thematic review

BAREing it all: the adoption of LXR and FXR and their roles in lipid homeostasis

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Abstract During the last three years there have been a plethora of publications on the liver X-activated receptors (LXRα, NR1H3, and LXRβ, NR1H2), the farnesoid X-activated receptor (FXR, NR1H4), and the pregnane X receptor (PXR, NR1I2) and the role these nuclear receptors play in controlling cholesterol, bile acid, lipoprotein and drug metabolism. The current interest in these nuclear receptors is high, in part, because they appear to be promising therapeutic targets for new drugs that have the potential to control lipid homeostasis. In this review we emphasize *i*) the role of LXR in controlling many aspects of cholesterol and fatty acid metabolism, *ii*) the expanded role of FXR in regulating genes that control not only bile acid metabolism but also lipoprotein metabolism, and *iii*) the regulation of bile acid transport/metabolism in response to bile acid-activated PXR.— Edwards, P. A., H. R. Kast, and A. M. Anisfeld. **BAREing it all: the adoption of LXR and FXR and their roles in lipid homeostasis. *J. Lipid Res.* 2002.** 43: **2–12.**

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INTRODUCTION

1. Nuclear receptors, hormone response elements, and activated transcription

Nuclear receptors, which include both non-steroidal and steroid receptors, bind to DNA *cis* elements, known as hormone response elements (HREs) and activate transcription of target genes. Most non-steroidal receptors, such as LXR, FXR, and PXR (liver X-activated receptor, farnesoid X-activated receptor, and pregnane X receptor, respectively), bind DNA as heterodimers with the obligate partner 9-*cis* retinoic acid receptor α (RXRα, NR2B1) (1).

HREs are usually composed of direct, inverted, or everted repeats (DR, IR, or ER, respectively) of the idealized sequence AGGTCA that are separated by a variable number (n) of nucleotides (where n = 1–6) to give DRn, IRn, or ERn hormone response elements (2). In general, each nuclear receptor preferentially binds to a limited number of HREs that have a particular spacing and orientation, thus providing specificity for the formation of the protein-DNA complex (2). Functional HREs have been identified in the proximal promoters of target genes, in distal enhancers that can be located ⩾22 kb from the transcription start site and in introns (see below).

LXR, FXR, PXR, and RXRα, like other members of this large superfamily of transcription factors, have a number of specific functional domains that usually include a poorly understood amino terminal transcriptional activation domain (AF-1), a DNA binding domain (DBD), a ligand binding domain (LBD), domains responsible for nuclear translocation and dimerization, and a transcriptional activation domain (AF-2) at the extreme carboxyl terminus (**Fig. 1**) (1, 3). In general, transcriptional activation is dependent on the entry of a specific ligand, usually a small lipophyllic molecule, into the cavity formed by the LBD of the nuclear receptor. Many, but not all, non-steroidal nuclear receptors are thought to be pre-bound to the HRE in a complex with corepressor proteins. Entry of the ligand into the LBD initiates changes in the conformation of the receptor that results in loss of corepressor proteins, recruitment of coactivator proteins and increased transcription (4). The role of the corepressor and coactivator proteins in controlling the condensed state of the DNA, via acetylation and deacetylation, has been reviewed recently, but is beyond the scope of the current article (4, 5).

2. Orphan nuclear receptors

Like many other nuclear receptors, LXR, FXR, and PXR were originally termed orphan nuclear receptors because their natural ligands were unknown at the time that they were initially cloned. With the recent identification of several physiological ligands that activate LXR, FXR, or PXR we can consider that these orphans have been "adopted". These

Abbreviations: apoC-II, apolipoprotein C-II; BARE, bile acid response element; FXR, farnesoid X-activated receptor; HRE, hormone response element; LXR, liver X-activated receptor; PXR, pregnane X receptor; RXR, 9-*cis* retinoic acid receptor; SREBP, sterol response element binding protein.

