved in preserving tight junction structure and function.

In addition to these functions, activation of TGR5 by LCA or the semi-synthetic BA, INT-777 (329, 410) (Table 2), promotes gallbladder smooth muscle cell relaxation (243, 254) (Figure 6). A similar phenotype is observed when BAs activate the intestinal FXR-FGF15 axis. *Fgf15*$^{-/-}$ mice have an empty gallbladder even in the fasted state when it is normally filled with bile (68). This phenotype is restored after FGF15 injection, causing rapid gallbladder filling without stimulation of the bile flow. Mechanistically, FGF15 induces relaxation of the gallbladder smooth muscle via cAMP-induced signaling (68). Both ileal FXR and cholangiocyte TGR5 signaling thus seem to converge on the same cAMP axis providing a unifying mechanism for coordinated regulation of gallbladder physiology.

A.3. BAs and hepatobiliary diseases

Given the broad role of BA receptors in coordinating bile homeostasis and biliary physiology, it is not surprising that impaired signaling is associated with the development of hepatobiliary diseases, ranging from cholestatic liver disorders, cholesterol gallstone disease (CGD) to other gallbladder-related conditions.

Cholestatic liver disorders. Cholestasis has diverse etiologies and can result from impaired bile secretion across the canalicular membrane of the hepatocytes (intrahepatic cholestasis), or from impaired bile flow secondary to bile duct pathology, as is the case for primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) (reviewed in (343, 360, 400)). Consistent with the etiology of cholangitis-related disorders, genome-wide association studies (GWAS) in PBC and PSC patients have highlighted a major role for immune-related genes (164). Of interest, a gene variant in TGR5 has been found in PSC patients and further research should confirm the precise role of TGR5 in this disease (174). Likewise, certain FXR single nucleotide polymorphisms (SNPs) can predispose to intrahepatic cholestasis of pregnancy (ICP) (422) and to progressive familial intrahepatic cholestasis (PFIC) (135), reinforcing the importance of a preserved FXR signaling to limit pathological BA overload. Consistent with these genetic findings, ample evidence exists for a beneficial role of FXR agonism in various preclinical models of cholestasis (extensively reviewed in (209, 298). Stimulation of FXR signaling restores bile flow, reduces BA synthesis and stimulates

phospholipid secretion, thereby decreasing the detergent capacity of BAs (109, 263, 328). Furthermore, part of these effects is also mediated by selective activation of intestinal FXR-FGF15/19 signaling (299), or by treatment with human FGF19 (299), or its nontumorigenic analogue M70, now referred to as NGM282 (269), and protect mice from cholestatic liver damage. Several of the FXR related therapeutics have been tested in PBC and PSC patients, as described in section V-C.

CGD and other gallbladder-related conditions. Quantitative trait loci (QTL) analysis in inbred mouse strains and human GWAS linked gene variants in the cholesterol transporter ABCG5/8 with gallstones (46, 139, 399, 451). Low-frequency variants associated with this disease were also found in genes controlling BA metabolism, including FXR, CYP7A1, ABCB11, APOB and the CCK receptor CCKAR, as well as the phospholipid transporter ABCB4 (reviewed in (164, 237)). Moreover, *Fxr*$^{-/-}$ mice fed a lithogenic diet exhibit several features that contribute to the development of CGD, including cholesterol supersaturation of bile, precipitation of cholesterol crystals, increased BA hydrophobicity and gallbladder inflammation, whereas activation of FXR by GW4064 prevents its development (302). The protective effect of FXR agonists in rodents is attributed to their ability to increase the hydrophilicity of the BA pool (81, 446) and to stimulate the secretion of BAs and phospholipids by inducing the expression of their transporters, BSEP and Mdr2 (302). In humans, both UDCA and CDCA are effective in reversing CGD (77, 97, 116, 140, 278, 293). However, the mechanisms by which these BA species confer protection differ, as UDCA, in contrast to CDCA, is a poor agonist for FXR. Recent reports revealed that OCA might confer a higher risk for gallstone formation when administered to CGD patients (3) or to NASH patients (466). Although the higher cholesterol saturation index and the elevated FGF19 levels in gallbladder likely account for this effect (3), a residual activity of OCA towards TGR5 could be another reason. TGR5 is highly expressed in the gallbladder epithelium where it exerts important cytoprotective actions (see section IV-A.2). Potential adverse effects however could arise upon chronic activation of the receptor. Gallbladder relaxation and increased size are prominent phenotypes of TGR5 agonism (43, 254). In line with these findings, mice lacking TGR5 are protected against gallstone formation (425). Although this represents a challenge for human therapeutics, tissue-specific targeting of TGR5 may overcome this undesired effect. Future clinical trials with next-generation TGR5 agonists will be needed to evaluate the exact impact of this pathway on biliary (patho)physiology.

B. BAs as integrators of nutrient availability and intestinal homeostasis

Since intestinal BA levels oscillate following a rhythm that is dictated by dietary intake, these molecules serve as a proxy for nutrient availability. It is thus not surprising that BA-responsive receptors in enterocytes and different types of intestinal cells, including enteric neurons, smooth muscle, and enteroendocrine cells, sense and relay nutrient availability to a physiological response.

FXR and TGR5 in particular modulate a series of events including fluid transport, hormone release, expression of transport proteins, intestinal motility, and secretory responses that enable the uptake and availability of nutrients, fluid, and ions along the gastrointestinal tract (Figure 6).

B.1. Secretion of enteroendocrine hormones

BAs are essential regulators of appetite- and metabolism-modulating gut hormones. The incretin, glucagon-like peptide-1 (GLP-1), has received major attention because of its therapeutic potential to lower blood glucose concentrations. GLP-1 is secreted from enteroendocrine L cells following food ingestion. While glucose and free fatty acids are established nutrient-derived triggers for GLP-1 secretion (307), BAs have been identified as equally potent postprandial stimulators. TGR5 is expressed in L cells and mediates BA-induced GLP-1 release both *in vitro* and *in vivo* through a cAMP-dependent mechanism (202, 268, 410) (Figure 7). In addition, dependent on the type of agonist, activation increases intracellular calcium levels (44, 319, 410). Receptor activation can also promote GLP-1 production by inducing its precursor preproglucagon (155). TGR5 is predominantly located on the basolateral membrane of L cells, suggesting that BAs first have to cross the intestinal epithelium before stimulating GLP-1 release (44, 49, 229). A similar mechanism is proposed for peptide YY (PYY) and neurotensin, whose secretion is also blunted in *Tgr5*$^{-/-}$ mice (229). The discovery of TGR5 agonists as GLP-1 secretagogues is currently used as a basis to identify regulatory nodes that would synergistically elevate endogenous GLP-1 levels. An interesting discovery in this respect is the functional synergism between TGR5 and GPCRs involved in fatty acid signaling, such as FFA1R (134, 160), which is consistent with the exacerbated response of BAs during high-fat diet (HFD) feeding (155, 410). Another way to enhance TGR5-mediated GLP-1 secretion is to combine TGR5 agonists with somatostatin receptor 5 antagonists, which would remove the brake on GLP-1 release (42). These discoveries suggest that targeting complementary signaling pathways is more effective than TGR5 activation alone in modulating the GLP-1 response. Contrary to TGR5, FXR is proposed to counteract GLP-1 signaling, either by blocking precursor synthesis via a mechanism involving carbohydrate-responsive element-binding protein (ChREBP) repression (413) or by reducing the expression and signaling of the short-chain free fatty acid receptor 2 (FFAR2) (95) (Figure 7). These studies would suggest that FXR most likely functions to regulate later phases of GLP-1 secretion. On the other hand, other studies showed that fexaramine-mediated FXR activation (325) (Table 2) as well as concurrent activation of both FXR and TGR5 pathways significantly induce GLP-1 secretion (324) by priming and enhancing TGR5 expression and signaling (324) (Figure 7). These unexpected observations reinforce the notion that both BA sensors are functionally required in the coordinate regulation of GLP-1 signaling.

B.2. Secretion of enterokines

As already outlined in section IV.A, FGF15/19 is an established ileal FXR target that signals to the liver to limit hepatic BA production (185). In addition to its regulatory role in BA homeostasis, FGF15/19 regulates several aspects of the hepatic postprandial response, including inhibition of gluconeogenesis (341) and increase in glycogen and protein synthesis (221). FGF15 furthermore coordinates a physiological feedback loop promoting gallbladder refilling after CCK-induced gallbladder emptying (68). FGF15/19 can also reach the brain where it exerts central metabolic actions including reduction of food consumption and the regulation of glucose homeostasis (238, 277, 300, 364). Pharmacological administration of this hormone promotes other beneficial actions including the increase in energy expenditure and fat mass loss, improvement of insulin sensitivity and decrease in blood triglycerides and cholesterol levels (120). However, abrogation of FGF15/19 signaling confers protection against diet-induced obesity (DIO) in *Klb*$^{-/-}$ mice due to changes in the BA composition (389). More details on these dedicated effects are described in the sections below.

B.3. Intestinal electrolyte and fluid balance

BAs are established regulators of colonic fluid balance and can both stimulate or inhibit electrolyte and fluid secretion, depending on the type of BA species and its abundance (reviewed in (161)). Chronic exposure to physiological concentrations of BAs inhibits the actions of Ca^{2+} and cAMP-dependent secretagogues, a process that likely involves BA receptors (204). FXR activation with GW4064 inhibits the Ca^{2+} and cAMP-dependent secretory responses (304) restoring the osmotic driving force for colonic fluid absorption. In addition, FXR stimulation attenuates diarrhea in a mouse model of ovalbumin-induced diarrhea and cholera toxin (CTX)-induced intestinal fluid accumulation (304). Since food is often absorbed together with water, this effect of BAs might represent a physiological role whereby food-triggered colonic delivery of BAs simultaneously stimulate water absorption. On the other hand, studies using colonic epithelial cell lines and primary isolated colonic crypts showed that supraphysiological concentrations of BAs increase intracellular Ca^{2+} levels, which in turn promote epithelial Cl^- secretion to drive intestinal fluid secretion (89, 90), causing a water secretory diarrhea. Three forms of BA diarrhea (BAD) exist, resulting from compromised ileal BA absorption associated with underlying bowel-related pathologies (type I or secondary BAD) (286), from BA overproduction due to decreased FGF15/19 levels (type II or primary, idiopathic BAD) or following cholecystectomy or other gastroenterological conditions (type III, miscellaneous) (206, 432). A prospective clinical study showed that FXR activation with OCA improves the consistency of the stool and diarrhea symptoms both in primary and in secondary BAD patients with short ileal resection (431). These data suggest that, in addition to the well-established BA sequestrants, FXR could be a promising target for the development of novel antidiarrheal therapeutics (reviewed in (206)).

