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Nuclear Orphan Receptors Control Cholesterol Catabolism

Minireview

David W. Russell
University of Texas Southwestern Medical Center
Dallas, Texas 75235

Over the last three decades, much progress has been made in understanding the mechanisms and regulation of the cholesterol supply pathways that cells and tissues use to sate their demands for this essential membrane component. But how does the body get rid of excess cholesterol and by what means is the catabolic process regulated? These questions are of medical as well as scientific importance because controlled elimination is one of the body's chief defenses against cholesterol accumulation and consequent heart attacks. Several recent papers address the essence of these questions and reveal potential physiological roles for three nuclear orphan receptors, the LXRα (or more properly NR1H2; Nuclear Receptors Nomenclature Committee, 1999), CPF (NR5A2), and FXR (NR1H4) proteins, in controlling the conversion of cholesterol into its bulk catabolic products, namely, the bile acids.

Included in this body of work is a paper in the May issue of *Molecular Cell* in which Wang et al. (1999) show that chenodeoxycholate, a bile acid derived from cholesterol, interacts with a nuclear hormone receptor, FXR, to suppress transcription from a reporter gene. In two papers appearing in *Science*, Makishima et al. (1999) and Parks et al. (1999) show that chenodeoxycholate interacts with FXR to suppress transcription of the cholesterol 7α-hydroxylase gene (*Cyp7a*), whose product catalyzes the rate-limiting step in bile acid synthesis from cholesterol. Ligand-activated FXR also induces transcription of *IBABP*, a gene encoding a transport protein that facilitates reuptake of bile acids in the small intestine. Together these results suggest that cholesterol metabolites control their synthesis in the liver through feedback suppression of *Cyp7a* and their transport across the intestine through feedforward induction of *IBABP*.

Physiology of Cholesterol Catabolism

About one-half gram of cholesterol is metabolized in the adult human liver each day by conversion into bile acids, predominantly cholate and chenodeoxycholate (Figure 1). After conjugation with the amino acids glycine or taurine, these endproducts are secreted via the bile ducts and gallbladder into the lumen of the small intestine where they act as detergents to emulsify dietary lipids and fat-soluble vitamins. These emulsified nutrients are taken up by enterocytes that line the proximal segments of the gut while bile acids continue to move distally. When the bile acids reach the end of the small intestine, they are taken up by a polytopic membrane protein termed the ileal bile acid transporter (encoded by the *IBAT* gene). About 95% of bile acids are retrieved by this route; the remaining 5% are lost to the colon and ultimately excreted from the body. The unrecovered bile acids are replaced by new synthesis in the liver, and this production (~0.5 g/day) accounts for almost all cholesterol breakdown in the body.

Enterocytes protect themselves from the detergent actions of intracellular bile acids by expressing the ileal bile acid binding protein (encoded by the *IBABP* gene), which facilitates the movement of the detergents across the cell and their secretion into the portal circulation. When they reach the liver, the bile acids are taken up by a transporter on the apical surface of the hepatocyte, and thereafter they are secreted into the bile duct to begin another round of the enterohepatic cycle.

Bile acids thus perform several functions in lipid physiology. First, their synthesis provides a disposal mechanism to counterbalance the cholesterol synthesis pathway and allow homeostasis to be achieved. Second, their detergent actions are essential within the intestine for the uptake of hydrophobic nutrients like fat-soluble vitamins, and within the liver for the solubilization of metabolites like bilirubin. Third, as detailed below, intermediates and endproducts of the bile acid pathway regulate the expression of genes that synthesize cholesterol, fatty acids, and bile acids themselves.

Pathways of Bile Acid Synthesis

In the early 1940s Konrad Bloch fed radiolabeled cholesterol to dogs and observed its conversion to polar metabolites that were excreted in the stool. These were identified as bile acids, which generally have three fewer carbon atoms than cholesterol. They also have multiple