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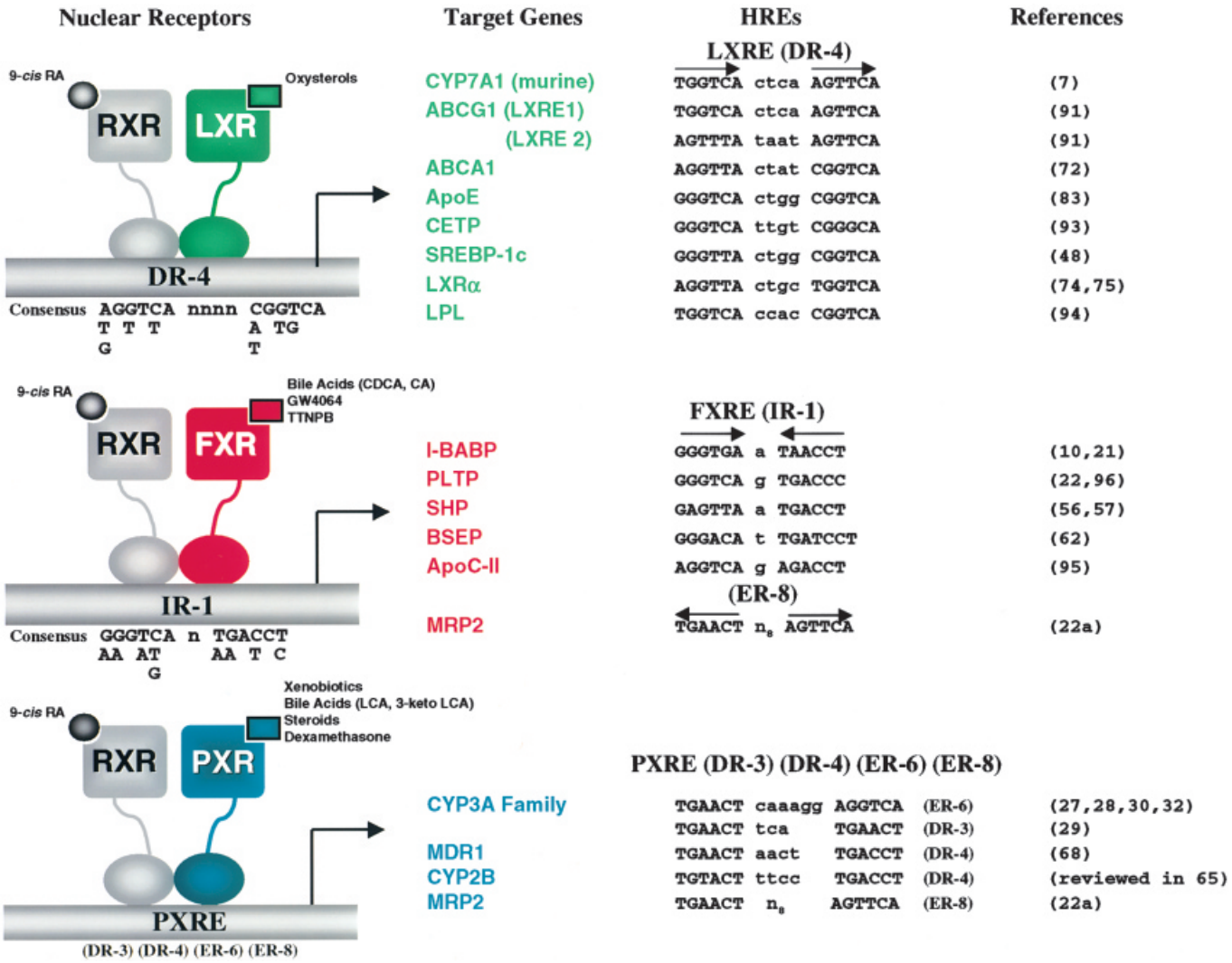

Fig. 1. LXR, FXR, and PXR (liver X-activated receptor, farnesoid X-activated receptor, and pregnane X receptor, respectively) target genes and their hormone response elements. The ligands that activate the indicated nuclear receptor heterodimers are shown in the cartoon on the left. The known target genes are shown opposite the corresponding heterodimer together with the nucleotide sequence of the hormone response element (LXRE, FXRE, PXRE). The consensus sequence for the LXRE (DR-4) and FXRE (IR-1) are shown on the left. The color of the target genes for LXR (green), FXR (red), and PXR (blue) are conserved in Figs. 1–3.

adoptions resulted from a series of elegant studies that identified *i*) oxysterols [e.g., 24(*S*),25-epoxycholesterol, 20(*S*)-, 22(*R*)-, 24(*S*)-, and 27-hydroxycholesterol] as activators of LXR (6–8), *ii*) primary bile acids, such as chenodeoxycholic acid (CDCA) and cholic acid (CA), as activators of FXR (9–11), and *iii*) the secondary bile acid, lithocholic acid (LCA) and its metabolite 3-keto LCA, as activators of PXR (12, 13) (Fig. 1). The identification of these natural ligands and the generation of mice with deletions in the genes encoding LXRα, LXRβ, FXR, and PXR have opened up new vistas linking the role of these receptors to regulatory functions.

3. Oxysterol- and bile acid-activated nuclear receptors (LXR, FXR, and PXR)

A. LXR. LXR was originally isolated from a human liver cDNA library and shown to be most highly expressed in this tissue (14). Subsequently, two genes were identified, LXRα and LXRβ (also called RLD-1 and OR-1, respectively), that encode highly conserved isoforms. LXRα is expressed in a tissue specific manner, whereas LXRβ is ubiquitously expressed (15). Both isoforms bind DNA as a heterodimer with the common partner RXR. LXR/RXR binds preferentially to hormone response elements (LXREs) that consist of two idealized hexanucleotide repeats (AGGTCA) separated by four nucleotides (DR-4) (Fig. 1) (14). Subsequently, screens were developed that led to the identification of specific oxysterols as activating ligands for LXR (6–8). The most potent oxysterols included 24(*S*),25-epoxycholesterol, 22(*R*)-hydroxycholesterol, and 24(*S*)-hydroxycholesterol (7, 8, 16).

Based on studies with LXR null mice and the identification of a limited number of LXR target genes (Fig. 1), it appears that LXR functions as a sensor of cellular oxysterols. Consistent with this proposal, all LXR target genes encode proteins that have major roles in controlling cholesterol and/or fatty acid homeostasis in a number of tis-

