

Nuclear Receptors and Lipid Physiology: Opening the X-Files

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Cholesterol, fatty acids, fat-soluble vitamins, and other lipids present in our diets are not only nutritionally important but serve as precursors for ligands that bind to receptors in the nucleus. To become biologically active, these lipids must first be absorbed by the intestine and transformed by metabolic enzymes before they are delivered to their sites of action in the body. Ultimately, the lipids must be eliminated to maintain a normal physiological state. The need to coordinate this entire lipid-based metabolic signaling cascade raises important questions regarding the mechanisms that govern these pathways. Specifically, what is the nature of communication between these bioactive lipids and their receptors, binding proteins, transporters, and metabolizing enzymes that links them physiologically and speaks to a higher level of metabolic control? Some general principles that govern the actions of this class of bioactive lipids and their nuclear receptors are considered here, and the scheme that emerges reveals a complex molecular script at work.

Nuclear receptors function as ligand-activated transcription factors that regulate the expression of target genes to affect processes as diverse as reproduction, development, and general metabolism. These proteins were first recognized as the mediators of steroid hormone signaling and provided an important link between transcriptional regulation and physiology. In the mid-1980s, the steroid receptors were cloned and found to exhibit extensive sequence similarity. The subsequent cloning of other receptor genes led to the unexpected discovery that there were many more nuclear receptor-like genes than previously suspected. Today, the human genome is reported to contain 48 members of this transcription factor family (1). This superfamily includes not only the classic endocrine receptors that mediate the actions of steroid hormones, thyroid hormones, and the fat-soluble vitamins A and D (2), but a large number of so-called orphan nuclear receptors, whose ligands, target genes, and physiological functions were initially unknown (3). Exciting progress has been made over the last several years to elucidate the role of these orphan receptors in animal biology. Here we review recent discoveries that suggest that unlike the classic

endocrine nuclear hormone receptors, many of the orphan receptors function as lipid sensors that respond to cellular lipid levels and elicit gene expression changes to ultimately protect cells from lipid overload.

The structural organization of nuclear receptors is similar despite wide variation in ligand sensitivity (Fig. 1). With few exceptions, these proteins contain an NH₂-terminal region that harbors a ligand-independent transcriptional activation function (AF-1); a core DNA-binding domain, containing two highly conserved zinc finger motifs that target the receptor to specific DNA sequences known as hormone response elements; a hinge region that permits protein flexibility to allow for simultaneous receptor dimerization and DNA binding; and a large COOH-terminal region that encompasses the ligand-binding domain, dimerization interface, and a ligand-dependent activation function (AF-2). Upon ligand binding, nuclear receptors undergo a conformational change that coordinately dissociates corepressors and facilitates recruitment of coactivator proteins to enable transcriptional activation (4).

The importance of nuclear receptors in maintaining the normal physiological state is illustrated by the enormous pharmacopoeia that has been developed to combat disorders that have inappropriate nuclear receptor signaling as a key pathological determinant. These disorders affect every field of medicine, including reproductive biology, inflammation, cancer, diabetes, cardiovascular disease, and obesity. Therefore, to maintain a normal physiological state, the spatial and temporal activity of nuclear receptors must be tightly controlled by tissue-specific expression of the receptors, as well as ligand availability. Interestingly, an evaluation of the

pathways involved in ligand availability reveals the existence of two distinctly different nuclear receptor paradigms.

The first paradigm is represented by the classic nuclear steroid hormone receptors (Fig. 1). Members of this group include the glucocorticoid (GR), mineralocorticoid (MR), estrogen (ER), androgen (AR), and progesterone (PR) receptors. Steroid receptors bind to DNA as homodimers, and their ligands are synthesized exclusively from endogenous endocrine sources that are regulated by negative-feedback control of the hypothalamic-pituitary axis (5). After synthesis, steroid hormones are circulated in the body to their target tissues where they bind to their receptors with high affinity (dissociation constant K_d = 0.01 to 10 nM). In vertebrates, the steroid receptor system evolved to regulate a variety of crucial metabolic and developmental events, including sexual differentiation, reproduction, carbohydrate metabolism, and electrolyte balance. The endocrine steroid receptors, their ligands, and the pathways they regulate have been the subject of decades of research, and their mechanism of action is well documented (5).

