BIOCHEMISTRY

 Volume 46, Number 47 November 27, 2007

Current Topics

Ligand Specificity of Nuclear Hormone Receptors: Sifting through Promiscuity†

Noa Noy*

Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4965

Received September 12, 2007; Revised Manuscript Received October 5, 2007

ABSTRACT: The superfamily of nuclear hormone receptors includes transcription factors that play key roles in regulating multiple biological functions during embryonic development and in adult tissues, as well as in many disease states. The quintessential characteristic of nuclear receptors, and the basis for the name of the family, is that their transcriptional activities can be regulated by small molecules, usually comprised of hydrophobic compounds. However, the endogenous ligands for approximately half of the members of the nuclear receptor family are unknown, and these receptors are thus designated as "orphan receptors". One class of orphan receptors encompasses receptors that display a broad ligand selectivity; i.e., they can promiscuously bind to and may be activated by multiple ligands. This characteristic complicates the identification of physiologically meaningful ligands that activate these receptors in vivo. Here, we discuss a few examples of promiscuous receptors and outline strategies that may be employed in shedding light on the nature of *bona fide* ligands for such receptors.

Nuclear hormone receptors comprise a family of transcription factors that regulate multiple facets of cellular behavior, including cell cycle progression, differentiation, apoptosis, and metabolism. These receptors thus play key roles in embryonic development as well as in tissue maintenance and remodeling in the adult. Forty-eight genes encoding nuclear receptors have been identified in the human genome, and using molecular phylogeny based on sequence homology, these receptors were classified into seven subfamilies (*1*) (Table 1). The transcriptional activity of most nuclear receptors is regulated by small molecules, usually comprised of cognate lipophilic compounds. However, while endogenous compounds that activate many receptors have been identified, approximately half of the members of the family remain "orphan receptors", denoting that their physiologically meaningful ligands are unknown. Of these, some may not be regulated by any ligand and thus may comprise true orphans. This mode is exemplified by NR4 subfamily member NURR1, in which the putative pocket ligand binding pocket is occupied by tightly packed bulky side chains, apparently excluding ligand binding (*2*). Ligands for other orphan receptors are simply unknown, and ongoing efforts by multiple groups aim to identify such compounds. A third class of orphan receptors includes receptors that display a broad ligand selectivity. In such cases, it is difficult to determine which, if any, of these ligands function as activators for the receptors in vivo. This review focuses on this latter type of receptors. Following a brief description of the mode of action of nuclear receptors, several promiscuous receptors will be discussed: the xenobiotic receptors PXR and CAR, the "master regulator" RXR, and the lipid sensors PPARs. Possible strategies for elucidating the nature of true

† Research in the author's laboratory has been supported by Grants CA68150 and CA107013 from the National Cancer Institute, Grant DK60684 from the National Institute of Diabetes and Digestive and Kidney Diseases, and Grant BCTR27606 from The Susan G. Komen Breast Cancer Foundation.

* To whom correspondence should be addressed: Department of Pharmacology, Case Western Reserve University School of Medicine, Biomedical Research Building, Rm 724, Cleveland, OH 44106. Telephone: (216)368-0302. Fax: (216)368-1300. E-mail: noa.noy@case.edu.