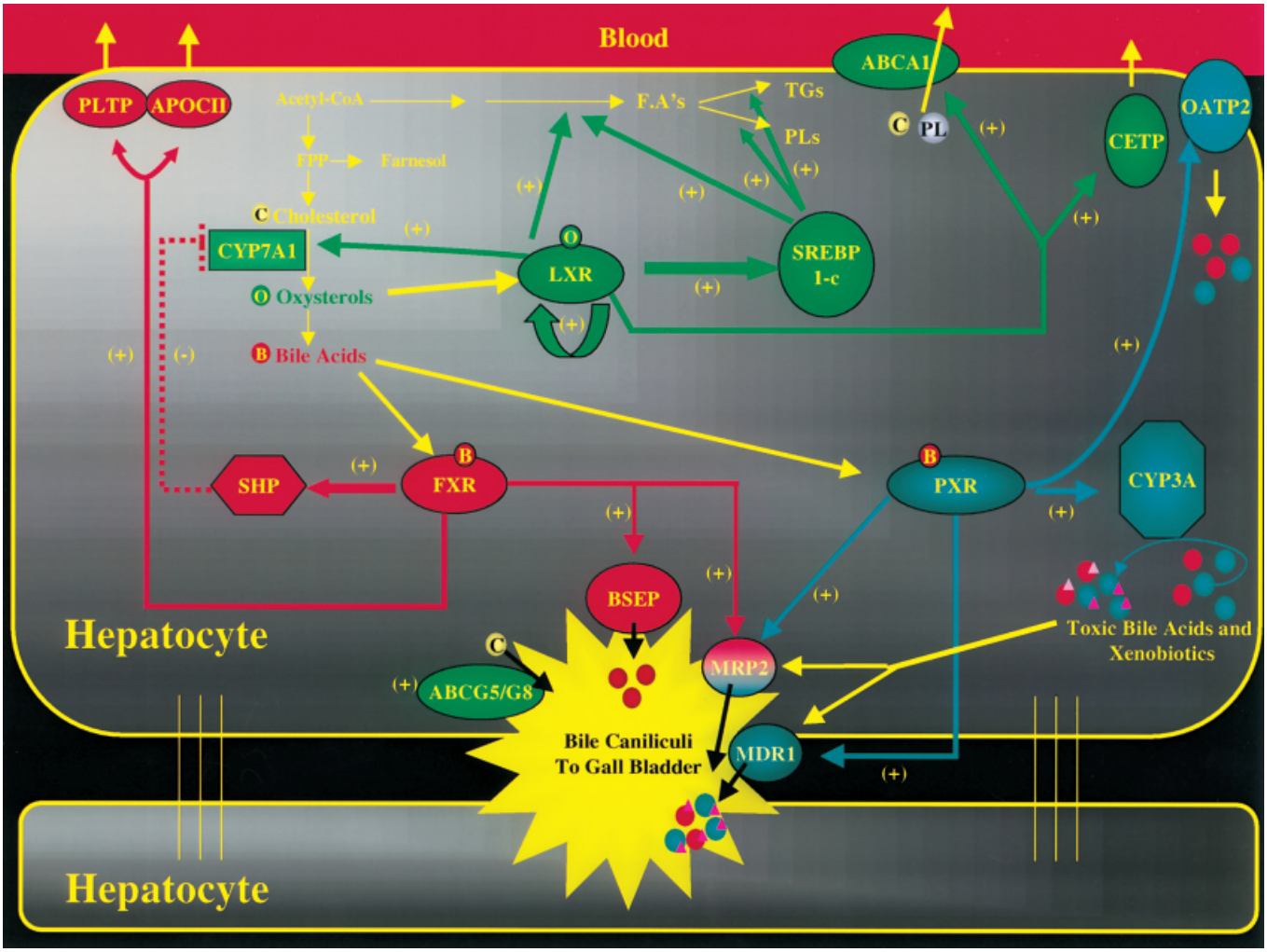

Fig. 2. LXR, FXR, and PXR regulate the hepatic expression of genes involved in lipid homeostasis. The conversion of acetyl-CoA to cholesterol or fatty acids (FA), phospholipids (PL), or triglycerides (TG)is indicated. The nuclear receptors LXR (green), FXR (red), and PXR (blue) are color-coded the same as their target genes. Transcriptional activation (+) or repression (−) is indicated. Bile acids (B), cholesterol (C), and oxysterols (O) are drawn as colored spheres. The cellular locations of ABCG1, ABCG5, and ABCG8 have not been established. FPP, farnesyl diphosphate. All other abbreviations are indicated in the text.

sues including the liver, intestine, macrophages and possibly adipose tissue (Fig. 1, **Fig. 2**, and **Fig. 3**). However, the relative importance of LXRα and LXRβ as sterol sensors and their specific roles in regulating gene expression is poorly understood. Recently, synthetic, highly potent LXR ligands were shown to have pronounced effects in vivo. For example, treatment of rodents with such agonists resulted in decreased cholesterol absorption and increased concentrations of plasma triglycerides and phospholipids (17).

B. FXR. Rat FXR was originally cloned using PCR and degenerate primers corresponding to the semi-conserved DNA binding domain of nuclear receptors (18). At the same time, murine FXR was isolated based on its interaction with RXR and, as a result, was originally referred to as RIP-14 (RXR interacting protein number 14) (19). In the original report, rat FXR was shown to be weakly activated by supraphysiological levels of the isoprenoid farnesol (hence the name FXR) (18). Farnesol is derived from the hydrolysis of farnesyl diphosphate in the isoprenoid biosynthetic pathway (Fig. 2) and had previously been shown to function as a signaling molecule in an unrelated pathway that controls the stability of HMG-CoA reductase (20). However, no direct interaction of farnesol with FXR or the LBD of FXR was ever demonstrated. More recent studies have shown that primary bile acids, such as CDCA or CA, bind to FXR in vitro, that this interaction occurs at physiological levels of the bile acids (EC_{50} of 10–15 μM), that this interaction results in recruitment of coactivators to the liganded FXR, and that there is a subsequent increase in the transcription of target genes (9–11). All of these properties are consistent with the hypothesis that CDCA and CA function to directly activate FXR in vivo.