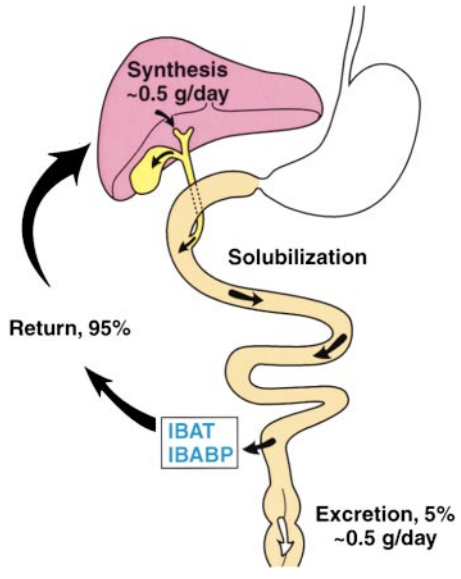

Figure 1. Bile Acid Physiology

Bile acids are synthesized in the liver and secreted via the bile duct and gallbladder into the lumen of the small intestine. They solubilize and thus facilitate the uptake of dietary lipids in the initial segment of the small intestine and then a majority are transported across enterocytes lining the distal segment of the gut by the actions of the ileal bile acid transporter (IBAT) and the ileal bile acid binding protein (IBABP). These bile acids are returned to the liver via the portal circulation. About 5% of the bile acid pool (0.5 g/day) escapes uptake and is excreted via the colon. This amount is replaced by new synthesis in the liver.

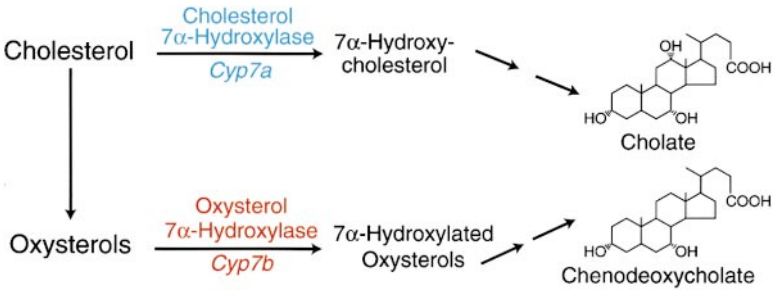

Figure 2. Pathways of Bile Acid Synthesis

Two pathways produce 7α-hydroxylated bile acids in the liver. The classical pathway begins with a cholesterol substrate and is initiated by cholesterol 7α-hydroxylase, the product of the *Cyp7a* gene. The alternate pathway begins after the conversion of cholesterol to one of several oxysterol substrates, which are then acted upon by oxysterol 7α-hydroxylase, the product of the *Cyp7b* gene. The 7α-hydroxylated intermediates arising from both enzymes are subsequently converted via multiple steps into primary bile acids like cholate and chenodeoxycholate.

hydrophilic substituents that render them water soluble and provide detergent properties (Figure 2). Workers over the ensuing 50 years elucidated the individual biochemical steps of the pathway by which cholesterol was converted into bile acids (reviewed in Russell and Setchell, 1992). The rate-limiting step was assigned to the first enzyme in this pathway, cholesterol 7α-hydroxylase. This enzyme is repressed by products of the pathway like chenodeoxycholate and cholate. In some species, like rats and mice, it is activated in a feedforward manner by dietary cholesterol.

During the last ten years, the genes encoding many enzymes in the bile acid synthesis pathway have been isolated, including that for cholesterol 7α-hydroxylase (*Cyp7a*), and mice lacking one or more of these genes have been produced. Studies of knockout mice have revealed consistencies and conundrums (reviewed in Schwarz et al., 1998). For example, 90% of *Cyp7a*$^{-/-}$ mice die during the first three weeks of life owing to generalized liver failure and deficiencies of fat-soluble vitamins, as expected given the physiological roles of bile acids described above. Unexpectedly, in the 10% of *Cyp7a*$^{-/-}$ animals that survive the newborn period, these pathological symptoms resolve and 7α-hydroxylated bile acids are present in the survivors. Since a half century of research had delineated only one metabolic pathway leading to 7α-hydroxylated bile acids, the biosynthetic origin of these compounds in the *Cyp7a*$^{-/-}$ mice was a mystery.

A second pathway of bile acid synthesis was subsequently defined that differed from the classical route in its early steps (Figure 2; Schwarz et al., 1998). This alternate pathway begins with the conversion of cholesterol into one of three oxysterols that contain a hydroxyl group at the 24, 25, or 27 carbon positions on the side chain. These intermediates are substrates for an oxysterol 7α-hydroxylase (encoded by the *Cyp7b* locus), which produces 7α-hydroxylated oxysterols that are funneled into the downstream steps of the classical pathway to produce bile acids. The importance of the alternate pathway has been demonstrated in two mammalian species: mice and humans. As mentioned above, this pathway allows mice to survive in the absence of the classical pathway of bile acid synthesis. Humans with mutations in the oxysterol 7α-hydroxylase gene have severe neonatal liver disease even though the cholesterol 7α-hydroxylase gene is intact.