10.1021/bi7018699 CCC: $37.00
Published on Web 11/06/2007

Table 1: Human Nuclear Receptors

subclass	name	ligand
NR1	thyroid hormone receptor (TRα, -*β*)	thyroid hormone
	retinoic acid receptor (RARα, -*β*, -*γ*)	retinoic acid
	peroxisome proliferator-activated receptor (PPARα, -*β*/*δ*, -*γ*)	orphan
	reverse erbA (Rev-erbα, -*β*)	orphan
	RAR-related orphan receptor α (RORα, -*β*, -*γ*)	orphan
	liver X receptor (LXRα, -*β*)	oxysterols
	farnesoid X receptor (FXRα; FXR*β* is a pseudogene)	bile acids
	vitamin D receptor (VDR)	vitamin D_3, bile acids
	pregnane X receptor (PXR)	xenobiotics
	constitutive androstane receptor (CAR)	xenobiotics
NR2	human nuclear factor 4 (HNF4α, -*γ*)	orphan
	retinoid X receptor (RXRα, -*β*, -*γ*)	9-*cis*-retinoic acid
	testis receptor (TR2, TR4)	orphan
	tailless (TLL)	orphan
	photoreceptor-specific nuclear receptor (PNR)	orphan
	chicken ovalbumin upstream promoter transcription factor (COUP-TF1, -TF2)	orphan
	ErbA2-related gene-2 (EAR2)	orphan
NR3	estrogen receptor (ERα, -*β*)	17*β*-estradiol
	estrogen receptor-related receptor (ERRα, -*β*, -*γ*)	orphan
	glucocorticoid receptor (GR)	cortisol
	mineralocorticoid receptor (MR)	aldosterone
	progesterone receptor (PR)	progesterone
	androgen receptor (AR)	testosterone
NR4	nerve growth factor-induced factor B (NGFIB)	orphan
	Nur-related factor 1 (NURR1)	orphan
	neuron-derived orphan receptor 1 (NOR1)	orphan
NR5	steroidogenic factor 1 (SF1)	orphan
	liver receptor homologous protein 1	orphan
NR6	germ cell nuclear factor (GCNF)	orphan
NR0	DSS-AHC critical region on the chromosome, gene 1 (DAX1)	orphan
	short heterodimeric partner (SHP)	orphan

endogenous ligands for promiscuous receptors will then be outlined.

Mode of Action of Nuclear Receptors

Nuclear receptors are modular proteins that contain several distinct functional domains (*3*). The amino-terminal domain (also termed the A/B region) mediates a ligand-independent transcriptional activation (AF-1). In different nuclear receptors, this region varies both in length and in sequence, and it is recognized by different accessory proteins. Adjacent to the A/B region is the DNA binding domain (DBD)[1] which contains two zinc finger DNA binding motifs (*4*). This region is highly conserved among all but two nuclear receptors, namely, DAX1 and SHP, which lack a DBD and are unable to directly associate with DNA. A third domain is a flexible hinge region which serves to connect the DBD to the carboxyl terminus of receptors, termed the ligand binding domain (LBD). The LBD is comprised of 12 helices and several *β*-turns. Eleven of the helices form a ligand binding pocket, while C-terminal helix 12 (H12) forms a flexible lid over the entrance to the pocket (*5*). In addition to an internal ligand binding pocket, the LBD contains surface regions that mediate multiple protein−protein interactions, including association with transcriptional coregulators (*6*, *7*), formation of dimers (*8*), and, in the case of one receptor, RXR, formation of tetramers (*9*, *10*). The LBD of nuclear receptors thus coordinates their ligand-dependent activation function (AF-2).

The transcriptional activities of nuclear receptors are exerted through binding to recognition DNA sequences [hormone response elements (HRE)] in regulatory regions of specific target genes. HRE are composed of polymorphic arrangements of the motif 5′-PuG(G/T)TCA, often in the form of two repeats (*1*, *11*, *12*). Indeed, while a few nuclear receptors, for example, SF1, function as monomers, most receptors bind to their cognate DNA in the form of dimers. Steroid receptors function as homodimers. In contrast, DNA binding by other receptors requires association with a common binding partner, the retinoid X receptor (RXR). Thus, the transcriptional activities of VDR, RAR, TR, and PPAR and some orphan receptors are exerted by their heterodimers with RXR. Consequently, while RXR itself can bind to DNA and regulate transcription as a homodimer, this receptor is also involved in multiple signaling pathways that converge at the genome and is often termed a “master regulator”. The partitioning of RXR between the different complexes in which it may participate appears to be controlled by cognate ligands. Apo-RXR self-associates into highly stable, transcriptionally silent tetramers, and these rapidly dissociate to yield active RXR homodimers upon binding of an RXR ligand (*9*, *10*, *13*, *14*). Formation of RXR heterodimers seems to be regulated by ligands for the partner receptors; e.g., it was reported that RXR−VDR and RXR−RAR complexes are stabilized in the presence of vitamin D_3 and all-*trans*-retinoic acid (RA), respectively (*15*, *16*). Specificity in gene targeting by different receptor dimers is

[1] Abbreviations: AF, activation function; CRABP, cellular retinoic acid binding protein; DBD, DNA binding domain; FABP, fatty acid binding protein; HAT, histone acetyltransferase; HDAC, histone deacetylase; HRE, hormone response element; iLBP, intracellular lipid binding protein; LBD, ligand binding domain; RA, all-*trans*-retinoic acid. For abbreveations of receptors, see Table 1.

determined by the orientations of the two recognition sequences, which can be arranged as direct, inverted, or palindromic repeats, and by the spacing between the repeats (*1*, *11*, *12*).