The hormone response element to which FXR/RXRα binds was originally termed an FXR response element (FXRE) (18). This element, however, has also been termed a BARE (bile acid response element), based in part on the more recent studies which indicate that bile acids are the natural ligands for FXR (10, 21). Earlier re-

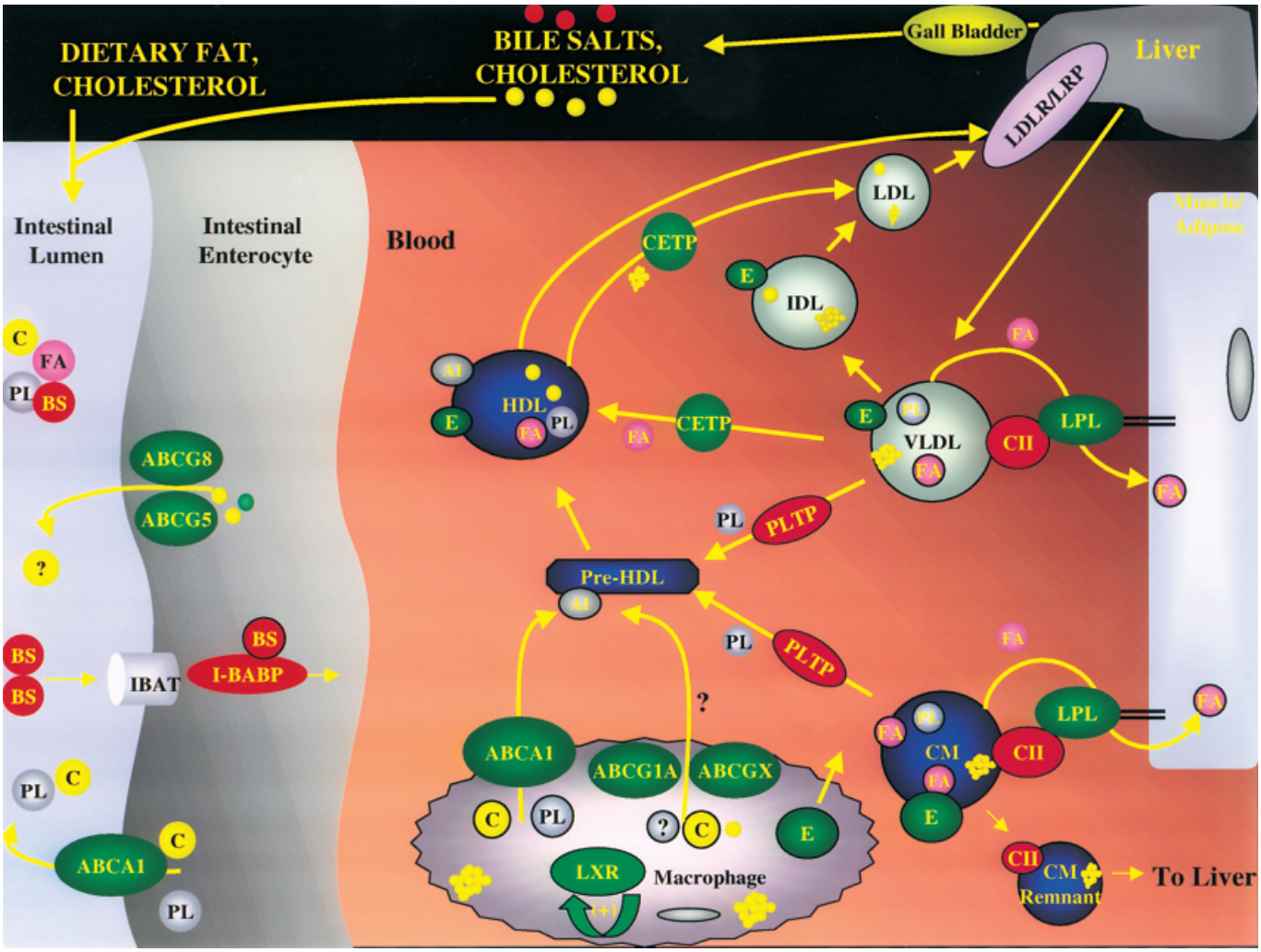

Fig. 3. LXR and FXR regulate genes involved in lipid absorption, excretion, and metabolism. The figure illustrates lipid absorption from the intestinal lumen into the villi, the metabolism of lipoproteins in the plasma and the movement of lipids out of macrophages or the liver. Genes that are activated by LXR are shown in green, while FXR target genes are shown in red. The membrane localization of ABCG1A, ABCGX, ABCG5, and ABCG8 are unknown. Other details are provided in the text. The following abbreviations are used: cholesterol (C, yellow droplets; FA, fatty acids; PL, phospholipids; BS, bile salts; IBAT, ileal bile acid transporter; I-BABP, ileal bile acid binding protein; AI, apolipoprotein A-I; E, apolipoprotein E; C-II, apolipoprotein C-II; PLTP, phospholipid transfer protein; LPL, lipoprotein lipase; CM, chylomicrons; CM remnant, chylomicron remnant; LDLR, LDL receptor; ABCG1A, ABCGX, represent different ABCG1 isoforms).

ports show that various degenerate forms of IR-1, DR-3, or DR-4 elements were bound by FXR/RXRα in vitro (18, 22). With one exception, all published FXR target genes contain one or more degenerate IR-1 elements in the proximal promoter or distal enhancers that function as an FXRE/BARE and are required for transcriptional activation (Fig. 1). In recent studies, we identified an ER-8 as a functional FXRE/BARE that is distinct from all previously identified nuclear hormone response elements (22a). Such an observation indicates that the nucleotide sequence of a functional FXRE/BARE may vary considerably.