Although acute exposure of natural and synthetic TGR5 agonists has been reported to reduce Cl^- secretion in rat colon (444), the role of TGR5 in coordinating intestinal fluid balance remains poorly

defined. Intriguingly, in the gallbladder, TGR5 rather stimulates Cl^- and bicarbonate excretion through activation of CFTR (207). CFTR is mutated in cystic fibrosis, a disease characterized by the production of abnormally viscous mucus in multiple organs, including the intestinal tract (144). Further studies are warranted to decipher the regulatory role of TGR5 in intestinal mucus formation.

B.4. Gut motility

BAs are well-established regulators of intestinal motility (112, 330, 388) and trigger differential responses according to the region in the gastrointestinal tract. BAs typically inhibit gastric emptying, slow down small intestinal transit to allow nutrient absorption by a process known as 'ileal brake', and finally stimulate colonic peristalsis and transit (17, 381). Conistent with these findings, jejunal BA infusion in healthy subjects delays small intestinal transit (330). Although some of these effects could be indirect by stimulating the release of regulatory peptides such as PYY or GLP-1 from L cells (17, 419) (see section IV-B.1), emerging evidence indicates that BAs can directly affect some of these processes through TGR5 activation (7, 205, 339). TGR5 is expressed in gastric smooth muscle, and its activation by the natural TGR5 agonist, oleanolic acid (Table 2), is proposed to cause gastric muscle relaxation via RhoA inhibition (350). In the colon, secondary BAs trigger the release of 5-HT and CGRP from enterochromaffin cells and intrinsic primary afferent neurons, respectively, thereby stimulating peristalsis (7). Consistently, *Tgr5*$^{-/-}$ mice suffer from a constipated phenotype and a reduction in the frequency of defecation (7). Physiologically, these mechanisms fit with the notion that BAs act as a proxy of nutrient availability. While being released during food intake, they act as signaling molecules to prime the different regions of the intestinal tract for optimal digestion and excretion. Following food consumption, BAs favor nutrient absorption by slowing down the small intestinal transit. Once accomplished, BAs in the distal part will stimulate peristalsis to promote defecation. Consistently, pathological alterations in BA metabolism are involved in the pathophysiology of constipation and diarrhea (reviewed in (19)).

C. BAs in metabolism and energy homeostasis

C.1. FXR as a regulator of lipid and nutrient metabolism

FXR plays a pivotal role in the regulation of intermediary metabolism by influencing the expression of numerous genes involved in hepatic glucose, lipid, and amino acid metabolism (Figure 4). The manifold studies summarized below underscore the importance of FXR as a molecular integrator of the nutritional state, thereby coordinating the fate of many nutrients. However, they also unveil the complexity of this regulatory framework as evidenced by the sometimes divergent results obtained from different *Fxr*$^{-/-}$ mouse models studied under specific metabolic conditions.

Lipid homeostasis. It has been known for a long time that treating gallstone patients with CDCA decreases hepatic VLDL production and serum TGs (374), while treating hypercholesterolemic patients with BA sequestrants increases circulating TGs (14). In mouse models of hypertriglyceridemia, BAs reduce VLDL secretion, serum TGs and counteract hepatic steatosis (448). These effects have been confirmed by subsequent studies where FXR agonists reduced circulating TGs (81, 324, 478) and steatosis (324). This beneficial remodeling of lipid metabolism is orchestrated by the FXR-SHP axis, which represses sterol regulatory binding protein-1c (SREBP1c), a master regulator of hepatic *de novo* lipogenesis (448) and by FXR-dependent interference of ChREBP binding to the liver pyruvate kinase (LPK) promoter (55) (Figure 4, left upper quadrant). In the liver, induction of the FXR-SHP axis also blunts hepatocyte nuclear factor 4 alpha (HNF4α), a master regulator of microsomal triglyceride transfer protein (MTP) and apolipoprotein B (ApoB) expression, two proteins important for VLDL secretion (163). Of note, FXR regulates several apolipoproteins known to impact lipoprotein lipase activity (72, 201) and reverse cholesterol transport (73, 127, 479), further contributing to the beneficial remodeling of lipid metabolism (extensively reviewed in (59)). As expected, whole-body *Fxr*$^{-/-}$ mice display an increase in circulating TGs (98, 142, 225, 236, 386) and cholesterol (98, 142, 225, 236, 251, 386) levels, together with an accumulation of hepatic lipid deposits (98, 271, 295, 386), and enhanced levels of lipogenic genes in the liver (98, 225, 271, 295, 373).

Despite the striking steatotic phenotype in whole-body *Fxr*$^{-/-}$ mice (386), it is still not fully established how FXR signaling keeps hepatic fat deposits in check. While a comparative study in liver- and intestine-specific *Fxr*$^{-/-}$ mice demonstrate that the liver is the principal site of BA-mediated protection against lipid accumulation (373), others show that FGF15/19 is sufficient to blunt SREBP-1c and hepatic lipogenesis (33, 295). Another report shows that intestinal FXR activation, in contrast, promotes SREBP-1c levels and lipid accumulation in the liver through ceramide-dependent mechanisms (193). Future studies will have to instruct which tissues play a predominating role in controlling hepatic fat accumulation, with potential contributions from immune cells deserving attention.

Glucose homeostasis. BAs are postprandial mediators of glucose homeostasis. Hepatic FXR activation reinforces the actions of insulin by inducing SHP which results in the inhibition of the gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase), in part through the repression of the nuclear receptors FOXO1 and HNF4α (54, 271, 459, 477) (Figure 4, left lower quadrant). Similar effects are observed when diabetic mice are subject to short-term treatment with the FXR agonist GW4064 (54). The intestinal FXR-FGF15/19-FGFR4 pathway also largely contributes to the abrogation of hepatic glucose production through an inter-organ signaling cascade that counteracts cAMP Response Element-Binding protein (CREB), a critical regulator of gluconeogenesis (341). The same intestinal axis inhibits GSK3 in the liver to sustain hepatic glycogen synthesis after the decline of insulin signaling (221). In a cell-

autonomous fashion, hepatic FXR activation decreases the transcription of LPK resulting in a shunt of glucose metabolites from glycolysis towards glycogen synthesis underscoring, once again, the complementary actions of hepatic and intestinal FXR activation (98). In line with these discoveries, the majority of studies reported that lean *Fxr*$^{-/-}$ mice suffer from reduced hepatic glycogen storage and reduced insulin sensitivity (53, 98). FXR also modulates glucose homeostasis directly in the pancreas where its activation induces glucose-stimulated insulin secretion in isolated pancreatic β-cells, an effect that is lost in islets from *Fxr*$^{-/-}$ mice (96, 340, 378). Contrary to physiological BA signaling, chronic pharmacological activation of FXR with OCA enhances glucocorticoid-induced gluconeogenesis during fasting (356).

Amino acid homeostasis. Sustained activation of FXR by OCA (Table 2) triggers the expression of amino acid catabolism and ammonium detoxification genes (281, 355). Conversely, the expression of the urea cycle rate-limiting enzyme carbamoyl phosphate synthetase I (CPS1) and other amino acid catabolizing enzymes is reduced in *Fxr*$^{-/-}$ mice (281, 355) (Figure 4, right lower quadrant). In addition, FXR activation in the intestine promotes *de novo* protein synthesis in the liver (221). Similar to its effect on glycogen synthesis, this process is mediated by the gut-liver FXR-FGF19-FGFR4 axis that triggers activation of the hepatic RAS/ERK signaling cascade and phosphorylation of the eukaryotic translation initiation factor 4B and 4E (eIF4B and eIF4E) (221). These proteins are components of the eIF4F complex whose phosphorylation promotes the initiation of translation (131). Binding of FGF19 to FGFR4 also promotes phosphorylation of ribosomal protein S6 (rpS6) which improves the efficiency of global protein synthesis by inducing cap-dependent translation (221).

C.2. BAs and the metabolic syndrome

Obesity is tightly associated with the development of insulin resistance and non-alcoholic liver disease (NAFLD). BAs and their receptors play a central role in the etiology of these obesity-related conditions. Despite the existence of discrepancies among studies, the use of diverse modulators of FXR and TGR5 signaling has significantly increased our knowledge of how BA signaling intersects with multiple pathways known to promote metabolic disease. As a result, novel promising therapies are emerging that target FXR or TGR5 signaling. Especially in the context of non-alcoholic steatohepatitis (NASH), an advanced form of NAFLD, significant progress is made (described in detail in section V-C). We provide here below an overview of the most significant pre-clinical studies in various mouse models of metabolic diseases. The link between BA signaling and cardiovascular disease, another condition of the metabolic syndrome, is described in section IV.D.

FXR in obesity and insulin resistance. While there is a fairly general consensus on the phenotype of chow-fed lean *Fxr*$^{-/-}$ animals, diverging findings are reported on the role of FXR during obesity. As an example, several groups reported that FXR disruption in obese mice attenuates body weight gain (345, 365, 476) and improves insulin sensitivity (345, 372, 476), however, the same disruption in lean mice worsens glucose tolerance (54, 271, 477). Gut-restricted inhibition of FXR activity with synthetic Gly-MCA (194), or natural tauro-βMCA (TβMCA) (250, 455) (Table 2), or alternatively by genetic disruption of intestinal FXR (193), protects from obesity and diet-induced glucose intolerance (194, 250, 455). Mechanistically, the beneficial effects of intestinal FXR antagonism were attributed to reduced ileal ceramide production that attenuates hepatic gluconeogenesis (455). Recent studies concluded that disruption of the FXR/SHP signaling axis in the liver also improves glucose and fatty acid metabolism when fed a HFD (2, 220). The exacerbation of obesity and insulin resistance in the setting of HFD feeding is consistent with FXR's role as a postprandial anabolic regulator of nutrient metabolism.

The effects of FXR activation by various synthetic agonists (Table 2) also led to discordant results in the setting of obesity. Long-term oral supplementation of GW4064 to obese and insulin-resistant mice exacerbated weight gain, dyslipidemia, and glucose intolerance (446), effects that can be attributed to the lower BA pool size following FXR activation. Conversely, FXR activation with the FXR/TGR5 dual agonist INT-767 induces a TGR5-dependent increase in GLP-1 secretion, leading to improved glucose and lipid homeostasis in diet-induced obese (DIO) mice (324). Of interest, a deuterated analog the intestine-specific agonist fexaramine (93), FexD, attenuated DIO and insulin resistance by increasing the thermogenic response in brown (BAT) and white (WAT) adipose fat and blunting gluconeogenesis (104). The same agonist also improved alcoholic liver disease through stimulation of the FXR/FGF15 axis (159), although others showed the existence of a cross-talk with TGR5 as a consequence of enhanced production of TLCA (325). Of interest, systemic administration of FGF19 to obese and diabetic mice induced an anti-diabetic effect (120). This could be in part coordinated by the CNS since activation of central FGF19 signaling reduces food intake (277, 364), body weight (238, 277, 364), and improves glucose homeostasis (238, 277, 300, 364) in rodent models of obesity. Consistent with these findings, a human clinical trial (Table 3) demonstrated that treatment with OCA (25 and 50 mg/day) increased insulin sensitivity by almost 25% in a cohort of patients with NAFLD and type 2 diabetes (T2D) (305).