The second nuclear receptor paradigm is represented by the adopted orphan nuclear receptors that function as heterodimers with the retinoid X receptor (RXR) (Fig. 1). Orphan receptors become adopted when they are shown to bind a physiological ligand. In contrast to the endocrine steroid receptors, the adopted orphan receptors respond to dietary lipids and, therefore, their concentrations cannot be limited by simple negative-feedback control (Fig. 2). Members of this group include receptors for fatty acids (PPARs), oxysterols (LXRs), bile acids (FXR), and xenobiotics [steroid xenobiotic receptor/pregnane X receptor (SXR/PXR) and constitutive androstane receptor (CAR)]. Furthermore, the receptors in this group bind their lipid ligands with lower affinities comparable to physiological concentrations that can be affected by dietary intake (>1 to 10 μ M). An emerging theme regarding these receptors is that they function as lipid sensors. In keeping with this notion, ligand binding to each of these receptors activates a feedforward, metabolic cascade that maintains nutrient lipid homeostasis by governing the transcription of a common family of genes involved in lipid metabolism, storage, transport, and elimination.

In addition to the adopted orphan receptors, there are four other RXR heterodimer

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receptors that do not fit precisely into either the feedforward or feedback paradigms mentioned. These include the thyroid hormone (TR), retinoic acid (RAR), vitamin D (VDR), and ecdysone (EcR) receptors (6–9). The ligands for these four receptors and the pathways they regulate employ elements of both the endocrine and lipid-sensing receptor pathways. For example, like other RXR heterodimer ligands, both retinoic acid and ecdysone are derived from essential dietary lipids (vitamin A and cholesterol, respectively), yet they are not calorogenic and the transcriptional pathways that these ligands regulate (i.e., morphogenesis and development) more closely resemble those of the endocrine receptors. Likewise, vitamin D and thyroid hormone require exogenous elements for their synthesis (sunshine for vitamin D, iodine for thyroid hormone), yet the ultimate synthesis of these hormones and the pathways they regulate are under strict endocrine control. Thus, it is possible that these four receptors provide an evolutionary segue, spanning the gap between the endocrine receptors and the adopted orphan receptors that have recently been shown to be lipid sensors.

The Lipid Metabolic Cascade of Orphan Nuclear Receptors

The development of strategies to identify natural orphan nuclear receptor ligands has led to the elaboration of a metabolic gene network that is transcriptionally regulated by their cognate receptors. Three families of proteins establish a positive feed-forward autoregulatory loop to maintain lipid homeostasis (Table 1). These families include (i) members of the cytochrome P450 (CYP) enzymes that catalyze various redox reactions to transform lipid ligands into inactive metabolites and facilitate their metabolic clearance (10); (ii) the intracellular lipid-binding proteins, a family of 14- to 15-kD proteins that buffer and transport hydrophobic ligands within cells (11); and (iii) the ATP-binding cassette (ABC) transporters, which shuttle their lipid ligands and precursors out of the cytosolic compartment into organelles or the extracellular environment (12). The coordinate regulation of these three gene families appears to be a particular feature of receptors that function as RXR heterodimers, especially the orphan receptors.

Retinoid X Receptors, the Common Heterodimer Partners

The most studied of the orphan nuclear receptor subfamilies are the retinoid X receptors (RXR α , β , γ). The identification of the vitamin A derivative, 9-*cis* retinoic acid, as an endogenous ligand for the RXRs represented the discovery of the first true orphan nuclear receptor ligand and ushered in the age of orphan nuclear receptors (13). Subsequent studies have shown that RXRs also can be

activated by a variety of dietary lipids, including docosahexaenoic acid (DHA), a toxic plant lipid called phytanic acid, and the insecticide derivative methoprene acid (3, 14). In terms of nuclear receptor signaling, one of the most important advances to come from the discovery of the RXRs was the finding that they function as obligate heterodimer partners for other nuclear receptors (13). Thus, RXRs typically do not function alone, but rather serve as master regulators of several crucial regulatory pathways. The evolution of the heterodimeric nuclear receptor has permitted a unique, but simple, mechanism for expanding the repertoire of lipid signaling pathways. Therefore, it is perhaps not surprising that the lipid-sensing receptors that have been identified thus far are all RXR heterodimers. The recognition that some RXR heterodimers are permissive for activation by RXR ligands has led to the finding that potent synthetic RXR agonists (called rexinoids) have dramatic effects on lipid homeostasis (15, 16).