Northern blot assays indicate that FXR transcripts in the rat are restricted to the liver, kidney, intestine (all involved in cholesterol/bile acid metabolism), colon, and adrenals (18). As discussed below, the original proposal that FXR might have a role in cholesterol/bile acid homeostasis has proven to be correct (Fig. 2) (18). However, the role of FXR in the adrenals is an enigma, since this organ is not known to be involved in any aspect of bile acid metabolism. The finding that androsterone, an intermediate in cholesterol/steroid metabolism, is both synthesized in the adrenals and induces the expression of a reporter gene under the control of an FXRE is certainly intriguing (23). Demonstration that androsterone, at physiological concentrations, can both bind FXR and activate endogenous FXR-target genes would provide further support for the proposal that this steroid is a natural agonist.

C. PXR. The secondary bile acid, lithocholic acid (LCA), is produced from the primary bile acid CDCA by a 7α-dehydroxylation pathway present in certain intestinal bacteria. In contrast to primary bile acids, secondary bile acids are poorly absorbed in the distal ileum. Since LCA has recently been shown to function as a ligand for PXR (see below), we have included a brief section on this nu-

clear receptor. Far more extensive reviews on PXR are available (24–26).

Murine PXR and its human homolog (hPXR) (also called the steroid and xenobiotic receptor, SXR, or the pregnane activated receptor, PAR) were first cloned in 1998 (27–30). In addition to LCA, a number of naturally occurring steroids, including pregnenolone, progesterone, androstanol, hyperforin (a component of St John's Wort), dexamethasone (a synthetic glucocorticoid), and various xenobiotics (e.g., rifampicin and phenobarbital) have been shown to activate PXR (Fig. 1) (29–32). The observation that a number of compounds differentially activate murine and human PXR is noteworthy as this may explain the varying responses of mice and humans to certain drugs.

The recent observations that the secondary bile acid LCA and its 3-keto metabolite (3-keto LCA) activate PXR are particularly relevant to this review. Accumulation of these natural compounds in the liver is associated with toxicity and cholestasis (12, 13). As discussed in detail below, the data suggest that PXR functions as a hepatic sensor for many xenobiotics, natural steroids and certain bile acids. Activated PXR then induces the expression of genes that are involved in hepatic uptake, metabolism and subsequent excretion of many of the same compounds (Figs. 1, 2).

THE ROLE OF LXR, FXR, AND PXR IN CELLULAR CHOLESTEROL AND BILE ACID METABOLISM

1. Intestine

A. Role of LXR in the intestine. Three genes (ABCA1, ABCG5, and ABCG8) that encode ATP-binding cassette (ABC) transporter proteins have been proposed as LXR target genes in the intestine (33–37). ABCG5 and ABCG8 appear to function by limiting the intestinal absorption of sterols (cholesterol and/or plant sterols) and enhancing the excretion of sterols from the liver into the bile (33, 35, 38). In contrast, ABCA1 appears to both facilitate the efflux of phospholipids and cholesterol from a variety of cells and also to limit cholesterol absorption in the intestine. As discussed below, these conclusions are based on the phenotype of patients or mice with mutations in these genes as well as the effects of activation of these genes by LXR agonists.

Patients with mutations in the ABCA1 gene have Tangier disease. These patients have little or no plasma HDL (35, 39–41). As expected, deletion of the ABCA1 gene in mice also results in a significant reduction in plasma HDL concentrations (42–44). However, it is perplexing that deletion of the ABCA1 gene in mice is reported to either decrease (45) or increase (42) the rate of absorption of cholesterol from the diet. Consistent with the latter study, it was recently reported that administration of a synthetic LXR agonist to mice resulted in increased intestinal expression of ABCA1 and a concomitant decrease in cholesterol absorption in wild-type but not LXRαβ double knockout mice (37). Based on these studies it has been proposed that ABCA1 may reduce cholesterol absorption by facilitating the efflux of cholesterol from the enterocyte back into the lumen. However, other LXR target genes, in addition to ABCA1, are also induced following the treatment of rodents with LXR agonists. Thus, we cannot rule out the possibility that these other LXR target genes have a role in decreasing sterol absorption. In addition, recent studies with cultured cells indicate that the primary role of ABCA1 is to promote phospholipid efflux to exogenous lipid-poor protein acceptors, such as apoA-I, and that the lipid/protein complex then functions as a sink to receive cellular cholesterol (see below). It remains to be established whether ABCA1 expressed in enterocytes also alters phospholipid movement and thus modulates cholesterol absorption.

Mutations in ABCG5 or ABCG8 result in sitosterolemia (33, 34). Patients with sitosterolemia have elevated levels of cholesterol and plant sterols, especially sitosterol, in both blood and tissues, and show evidence of premature coronary atherosclerosis (33, 34, 38). These increased levels appear to result from hyper absorption of sterols (cholesterol and plant sterols) and a defect in the excretion of plant sterols from the liver into bile (Fig. 3). Since patients with sitosterolemia have mutations in either ABCG5 or ABCG8, it seems likely that these transporters form a functional heterodimer (33). Identification of both the cellular location of these proteins and the substrate that they transport would provide critical information that is currently lacking.

It has been suggested that ABCA1 and ABCG5/ABCG8 function to pump phospholipids and/or sterols out of intestinal enterocytes, hepatocytes and/or macrophages (Figs. 2, 3). Since these three genes are all activated by LXR there is considerable clinical interest in determining whether treatment with LXR agonists will be beneficial as a result of either decreasing cholesterol absorption or increasing the efflux of lipids from macrophage foam cells in the artery wall.