TGR5 in obesity and insulin resistance. The role of TGR5 in enhancing lipid catabolism has been extensively studied in the setting of DIO. The first indication for such a role came from the observation that chronic supplementation of CA protects mice against DIO and insulin resistance by enhancing local thyroid signaling and mitochondrial activation in BAT and muscle (447) (Figure 8). This was further corroborated by the finding that dietary supplementation of HFD with INT-777 improves glycemic control, reduces liver steatosis, protects against weight gain (410) and induces beiging of the subcutaneous WAT (427). The beiging phenotype was associated with enhanced

mitochondrial biogenesis and function, along with marked lipolysis and fatty acid oxidation in response to environmental cues such as cold exposure or high caloric intake (427) (Figure 8). Similarly, TGR5 activation with the specific agonist BAR501 (Table 2) prevented DIO and increased the expression of thermogenic genes in BAT and WAT (51). In humans, CDCA oral supplementation increased energy expenditure and BAT activity (45). *In vitro* assays showed that CDCA induces mitochondrial uncoupling through TGR5 in brown adipocytes isolated from healthy women (45), confirming the results obtained in mice. However, genetics plays an important role in regulating the BA-dependent thermogenic effect, as demonstrated by the resistance of 129S6/SvEvTac mice to the beneficial effects of CA on body weight loss (117).

In addition to stimulating energy expenditure, which indirectly restores insulin resistance, TGR5 also directly regulates glucose homeostasis through its impact on enteroendocrine and immune cells of the myeloid lineage (described in sections IV-B.1 and IV-D.2, respectively). Administration of TGR5 agonists (410) or intestine-selective activators (108, 242) increases circulating GLP-1 levels and improves glucose tolerance in obese and insulin-resistant mice. Conversely, nutrient-dependent GLP-1 secretion and glucose homeostasis are impaired in whole-body *Tgr5*$^{-/-}$ mice (410). BA sequestrants furthermore improve glucose homeostasis through TGR5-dependent GLP-1 release (155, 342). Treatment with the BA sequestrant, colestimide, reduces body weight gain and increases insulin sensitivity in DIO mice, possibly via TGR5 (449). More recently, it was demonstrated that TGR5 contributes to exercise-induced improvement of muscle function (367) and ameliorates glucose homeostasis by increasing insulin responsiveness in skeletal muscle (178).

FXR and TGR5 in NAFLD. NAFLD is a common disease affecting more than 70% of the obese and diabetic population worldwide. It includes a spectrum of liver conditions ranging from simple steatosis to more advanced NASH, which in the later stages can culminate into endstage liver fibrosis and cancer. Although its etiology is complex and not fully elucidated, accumulating evidence indicates that BA levels and signaling are profoundly disrupted in NAFLD (15, 65, 195, 347). FXR is a master regulator of hepatic lipid homeostasis (section IV-C.1) and disruption of whole-body FXR signaling is associated with enhanced NAFLD susceptibility. Consistent with these findings, hepatic lipid accumulation is inversely correlated with FXR expression in NAFLD mouse models or patients (267, 461) and *Fxr*$^{-/-}$ mice show marked steatosis (386), hepatic inflammation and spontaneous progression to NASH and hepatocellular carcinoma (HCC) (84, 460). Conversely, FXR activation by CA (448), OCA (71), GW4064 (477)), WAY-362450 (262, 474) or the intestine-restricted FXR agonist fexaramine (104) (Table 2), significantly dampens fat accumulation in the liver and protects against the development of NAFLD, and other hepatobiliary diseases as described in section V-C. Of note, blockage of FXR signaling by genetic disruption of the receptor in the intestine (193), or by administration of the FXR antagonist Gly-MCA (194), also protects against steatosis. These paradoxical results may be explained by the fact that some FXR agonists, such as fexaramine, also impact on TGR5 signaling outside of the enterohepatic axis (104). Indeed some of the effects of

665 fexaramine, especially those related to browning, were blunted in *Tgr5*$^{-/-}$ mice suggesting an indirect,
666 yet secondary BA-dependent, activation of this receptor by fexaramine (104, 325). These results
667 would be in line with the known role of TGR5 activation in browning (427). Alternatively, the inhibition
668 of intestinal FXR by Gly-MCA could also impact on the gut microbiome composition and thereof on
669 global metabolic health. Indeed, FXR antagonism in the intestine results in a stimulation of BA
670 synthesis that protects against obesity and insulin resistance (321). This would be in line with the
671 established association between gut dysbiosis and NAFLD (38, 136).
672 Similar to FXR, TGR5 activation by INT-777 or BAR501 blunts hepatic lipid accumulation in mouse
673 models of obesity (52, 83, 410). As expected, combined pharmacological activation of TGR5 and
674 FXR with the dual agonist INT-767 reverses the progression of several hallmarks of NAFLD and
675 NASH (74, 176, 189, 285, 324). Of interest, TGR5/FXR dual agonism shows increased efficacy than
676 OCA in reducing hepatic steatosis and liver damage in mouse NASH models (362).
677
678 D. BAs in immune homeostasis
679
680 Based on their detergent properties and capacity to disrupt cellular membranes, BAs were initially
681 categorized as pro-inflammatory agents. This was furthermore supported by the knowledge that BA
682 accumulation caused by bile duct obstruction or liver disease leads to hepatic inflammation (270,
683 412) and that systemic accumulation of BAs can damage extrahepatic tissues, particularly the kidney
684 (107). Conversely, BAs can also elicit potent anti-inflammatory responses, as first demonstrated in
685 patients with jaundice and elevated levels of circulating BAs, who experienced significant alleviation
686 of rheumatic symptoms (162). Furthermore, recent evidence suggests that activation of BA receptors
687 exerts anti-inflammatory effects in multiple inflammatory diseases, including experimental
688 autoimmune encephalomyelitis (167, 249), atherosclerosis (143, 152, 158, 296, 297, 337, 478) and
689 hepatic inflammation (105, 217, 285, 441, 442, 462, 474).
690
691 D.1. Immuno-regulatory properties of FXR
692
693 Compared to its abundant expression in enterocytes and hepatocytes, FXR is only discretely
694 expressed in immune cells (375). Nonetheless, anti-inflammatory responses have been reported in
695 peripheral blood mononuclear cells (PMBCs), in mouse and human myeloid cells, in dendritic cells
696 (DCs), and in hepatic natural killer T cells after exposure to FXR agonists (50, 288, 426, 462). Based
697 on these findings, several studies have been conducted to elucidate its role in modulating diseases
698 that are triggered by a disturbed immune balance. Despite these efforts, it is not yet established
699 whether the anti-inflammatory actions of FXR arise from its cell-autonomous actions on immune cell
700 modulation or rather indirectly from its potent BA and lipid lowering effect, the latter being essential
701 in protecting the tissues from lipid-induced toxicity.
702

Liver disease and NASH. The first tangible indications of an anti-inflammatory role of FXR and its signaling in the liver were derived from *in vivo* studies. *Fxr*$^{-/-}$ mice exhibit a higher incidence of hepatic carcinogenesis (219, 460), and suffer from exacerbated hepatic inflammation and necrosis in a model of autoimmune hepatitis (289), or after LPS-induced inflammation (441, 462). To what extent these phenotypes result from an excessive accumulation of BAs or hepatic lipid deposits is not fully established. Mechanistic studies proposed that the hepatic inflammatory phenotype is triggered by a de-repression of NF-κB, a master transcriptional regulator of pro-inflammatory cytokines (441). FXR is also tightly connected with inflammation and cholestasis. Activation by GW4064 ameliorates cholestatic liver damage in rats (263), and ischemia/reperfusion-induced hepatic damage by upregulating SHP in Kupffer cells (196). BAs and FXR furthermore modulate sepsis via control of the NLRP3 inflammasome, which could be relevant for cholestasis-associated sepsis. Cholestasis is a common complication in patients with sepsis and significantly increases mortality rate (401). In this pathological context, BAs have been proposed to act as a new class of danger-associated molecular pathways (DAMPs), triggering a hyperinflammatory response via activation of both signal-1 and -2 of the NLRP inflammasome (153). Remarkably, FXR would act as a negative regulator of the NLRP3 inflammasome via direct physical interaction with NLRP3 and caspase 1, hence preventing its assembly in the mitochondria of activated macrophages (153). Additionally, FXR could also inhibit the inflammasome indirectly by reducing endoplasmic reticulum (ER) stress in hepatocytes (150). Of note, engineered FGF19 analog treatment also blunts hepatic inflammation, along with a significant amelioration of cholangiopaties and NASH in mouse models (481, 482). The protective role of FXR/FGF19 signaling in different immune-related liver diseases, including NASH, PBC and PSC, has been confirmed in patients, as described in section V-C.

Intestinal inflammation. Several mouse models of colitis, including dextran sulfate sodium (DSS)- and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis demonstrated that functional disruption of FXR increases, whereas small molecule FXR agonist treatment suppresses, mucosal inflammation (124, 280) Similar phenotypes were found in two independent models of intestinal hyperpermeability and inflammation, i.e. cholestatic liver injury (428), and ischemia-reperfusion injury (56). Mechanistically, colons from mice with DSS-induced colitis treated with OCA displayed reduced pro-inflammatory cytokine (mainly IL-1β, IL-6) and chemokine (CCL2) expression (124). OCA also repressed TLR4-induced pro-inflammatory gene expression in intestinal epithelial cells (IECs) (426) and attenuated inflammatory cytokine and chemokine expression in cultured human CD14+ monocytes and DCs (124). Thus, FXR appears to limit mucosal inflammatory responses by acting on both IECs and innate immune cells. Of note, intestinal FXR expression was decreased in patients with colitis and its activation reduced disease severity (308, 313, 426). More recently, the bacteria-derived secondary BA, 3β-hydroxydeoxycholic acid (isoDCA), was shown to further refine the immunological balance of the colon by limiting FXR activity in DCs to allow the differentiation of

pTreg cells (50). This highlights the complex interplay between BAs, the gut microbiome, and FXR, a topic discussed in detail in section IV-F.3.

Cardiovascular disease. The role of FXR in atherosclerosis is still under debate. An increase in the atherosclerotic lesion area and an altered plasma lipid profile was observed in a *Fxr*$^{-/-}$ *ApoE*$^{-/-}$ double knockout (DKO) mouse model fed a HF/HC diet (152). Conversely, two other studies showed reduced atherogenic lesion size after FXR disruption in mice on a *Ldlr*$^{-/-}$ or *ApoE*$^{-/-}$ background, together with unexplained differences in plasma lipids (143, 478). Studies using FXR agonists, however, observe protection against lesion formation in *ApoE*$^{-/-}$ or *Ldlr*$^{-/-}$ atherosclerosis prone mice (158, 288). FXR signaling is also functional in vascular smooth muscle cells (VSMCs) (35) and its stimulation blunts the inflammatory response and reduces cell migration. Mechanistically, FXR induces SHP and inhibits the expression of both cyclooxygenase-2 and inducible nitric oxide synthase (256).

