REVIEW

Orphan Nuclear Receptors: Shifting Endocrinology into Reverse

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Steroid and thyroid hormones and vitamin A metabolites (retinoids) regulate the expression of complex gene programs by binding to members of the nuclear receptor family of ligand-activated transcription factors. The nuclear receptor family also includes many "orphan" members that currently lack known ligands but that represent candidate receptors for new hormones. Recently, natural and synthetic ligands have been identified for several orphan receptors and used to dissect their biological roles. This "reverse endocrinology" strategy has resulted in the discovery of unanticipated nuclear signaling pathways for retinoids, fatty acids, eicosanoids, and steroids with important physiological and pharmacological ramifications.

Nuclear receptors have critical roles in nearly every aspect of vertebrate development and adult physiology by transducing the effects of small, lipophilic hormones into transcriptional responses (*1*–*4*). Members of the nuclear receptor family share several structural features including a central, highly conserved DNA binding domain (DBD) that targets the receptor to specific DNA sequences, termed hormone response elements. The COOH-terminal portion of the receptor includes the ligand binding domain (LBD), which interacts directly with the hormone. Embedded within the LBD is a hormone-dependent transcriptional activation domain. The LBD serves as a molecular switch that recruits coactivator proteins and activates the transcription of target genes when flipped into the active conformation by hormone binding. The integration of the different domains within the receptor results in the exquisite specificity of the hormonal response.

The term "orphan receptor" was coined a decade ago to describe gene products that appeared to belong to the nuclear receptor family on the basis of sequence identity but that lacked identified hormones. Orphan receptors have been identified in most metazoans, including about 40 encoded by distinct genes in humans (*3*, *4*). Along with the orphan receptors has come the exciting new concept of "reverse endocrinology" (Fig. 1). Historically, new hormones were discovered through analysis of their effects on physiological or developmental processes. The purified hormone was subsequently used to identify its partner receptor. This classic approach established the field of endocrinology and led to many key insights into physiology and disease. The cloning of the orphan receptors ushered in a new era in which this process is reversed, and the orphan receptors are used to search for previously unknown hormones. The hormones themselves can then be used as chemical tools to elucidate the biology of the signaling pathway. Reverse endocrinology was first successfully applied to the retinoid X receptor (RXR) family of nuclear receptors. Identification of the vitamin A metabolite 9-cis retinoic acid as a high-affinity ligand for the three RXR subtypes (*5*) substantially increased our understanding of the biological processes regulated by retinoids.

In this review, we focus on recent progress in understanding hormone signaling through the use of reverse endocrinology. Five families of mammalian orphan nuclear receptors are discussed: the peroxisome proliferator-activated receptors (PPARs), the liver X receptors (LXRs), the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), and the farnesoid X receptor (FXR). The work on these orphan receptors illustrates how the use of natural and synthetic ligands as chemical tools has uncovered new hormone signaling pathways and provided insights into the regulation of glucose, lipid, and drug metabolism.

Peroxisome Proliferator-Activated Receptors: Fatty Acid and Eicosanoid Receptors

Energy homeostasis represents a delicate balance between energy intake and dissipation. In humans, a shift in this balance toward excessive energy intake is associated with a number of prevalent metabolic disorders such as obesity, atherosclerosis, and type 2 diabetes. The PPAR family of orphan receptors has been the subject of intense investigation during the past several years because of the fundamental roles that these nuclear receptors have in regulating energy balance (*6*). The PPAR family comprises three closely related gene products—PPARα, PPARγ, and PPARβ/δ—and is so named because PPARα is activated by chemicals that elicit increases in the number and size of peroxisomes when administered to rodents (*7*). The three PPARs have very different expression patterns: PPARα is most abundantly expressed in liver, kidney, heart, and muscle; PPARγ is most abundantly expressed in fat cells, large intestine, and cells of the monocyte lineage; and PPARβ/δ is expressed in nearly all tissues (*6*). These contrasting expression patterns provided one of the first hints that the three PPARs subserve distinct biologies.