B. Role of FXR in the intestine. The first gene to be identified as a direct target of ligand-activated FXR was I-BABP (ileal bile-acid binding protein) (10). An IR-1 was identified in the proximal promoter and shown to function in transcriptional activation of the I-BABP gene in response to FXR and bile acids (10, 21). I-BABP is a soluble protein that is expressed in enterocytes and binds bile acids. Consequently, I-BABP may limit the free concentration of bile acids intracellularly and thus limit bile acid-induced toxicity (Fig. 3). The identification of I-BABP as an FXR target gene is consistent with the proposal that FXR plays a central role in regulating bile acid metabolism.

2. Liver

A. Role of LXR in the liver. Hepatic LXR target genes identified to date function to control bile acid synthesis, and metabolism, cholesterol movement, fatty acid synthesis, and lipoprotein metabolism (Figs. 1–3). The availability of mice in which one or both LXR gene(s) have been deleted (LXRα$^{-/-}$, LXRβ$^{-/-}$, or LXRα/β$^{-/-}$) has been particularly useful in understanding the function of these two nuclear receptors. The results of a number of studies

demonstrate that LXRα is necessary for mice to respond appropriately following the administration of cholesterol-enriched diets (46). One important target is the gene encoding cholesterol 7α-hydroxylase.

The hepatic enzyme cholesterol 7α-hydroxylase catalyses the rate limiting reaction in the conversion of cholesterol to bile acids in the classic (neutral) pathway, and consequently plays a critical role in cholesterol and bile acid homeostasis (47). This enzyme is encoded by the highly regulated CYP7A1 gene (47). An alternative pathway has been described that involves the hydroxylation of oxysterols by oxysterol 7α-hydroxylase (CYP7B) prior to their conversion to primary bile acids (47). Recent studies have shown that the rodent CYP7A1 gene is activated by LXR and oxysterols by a process that depends on a functional LXRE in the proximal promoter of the gene (46) (Figs.1, 2). Consistent with these observations, administration of a diet supplemented with high levels of cholesterol increases the expression of CYP7A1, bile acid synthesis and excretion in wild-type mice, but not in LXRα$^{-/-}$ or LXRα/β$^{-/-}$ mice (46, 48, 49). As a result, LXRα/β$^{-/-}$ and LXRα$^{-/-}$ mice, but not wild-type or LXRβ$^{-/-}$ mice, accumulate cholesterol to toxic levels in the liver (46, 49). The physiologically important oxysterol(s) that function as LXR activators and are presumably generated from dietary cholesterol, remain to be identified. Prime candidates include 24(*S*),25-epoxycholesterol, 20(*S*)-hydroxycholesterol, 22(*R*)-hydroxycholesterol and 27-hydroxycholesterol. The proximal promoter of the human CYP7A1 gene does not contain an LXRE and consequently it is not activated by oxysterols or LXR. Thus, the regulation of the murine and human genes are not identical.

Other LXR target genes that are induced in the liver include ABCA1, ABCG5, and ABCG8. Additional studies should help to determine whether these ABC transporter proteins facilitate the efflux of specific phospholipids, cholesterol and/or plant sterols from the liver into either the blood or the bile.

In the last few months it has become apparent that LXR plays a critical role in activating genes involved in fatty acid synthesis (Figs. 1, 2). Presumably, the increased levels of fatty acids are then made available for esterification of excess cholesterol and for the synthesis of triglycerides and phospholipids. In this regard, it has long been known that excess cellular cholesterol is rapidly esterified with an unsaturated fatty acid to form cytoplasmic cholesteryl ester lipid droplets and that this reaction is catalyzed by the membrane-bound enzyme acyl-CoA:cholesterol acyltransferase (ACAT) (50). Interestingly, LXR has recently been shown to activate the sterol regulatory element-binding protein 1c (SREBP-1c) gene, which encodes a transcription factor that is itself critical for the increased expression of a number of genes involved in the biosynthesis and esterification of unsaturated fatty acids (Figs. 1, 2) (51, 52). Such SREBP-1c-activated genes include acetyl-CoA synthetase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase, glycerol phosphate acyltransferase and CTP:phosphocholine cytidylyltransferase (Fig. 2) (53–55). Surprisingly, oxysterols selectively suppress the nuclear levels of SREBP-2, as compared to SREBP-1 (51, 52). As a result, SREBP-2-dependent genes (e.g., LDL receptor, HMG-CoA reductase) are repressed by oxysterols whereas hepatic SREBP-1c-dependent genes are activated.

B. Role of FXR in the liver. FXR is highly expressed in the liver where it appears to function as a bile acid sensor. Earlier studies had shown that administration of bile acids to rodents repressed the expression of hepatic CYP7A1. Subsequent studies demonstrated that the repression of CYP7A1 was indirect and involved two other nuclear hormone receptors named SHP (NR0B2) and LRH-1 (NR5A2). It was shown that *i*) the gene encoding SHP was activated directly by FXR (an FXRE/BARE was identified in the proximal promoter of the SHP gene), *ii*) there was an increase in SHP protein, and *iii*) the SHP protein formed a complex with, and inactivated, the transcription factor LRH1/CPF (56, 57). Since LRH1 is required for transcription of CYP7A1 (58), one net result of activating FXR and SHP expression was a decrease in the mRNA, protein and activity of CYP7A1 (Figs. 1, 2). Bile acids also repress the synthesis of cholic acid by repressing the transcription of CYP8B1 (sterol 12α-hydroxylase) via a similar mechanism that also involves the inactivation of LRH1 by SHP (59). Thus, bile acids function as end product inhibitors and repress the synthesis of both chenodeoxycholic acid and cholic acid as a result of the decreased expression of CYP7A1 and CYP12B1, respectively (Fig. 2). Alternative mechanisms of regulation of CYP7A1 have also been reported that involve the bile acid-dependent induction of cytokines from Kupffer cells and the subsequent cytokine-mediated suppression of CYP7A1 in adjacent hepatocytes (60). Proof of this model will require studies with SHP null mice.