D.2. Immuno-regulatory properties of TGR5

TGR5 is highly expressed in cells of the myeloid lineage (203), and is considered a negative modulator of inflammation (reviewed in (334) and Figure 8). In addition to its cross-talk with master regulators of inflammation, such as NF-κB and C/EBPβ (discussed below), indications exist that the BA-TGR5 axis shift macrophages towards a more regulatory and anti-inflammatory phenotype (34, 171, 285, 333, 433). The anti-inflammatory role of TGR5 signaling in monocytes and macrophages profoundly modulates the physiology of metabolic tissues, such as liver, adipose tissue, and vasculature, and consequently influences the development of NASH, T2D and atherosclerosis.

Liver disease and NASH. Stimulation of TGR5 in myeloid cells exerts potent immunosuppressive actions and dampens NF-κB-mediated cytokine expression in mouse models of LPS-induced hepatic inflammation (442). Furthermore, dual activation of FXR and TGR5 by INT-767 improved NAFLD and increased the number of intrahepatic anti-inflammatory monocytes, suggesting that both receptors act hand in hand to reduce liver inflammation (285). More recently, it was demonstrated that TGR5 agonism by itself can rescue the hepatic and vascular damage caused by exposure to a high fat-high fructose diet (52). TGR5-dependent suppression of cytokine production in Kupffer cells is furthermore potentiated following bile duct ligation in rats, indicating a protective role of TGR5 in cholestasis-induced liver injury (208).

Intestinal inflammation. TGR5 activation by natural BAs or INT-777 suppresses LPS-induced inflammatory cytokine expression, whereas these responses are elevated and unresponsive to BAs in macrophages lacking TGR5 (70, 464). In mouse models of TNBS- or DSS-induced colitis, TGR5 activation attenuates the symptoms of inflammatory bowel disease (IBD), whereas functional

disruption of TGR5 impairs intestinal barrier and exacerbates the inflammatory response (70, 126). In addition, activation of TGR5 by BAR501 was shown to switch mucosa-associated macrophage phenotypes from M1 (pro-inflammatory) to M2 (tissue-protective) during chemically induced colitis (34). In this setting, TGR5 activation reduced the
expression of proinflammatory cytokines (TNFα, IL-1b, IL-6, and IFN-γ) and increased the expression of anti-inflammatory cytokines (TGF-β and IL-10) (34). Induction of IL-10 in macrophages allowed the recruitment of Treg cells to inflamed colonic tissue (34). These TGR5-mediated anti-inflammatory pathways not only acutely suppress innate immune responses, but also guide the downstream priming of inflammatory T cell responses. In particular, activation of TGR5 by BAs directs the differentiation of monocytes toward tolerogenic DCs that secrete low levels of TNFα and IL-12, cytokines required for the priming of pro-inflammatory Th1 responses (182). In addition, *ex vivo* treatment of mucosa-associated macrophages isolated from Crohn's disease patient biopsies reduced inflammatory cytokine expression, including TNFα (464), suggesting that disturbed BA circulation and/or metabolism during chronic intestinal inflammation may limit endogenous TGR5 activation.

Cardiovascular and metabolic disease. The role of TGR5 as an anti-inflammatory mediator has also been studied in the context of atherosclerosis. *In vivo* experiments showed that INT-777 supplementation blunts the development of atherosclerosis in *Ldlr*$^{-/-}$ mice fed a HC diet. This anti-atherogenic effect is driven by cAMP induction, followed by repression of NF-κB-activation and cytokine production in macrophage resident plaques (337). Similar results were observed in *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ treated with the dual FXR/TGR5 agonist INT-767 (297), an effect that was lost in the triple *Ldlr*$^{-/-}$, *Fxr*$^{-/-}$ and *Tgr5*$^{-/-}$ mice (296). In other metabolic tissues, additional mechanisms contribute to the TGR5-mediated anti-inflammatory effects. For instance, insulin resistance is exacerbated by increased inflammation, especially in white fat depots. TGR5 activation of adipose tissue macrophages reduces LPS-induced chemokine expression and protects DIO animals from adipose tissue-associated insulin resistance (333) (Figure 8). Specifically, INT-777 treatment of macrophages activates mTOR complex 1 (mTORC1) which induces the differential translation of the liver-enriched inhibitory protein (LIP) to reduce cytokine transcription and macrophage migration (333). In line with these observations, a recent study demonstrated that activation of the TGR5-cAMP-PKA pathway in innate macrophages induces phosphorylation and degradation of the NLRP3 inflammasome, resulting in an improvement of insulin sensitivity and glucose tolerance (141).

Antiviral response and senescence. In addition to its role in macrophages, TGR5 has been suggested to control antiviral innate immunity through the activation of an AKT/IRF3 (457) and the β-arrestin-SRC signaling axis (175). Furthermore, TGR5 modulates cytokine levels in the context of cell proliferation and senescence, as INT-777 prevents IL-1β induction of cell senescence in human chondrocytes (177), indicating that the anti-inflammatory effect of TGR5 signaling is wide-spread.

E. BAs in tissue plasticity and remodeling

Tissue plasticity and regeneration are dynamic processes that involve multiple signaling pathways. Several lines of evidence suggest that BAs are implicated in liver regeneration through modulation of FXR and TGR5 signaling. In addition, BA signaling contributes to cellular reprogramming, a process with great potential for the treatment of hematopoietic, immune and metabolic diseases.

E.1. BAs in liver regeneration

The liver is one of the few organs that can robustly regenerate itself in response to partial ablation or injury. BAs, together with cytokines and growth factors, activate specific signaling pathways and gene expression programs essential for hepatic regeneration. Several studies demonstrated an inhibition of liver regeneration following interruption of the normal enterohepatic biliary circulation (314, 418). BAs were also found to directly stimulate hepatocyte proliferation (24) and a study based on a 70% partial hepatectomy (PHx) mouse model demonstrated that low doses of BAs promote liver regeneration (179) (Figure 9). Conversely, reduction of total BA levels with BA sequestrants delayed liver regeneration (179). FXR and TGR5 display distinct roles in this complex adaptive process.

Hepatocyte FXR and liver regeneration. FXR was initially described as the master regulator of BA-mediated homeotrophic liver growth during hepatic regeneration (179). Enhanced activity of both intestinal and hepatic FXR contributes to this process (Figure 9). The first physiological change during PHx is the redistribution of portal and arterial blood supply to the remnant liver in place of the entire organ. As a result, hepatocytes become exposed to a ~3-fold greater amount of regenerative factors, including BAs that are mainly supplied by the portal vein (292). This leads to a robust activation of hepatic FXR that induces the expression of the forkhead box M1b (FOXM1B), a key transcription factor controlling hepatocyte DNA replication during liver regeneration (60, 179). FXR post-translational modifications can also impact on hepatocyte regeneration as illustrated by SIRT1-mediated deacetylation of FXR that inhibits its activity and impairs hepatocyte proliferation after PHx (125). In addition to directly stimulating hepatocyte proliferation, FXR activation promotes the active efflux of BA from hepatocytes through the transcriptional upregulation of dedicated transporters (described in section IV-A.1) (12). BA efflux from the remnant liver is further reinforced by coordinated activation of TGR5 in cholangiocytes (327) (see section below). This harmonized mechanism helps to protect the remnant liver from apoptosis triggered by the cytotoxic effects of BA overload (327, 443) and leads to an acute, but temporary, increase in BA levels in the intestine and systemic circulation (310). In turn, BAs activate intestinal FXR that stimulates FGF15 secretion whose signaling is also implicated in liver regeneration. Mechanistically, the FGF15/FGFR4 axis

reinforces the effects of hepatic FXR activation by upregulating FOXM1B and its downstream mitogenic target genes (*Cdc25b*, *Ccnd1*, and *Pcna*) (420). In addition, FGF15 helps to protect hepatocytes against apoptosis triggered by high levels of BAs by suppressing *Cyp7a1* transcription and thus, *de novo* BA synthesis (473). Consequently, *Fgf15*$^{-/-}$ mice were found to suffer from severe liver injury and exacerbated mortality after PHx. This effect is attenuated upon treatment with the BA sequestrant, cholestyramine, or after adenoviral delivery of FGF15 (420). Kong et al. (224) observed similar effects in *Fgf15*$^{-/-}$ mice and identified additional mitogenic pathways controlled by this growth factor. *Fgf15*$^{-/-}$ mice exhibited reduced activation of the JNK and p38 pathways confirming earlier reports on FGF15-induced MAPK signaling (226). The mutant mice also suffered from reduced activation of the JAK/STAT pathway (226), a pathway known to be activated during liver regeneration (76, 255). Mechanistically, the mitogenic effects of STAT3 appear to be mediated by FOXM1B (287, 317) suggesting that both cell-autonomous (FXR-mediated) and non-cell autonomous (FGF15-mediated) effects of BAs on proliferation converge on this transcription factor. Interestingly, selective activation of intestinal FXR or treatment with FGF19 also reduces inflammation and liver necrosis after obstructive cholestasis induced by bile duct ligation (299).

In addition to their role in pathological settings, BAs have been reported to mediate FXR-dependent hepatocyte proliferation under physiological conditions. For instance, during pregnancy, FXR is important for fetal liver growth and its loss of function reduces fetal liver enlargement (294).

Cholangiocyte TGR5 and liver regeneration. While the role of FXR in liver regeneration has been extensively studied, less is known about TGR5 in this process. Nonetheless, TGR5 is an important player involved in cholangiocyte proliferation while simultaneously protecting the liver against BA overload. A study demonstrated that PHx was followed by cholestasis and hepatocyte necrosis and markedly delayed liver regeneration in *Tgr5*$^{-/-}$ mice (327). At the molecular level, the mechanisms through which TGR5 protects the remnant liver from BA cytotoxicity are different and complementary from those of FXR. The origin of this difference lays in the divergent patterns of expression of these two receptors with FXR being highly expressed in hepatocytes, while TGR5 is especially enriched in the gallbladder and biliary tract (207) where it contributes to adapt bile composition in ions after PHx. Indeed, in cholangiocytes TGR5 controls CFTR-dependent Cl^- secretion (207) and the observed TGR5-dependent increase in biliary HCO_3^- and Cl^- output after PHx likely constitutes an adaptive mechanism to enhance bile secretion, fluidity, and consequently protect the overloaded remnant liver from BA toxicity (327) (also discussed in III-A.2). In line with the idea that TGR5 contributes to increase bile turnover during liver regeneration is the observation that TGR5 facilitates BA elimination in the urine, protecting the entire organism from BA overload (327). Another mechanism through which TGR5 protects the liver from BA toxicity during regeneration is through its control of BA hydrophobicity. Several studies demonstrated that *Tgr5*$^{-/-}$ mice exhibit a more hydrophobic BA composition (92, 254, 327), which exacerbate hepatocyte injury immediately after PHx. Accordingly, liver injury in *Tgr5*$^{-/-}$ hepatectomized mice can be rescued by BA resins (327).