Peroxisome Proliferator-Activated Receptors, the Fatty Acid Sensors

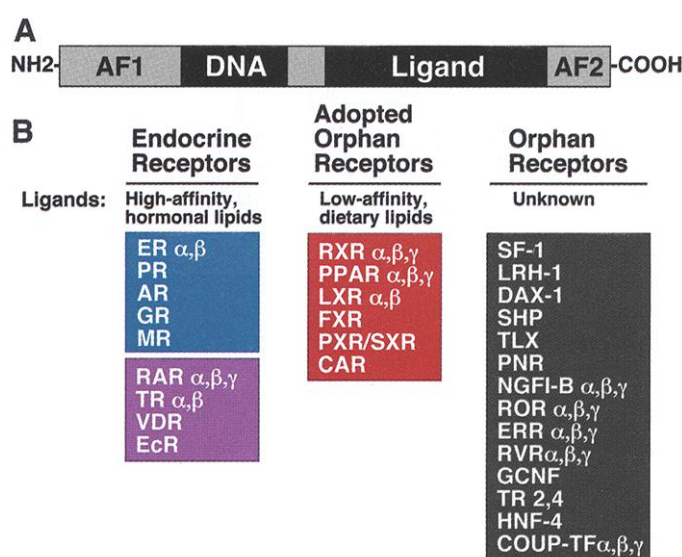
The peroxisome proliferator-activated receptors (PPAR α , γ , δ) are activated by polyunsaturated fatty acids, eicosanoids, and various synthetic ligands (17). Consistent with their distinct expression patterns, gene-knockout experiments have revealed that each PPAR subtype performs a specific function in fatty acid homeostasis.

Over a decade ago, PPAR α was found to respond to hypolipidemic drugs, such as fibrates. Subsequently, it was discovered that fatty acids serve as their natural ligands. Together with the analyses of PPAR α -null

mice, these studies established PPAR α as a global regulator of fatty acid catabolism. PPAR α target genes function together to coordinate the complex metabolic changes necessary to conserve energy during fasting and feeding. In the fatty acid metabolic cascade, PPAR α activation up-regulates the transcription of liver fatty acid-binding protein, which buffers intracellular fatty acids and delivers PPAR α ligands to the nucleus (18). In addition, expression of two members of the adrenoleukodystrophy subfamily of ABC transporters, ABCD2 and ABCD3, is similarly up-regulated to promote transport of fatty acids into peroxisomes (19) where catabolic enzymes promote β -oxidation. The hepatocyte CYP4A enzymes complete the metabolic cascade by catalyzing ω -oxidation, the final catabolic step in the clearance of PPAR α ligands (20) (Table 1).

PPAR γ was identified initially as a key regulator of adipogenesis, but it also plays an important role in cellular differentiation, insulin sensitization, atherosclerosis, and cancer (21). Ligands for PPAR γ include fatty acids and other arachidonic acid metabolites, antidiabetic drugs (e.g., thiazolidinediones), and triterpenoids. In contrast to PPAR α , PPAR γ promotes fat storage by increasing adipocyte differentiation and transcription of a number of important lipogenic proteins. Ligand homeostasis is regulated by governing expression of the adipocyte fatty acid-binding protein (A-FABP/aP2) and CYP4B1 (22). In macrophages, PPAR γ induces the lipid transporter ABCA1 through an indirect mechanism involving the LXR pathway (see below), which in turn promotes cellular efflux of phospholipids and cholesterol into high-density lipoproteins (23, 24).

Fig. 1. The nuclear receptor superfamily. (A) Schematic structure of a typical nuclear receptor is shown (see text for details). (B) Nuclear receptors can be subdivided into three or four groups, depending on the source and type of their ligand. Receptors with known physiological ligands are shown in color, and current orphan receptors are shown in gray. Included in the orphan receptor list are proteins (e.g., ERRs, HNF4) that have been shown to bind compounds under non-physiological conditions (65–67). The receptors shown include all 48 human receptors and the insect ecdysone receptor (EcR), which is the only nonvertebrate nuclear receptor with a known ligand. For a detailed list of nuclear receptors, their standard nomenclature, and a species comparison, see (7).



Despite our understanding of the roles of PPAR α and γ , knowledge of the function of PPAR δ has emerged more slowly (17). Ligands for PPAR δ include long-chain fatty acids and carboprostacyclin. The recent identification and study of synthetic, high-affinity PPAR δ ligands also suggest a role for this receptor in lipid metabolism (25, 26). Pharmacological activation of PPAR δ in macrophages and fibroblasts results in up-regulation of the ABCA1 transporter, and because of its widespread expression, PPAR δ may affect lipid metabolism in peripheral tissues (25, 26). Consistent with this notion, PPAR δ -null mice are growth retarded and have reduced adipocyte mass and myelination in their central nervous system (27, 28).