Notably, some receptors, e.g., GR, are cytosolic in the absence of ligand, and they move to the nucleus upon ligation (*17*). In contrast, other receptors, e.g., PPAR, are predominantly nuclear and are bound to DNA in the absence of a cognate ligand (*18*). These observations raise the question of how receptor ligands, which are hydrophobic in nature, reach the nucleus. While such delivery mechanisms remain incompletely understood, it has been demonstrated that some nuclear receptor ligands are shuttled from the cytosol to the nucleus by particular members of a family of proteins called intracellular lipid binding proteins (iLBP), which deliver ligands to specific nuclear receptors. ILBPs that were reported to function in this capacity include cellular retinoic acid binding protein-II (CRABP-II), which delivers RA to RAR, and the fatty acid binding proteins (FABP) FABP3, FABP4, and FABP5, which shuttle ligands to PPARα, PPARγ, and PPARβ/δ, respectively (*19*−*24*). These iLBPs are cytosolic in the absence of ligand, and they mobilize to the nucleus upon binding of ligands that serve as activators for their cognate receptors. In the nucleus, the binding proteins directly associate with the respective receptors to form a complex through which the ligand is "channeled" to the receptor. Interestingly, while all FABPs bind multiple lipid compounds with similar affinities, only a narrow spectrum of ligands induces their nuclear localization. Specifically, the ligand selectivity in activation of the nuclear import of these FABPs appears to correspond to the ligand selectivity of their cognate receptors. For example, FABP4 associates with compounds that activate all three PPAR isotypes, but it undergoes nuclear localization only upon binding of PPARγ activators (*25*, *26*). Specific FABP/PPAR pairs thus tightly cooperate in regulating the transcriptional activities of their shared ligands.

Transcriptional activation by nuclear receptors involves ligand-controlled association with various accessory proteins that act as coactivators or corepressors (for reviews, see refs *7* and *27*). Receptors that are nuclear and can bind to DNA in the absence of ligand associate with a corepressor complex that includes histone deacetylases (HDACs), enzymes that catalyze histone deacetylation, leading to a condensed chromatin structure and transcriptional repression. Upon ligand binding, C-terminal helix H12 of the receptors' LBD undergoes a dramatic conformational rearrangement, resulting in dissociation of corepressors and in the formation of protein surfaces that allow for recruitment of coactivator complexes. Multiple coactivator complexes are involved in transcriptional activation mediated by nuclear receptors. These include ATP-dependent chromatin remodeling factors, which facilitate sliding of nucleosomes, complexes containing histone acetyltransferases (HATs), which loosen chromatin structure, and mediator complexes, which form a bridge between a receptor and the general transcription machinery. Other proteins that associate with coactivator complexes catalyze various post-translational modifications of histones and other factors, including the receptors themselves. Hence, coactivator complexes comprise a multifaceted array of regulatory proteins that control transcriptional activation as well as termination of transcriptional signaling brought about by post-translation modification of receptors and coactivators, leading to their degradation by the proteasome (*28*, *29*). Overall, transcriptional repression/activation by nuclear receptors and "switching-off" hormonal signals involve ligand-controlled alternate usage of different classes of coregulatory proteins.