Consistently, an enlargement of a hydrophobic BA pool was also associated with an inhibition of liver regeneration in a model of PHx (125).

BA feeding is also known to induce cholangiocyte proliferation (8), an effect later on attributed to their potential to activate TGR5 (353) (Figure 9). Mechanistically, conjugated LCA and TGR5-selective agonists were shown to induce cholangiocyte proliferation through elevation of reactive oxygen species (ROS) and SRC-mediated EGFR transactivation. Subsequent MAPK phosphorylation induced proliferation in *Tgr5*$^{+/+}$, but not *Tgr5*$^{-/-}$ derived cells (353). Similar interactions between BA signaling and transactivation of the EGFR have been reported to control the proliferation of other cell types (258, 309, 391, 450) suggesting that it may represent a universal and novel mitogenic branch of BA signaling.

E.2. BAs in differentiation and cellular reprogramming

FXR in stromal cell differentiation. Mesenchymal stromal cells give rise to osteoblasts and adipocytes. FXR activation regulates this process by promoting both osteoblast (67, 183) and adipocyte differentiation (1). The pro-adipogenic phenotype was attributed to a synergism with the master regulator of adipogenesis PPARγ and to the suppression of the Wnt/β-catenin signaling pathway (1). Surprisingly, the FXR antagonist, guggulsterone (421), impairs osteoblast differentiation but induces the expression of adipogenic markers, suggesting a role for FXR in the regulation of the osteoblast/adipocyte balance (183). Altogether these studies show that FXR disruption significantly impacts diverse aspects of bone homeostasis and adipogenesis. A recent study demonstrates that overexpression of FXR in WAT alters its architecture underscoring the need for a tight regulation of FXR expression/activity in white fat (423).

TGR5 in adipocyte reprogramming. The white-to-brown conversion of adipose tissue, a phenomenon referred to as beiging, is a dynamic process involving the genetic rewiring towards a mitochondrial phenotype. Beiging is triggered by chronic cold exposure and β-adrenergic stimulation, and multiple cell types, including adipocyte precursors, immune cells and mature adipocytes, participate in this process (69). TGR5 is required for the emergence of beige adipocytes within WAT upon cold exposure (427) (Figure 8). Pharmacological stimulation of TGR5 induced beige remodeling during HFD feeding (51, 427), independently of adrenergic stimulation (427). Mechanistically, TGR5 activation leads to lipolysis, mitochondrial fission and mitochondrial biogenesis over time (427). These results highlight a critical role of the TGR5 signaling axis in mitochondria dynamics that could underlie a general feature linked to cell differentiation and/or reprogramming.

E.3. BAs as components of the intestinal stem cell niche

Epithelial cells of the intestine undergo rapid renewal to counteract intestinal damage and disruption of the barrier function (128). Renewal and patterning of the intestinal epithelium are carried by intestinal stem cells (ISCs) that reside at the bottom of intestinal crypts (128). These crypts define an ISC niche that regulates the balance between self-renewal and cell fate specification (128). Recent studies demonstrated that BAs constitute an intrinsic regulatory component of the ISC niche capable of regulating both the renewal and the specification of ISCs (268, 396). In particular, BAs were shown to foster epithelial regeneration under both physiological and colitis-induced conditions by activating TGR5 in the ISC compartment through a mechanism involving yes-associated protein 1 (YAP) and its upstream regulator SRC. Importantly, endogenous BA release in the intestinal lumen was found to be sufficient to coordinate ICS renewal and proliferation (396). These findings suggest that the physiological cycles of food intake followed by discharge of BAs in the intestinal lumen represent an intrinsic stimulus that dictates ISC proliferation rhythms to sustain daily regeneration (396). BAs also seem to play a role in the patterning of the intestinal epithelium as another study reported that the BAs/TGR5 axis regulates L-cell differentiation and abundance in the intestinal epithelium (268). *In vivo,* TGR5 activation elevated GLP-1 secretory capacity and improved glucose tolerance (268). While BAs appear to be an integral component of the ISC niche, their levels and chemical structure must be kept in check as both quantitative and qualitative changes in the BA pool can initiate and drive the proliferation of cancer ISCs (121). Indeed, BA species known to antagonize FXR (mainly TβMCA and DCA) were shown to induce proliferation and DNA damage in *Lgr5*$^{+}$ cancer stem cells (121). Therapeutically, FexD, a gut-biased FXR agonist, delayed tumor progression and profoundly increased survival in APC$^{min/+}$ mouse models of adenoma and adenocarcinoma (121), suggesting that restoring a healthy BA balance might represent a therapeutic approach to treat colorectal cancer.

F. BAs, gut microbiota, and host metabolism

The gut is home to one of the most complex eco-systems known, the gut microbiome, which impacts on nearly every aspect of physiology (reviewed in (376)). How the host and the microbiome communicate with one another is an intense area of research. Because the BA pool is generated by the host and actively modulated by intestinal bacteria, these molecules are key in mediating this symbiotic communication (Figure 6). Furthermore, BAs actively shape the microbiome at the highest taxonomic levels. This interlinked dependence turns the BA-microbiome axis into a chief determinant of health and disease.

F.1. The gut microbiome shapes the BA pool

Microbial conversion of primary into secondary BAs increases BA diversity and promotes hydrophobicity of the BA pool (430). Several studies using germ-free (GF) or antibiotic-treated

968 rodents revealed both a quantitative and a qualitative modification of the BA pool, characterized by
969 an overall enlargement of the BA pool size and an increased ratio of primary conjugated to secondary
970 BAs (213, 370, 377, 406). In the host, TGR5 signaling is particularly affected by the microbiome as
971 the secondary BAs, LCA and DCA, and their conjugated forms are potent TGR5 activators. In fact,
972 microbial transformation of primary to secondary BAs represents the most direct link between BA
973 7α-dehydroxylating gut bacteria and host health. This is exemplified in humans, who unlike rodents,
974 cannot reconvert secondary BAs into primary ones by hepatic 7α-hydroxylation (357). Therefore,
975 dietary or pharmacological interventions (e.g. antibiotics) that modulate the proportion of intestinal
976 bacteria with 7α-dehydroxylation activity alter the availability of endogenous TGR5 ligands (123). In
977 fact, dietary modification including changes in protein (445) or fat (88, 197) source, can modulate
978 the gut microbiota composition and consequently the intestinal BA pool. In turn, major changes in
979 the BA pool, such as those triggered by antibiotics, can influence whole-body energy and glucose
980 homeostasis (Figure 6) (470). Other environmental cues, including cold exposure (64, 452, 485)
981 induce similar phenotypes (Figure 6). In line with these findings, a recent study demonstrated that
982 broad-spectrum antibiotics given to healthy adults prior and subsequent to seasonal influenza
983 vaccination significantly impaired H1N1-specific neutralization (146). This was accompanied by a
984 1,000-fold reduction in serum secondary BAs, which was highly correlated with AP-1/NR4A signaling
985 and inflammasome activation suggesting an involvement of TGR5 signaling in systemic immune
986 homeostasis as described in section IV-D.2 (146).
987 The microbiome also directly affects quantitative aspects of the BA pool size which is nearly doubled
988 in GF mice (370). The mechanisms explaining this effect are only starting to be understood and
989 involve the gut microbiome-mediated shift of BA composition from FXR antagonists to agonists. For
990 instance, the absence of microbiota resulted in the accumulation of BAs with FXR antagonizing
991 properties, such as TβMCA, which were identified as the main endogenous FXR antagonists that
992 could not be metabolized in the absence of intestinal bacteria (370). Conistent with the obesigenic
993 phenotype of mice after long-term FXR agonist treatment (446), accumulation of TβMCA enhanced
994 BA synthesis and recycling (370), which in turn contributed to the resistance against DIO observed
995 in GF *Fxr*$^{-/-}$ mice (321). Similarly, administration of glycine-β-MCA (Gly-MCA), a β-MCA analog
996 resistant to bacterial deconjugation, improves metabolic homeostasis by inhibiting FXR specifically
997 in the intestine (194). Of note, novel gut microbiome-produced secondary BAs have recently been
998 discovered that would act as FXR agonists (348). In addition to the well-studied BA deconjugating
999 activity, gut bacteria of the *Clostridium* species were shown to conjugate cholate with phenylalanine,
1000 tyrosine and leucine through a yet unknown enzymatic reaction (348). These novel BA conjugates
1001 are absent upon antibiotic treatment and seem to be increased in HFD-fed mice and IBD patients
1002 (348), indicating a potential role of these metabolites in metabolic and inflammatory disorders.
1003 Antioxidants and drugs can similarly impact on the BA pool because of their effects on BA
1004 metabolizing members of the microbiome. For instance, remodeling of the gut microbiota with the

antioxidant tempol increases TβMCA levels, resulting in an inhibition of intestinal FXR signaling and a decrease in obesity (250). Probiotic supplementation can also positively or negatively modulate BA synthesis in the liver. Treating mice with the VSL#3 probiotic formulation increased BA deconjugation and fecal excretion along with an induction of hepatic BA synthesis (85). Conversely, *Lactobacillus rhamnosus GG* supplementation reduced hepatic BA levels by promoting the synthesis of FXR antagonists which prevented excessive BA-induced liver injury and fibrosis in mice (264). Finally, it should be noted that the glucose-lowering effects of metformin are, in part, mediated by the intestinal reduction of *Bacteroides fragilis* leading to an increase in the production of the intestinal FXR antagonist glycoursodeoxycholic acid (GUDCA) (404). Conversely, certain pathological conditions can lead to a microbiota-host cross-talk in which the modified BA profile will propel the disease. For instance, progressive hypercholanemia during pregnancy was recently reported to originate from an altered microbiome associated with a lowering of ileal FXR activity, and subsequent enhancement of hepatic BA synthesis leading to an elevation of circulating BAs (316). UDCA can treat this condition but only in women with a microbiome characterized by a high ratio of *Bacteroidetes* to *Firmicutes* (316). In these women, it is suggested that UDCA could be converted to CDCA leading to an activation of ileal FXR and to an increase of the FGF19-mediated enterohepatic feedback on BA production (315). On the other hand, UDCA has also been described as an FXR antagonist able to increase BA synthesis and reduce FGF19 levels in obese patients (306). Similarly, a *Clostridia*-rich microbiota and their BA metabolites, including UDCA, were shown to suppress intestinal FGF19 expression contributing to excessive BA excretion in patients with diarrhea-predominant irritable bowel syndrome (480). Remodeling of the gut microbiota and alteration of the BA profile also takes place after chronic alcohol consumption (159). In turn, this altered BA pool was shown to promote alcoholic liver disease that could be ameliorated by treating alcoholic mice with the intestine-restricted FXR agonist fexaramine or by overexpression of a non-tumorigenic FGF19 variant (159). Finally, the metabolic benefits of surgical interventions also seem to depend on changes in the gut microbial communities and affect FXR-dependent processes (365). This topic is further developed in section V-B.