Liver X Receptors, the Sterol Sensors

In addition to its expression in the liver, LXR α is also abundantly expressed in other tissues associated with lipid metabolism, including adipose, kidney, intestine, lung, adrenals, and macrophages, whereas LXR β is ubiquitously expressed (29). The LXRs are activated by naturally occurring oxysterols including 24(S)-hydroxycholesterol (brain), 22(R)-hydroxycholesterol (adrenal), 24(S),25-epoxycholesterol (liver), and 27-hy-

droxycholesterol (human macrophage) (29, 30). Evidence also suggests that LXR activation can be antagonized by other small lipophilic agents, including 22(S)-hydroxycholesterol, certain unsaturated fatty acids, and geranylgeranyl pyrophosphate (31–33).

LXRs act as cholesterol sensors that respond to elevated sterol concentrations, and transactivate a cadre of genes that govern transport, catabolism, and elimination of cholesterol (29). LXRs also regulate a number of genes involved in fatty acid metabolism (34, 35). In the LXR metabolic cascade (Table 1), several sterol transporters have been identified as targets, including ABCA1, ABCG1, ABCG4, ABCG5, and ABCG8 (16, 36–39). ABCA1 is a monomeric transporter that resides in the plasma membrane of tissues, including liver, intestine, placenta, adipose, and spleen. ABCA1 transports phospholipids and cholesterol and is believed to be the rate-limiting step in reverse cholesterol transport (40). The dimeric transporters ABCG1, ABCG4, ABCG5, and ABCG8, are likely to be associated with membranes of intracellular organelles and have all been implicated in the intracellular trafficking of sterols in macrophages (for ABCG1 and perhaps ABCG4), and in liver and small intestine (for ABCG5

and ABCG8). Mutations in the genes for ABCA1 and ABCG5/G8 result in two disorders in cholesterol metabolism: Tangier disease and sitosterolemia (39, 40). These genes therefore play pivotal roles in the cellular flux of lipids from macrophages, and the biliary secretion and intestinal absorption of sterols.

No cytosolic binding proteins have yet been identified as target genes of the LXRs, although one or more of the newly described oxysterol-binding proteins may fulfill such a role. However, in rodents, the CYP enzyme cholesterol 7 α -hydroxylase (CYP7A1) has been shown to be an important LXR target gene. CYP7A1 encodes the rate-limiting enzyme in the neutral bile acid biosynthetic pathway and is one of the principle means for eliminating cholesterol from the body. Mice lacking LXR α fail to increase production of CYP7A1 and exhibit profound liver accumulation of cholesterol esters (41). Mice lacking only LXR β do not exhibit this alteration in bile acid metabolism, suggesting that the two LXRs may subserve distinct biological roles (42).

The human LXR α gene is itself a target of the LXR signaling pathway (43, 44). Particularly in macrophages, the autoregulation of LXR α would be an important way to amplify the cholesterol catabolic cascade.