Examples of Promiscuous Receptors

PXR and CAR. Pregnane X receptor [PXR; also termed steroid and xenobiotic sensor (SXR)] and constitutive androstane receptor (CAR) are members of the NR1 receptor subfamily which are activated by a variety of xenobiotics (for recent reviews, see refs *30* and *31*). These receptors coordinate detoxification processes by inducing the expression of genes involved in degradation and secretion pathways, including cytochrome P450 enzymes, uridine diphosphoglucuronosyltransferases, glutathione *S*-transferases, and sulfotransferases. Both CAR and PXR also regulate the expression of pumps that mediate secretion of toxic compounds, such as multidrug resistance proteins. Notably, while PXR and CAR regulate the expression of some distinct target genes, they also display overlapping activities, and they appear to be activated by some of the same ligands. Hence, toxin recognition and induction of detoxification pathways by the two receptors appear to be partially redundant.

PXR is activated by various endogenous steroids, including dexamethasone, estradiols, progesterone, and the progesterone precursors, pregnanes. However, binding affinities of PXR for endogenous steroids are on the order of 10 μM (*32*), many orders of magnitude higher than the concentrations of these compounds in vivo, suggesting that these compounds are unlikely to function as endogenous ligands. This receptor is also activated by a large array of synthetic steroid receptor agonists or antagonists (for a list, see ref *30*). Other proposed ligands for PXR include the cholesterol-derived compound 5β-cholestane-3α,7α,12α-triol (*33*), and xenobiotics such as rifampicin and phenobarbital. Examination of the crystal structures of the PXR−LBD complex reveals a 60-residue insert, configured as two β-strands and an α-helix adjacent to the ligand binding pocket, which is not present in other receptors (*34*). It has been suggested that this insert can facilitate a large expansion of the ligand binding pocket, thereby enabling binding of ligands of varied sizes. Indeed, the volume of the PXR pocket can vary between ~1300 Å^3 in the absence of ligand and >1600 Å^3 in the presence of a large ligand such as rifampicin (*35*). Considering the nature of the PXR target genes and the broad ligand binding selectivity of the receptor, it is usually suggested that it serves as an overall sensor of xenobiotics, acting to induce general detoxification responses (*36*).

A distinctive characteristic of CAR is that it is constitutively active in the absence of exogenously added ligands (*37*). Reported crystal structures of this receptor suggest that its constitutive activity emanates from a short and rigid H12, a short H11, and hydrogen bonds between the C-terminus and residues in helices H4 and H10, allowing the AF-2 helix, H12, to remain in an active configuration in the absence of ligand (*38*, *39*). Various biological compounds, e.g., 17β-estradiol, can activate CAR further, while others, e.g., progesterone, function as antagonists and repress its basal activity (*40*). It has been suggested that, while PXR is central

for sensing xenobiotics, CAR may mediate the transcriptional activities of endogenous biological compounds. However, the exact nature of these endogenous activators and whether any of these comprise specific high-affinity ligands remain unknown.

Peroxisome Proliferator-Activated Receptors (PPARS). Three PPAR subtypes, encoded by three separate genes, are known to exist, PPARα, PPARβ/δ, and PPARγ, and these are often termed the "lipid sensor" receptors. PPARα is expressed in liver, heart, muscle, and kidney, where it regulates fatty acid catabolism. Indeed, activation of PPARα lowers serum lipid levels, and synthetic ligands that activate this receptor are used as therapeutic agents in treatment of hyperlipidemia (*41*). PPARβ/δ is broadly expressed and is involved in various biological activities, including neuronal development (*42*), inflammation (*43*), skeletal muscle lipid oxidation (*44*), keratinocyte differentiation, and wound healing (*45*). It has been suggested that activation of PPARβ/δ is protective against obesity and insulin resistance and that this receptor may be a target for new strategies in treatment of type II diabetes (*46*). PPARγ is expressed predominantly in adipose tissue and macrophages, where it is involved in adipocyte differentiation, regulation of sugar and lipid homeostasis, and control of inflammatory responses (*47*). Thiazolidinediones, synthetic compounds that activate PPARγ, are in current use as antidiabetic drugs (*48*).