Insight into the biology of PPARα has been gleaned from the discovery that this receptor is the molecular target for the fibrates, drugs that are widely prescribed for the reduction of high triglyceride levels, a risk factor for coronary heart disease (*7*) (Fig. 2). The fibrates lower triglyceride levels through effects on both the production and clearance of triglyceride-rich lipoproteins. The fibrates also have the beneficial effect of increasing circulating amounts of high density lipoproteins. At the molecular level, fibrates act as PPARα ligands to regulate the transcription of a large number of genes that affect lipoprotein and fatty acid (FA) metabolism (*6*–*8*). Mice lacking functional PPARα exhibit abnormalities in triglyceride and cholesterol metabolism, do not respond to fibrates, and become obese with age (*9*). Thus,

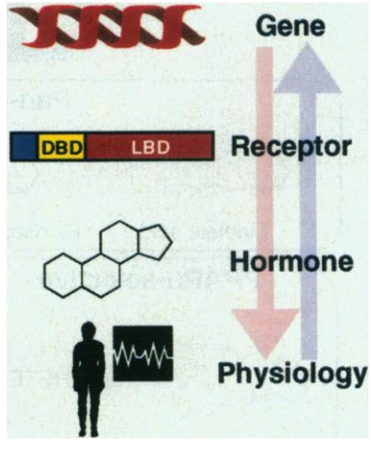

Fig. 1. Reverse endocrinology. Traditionally, hormones were identified on the basis of their biological effects (blue arrow). In reverse endocrinology (red arrow), this process is reversed, and the orphan receptors are used to identify the hormones and their associated biology. DBD, DNA binding domain; LBD, ligand binding domain.

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PPARα is a key regulator of intra- and extracellular lipid metabolism.

Like PPARα, our understanding of PPARγ has been enhanced by the finding that this orphan receptor is the molecular target for a class of drugs—in this case, the antidiabetic thiazolidinediones (TZDs), including the recently approved drug Rezulin (Fig. 2) (*10*). The TZDs were originally developed for the treatment of type 2 diabetes on the basis of their ability to lower glucose levels in rodent models of insulin resistance. These drugs also have the beneficial effect of lowering circulating levels of FAs. The finding that TZDs mediate their therapeutic effects through direct interactions with PPARγ established this orphan receptor as a key regulator of glucose and lipid homeostasis (*11*). PPARγ is abundantly expressed in fat cells (*12*) and functions as a master regulator of adipocyte differentiation (*13*). The connection between PPARγ and the TZDs thus provided evidence for an unexpected link between adipocyte biology and glucose homeostasis.

What are the natural hormones for the PPARs? Physiological concentrations of various FAs, including those that vary in both chain length and degree of saturation, bind and activate the PPARs (*14*, *15*). The PPARs are also activated by certain cyclooxygenase and lipoxygenase metabolites of polyunsaturated FAs (*15*, *16*) (Fig. 2). Given the established roles of the PPARs in lipid homeostasis, the idea that FAs or FA metabolites serve as natural PPAR ligands is appealing. Structural analyses of the PPARγ and PPARβ/δ LBDs reveal that their ligand-binding pockets are roughly three times larger than those of other nuclear receptors and are sufficiently large to allow FAs to bind in multiple conformations (*17*). These findings suggest that the PPARs may have evolved to serve as lipid sensors and recognize a number of different FAs and FA metabolites rather than a single high-affinity hormone. However, this idea remains speculative, and the search for a high-affinity, natural PPAR ligand continues.

The fibrates and TZDs have been used as chemical tools to uncover other biological roles for the PPARs. PPARγ regulates the differentiation of monocytes and macrophages (*18*). Furthermore, activation of either PPARα or PPARγ suppresses the expression of inflammatory cytokines and other downstream markers of inflammation in various cell types (*19*). Finally, PPARγ can have either positive or negative effects on the cell cycle: Activation of PPARγ inhibits the proliferation of certain cell lines and blocks the growth of some tumors in mice (*20*); however, TZDs promote polyp formation in a strain of mice that is genetically predisposed to colon cancer (*21*). There is clearly still much to be learned about the PPARs, including the function of the broadly expressed PPARβ/δ subtype.