Studies with FXR null mice provided additional data that support the proposal that FXR is an important sensor of bile acids (61). For example, FXR null mice exhibited severe wasting or death following the inclusion of cholic acid in the diet (61). One putative FXR target gene identified in the study with FXR null mice encoded BSEP (bile salt export pump), which has recently been characterized as a true FXR target (62). BSEP (ABCB11) is a member of the ABC superfamily of transporters that is located on the canalicular membrane of hepatocytes where it facilitates the transport of bile acids from the hepatocyte into the bile (Fig. 2) (63, 64). Thus, identification of FXR target genes is consistent with an important role of this nuclear receptor in regulating *i*) bile acid synthesis in the liver (via SHP), *ii*) excretion of bile acids into the bile (BSEP), and *iii*) the re-uptake of bile acids from the intestinal lumen (I-BABP) (Figs. 2, 3).

C. Role of PXR in the liver. Recent studies have demonstrated that mRNA levels of the murine sodium-independent organic anion transporting polypeptide (OATP2) and specific cytochrome P450 (CYP) genes such as CYP3A and CYP2B are induced when hepatocytes are incubated with LCA, 3-keto LCA, or a number of other PXR ligands (Figs. 1, 2) (12, 13). CYP genes encode enzymes involved in hydroxylation and metabolism/inactivation of numerous drugs, xenobiotics, and some bile acids (65). Rodent OATP2 functions to transport organic anions and sulfated

and glucuronidated bile acids from the blood into hepatocytes, whereupon these compounds may be hydroxylated by CYP3A prior to their excretion in the bile (Fig. 2) (66, 67). Interestingly, the excretion of many of these compounds into the bile occurs via the transmembrane transporter MDR1 (ABCB1) located on the cannalicular membrane (Fig. 2) (68). Since MDR1 is also induced by PXR, it appears that this nuclear receptor activates genes involved in the hepatic uptake of anions from the blood, their hydroxylation and their subsequent excretion.

Activated PXR, like activated FXR, results in repression of CYP7A1 (12). However, the mechanism of this repression remains to be determined. Nonetheless, CYP7A1 appears to be a central target for multiple nuclear receptors; CYP7A1 expression is repressed by both bile acid-activated PXR and FXR, and induced by oxysterol-activated LXR. Such data suggest that in mammals, the rate of conversion of cholesterol to bile acids is a particularly important process.

Studies with $PXR^{-/-}$ mice and $PXR^{-/-}$ mice that overexpress constitutively activated human PXR ($PXR^{-/-}$ $SXR\text{-}VP16^{+/+}$) have been particularly informative in defining the roles of murine and human PXR in the liver (12, 69). The results indicate that PXR has a key role in protecting mice from xenobiotic toxicants (69) or from hepatotoxic bile acids (12, 13). Presumably, PXR regulates the expression of hepatic genes that are involved in clearance of toxic compounds, such as LCA, from the blood and their subsequent metabolism and excretion into the bile (Fig. 2). Recently, we identified one gene (MRP2; ABCC2) involved in this excretion process that, surprisingly, is activated by PXR, FXR, and CAR (constitutive androstane receptor) (22a). This latter result emphasizes the overlapping roles of multiple nuclear receptors.

3. Macrophages

Macrophages express LXRα and LXRβ. In contrast, neither FXR nor PXR appear to be expressed in this cell type. Incubation of human or murine macrophages with either oxysterols or synthetic LXR agonists results in increased expression of ABCA1 (70–72), ABCG1 (73), apolipoprotein E (22), and LXRα (74, 75). Functional LXREs have been identified in the promoters or enhancers of each of these genes (Fig. 1). Interestingly, LXR induces the LXRα gene itself in human (but not murine) macrophages by a process that is dependent on an LXRE in a distal enhancer (74, 75) (Figs. 2, 3). In addition, the LXRα gene is induced by PPARγ and PPARα (76–78) that may in turn be activated by the newly synthesized unsaturated fatty acids.

The LXR-dependent increased expression of ABCA1 has been shown to function in the increased efflux of phospholipid and cholesterol from the cells to exogenous lipid-poor proteins (Fig. 3) (79, 80). Such acceptors include apolipoprotein A1 and apolipoprotein E (81, 82). Since apoE expression is increased in macrophages following activation of LXR, the secreted apoE protein may function as an acceptor for effluxing phospholipids and cholesterol (83). Recent studies indicate that ABCA1 may mediate the efflux of cellular phospholipids to extracellular protein acceptors as the primary event (84, 85), and that cellular cholesterol, possibly derived from late endosomes/lysosomes, subsequently effluxes to the preformed extracellular phospholipid/protein complex (86, 87).

Our current ideas about the importance of ABCA1 come from the observations that HDL is virtually absent from the blood of patients or mice containing mutations in both ABCA1 alleles. The demonstration that transgenic mice, expressing the human ABCA1 gene from a BAC clone, have elevated HDL levels (88) provides additional evidence that the ABCA1 protein has a critical role in controlling HDL levels.