F.2. The BA pool shapes the gut microbiome.

The effects of the microbiome on the BA pool are bidirectional since BAs also modulate the gut microbiota composition. Similar to HFD feeding which stimulates bile secretion (352), BAs reshape the microbial landscape and shift the ratio of bacterial phyla (187) through both direct antimicrobial effects (27), and indirect effects mediated by FXR-induced antimicrobial program (186). The antimicrobial properties of BAs are a function of their hydrophobicity. DCA, for instance, is a more potent antimicrobial agent than CA, owing to its high hydrophobicity and detergent properties on bacterial membranes (405). DCA promotes the survival of microbe populations that resist BA-induced membrane damage (233). Complex and significant changes in the gut microbiome are

observed when rats are fed BAs. A high-CA (5 mmol/kg) diet profoundly alters the gut microbiome both at the taxon- and phylum-level (187), with significant inhibition of the *Bacteroidetes* and *Actinobacteria*, two of the three major phyla reported in human microbiomes (187). Consequently, expansion of the *Firmicutes,* in particular *Clostridium cluster XVa,* increased the number of DCA-producing bacteria highlighting the bi-directionality of the BA–microbiome axis (187). While the potential contribution of BA-responsive receptors to this phenotype was not investigated in this study, it is now recognized that the effects of BAs on the microbial landscape can also be mediated through these receptors. It was recently demonstrated that FXR activation by OCA reduces endogenous BA levels and increases the proportion of $Gram^+$ bacteria (115), demonstrating that the human microbiome can dynamically respond to BA modulation. Modulating the BA pool can also be a therapeutic strategy in the fight against intestinal infections, in particular nosocomial infections caused by *Clostridium difficile* (CDI). This infection often arises following the depletion of intestinal bacterial species after antibiotic treatment (409). In this context, *Clostridium scindens*, a species able to 7α-dehydroxylate BAs, was identified to confer protection by generating secondary BAs that block the germination of *Clostridium difficile* spores (47). Similarly, LCA was recently demonstrated to lock Vancomycin-resistant Enterococcus bacteria in diplococcal mode, impairing their biofilm formation, and increasing their susceptibility to the antibiotic daptomycin demonstrating that BAs not only select bacteria but also actively shape their morphotype (284). Thus, BA pool size and composition appear to be some of the most important host factors in regulating gut microbial density, community, and structure in humans.

F.3. The BA-microbiome axis modulates intestinal immunity along the gastrointestinal tract.

Immune cells at the mucosal surface of the gut are challenged with the rapid detection and elimination of pathogenic microorganisms, while also maintaining tolerance toward commensal bacteria (28). Genetic or environmental insults can disrupt this balance and precipitate chronic intestinal inflammation characteristic of IBD (276). The BA-microbiome axis finely shapes intestinal inflammation along the gastrointestinal tract by defining a series of unique immunoregulatory microenvironments. In the ileum, high (millimolar) concentrations of conjugated primary BAs prevent bacterial overgrowth through both direct antimicrobial effects (reviewed in (27)), and indirect, FXR-mediated, induction of an antimicrobial program (186). In the colon, the immunological balance requires further adjustments during microbial colonization when immune cells need to develop tolerance toward commensal bacteria (28). The BA-microbiome axis plays a key role in this process as the bacteria-derived secondary BA 3β-hydroxydeoxycholic acid (isoDCA) limits FXR activity in DCs to diminish their immunostimulatory properties (50). The anti-inflammatory phenotype acquired by DCs, in turn, allows the differentiation of pTreg cells that help dampen immune responses during bacterial colonization (50). In the colon, microbiome-derived secondary BAs can also maintain a healthy pool of $FOXP3^+$ $ROR\gamma^+$ Treg cells by selectively activating VDR signaling (395). Colonic,

microbiome-derived BAs further modulate TGR5 activity in DCs to instruct tolerance toward commensal microbes. Specifically, BA-dependent activation of TGR5 by secondary BAs channels the differentiation of human monocytes into tolerogenic DCs that secrete low levels of TNFα and IL-12 cytokines (182). Similarly, two BA microbial metabolites were recently shown to fine-tune intestinal immunity (151). 3-oxo-LCA blocked TH17 differentiation via retinoid-related orphan receptor-γt (RORγt) while isoallo-LCA increased Treg differentiation through a mitochondrial ROS-FOXP3-dependent signaling (151), possibly by activating TGR5 (Figure 8). These data suggest that the host-BA-microbiome axis defines a BA-mediated, pan-genomic network of communication. Immunological tolerance towards commensal bacteria is instructed by the microbiome itself through complex modifications of the host's BA profile. Disturbance of this BA-based communication network can propel the development of inflammatory diseases. Indeed, reduced microbial metabolism of primary BA precursors into secondary BA products during states of dysbiosis negatively impacts on TGR5 signaling during intestinal inflammation, as observed in IBD (78, 387). Therapeutically, restoration of secondary BA levels directly through rectal administration (387) or indirectly through administration of a hydrolyzed protein diet (437) can help in the management of these diseases.

G. BAs and aging

Aging is defined as the progressive decline of cellular and ultimately organismal function. Although BAs are known to be beneficial in the treatment of chronic metabolic and inflammatory disorders, their effect on lifespan remains elusive in mammals. There are, nonetheless, indications that steroid acids with BA-like features or bona fide BAs can regulate longevity in *C. elegans* (129, 272, 303, 380). The first tangible evidence for a role of BAs in lifespan regulation stems from high-throughput screens in yeast in which the secondary BA LCA was identified to extend the chronological lifespan in a calorie restriction-independent fashion (133). Another report proposed that intracellular LCA modifies the inner mitochondrial membrane lipidome to enlarge mitochondria and increase the number of disconnected cristae (26). This remodeling would enhance respiration, ATP synthesis and production of ROS, resulting in a global increase of mitochondrial long-term stress resistance (26, 48).

In mammals, age-related changes in the BA composition of bile (246), liver and serum (122) have been reported. Although the nature of these modifications differs according to various factors, including gender, aging is mainly associated with a decline in BA levels (118). In further support of this notion, long-lived dwarf mice (*Ghrhr*$^{lit/lit}$), characterized by a defect in growth hormone/IGF-1 signaling, exhibit an enlarged BA pool size (9). Of interest, xenobiotic detoxification is enhanced in these mice, most likely through a BA-mediated mechanism (9). Moreover, CA administration in wild-type mice mimics the changes in drug-metabolizing enzymes observed in *Ghrhr*$^{lit/lit}$ mice, suggesting that the xenobiotic stress response induced by BAs could contribute to extending lifespan (10). Remarkably, methionine restriction was found to extend healthspan and lifespan of progeroid mice

by normalizing a dysfunctional BA pool (22). The same phenotype could be recapitulated by dietary intervention with CA (22). Premature aging was also delayed in progeroid mice by fecal microbiota transplantation of healthy wild-type mice (23), and the reconstitution of the secondary BA pool size was identified as a mechanism that accounts for the prolonged lifespan. Although these observations point to a role for TGR5 in this process, its exact role remains to be identified. It is however noteworthy that the expression of FXR and TGR5 declines with age and that dual agonists for TGR5 and FXR delay age-related kidney deterioration (439), as well as osteoporosis, another age-related disease (257). The intricate relationship between BA signaling, healthspan and longevity thus seems to represent an interesting area of future investigations that will undoubtedly shed light on how BAs modulate lifespan.

V. Strategies to modulate BA signaling

A. Physiological and environmental cues

Food ingestion and circadian rhythmicity are well-established physiological cues that coordinate BA homeostasis. Recent evidence, however, indicates that multiple environmental factors dramatically alter this tightly regulated process (Figure 6). Often, these effects imposed by the environment go along with changes in the gut microbiota. In the last decade, the role of HFD feeding on microbiota-host interactions has been the focus of intense research. In addition to its marked impact on the gut microbial community (18), HFD significantly influences the BA pool size and composition (121). The consumption of HFD increases the synthesis of CA and DCA and decreases the levels of CDCA in healthy subjects (36). In rodents, secondary BAs are significantly higher in HFD compared to CD fed controls (102, 121). Cold exposure also dramatically alters the microbiome and counteracts metabolic disease. This was first illustrated by the observation that bacteria transplanted from cold-exposed mice improve the metabolic outcome of recipient mice (64) and that BAs could play a role in this process (452, 485). Cold exposure increased the ratio of conjugated BAs (452, 485), and enlarged the BA pool through selective induction of the alternative BA synthesis pathway (452). In support of the latter finding, *Cyp7b1*$^{-/-}$ mice were unable to adjust their BA pool and displayed lower body temperature after cold challenge. A similar phenotype on body temperature was observed in adipose-specific *Tgr5*$^{-/-}$ mice, suggesting that an adequate thermogenic response requires TGR5 in adipocytes (427). Furthermore, one of the main BA species to be increased in response to cold is the FXR antagonist, TβMCA (485). Altogether, these results suggest that cold, as an environmental cue, impacts the gut microbiota in such a way that it induces TGR5 signaling while concomitantly attenuating FXR activation. Another environmental factor that enhances energy expenditure is exercise. Morville et al., observed that several BAs are significantly altered following endurance and resistance exercise. Amongst those, the TGR5 endogenous agonists LCA and DCA were

consistently induced (301). The hallmarks of exercise-induced phenotypes, such as increased energy expenditure and improved glucose tolerance, may hence be coordinated, at least in part, by an activated TGR5 signaling pathway.

B. Surgical interventions

Roux-en-Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG) and bile diversion to the ileum (GB-IL) are surgical procedures that promote weight loss and induce a rapid remission of T2D in patients. Although the mechanistic basis for this phenomenon is not fully established, elevated concentrations of circulating incretin hormones is a hallmark that may contribute to these metabolic improvements (reviewed in (138)). In 2009, Patti et al., demonstrated for the first time that serum BAs are also significantly elevated in patients following RYGB and proposed TGR5 as a putative mechanism by which improvements in glucose and body fat management can be achieved (326), later confirmed by other studies (5, 385). A subsequent study confirmed that DCA, a very strong TGR5 agonist, is increased in patients 24-months after RYGB, while UDCA and its conjugated forms are the most changed BAs one month after surgery (5). While some studies reported TGR5 as a mediator of the RYGB-mediated metabolic improvements, including GLP-1 secretion (471), others failed to confirm these findings (154). Furthermore, although there is agreement that activation of TGR5 improves glucose response and attenuates fatty liver disease (91, 283), controversy exists relative to its role in energy expenditure in the context of VSG. A study using a DKO model of TGR5 and glucagon receptor suggested that TGR5 is not critical for the secretion of proglucagon-derived peptides (322). While sustained elevation in circulating BAs is a phenotypic consequence of all bariatric procedures, they are also typified by a relocation of BA delivery to more distal segments of the small intestine and an induction of the ileal signaling factor and FXR target, FGF19 (344). Two independent studies demonstrated that DIO germline or intestine-specific *Fxr*$^{-/-}$ mice can no longer recapitulate the metabolic improvements observed after VSG (365) or GB-IL (4). Further studies are required to identify the missing link between gastric bypass surgeries, FXR, and weight loss, but dynamic alterations of the BA pool and the gut microbiome seem to play a key role in this process (199) (Figure 6). Consistently, fecal microbiota transplantation from postbariatric donors improved metabolic parameters in patients with metabolic syndrome (82).