A New Generation of Steroid Hormone Receptors

Scientists have been fascinated for centuries by the profound biological effects of what we now know to be steroid hormones. By the mid-1900s, a number of different steroid hormones had been isolated and characterized, including sex steroids (progesterone, estrogen, and testosterone), adrenal steroids (cortisol and aldosterone), and the secosteroid vitamin D. During the 1980s, receptors were cloned for each of these nuclear-acting steroid hormones (*1*). Although there was no reason to believe a priori that additional steroid hormone signaling pathways existed, there is now compelling evidence that several of the orphans, including LXR, PXR, CAR, and FXR, are steroid receptors (Fig. 3). The discovery of these signaling pathways reveals a broader biological role for steroid hormones than was previously appreciated.

Liver X receptors. Although cholesterol is an essential membrane component in mammals, in excess it can cause medical disorders such as atherosclerosis and gallstones. Thus, intra- and extracellular amounts of cholesterol must be precisely regulated. The conversion of cholesterol to hydrophilic bile acids in the liver is a major pathway for its elimination from the body. Dietary cholesterol increases transcription of the cholesterol 7α-hydroxylase (CYP7a) gene, which encodes the enzyme responsible for the rate-limiting step in the conversion of cholesterol to bile acids. This feed-forward regulatory pathway ensures the catabolism of excess cholesterol.

Characterization of the orphan receptor LXRα provided the molecular link between dietary cholesterol and the regulation of CYP7a gene transcription. LXRα is abundantly expressed in the liver (*22*) and binds as a heterodimer with RXR to a DNA response element in the CYP7a gene promoter (*23*). Cholesterol does not activate LXRα directly. Instead, two oxysterols, 24(*S*),25-epoxycholesterol and 24(*S*)-hydroxycholesterol, bind and activate LXRα at physiological concentrations (*23*, *24*). 24(*S*)-hydroxycholesterol is a direct metabolite of cholesterol, whereas 24(*S*),25-epoxycholesterol is generated by a shunt pathway in the cholesterol biosynthetic pathway (*25*). Hepatic amounts of both oxysterols increase in response to dietary cholesterol (*25*). Mice lacking LXRα are viable and appear normal when fed a diet containing small amounts of cholesterol (*26*). However, when fed a cholesterol-rich diet that has no adverse effects on wild-type animals, the LXRα null mice accumulate enormous amounts of cholesterol esters in their livers, which eventually results in impaired hepatic function (*26*). As expected, CYP7a gene transcription is not increased in the LXRα null animals in response to dietary cholesterol (*26*). Thus, the accumulation of cholesterol in the livers of LXRα null animals is at least in part a consequence of their inability to convert cholesterol into bile acids. These findings establish LXRα as an oxysterol receptor that regulates the catabolism of excess dietary cholesterol.