In contrast to ABCA1, there is much less information on the function of ABCG1. The results of studies with cultured cells treated with antisense oligonucleotides to ABCG1 suggest that this protein may be involved in controlling the efflux of cellular cholesterol to HDL and/or the secretion of apoE (89, 90). ABCG1 has been reported to reside in the endoplasmic reticulum and Golgi membranes (89). However, recent studies have reported that multiple ABCG1 transcripts are produced as a result of the use of alternative promoters and alternative RNA splicing; translation of these transcripts produces multiple ABCG1 proteins with significantly different amino termini (Fig. 3) (91, 92). It is possible that these alternative forms of the ABCG1 protein combine to form different heterodimers with related transport functions. Consequently, additional studies will be required to determine both the tissue and cellular location and function of each of these ABCG1 isoforms.

4. The role of LXR and FXR in lipoprotein metabolism

A. Role of LXR in lipoprotein metabolism. As discussed above, plasma lipid levels are also modulated by LXR agonists, presumably in part as a result of the activation of SREBP-1c and subsequent increases in the rate of synthesis of fatty acids, triglycerides and phospholipids (Fig. 2). In addition, increased expression of ABCA1 and ABCG5/ABCG8 presumably alters sterol absorption and the efflux of cellular cholesterol and phospholipid from liver and macrophages into the plasma or bile. (Figs. 2, 3). In addition, CETP and lipoprotein lipase (LPL) genes are also directly activated by oxysterols and LXR (93, 94). Since CETP functions to facilitate the transfer of cholesteryl esters between plasma lipoproteins, and while LPL catalyses the hydrolysis of lipoprotein triglycerides, it is clear that LXR modulates lipoprotein metabolism (Figs. 2, 3). Interestingly, the expression of apolipoprotein C-II (apoC-II), the obligate cofactor for LPL, is induced by FXR (Figs. 1–3) (95). We would predict that additional LXR target genes will be identified that directly control different aspects of lipoprotein metabolism.

B. Role of FXR in lipoprotein metabolism. Based on studies with FXR null mice and the identification of a limited number of FXR target genes, it has become evident that this nuclear receptor controls specific aspects of lipoprotein metabolism. The idea that FXR might have such a role came when the second FXR target gene was identified and shown to encode PLTP(phospholipid transfer protein)

(22, 96). PLTP is a secreted protein that facilitates the transfer of phospholipids and cholesterol from triglyceride-rich lipoproteins to HDL and consequently modulates the concentration of plasma HDL (Figs. 1–3) (97). More recent studies that utilized FXR-overexpressing cells and suppression subtraction hybridization identified apoC-II as an additional FXR target gene (Figs. 1–3) (95). Induction of hepatic apoC-II by activated FXR was dependent on the presence of IR-1 elements (FXRE/BARE) (95) contained within two distal enhancers that have been termed Hepatic Control Regions (HCR.1 and HCR.2) (98). Interestingly, these 319 bp HCRs are located within 11 kb and 22 kb 5′ of the apoC-II gene transcriptional start site, and are critical for the liver specific expression of a number of genes including apoC-II (99). Preliminary studies also show that hepatic apoE mRNA levels also increase following the activation of FXR by bile acids (Kast et al., unpublished observations). Since apoE is located in a gene cluster that includes apoC-I, apoC-II, and apoC-IV (99), we hypothesize that the FXREs/BAREs in HCR.1 and HCR.2 may be involved in the regulated transcription of multiple apolipoproteins. As PLTP, apolipoprotein C-II and apolipoprotein E are all secreted into the blood and are known to be involved in the metabolism of plasma lipoproteins, these data suggest that bile acid-activated FXR has a central role in regulating plasma lipid levels (Figs. 2, 3). Consistent with this proposal, the administration of either a synthetic FXR ligand (GW4064) (100) or a natural FXR ligand (cholic acid) (95) to rodents results in a significant decline in plasma triglyceride levels. The finding that administration of a cholesterol-rich diet to FXR null mice results in elevated levels of proathergenic lipoproteins (61) provides additional support for the idea that FXR has an important role in controlling normal plasma lipoprotein levels.

SUMMARY

It has been less than four years since natural ligands for LXR, FXR, and PXR were identified. The rapid increase in our understanding of these three receptors in this short time is a direct result of finding the "holy grail" (i.e., the natural ligands) and generating mice in which the genes encoding LXR, FXR, or PXR have been deleted. The data from these studies are consistent with the proposal that, under a variety of dietary conditions, the control of lipid homeostasis is dependent upon cross talk between these three nuclear receptors. For example, excess uptake of cholesterol from the diet results in the hepatic production of oxysterols, activation of LXR, increased expression of CYP7A1, and enhanced production of primary bile acids. In turn, bile acid-activated FXR enhances the expression of genes involved in bile acid excretion (BSEP) and reabsorption (I-BABP), while inhibiting the expression of additional CYP7A1 (via SHP). At the same time, hepatic PXR, activated by LCA generated in the intestine, represses CYP7A1 and activates genes involved in bile acid metabolism (CYP3A).

In addition, the studies cited in this review demonstrate that the metabolism of plasma lipoproteins is also significantly affected by these nuclear receptors as a result of the increased expression of apoC-II, apoE and PLTP (by FXR), and CETP and LPL (by LXR). Despite these new insights into the roles of LXR, FXR, and PXR, and despite the spectacular scientific inroads that have recently been made, it is quite clear that our knowledge is far from complete. The future is likely to be no less exciting and stimulating as investigators attempt to unravel the roles of LXR, FXR, and PXR as regulators of lipid metabolism.

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