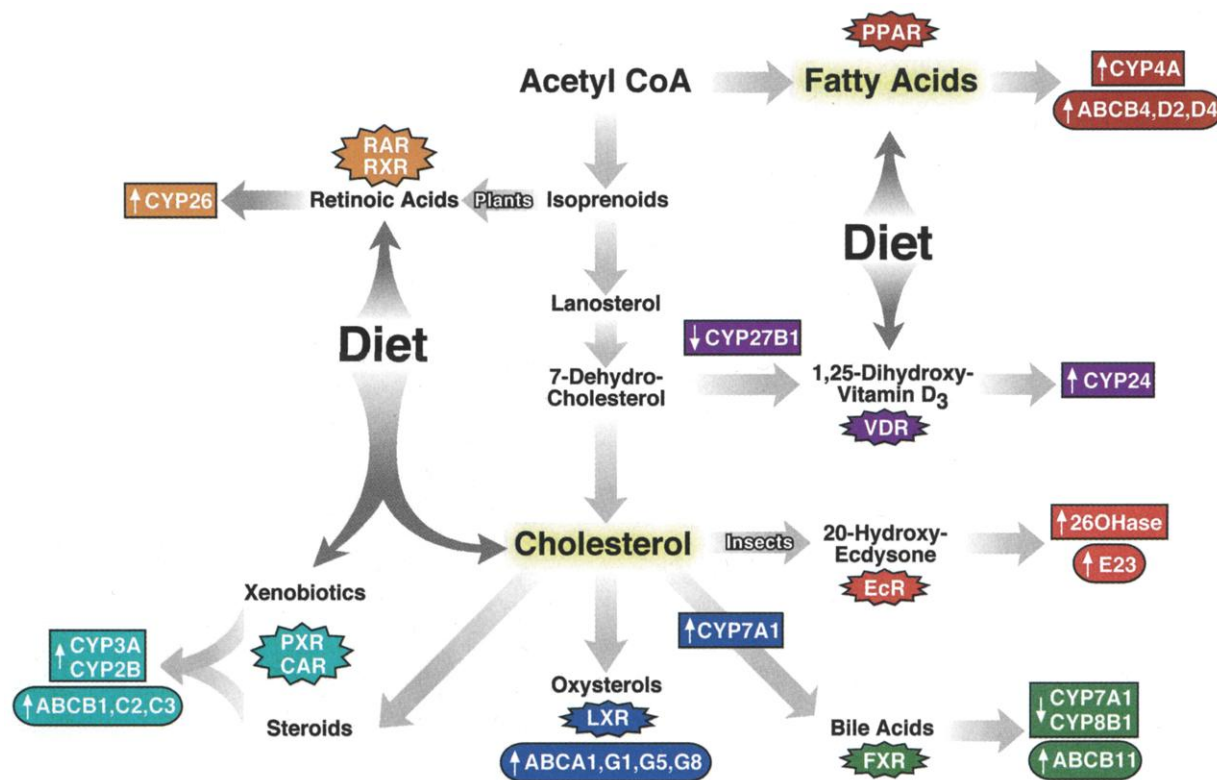


Fig. 2. Metabolic pathways for the acquisition and elimination of nuclear receptor ligands. With the exception of thyroid hormones and some xenobiotics, all nuclear receptor ligands are derived from the biosynthetic pathways that generate cholesterol and fatty acids from acetyl coenzyme A (Acetyl CoA). Ligands (or their lipid precursors) for the RXR heterodimer receptors are also acquired from the diet. For the sake of simplicity, several intermediary steps in these pathways

have been condensed and are not shown [for a detailed analysis see (68)]. Receptors are shown next to their ligands and are color-coded along with the genes they regulate. Shown are the CYP enzymes (in boxes) and ABC transporters (in ovals) that are regulated up or down (arrows) by their cognate ligands to prevent further synthesis (feedback loop) or increase catabolism (feed-forward loop). See text for abbreviations and details.

Farnesoid X Receptor, the Bile Acid Sensor

Although supraphysiological concentrations of the cholesterol precursor farnesol can weakly activate FXR, the relevant biological ligands for FXR are now known to be certain bile acids, including chenodeoxycholic acid, cholic acid, and their respective conjugated metabolites (45). FXR is highly expressed in the enterohepatic system, where it acts as a bile acid sensor that protects the body from elevated bile acid concentrations.

A number of *in vitro* and *in vivo* studies using mouse models have elucidated the FXR gene regulatory cascade (46–48) (Table 1). FXR activation results in the up-regulation of ABCB11 (also known as the bile salt efflux pump, BSEP), a bile acid transporter that increases the flow and secretion of these detergent-like molecules into bile, where they are required for the solubilization and absorption of lipids and fat-soluble vitamins in the intestine (48, 49). In the enterocytes of the ileum, bile acids are efficiently reclaimed for return to the liver. In these ileal enterocytes, bile acids induce the expression of a cytosolic binding protein

called IBABP (ileal bile acid binding protein), another FXR target gene that has been proposed to buffer intracellular bile acids and promote their translocation into the portal circulation (50). In the liver, bile acid activation of FXR represses transcription of the key CYP genes involved in bile acid synthesis (Fig. 2 and Table 1). Much of this feedback repression is due to FXR-mediated up-regulation of SHP (small heterodimer partner), an atypical orphan nuclear receptor that functions as a transcriptional repressor (46, 47). SHP interacts with and represses LRH-1, an orphan nuclear receptor that is required for liver-specific expression of CYP7A1 and sterol 12 α -hydroxylase (CYP8B), the enzyme responsible for the synthesis of trihydroxy-bile acids, such as cholic acid. Thus, FXR uses a rather unique variation on the ligand sensor cascade to maintain bile acid homeostasis.