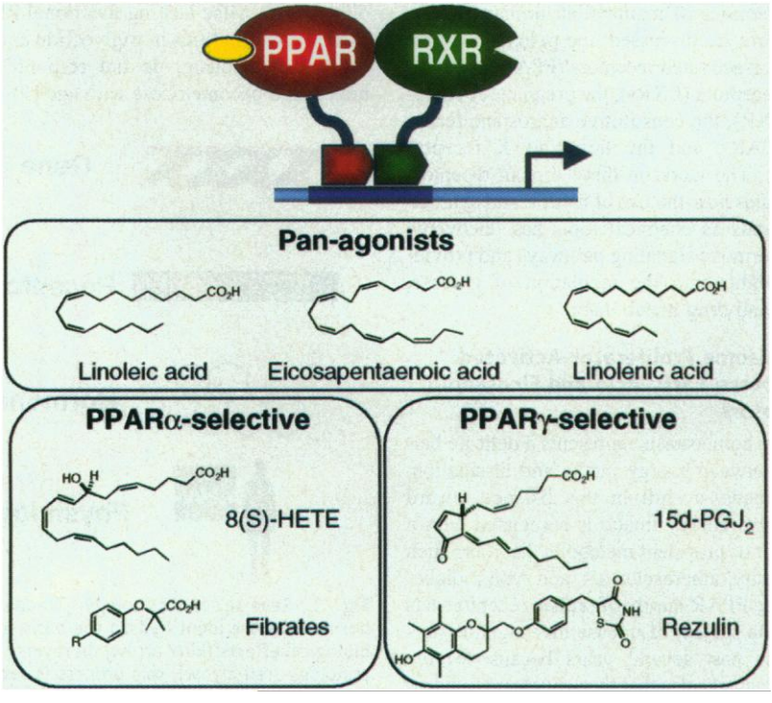

Fig. 2. Fatty acids, eicosanoids, and drugs are PPAR ligands. Molecules that function as ligands for all three PPAR subtypes (pan-agonists) or are selective for either PPARα or PPARγ are shown. HETE, hydroxyeicosatetraenoic acid; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2.

In addition to CYP7a, the expression of a number of other genes that participate in cholesterol and FA homeostasis is dysregulated in the LXRα null mice (*26*), suggesting additional functions for this orphan receptor. The biological role of a closely related orphan receptor, LXRβ (*27*, *28*), which is expressed in many tissues and activated by the same oxysterols as LXRα, remains unclear.

Pregnane X receptor. The efficient detoxification of harmful xenobiotics is essential to the survival of all organisms. Members of the cytochrome P450 (CYP) superfamily of monooxygenases are crucial for the detoxification of most xenobiotics, including various environmental pollutants, carcinogens, and drugs. The CYPs are also responsible for the oxidative metabolism of endogenous compounds, including many steroid hormones. The CYP3A4 isozyme is of particular significance from a medical perspective because it is involved in the metabolism of roughly 50% of all drugs (*29*). Transcription of the CYP3A4 gene is substantially increased in the liver and intestine by a number of widely used drugs, including antibiotics, antimycotics, glucocorticoids, and the statin class of HMG-CoA reductase inhibitors (*29*). This transcriptional activation of CYP3A4 gene expression, coupled with the broad substrate specificity of the CYP3A4 enzyme, constitute the molecular basis for a number of important drug-drug interactions in patients taking multiple medications.

The molecular mechanism through which transcription of the CYP3A4 gene is increased by these structurally unrelated drugs had remained unclear until an orphan nuclear receptor termed the pregnane X receptor (PXR) was characterized (*30*, *31*). PXR is activated by the array of compounds that increase CYP3A4 gene transcription and binds as a heterodimer with RXR to a xenobiotic response element in the CYP3A4 gene promoter. Furthermore, PXR is selectively expressed in the liver and intestine, the same tissues in which CYP3A4 gene expression is induced. These data indicate that a single orphan nuclear receptor accounts for the regulation of CYP3A4 gene expression by structurally diverse xenobiotics. An important pharmaceutical implication of these findings is that PXR binding and activation assays can now be used to predict which compounds will induce CYP3A gene expression and interact with other drugs.

Naturally occurring steroids have been identified that are efficacious activators of PXR (*30*, *31*). The most potent compounds are C21 steroids (pregnanes) such as the progesterone metabolite 5β-pregnane-3,20-dione, but corticosteroids and estrogens also activate PXR. Because the concentrations of individual steroids required to activate PXR are greater than those detected in biological samples, it remains to be determined whether PXR has a high-affinity ligand or instead functions as a more generalized steroid sensor (*31*). These findings suggest the existence of a regulatory pathway whereby the accumulation of steroidal PXR ligands results in increased CYP3A transcription and steroid catabolism, perhaps providing a mechanism for the elimination of excess steroid hormones from the body. Thus, in addition to serving as a xenobiotic sensor, PXR is also likely to have important implications in the regulation of steroid homeostasis.