In addition to producing bile acids, the alternate pathway performs another important regulatory function by inactivating oxysterols. When this pathway is disabled by mutation of the oxysterol 7α-hydroxylase gene, oxysterols accumulate to very high levels and disrupt gene expression in the liver. Some of these compounds turn off the transcription of genes specifying lipid-metabolizing enzymes while others turn on genes in this network. For example, the addition of 25-hydroxycholesterol to cells or overexpression of the gene encoding the biosynthetic enzyme for this oxysterol blocks the activation of sterol regulatory element binding proteins or SREBPs (Lund et al., 1998). SREBPs are bHLH-Zip transcription factors that regulate the expression of many genes in the cholesterol supply and fatty acid synthesis pathways (Brown and Goldstein, 1997). Another oxysterol, 24-hydroxycholesterol, enhances the transcription of several genes by activating a nuclear receptor.

LXRα, an Oxysterol Receptor, Activates Cyp7a *Transcription*

When rats and mice consume large amounts of dietary cholesterol, they respond in part by synthesizing more bile acids through the cholesterol 7α-hydroxylase pathway. The increase in catabolism is driven by augmented transcription of the *Cyp7a* gene. Using elegant combinations of classical hormone hunting and molecular biological methods, three groups showed that activation of the nuclear orphan receptor LXRα by cholesterol metabolites increased *Cyp7a* transcription (Janowski et al., 1996; Forman et al., 1997; Lehmann et al., 1997). Transfection studies with the LXRα response element from the *Cyp7a* gene reveal that the most active ligands in this regard are the oxysterol 24-hydroxycholesterol, and 24,25-epoxycholesterol. The epoxide is probably the physiologically relevant activator since levels of this sterol are high in the liver (Lehmann et al., 1997). On the other hand, 24-hydroxycholesterol is synthesized in the brain and rapidly metabolized in the liver (Lund et al., 1999).

The crucial role of LXRα in regulating cholesterol catabolism is dramatically illustrated in mice with a targeted disruption of this gene. When fed high cholesterol diets, LXRα$^{-/-}$ mice fail to induce *Cyp7a* transcription and accumulate enormous amounts of cholesterol in the liver (Peet et al., 1998). The discoveries that LXRα is activated by oxysterols and that *Cyp7a* is a target for this receptor neatly explain one form of feedforward regulation in the classical pathway of bile acid synthesis.

CPF, an Orphan Receptor, Also Activates Cyp7a *Expression*

The cholesterol 7α-hydroxylase gene is only expressed in the liver (Russell and Setchell, 1992). Transfection

studies with the *Cyp7a* promoter have implicated several well-known transcription factors in this tissue-specific expression (Chiang, 1998); but interpretation of these results is complicated by the potential artifacts arising from overexpression. LXRα is expressed in several tissues, making it an unlikely mediator of liver-specific *Cyp7a* expression.

A strong candidate for this activity has been identified recently using combined molecular biological and biochemical approaches. Nitta et al. (1999) defined a liver-specific response element in *Cyp7a* by DNase I hypersensitivity mapping and then isolated the transcription factor that interacts with this sequence. cDNA cloning revealed it to be a mammalian homolog of the *Drosophila fushi tarazu F1* gene, which the authors named CPF (*Cyp7a* promoter binding factor). This protein is also referred to as FTF and LRH-1 in the literature. CPF and fushi tarazu F1 are orphan nuclear receptors that bind DNA as monomers and activate the transcription of target genes. Interestingly, they are closely related to steroidogenic factor 1, which activates genetic pathways that convert cholesterol to steroid hormones in the adrenal gland and gonads (Parker and Schimmer, 1997). The CPF- and LXRα-binding sites in the *Cyp7a* promoter are located about 70 bp apart, which suggests that simultaneous binding of both proteins is possible and perhaps even required for maximal induction of the gene.

FXR, a Bile Acid Receptor, Suppresses Cyp7a *Transcription*

When the bile acid pool size in an animal is increased by dietary bile acids, transcription from *Cyp7a* is decreased and that from *IBABP* is increased. Conversely, reducing the bile acid pool size by the use of drugs, or by surgical intervention to prevent their return to the liver, causes an increase in *Cyp7a* transcription and a decrease in *IBABP* transcription. The new papers in *Molecular Cell* and *Science* illustrate in convincing fashion that FXR is the bile acid receptor mediating this regulation.