CAR and PXR/SXR, the Xenobiotic Sensors

To protect the body against foreign chemicals (xenobiotics) and the buildup of toxic endogenous lipids, two nuclear receptors function in

this metabolic cascade to regulate detoxification and elimination (Table 1). The constitutive androstane receptor (CAR) mediates the response to a narrow range of phenobarbital-like inducers (51). In contrast, the human steroid xenobiotic receptor (SXR) or its rodent ortholog, the pregnane X receptor (PXR), respond to many prescription drugs, environmental contaminants, steroids, and toxic bile acids (52). Consistent with their role as xenobiotic sensors, both receptors are expressed primarily in liver and small intestine.

Although CAR was initially proposed to be constitutively active, ligands with negative (androstanes) and positive (phenobarbital) effects were soon found (51). CAR binds to and activates the CYP2B promoter in response to phenobarbital-like molecules, the pesticide 1,4-bis[2-(3,5-dichloropyridyloxy)]-benzene (TCPOBOP), certain androgens, and the muscle relaxant drug zoxazolamine. Genetic disruption of the mouse CAR gene abolishes induced CYP2B expression, resulting in increased serum levels of nonmetabolized products (53). In terms of the metabolic cascade model, no cytoplasmic binding proteins have yet been identified that generally bind xenobiotics, although phenobarbital does induce the expression of ABCC3, a member of the multidrug resistance-related protein subfamily (54).

The CYP3A enzyme is responsible for metabolizing and clearing over 60% of clinically prescribed drugs, and its induction plays a pivotal role in the clearance of hepatotoxic bile salts. CYP3A gene expression is induced by a large variety of xenobiotic compounds through SXR/PXR activation (55). Confirmation that SXR and PXR act as xenobiotic receptors comes from mouse knockouts of PXR that abolish both CYP3A inducibility and the protection of liver from the effects of toxic compounds (56, 57). Consistent with the lipid metabolic cascade model, two xenobiotic transporters, ABCB1 (or MDR1) and ABCC2 (or MRP2), are up-regulated in hepatocytes and intestinal cells by SXR activators and chemotherapeutic agents, such as Taxol (58, 59). Thus, this part of the regulatory circuit plays an important role in drug resistance. Taken together, the xenobiotic activation of CAR and SXR/PXR induces a positive feed-forward loop that aids in clearance of foreign chemicals and thereby resets the xenosensors for another round of signaling.

Other RXR Heterodimer Receptors

The orphan members that function as RXR heterodimers appear to rely heavily on feed-forward pathways to maintain ligand homeostasis through increased catabolism and elimination (Fig. 2 and Table 1). However, as mentioned above, aspects of this metabolic regulatory cascade are also used by other nuclear receptors to limit ligand concentrations. For example, the insect ecdysone receptor (EcR) functions as an endocrine regu-

Table 1. The nuclear receptor ligand metabolic cascade. The RXR heterodimers, their ligands, and regulated target genes are shown (see text for details). Question-marks (?) indicate that a member of this family has not yet been identified as a target for this ligand/receptor. Arrows denote whether the gene is up- or down-regulated by its cognate ligand. CYP, cytochrome P450; ABC, ATP-binding cassette.

Nuclear receptor		Ligand	CYP enzyme	Cytosolic binding protein	ABC transporter
Retinoid X receptors*	RXR α,β,γ	9- <i>cis</i> Retinoic acid	–	–	–
Peroxisome proliferator-activated receptors	PPAR α	Fatty acids Fibrates	↑ CYP4A1 ↑ CYP4A3	↑ L-FABP	↑ ABCD2, ABCD3 ↑ ABCB4
	PPAR δ	Fatty acids Carboprostacyclin	(?)	(?)	(?)
	PPAR γ	Fatty acids Eicosanoids Thiazolidinediones	↑ CYP4B1	↑ ALBP/aP2 ↑ H-FABP	(?)
Liver X receptors	LXR α,β	Oxysterols	↑ CYP7A1	OSBPs?	↑ ABCA1, ↑ ABCG1, ABCG4 ↑ ABCG5, ABCG8
Farnesoid X receptor	FXR	Bile acids	↓ CYP7A1 ↓ CYP8B1	↑ IBABP	↑ ABCB11
Xenobiotic receptors	SXR/PXR	Xenobiotics Steroids	↑ CYP3A ↑ CYP2C	(?)	↑ ABCB1, ABCC2
	CAR	Xenobiotics Phenobarbital	↑ CYP2B ↑ CYP2C	(?)	↑ ABCC3
Ecdysone receptor	EcR	20(OH)-ecdysone	↑ 26-(OH)ase	Hexamerins	↑ E23
Retinoic acid receptors	RAR α,β,γ	Retinoic acids	↑ CYP26A1	↑ CRABPII ↑ CRBPI	(?)
Vitamin D receptor	VDR	1,25(OH) ₂ -vitamin D ₃	↑ CYP24 ↓ CYP27B1	(?)	(?)