Constitutive androstane receptor. The orphan receptor CAR has strong transcriptional activity in cell-based assays in the absence of any exogenously added ligand (*32*). The transcriptional activity of CAR is blocked by the testosterone metabolites 3α,5α-androstenol and 3α,5α-androstanol (*33*). One explanation for these findings is that the androstanes antagonize an endogenous CAR ligand produced by the cells. Alternatively, CAR may be active in the absence of a ligand and deactivated upon hormone binding. The finding that 3α,5α-androstenol and 3α,5α-androstanol cause the dissociation of CAR from coactivator proteins in a cell-free assay (*33*) supports the latter hypothesis. Because many of the remaining orphan receptors are transcriptionally active in cell-based assays in the absence of exogenously added hormone, ligand-mediated receptor deactivation may represent a general mechanism of nuclear hormone action.

The physiological relevance of the CAR androstane signaling pathway remains to be determined. However, CAR is abundantly expressed in the liver (*32*) and was recently implicated as a transcriptional regulator of the steroid hydroxylase CYP2B gene (*34*). These data raise the possibility that CAR, like PXR, influences steroid homeostasis through transcriptional regulation of specific members of the CYP superfamily.

Farnesoid X receptor. The rat ortholog of FXR was originally shown to be weakly activated by farnesol, a product of the mevalonate pathway (*35*). However, farnesol does not activate the mouse and human FXR orthologs (*36*). FXR is also activated by the synthetic retinoid TTNPB and superphysiological concentrations of all-trans retinoic acid (*36*). Recently, FXR was found to serve as a receptor for physiological concentrations of several bile acids, among which chenodeoxycholic acid (CDCA) is the most potent (*37*). CDCA regulates the expression of several genes that participate in bile acid homeostasis, including those encoding CYP7a and the intestinal bile acid-binding protein. FXR is abundantly expressed in tissues through which bile acids circulate, including the liver, intestine, and kidney (*28*, *35*). Thus, FXR is proposed to be a nuclear bile acid receptor.

Conclusions

Several important themes have emerged from the application of reverse endocrinology to the orphan nuclear receptors. First, some of the natural orphan receptor ligands, including FAs and oxysterols, are likely to be produced in the same cells as their cognate receptors. Thus, molecules that were previously thought of as metabolic intermediates or by-products are in fact "intracrine" signaling molecules (*3*). These signaling networks provide a mechanism for tightly coupling metabolic pathways with changes in gene expression. A second theme is that steroids have an even broader role in vertebrate physiology than was previously antici-