The common conclusions of these studies can be summarized as follows. First, several bile acids are potent and selective activators of FXR-mediated transcription, a finding originally reported at several meetings by Cary Weinberger (NIEHS). Second, these compounds activate FXR at concentrations that are found in liver and intestinal cells. Third, bile acids act through FXR and not by way of its heterodimerization partner, RXR (the NR2B class of nuclear receptors). Fourth, more hydrophilic bile acids, including those conjugated with glycine or taurine, require the presence of a plasma membrane transporter like IBAT (Figure 1) to gain access to FXR in the cell's interior. Fifth, in the presence of bile acids but not in their absence, FXR interacts with members of the p160 family of transcriptional coactivator proteins. In these respects, bile acid activation of FXR closely parallels the actions of ligands for the classical nuclear hormone receptors.

Each paper also makes unique contributions to our understanding of the relationship between nuclear receptors and cholesterol catabolism. Wang et al. (1999) show that FXR and bile acids antagonize the action of LXRα on a reporter gene. The significant conclusion of this experiment is that bile acid–mediated suppression of transcription may occur through antagonism of the positive actions of LXRα. In the future it will be important

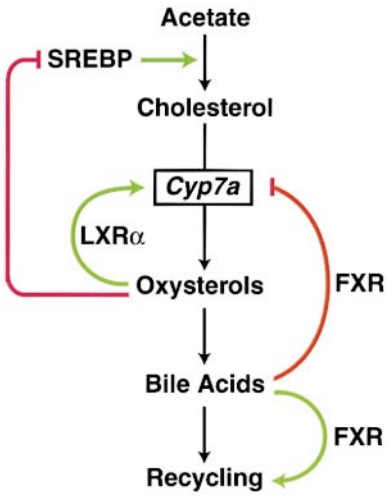

Figure 3. Regulatory Loops in Cholesterol Metabolism

Black arrows indicate metabolic pathways involving cholesterol and bile acids. The cholesterol 7α-hydroxylase target gene (*Cyp7a*) is boxed. Green arrows indicate positive regulation. Red brakes indicate negative regulation.

to determine the relative contributions of oxysterols versus bile acids in regulating gene expression. Whether antagonism occurs on the DNA, by protein–protein interaction, or by competition for the shared RXR subunit must also be examined.

Parks et al. (1999) used a fluorescence-based peptide binding assay originally described by Zhou et al. (1998) to identify chenodeoxycholate as a ligand for the FXR receptor. This assay measures ligand-dependent interactions between receptors and fluorescently labeled peptides derived from the sequences of coactivator proteins. It is sensitive, lends itself to automated methods of high throughput screening, and is almost certainly being incorporated into the drug discovery programs of many large and small pharmaceutical companies.

And what about the Makishima et al. (1999) paper? These investigators show that FXR is both an activator and a suppresser of transcription and that the choice between these two opposing actions depends on the target gene. Thus, in the presence of bile acids, FXR suppresses transcription from a *Cyp7a* reporter gene, while it activates transcription from the *IBABP* promoter. It is from these results and the physiological pathway of Figure 1, that we infer FXR is a global regulator of bile acid metabolism, modulating both synthetic output in the liver and recycling in the intestine.

The Next Step?

The multiple roles of metabolites and nuclear orphan receptors in regulating cholesterol homeostasis are summarized in Figure 3. A complex interplay of intermediates and transcription factors serves to regulate the expression of dozens of genes, all in an attempt to control the levels of potentially toxic but essential hydrophobic molecules.

Perhaps the most hotly anticipated experiment in the field will be the characterization of FXR knockout mice. We can only guess at their phenotype. It seems likely that they will suffer unregulated production of bile acids

through the cholesterol 7α-hydroxylase pathway. Whether this outcome will result from the loss of feedback regulation mediated by FXR, the unopposed positive action of LXRα, or the reduced expression of intestinal *IBABP* causing a decreased return of bile acids to the liver, will have to be determined. Regardless of the phenotype of $FXR^{-/-}$ mice, this body of work represents a major advance in lipid biology. Like any series of important findings, it raises additional questions and provides the tools with which to answer them.

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