C. Pharmacological interventions

In addition to surgery, a series of FDA-approved chemicals exists that modulate BA signaling thereby improving metabolic disorders. The oldest group of BA-modifying drugs are the BA sequestrants, initially designed to interrupt the enterohepatic circulation. BA sequestrants are effective in lowering LDL cholesterol and inducing GLP-1 release, by promoting the hepatic conversion of elevated cholesterol levels into BAs and by coordinately modulating intestinal FXR and TGR5 activities.

IBAT/ASBT inhibitors have a similar mechanism of action preventing BA re-uptake across the intestinal epithelium (extensively reviewed in (230, 397, 414). Only recently, antibiotics have regained new interest, not so much because of their impact on the size, but rather on the composition of the BA pool (Figure 6). The decrease in secondary BAs after short-term use of antibiotics was recently shown to reduce serum glucose and triglyceride levels (232). However, caution should be taken when developing therapeutic strategies as the same antibiotic-driven reduction in secondary BAs was linked to the development of cholestasis in pediatric patients (454), and to *Clostridium difficile* outgrowth in the large intestine (408).

Therapies using natural BAs, such as UDCA, have proven to be successful in a subset of patients with cholestatic disorders (32). UDCA is the first-line therapy for PBC, and is effective in approximatively 60% of patients (318). While its efficacy is still debated for PSC (241) and NAFLD/NASH (16), the UDCA-homologue 24-norursodeoxycholic acid (*nor*UDCA) seems to be effective for PSC (106). Finally, promising therapeutic opportunities with selective FXR and/or TGR5 modulators have made their appearance. Several selective and dual agonists, but also antagonists, have been developed (Table 2) and tested in human subjects for their ability to prevent or delay cholestatic liver disorders, obesity, T2D, NASH, atherosclerosis, and IBD, as described above. While to date only one TGR5 agonist has been studied in T2D patients with unexpected outcomes on glucose management (168), numerous FXR agonists have been tested in clinical trials (Table 3). Of these, the most advanced is the CDCA semi-synthetic derivative, OCA (328), which has been FDA-approved as second-line therapy for UDCA-unresponsive or -intolerant PBC patients (416). OCA treatment blunts cholestasis and inflammation in PBC patients (166, 227, 312, 416), and stabilizes or even improves hepatic damage and fibrosis (39). While the most common adverse effect of OCA is pruritus, severe non-hepatic ascites and varices can occur in a minority of patients, and worsening of liver disease in cirrhotic patients has been reported (99). In non-cirrhotic NASH patients, however, OCA treatment is beneficial and diminishes liver steatosis, inflammation and fibrosis, while enhancing insulin sensitivity (305, 311, 466). FXR activation, however, also increased total and LDL cholesterol and decreased HDL cholesterol levels in NASH patients (311)) and healthy volunteers (331), warranting long term studies to further assess the clinical relevance of this dyslipidemia. Phase III clinical trials (REGENERATE AND REVERSE) are currently ongoing to assess clinical outcomes and long-term safety, as well as OCA efficacy in cirrhotic NASH patients (REVERSE trial). Interestingly, similar improvements on fibrosis were reported in patients after a 3-year follow-up, suggesting long-term clinical benefits (351, 466).

In addition to the steroidal OCA compound, which is the most advanced in the clinic, several non-steroidal FXR agonists, including Tropifexor (417), Nidufexor (66), EDP305 and Cilofexor (Table 2), have reached the phase II clinical test stage and have the potential to become novel therapeutic agents for NASH (323), PSC (415) and PBC (417). Likewise, the FGF19 analog, NGM282, has also been evaluated in clinical trials and has proven efficacy in PSC (165), PBC (282) and NASH (86, 156, 157).

VI. Undesired side effects of BA signaling

BAs and cancer. Exposure to elevated BA levels has been linked with higher cancer incidence in several digestive organs. Already in 1940, BAs were demonstrated to be inducers of cancer in rodents that were subcutaneously injected with DCA (75). The consensus hence was that BAs, especially hydrophobic species, were tumor-promoting molecules. Subsequently, several pathways linking BAs to cancer were identified, including oxidative stress with DNA damage and genomic instability, apoptosis, and interactions with gut microbiota (reviewed in (192)). These mechanisms can also be secondary to environmental stimuli (diet, lifestyle, exposure to environmental toxins) and affect predominantly the hepato-gastrointestinal tract (reviewed in (192)), especially the liver (465), biliary tract (223), and colon (121). The main mechanisms involved are the increased intracellular production of reactive oxygen and nitrogen species (30) and the altered expression of tumor suppressor/promoting genes (438). In this process, the degree of hydrophobicity dictates the oncogenic potential of BAs as illustrated by the fact that in the liver, feeding various concentrations of BAs produced the following hepatotoxicity: UDCA<CA<CDCA<DCA<LCA (394). Consistently, *Fxr*$^{-/-}$ mice develop spontaneous liver cancer because of increased levels of BAs (460) and lowering their BA pool with cholestyramine significantly inhibits tumor lesions (460). While intestine-restricted FXR agonists are usually seen as having positive therapeutic impacts, it should be noted that chronically elevated levels of circulating FGF19 are linked with liver cancer (482). The pro-tumorigenic effects of FGF19 are due to a non-cell autonomous activation of IL-6/STAT3 signaling (484). Interestingly, an FGF19 engineered analog NGM282, which differs from wild-type FGF19 in the amino terminus, retains the ability to repress *Cyp7a1* expression without triggering the activation of STAT3, eliminating FGF19-associated tumorigenicity (157, 481, 483). Recently, a direct link between BAs and cancer progression was described. TβMCA was shown to not only initiate colorectal cancer through DNA damage but also to actively promote cancer stem cell proliferation via inhibition of FXR activity in *Lgr5*$^{+}$ intestinal stem cells (121). Therapeutically, restoring FXR activation with FexD, a gut-biased FXR agonist, delayed tumor progression and profoundly increased survival of APC$^{min/+}$ mouse models of adenoma and adenocarcinoma (121). Similarly, the growth of lymph node-metastatic melanoma was shown to depend on BA-mediated activation of YAP (245). Unexpectedly, this study suggests that lymph node-metastatic tumors themselves can upregulate *Cyp7a1* and produce BAs in an autocrine manner to further stimulate their own growth (245). Importantly, evidence also exists in support of an oncoprotective role for BAs. While some studies involve direct effects of BAs on cancerous cells (248, 332, 335), a new report demonstrated that the gut microbiome can use BAs to shape immunity against liver cancer (270). In this study, the authors demonstrated that microbiome-mediated primary-to-secondary BA conversion triggers

CXCL16 expression in liver sinusoidal endothelial cells enabling the recruitment of natural killer T cells to mediate liver-selective tumor inhibition (270).

BAs and adverse cardiovascular outcomes. Despite the established benefits of BA signaling in cardiometabolic homeostasis (see section IV-D), elevated BAs can be cardiotoxic and lead to progressive cardiomyopathy (reviewed in (424)). Conjugated BAs, in particular TCA, furthermore induce arrhythmic contractions in human atria (349), underscoring once more the potential detrimental action of BAs in disease. While BA responsive receptors are expressed in the heart, their contribution to human cardiac disease is not completely understood. FXR activation triggers apoptosis in cardiomyocytes while conversely, inhibition of FXR is protective against ischemia-induced cardiac insults (346). In addition, long-term FXR activation by OCA in humans can elevate LDL cholesterol levels (311). This unfavorable and atherogenic serum lipid profile may originate from the ability of FXR to blunt BA synthesis and LDL clearance via repression of *Cyp7a1* (59) and hepatic pro-protein convertase subtilisin/kexin type 9 (*Pcsk9*) expression (130, 240), respectively. However, HDL-cholesterol is also decreased (311). The FXR-dependent repression of apolipoprotein A-1 (ApoA-I) (73) and paraoxonase 1, involved in the inactivation of pro-atherogenic lipids (145, 382), may contribute to this effect and ultimately lead to long-term adverse clinical outcomes. Further studies will be needed to fully understand the underlying mechanisms.

Although less studied, cardiovascular concerns have been raised for TGR5 as well. TGR5 has been proposed to mediate cardiac hypertrophy in a mouse model of liver injury by triggering AKT signaling (87), and reflex tachycardia (as a result of reduced vascular tone and blood pressure) has been observed in dogs treated with a synthetic TGR5 agonist (119). Other studies, however, attribute a cardioprotective role to TGR5 by improving the myocardial response to cardiac stress (100), as well as by reducing atherosclerosis (337). In line with this, LCA negatively correlates with atheroma presence in patients and its levels can predict the disease (94). Dedicated studies and clinical trials will be required to identify the exact impact of TGR5, as well as the other non-canonical BA receptors, such as the muscarinic receptors, on cardiovascular risk.

BAs and pruritus. Although itching can be seen as a protective reflex to remove pathogens and skin irritants, chronic pruritus is associated with pathological states and significantly impacts the quality of life. TGR5 is expressed in peripheral neurons of the dorsal root ganglia where its activation stimulates the release of neuropeptide transmitters of itch. The TGR5-dependence of this effect was proven *in vivo* where DCA treatment induced spontaneous scratching in *Tgr5*$^{+/+}$ but not in *Tgr5*$^{-/-}$ mice (6). Later, it was reported that the neuronal hyperexcitability followed by TGR5 stimulation is mediated through activation of the transient receptor potential ankyrin 1 (TRPA1) channel, required for the acute pruritogenic response (260). However, it should be mentioned that TGR5 activation in the dorsal root innervation can also attenuate pain through an opioid-dependent mechanism (6) thus blunting the perception of what was initially considered as an unfortunate side effect. Of note, BA

derivatives predominantly targeting FXR, such as OCA, can also trigger itching (311). Furthermore, no adverse effects of itching were observed in humans with the selective TGR5 agonist, SB-756050 (168). The mechanism underlying pruritus may, therefore, be more complex than originally proposed.

VI. Final remarks and future perspectives

BA signaling has many beneficial roles as it enables tissues to adapt to environmental, nutritional, and physiological cues. However, this signaling can also become maladaptive, especially when the tight feedback regulation of BA synthesis is compromised to the point that BAs become cytotoxic. Several diseases and conditions, as diverse as cholestasis, fibrosis, cardiomyopathy, gallbladder stones, cancer, and pruritus, have been associated with an uncontrolled rise in BA concentrations or observed after BA treatment. Whether these correlate with human pathologies are the focus of intense research aimed at better understanding the molecular basis of BA-induced disease progression. However, the field should remain cautious about the contrasting features of BAs, swiftly fluctuating between good and bad.