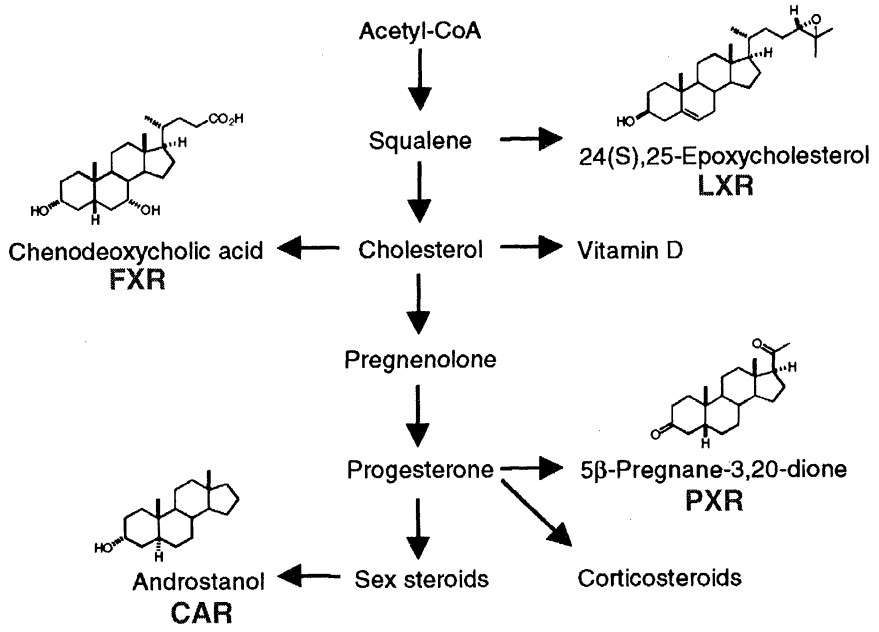

Fig. 3. Orphan receptors reveal steroid hormone signaling pathways. Hormones for LXR, FXR, PXR, and CAR are derived from the mevalonate pathway as indicated.

pated. LXRα is an oxysterol receptor that serves as a cholesterol rheostat, regulating the conversion of excess cholesterol to bile acids for removal from the body. The finding that FXR is a bile acid receptor suggests that this orphan also contributes to cholesterol homeostasis. The discovery that PXR and CAR are steroid receptors that modulate the expression of steroid hydroxylases suggests a mechanism for regulating the amounts of steroid hormones. Thus, these orphan receptors not only function as steroid receptors but also regulate key steps in steroid metabolism. A final theme is that orphan receptors represent a tremendous opportunity in terms of understanding and treating human disease. Historically, nuclear receptors have been important drug targets. The discovery that some orphan receptors regulate key metabolic pathways suggests that they will be useful targets for intervention in disease processes. We now know that the inadvertent activation of other orphan receptors can contribute to detrimental side effects of drugs. Thus, knowledge of orphan receptor signaling pathways will be important both for the discovery of new drugs and for minimizing the side effects of these compounds.

The first decade of orphan nuclear receptor research has yielded a large number of new family members and many tantalizing clues as to their biological functions. However, ligands have been identified for only a handful of the orphans. Given the large number of remaining orphan nuclear receptors and the recent advances in combinatorial chemistry, high-throughput screening, and functional genomics, the next decade of reverse endocrinology promises an explosion in our understanding of nuclear hormone signaling pathways.

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REVIEW

Cryptochromes: Blue Light Receptors for Plants and Animals

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Cryptochromes are blue, ultraviolet-A photoreceptors. They were first characterized for *Arabidopsis* and are also found in ferns and algae; they appear to be ubiquitous in the plant kingdom. They are flavoproteins similar in sequence to photolyases, their presumptive evolutionary ancestors. Cryptochromes mediate a variety of light responses, including entrainment of circadian rhythms in *Arabidopsis*, *Drosophila*, and mammals. Sequence comparison indicates that the plant and animal cryptochrome families have distinct evolutionary histories, with the plant cryptochromes being of ancient evolutionary origin and the animal cryptochromes having evolved relatively recently. This process of repeated evolution may have coincided with the origin in animals of a modified circadian clock based on the PERIOD, TIMELESS, CLOCK, and CYCLE proteins.

In an early description of a biological response to blue light, Charles Darwin noted that the heliotropic movement of plants was eliminated if the light was first filtered through a solution of potassium dichromate (*1*). As passage through a dichromate solution reduces the blue content of the radiant light, this experiment demonstrated that plants were selectively sensing the blue region of the spectrum. It is now realized that this ability to sense and respond to blue light (400 to 500 nm) is widespread throughout the biological kingdom. Other examples of such responses include the production of anthocyanins and carotenoids in plants and fungi and the entrainment of behavioral rhythms in flies and mammals. The action spectrum of many responses to blue light is similar to the absorption spectrum of flavins, which prompted Galston to postulate the involvement of a flavoprotein (*2*). However, for several decades the nature of this photoreceptor continued to be hotly debated—some argued in favor of a flavoprotein, and others speculated that the photoreceptor contained a ca-