*RXRs serve as common heterodimer partners with other receptors.

lator of insect molting as a heterodimer with ultraspiracle (the insect homolog of RXR) (9). However, like its vertebrate counterparts, EcR also up-regulates the expression of genes that reduce elevated ligand concentration by catabolism and efflux. One of these genes is E23, an ABC transporter that effluxes ecdysteroids from *Drosophila* cells (60). Administration of EcR agonists also leads to the induction of a 26-hydroxylase, a cytochrome P450 protein that produces inactive ecdysteroid metabolites (61). Likewise, the vertebrate retinoic acid receptors, RAR α , β , and γ , coordinately up-regulate two cytosolic binding proteins, cellular retinoic acid binding protein (CRABP) and cellular retinol binding protein (CRBP), which control the availability and transport of vitamin A-derived ligands (62). The RARs also up-regulate expression of the cytochrome P450 enzyme CYP26A1, which deactivates the most potent ligand, all-*trans* retinoic acid (63). Finally, the 1,25-dihydroxyvitamin D receptor (VDR) similarly up-regulates expression of a 24-hydroxylase (CYP24), which inactivates the active vitamin D hormone, as well as down-regulates the expression of the 1 α -hydroxylase (CYP27B1), the enzyme that produces the vitamin D hormone (8). The remaining RXR heterodimer partner, the thyroid hormone receptor (TR), does not appear to rely on the metabolic cascade and more closely resembles the endocrine steroid receptors in its function. In the future, it will be interesting to see whether other aspects of the ligand regulatory cascade are controlled by these and other nuclear receptors.

Perspectives

This review has explored the concept that nuclear receptor signaling is the product of a series of scripted events that can be understood in terms of molecules and mechanisms that together compose a regulatory network. This evolving understanding of the pathways through which nuclear receptors govern lipid metabolism provides the basis for several important directions of future research, including recognition of new physiological pathways and the development of new and better medicines. Knowledge of the transporters, binding proteins, and catabolic enzymes that regulate the fate of a given receptor's endogenous ligand may enable the identification of novel ligands (i.e., drugs) with desirable pharmacokinetic profiles. The organizational scheme proposed here reveals that nuclear receptors function as effective regulators of lipid metabolism by affecting the synthesis of key enzymes that control the intensity, duration, and direction of numerous metabolic steps (see Table 1). Perhaps this is no better illustrated than by the finding that the xenobiotic receptor SXR is the transcriptional mediator of CYP3A, the enzyme responsible for catabolic clearance of most

drugs and a number of important drug-drug interactions. By screening for the ability of other nuclear receptor agonists (or antagonists) to affect SXR, one should be able to predict the metabolic fate of these compounds in vivo.

A second area of future research that continues to generate much interest is the large number of orphan nuclear receptors that are still without known ligands (Fig. 1). A question still open for debate is whether or not the transcriptional activities of the remaining nuclear orphan receptors are, like the original members of the family, also regulated by the competitive binding of a reversible endogenous ligand. An alternative hypothesis that is gaining support from structural studies suggests that many of these orphan receptors may never exist as apo-receptors. Instead, it is believed these nuclear receptors may constitutively bind to an abundant cellular lipid (e.g., a fatty acid), which becomes an integral part of the ligand-binding domain and helps to stabilize the receptor for interactions with other proteins (64). While this debate rages on, several promising new studies suggest that the activity of at least some of these proteins [e.g., ERRs (estrogen-related receptors)] can be pharmacologically manipulated to regulate previously unknown metabolic and developmental cascades (65, 66). Thus, through the hunt for novel bioactive lipids and the illumination of the genetic network of target genes, the mysteries sealed within the X-files of the orphan nuclear receptors will be revealed. The truth is out there.

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