The prime effectors of BA signaling are the receptors, FXR and TGR5, that evolved often complementary functions. Their balanced contributions translate the signals conveyed by the many different BAs to shape not only cellular responses but also tissues and even entire systems to the quality and quantity of BAs. There are still many challenges ahead to grasp the full complexity of BA signaling and their role in many contexts are only starting to be elucidated. For instance, we are only on the verge of understanding how the gut microbiome affects BA composition and levels, which constitutes a prime way for the microbiome to synchronize a wide range of physiological processes. There is still controversy about the metabolic benefits of intestinal FXR agonists versus antagonists, and a more in-depth analysis of its impact on the microbiota will be needed to fully elucidate the intricate interplay of microbial and host factors. Likewise, we know very little about the signaling roles of BAs in the brain, although bile has been postulated to affect our mood since ancient times. Furthermore, we are only starting to understand how convergent signaling by two BA receptors controls cellular processes as fundamental as cell proliferation, differentiation, and death. In this respect, the discovery that BAs influence stem cell homeostasis opens a new field that may fuel novel opportunities in regenerative medicine. Finally, from an evolutionary point of view, we still need to understand the impressive species-specific differences in BA production and signaling pathways.

While many aspects of BA signaling still need to be deciphered, the first therapeutics targeting FXR are making their way into the clinic. Likewise, it is expected that TGR5-based therapies for targeted diseases will soon arise, although a creative approach will be needed to generate compounds with a more restricted bioavailability and/or activity. Similarly, OCA, the current FDA-approved FXR

agonist for the treatment of PBC is safe and effective, but the existence of undesired side-effects urges the development of next-generation drugs with fewer side effects. Overall, given the wide distribution and numerous actions of FXR and TGR5, the future of these molecules will lie in the development of selective FXR and TGR5 modulators, whose activities should be tailored to target only a set of functions that are relevant to the type of disease. In sum, drugs targeting BA signaling have a bright future and the continuing efforts on studying the impact of changing BA signaling pathways in humans will be extremely useful to translate our emerging knowledge on BA physiology in model organisms into clinical benefits.

VII. References

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VIII. Figure legends

Figure 1. BA synthesis and transport. A: Scheme depicting the main biochemical transformations during BA synthesis in liver. Primary BAs (white rectangles with dashed lines) are produced from cholesterol by the classic or alternative pathway. BA 7α-hydroxylation is catalyzed by CYP7A1 (classic pathway) or CYP7B1 (alternative pathway). Sterol ring modification is mainly catalyzed by HSD3B7 and CYP8B1, while side-chain oxidation and shortening requires CYP27A1. BAs are then conjugated (grey rectangles) in the liver, released in the gut where they are modified by the gut microbiome into secondary BAs (white rectangles) and recycled back to the liver where they are re-conjugated. **B:** Summary of sites of hydroxylation on steroid nucleus of BA species indicated in panel A. **C:** Schematic representation of the main BA transporters in the enterohepatic system.
BAs, bile acids; CYP7A1, cholesterol 7α-hydroxylase; CYP27A1, sterol 27-hydroxylase; HSD3B7, hydroxy-delta-5-steroid dehydrogenase; CYP7B1, oxysterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CA, cholic acid; CDCA, chenodeoxycholic acid; αMCA, alpha-muricholic acid; βMCA, beta-muricholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TαMCA, tauroalpha-muricholic acid; TβMCA, taurobeta-muricholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid, HCA, hyocholic acid; MDCA, murideoxycholic acid; ωMCA, omega-muricholic acid; HDCA, hyodeoxycholic acid; C6, carbon 6; C7, carbon 7; C12, carbon 12; H, hydrogen; α-OH, alpha hydroxyl group; β-OH, beta hydroxyl group; OSTα, organic solute transporter α; OSTβ, organic solute transporter β; MRP3, multidrug resistance protein 3; MRP4, multidrug resistance protein 4; MRP2, multidrug resistance protein 2; ASBT, apical sodium-dependent BA transporter; BSEP, bile acid export pump; I-BABP, ileal bile acid binding protein; NTCP, sodium-dependent taurocholate co-transporting polypeptide; OATP1, organic anion-transporting polypeptide 1.

Figure 2. Triple action of BAs. The chemical structure of BAs highlights the presence of a hydrophobic and a hydrophilic side (left panel) that allow BAs to act as detergents that facilitate intestinal lipid absorption. BAs also act as substrates for the gut microbiome (middle panel) and control multiple cellular processes through the activation of dedicated nuclear and membrane receptors, such as FXR and TGR5, respectively (right panel).
TGR5, Takeda G-protein receptor 5; FXR, farnesoid X receptor.

Figure 3. BA receptors and signaling. A: Table depicting the BA-responsive nuclear and membrane receptors. **B:** Molecular mechanisms and signaling cascades by which TGR5 and FXR relay BA signals into adaptive cellular responses.
FXR, farnesoid X receptor; VDR, vitamin D3 receptor; PXR/SXR, pregnane X receptor/steroid and xenobiotic-sensing receptor; CAR, constitutive androstane receptor; TGR5, Takeda G-protein receptor 5; S1PR2, sphingosine 1-phosphate receptor 2; FPR, formyl-peptide receptor; mAChR, muscarinic acetylcholine receptor; SRC-1, steroid receptor coactivator 1; PGC-1α, peroxisome-proliferator-receptor (PPAR)-γ coactivator-1α; CARM-1, coactivator-associated arginine (R) methyltransferase-1; PMRT-1, protein arginine (R) methyltransferase-1; EPAC, exchange protein directly activated by cAMP; PKA, protein kinase A; mTOR, mechanistic target of rapamycin; ERK1/2, extracellular signal-related kinase 1/2; RXRα, retinoic acid receptor α; PTM, post-translational modification.

Figure 4. FXR-mediated BA signaling in hepatocytes. Molecular mechanisms by which FXR controls multiple metabolic processes in hepatocytes. Hepatic FXR and intestinal FXR (through FGF15/19 release and activation of the FGFR4-β-KLOTHO signaling) synergize in the control of lipid, glucose and amino acid homeostasis, as well as in the feedback regulation of BA synthesis. FXR target genes are highlighted in blue.
FXR, farnesoid X receptor; FGF15/19, fibroblast growth factor 15/19; FGFR4, fibroblast growth factor receptor 4; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; SCD1, stearoyl-CoA desaturase-1; SREBP1c, sterol regulatory binding protein 1c; LPK, liver pyruvate kinase; ChREBP, carbohydrate-responsive element-binding protein; SHP, small heterodimer partner; VLDL, very low density lipoprotein; MPT, microsomal triglyceride transfer protein; ApoB, apolipoprotein B; HNF4α, hepatocyte nuclear factor 4 alpha; SHP2, Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2; ERK, extracellular signal-regulated kinase; CYP7A1, cholesterol 7α-hydroxylase; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose 6-phosphatase; CREB, cAMP-response element binding protein; GSK3, glycogen synthase kinase 3; eIF4B, eukaryotic translation initiation factor 4B; eIF4E, eukaryotic translation initiation factor 4E; rpS6, ribosomal protein S6.

Figure 5. Transport of bile components in the enterohepatic organs. Schematic of the main bile component transporters in the enterohepatic system. FXR target genes are highlighted in blue.

BA, bile acid; NTCP, sodium-dependent taurocholate co-transporting polypeptide; OATP1, organic anion-transporting polypeptide 1; OSTα, organic solute transporter α; OSTβ, organic solute transporter β; MRP3, multidrug resistance protein 3; MRP4, multidrug resistance protein 4; BSEP, bile acid export pump; MRP2, multidrug resistance protein 2; ABCG5, ATP-binding cassette sub-family G member 5; ABCG8, ATP-binding cassette sub-family G member 8; MDR2/3, multidrug-resistant protein 2/3; ASBT, apical sodium-dependent BA transporter; MDR1, multidrug-resistant protein 1; I-BABP, ileal bile acid binding protein.

Figure 6. TGR5- and FXR-mediated BA signaling in the enterohepatic organs. Physiological and environmental cues, as well as disease or disease intervention (grey rectangles on top), modulate gut microbiome and BA pool size/composition to control TGR5 and FXR signaling in the various cell types of the enterohepatic system. These receptors act in a synergistic (one arrow) or complementary manner (two arrows) to regulate the physiological processes indicated in the green rectangles. Green arrows indicate an increase while red arrows indicate a reduction.
TGR5, Takeda G-protein receptor 5; EEC, enteroendocrine L cell; GLP-1, glucagon-like peptide-1; CGRP, calcitonin gene-related peptide; FXR, farnesoid X receptor; BA, bile acid; TG, triglyceride; VLDL, very low density lipoprotein; FGF15/19, fibroblast growth factor 15/19; H_2O, water; TICE, transintestinal cholesterol excretion.

Figure 7. TGR5- and FXR-mediated BA signaling in the enteroendocrine L cell. Molecular mechanisms and signaling cascades by which FXR and TGR5 control preproglucagon (*Gcg*) gene transcription and GLP-1 secretion in intestinal enteroendocrine (EEC) L cells.
EEC, enteroendocrine L cell; SGLT1, sodium-glucose cotransporter 1; FXR, farnesoid X receptor; Gcg, glucagon; ChREBP, carbohydrate-responsive element-binding protein; Ffar2, free fatty acid receptor 2; Gbpar1, G protein-coupled bile acid receptor 1; PC1/3, prohormone convertase 1/3; FFAR1/2, free fatty acid receptor 1/2; Ca^{2+}, calcium; TGR5, Takeda G-protein receptor 5; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; AC, adenylyl cyclase; PKA, protein kinase A; GLP-1, glucagon-like peptide-1.

Figure 8. BA-TGR5 signaling in adipose tissue and immune cells. Physiological and environmental cues (grey rectangles on top) modulate gut microbiome and BA pool size/composition to control TGR5 signaling in the depicted cell types and regulate the physiological processes indicated in the green rectangles. Green arrows indicate an increase while red ones indicate a reduction.
TGR5, Takeda G-protein receptor 5; T_{reg}, Regulatory T cell; T_H17, T helper 17 cell.

Figure 9. BA signaling in liver regeneration. Molecular mechanisms by which sudden rise in BA concentration following partial hepatectomy coordinate liver regeneration. FXR and TGR5 play complementary roles in stimulating proliferation in hepatocytes and cholangiocytes, respectively. FXR, farnesoid X receptor; FOXM1B, forkhead box M1b; JAK/STAT, janus kinase/signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; FGF15/19, fibroblast growth factor 15/19; FGFR4, fibroblast growth factor receptor 4; ROS, reactive oxygen species; SRC, steroid receptor coactivator; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; TGR5, Takeda G-protein receptor 5.