PPARs display a broad ligand selectivity, and they bind multiple fatty acids and fatty acid metabolites. Among these, it has been proposed that 8(*S*)-HETE and leukotriene B4 (LTB4) serve as endogenous ligands for PPARα (*49*) and that 15-deoxy-Δ12,14-prostaglandin J2 (PGJ2) activates PPARγ (*50*) The nature of endogenous ligands for PPARβ/δ may be more enigmatic. The ligand binding pocket of this receptor is as large as that of unliganded PXR, with an entrance area of approximately 100 Å that can become even larger due to the flexibility in surrounding helices (*51*). The extensive size of the pocket is consistent with the receptor's promiscuous ligand binding characteristic and raises the possibility that it may be activated by multiple compounds. Reported X-ray crystal structures of the recombinant PPARβ/δ−LBD complex revealed that the protein was purified with bound bacterial fatty acids such as eicosapentaenoic acid (EPA) (*51*), 11(*Z*)-octadecenoic acid, palmitic acid, and stearic acid (*52*). Like the other PPAR isotypes, biological compounds that can activate PPARβ/δ include various leukotrienes and prostaglandins. It was also recently reported that PPARβ/δ is activated by the vitamin A metabolite RA, a compound that has long been known to serve as a ligand for a different nuclear receptor, namely RAR (*23*), and that was also reported to act as an antagonist for RORβ (*53*). Hence, while all three PPARs can be activated by multiple fatty acid derivatives, it remains unclear whether any of these are true PPAR ligands in vivo.

Retinoid X Receptors (RXRs). The three RXR subtypes, RXRα, RXRβ, and RXRγ, all bind to and are potently activated by the RA isomer 9-*cis*-retinoic acid (9cRA), which is the best characterized ligand for these receptors. However, the relevance of this compound for activation of RXR in vivo, and thus its identification as the endogenous ligand, remain controversial. Several other ligands were suggested to bind to and activate RXR. For example, RXR can be activated by RA, albeit with an efficacy significantly lower that that of the 9-*cis* isomer. It was also reported that this receptor can be activated by phytanic acid, a dietary fatty acid produced by chlorophyll catabolism (*54*, *55*), and by docosahexaenoic acid (DHA, C22:6), a polyunsaturated fatty acid which is highly enriched in brain and retina (*56*). Notably, the affinity of RXR for these ligands varies widely. While the K_d of this receptor for 9cRA is ∼15 nM (*57*), the DHA concentrations required for RXR activation in the context of reporter assays were reported to be higher than 100 μM (*56*). Accordingly, it was reported that the potency by which these ligands induce coactivator recruitment by RXRβ occurs in the following order: 9cRA > RA > phytanic acid > DHA (*58*). Their relevance for the biological functions of RXR will thus depend on the endogenous concentrations of these compounds. The concentration of 9cRA in vivo has not been determined with precision and appears to be quite low and often undetectable in various tissues. DHA is highly enriched in brain and sperm but is present at very low levels in other tissues. These considerations raise the possibility that RXR (and perhaps other promiscuous receptors) may be activated by different ligands in different tissues and/or under different physiological conditions.

Strategies for Identifying Endogenous Ligands for Promiscuous Receptors

Promiscuous nuclear receptors, such as those discussed here, may bind and become activated by several, and sometimes numerous, compounds. These observations complicate clear identification of physiologically meaningful ligands that serve to activate them in vivo. It is possible that some of these receptors are truly promiscuous, i.e., have evolved to respond to classes of compounds rather than to specific ligands. However, it is also possible that some apparently promiscuous receptors do possess a selectivity toward particular compound(s), but the nature of their *bona fide* ligands has not been elucidated. Various considerations and experimental approaches may be complementarily employed in the ongoing efforts to identify such ligands and to distinguish between irrelevant compounds and ligands that serve as the endogenous activators for particular receptors.

Examining Three-Dimensional Structures of Nuclear Receptors. Determined crystal structures of nuclear receptors and of receptor−ligand complexes have provided new knowledge into candidate ligands, as well as into atomic-level details of the mechanisms by which ligands modulate receptor activities. As discussed above, the structures of unliganded PXR and of complexes of this receptor with several ligands yielded insights into the unique structural features that enable this receptor to accommodate multiple compounds. This information raises the level of confidence that PXR is indeed a promiscuous receptor, i.e., tailored to respond to several classes of compounds rather than to a single high-affinity ligand. X-ray crystallography also provided a strong rationale for classifying NURR1 as a true orphan whose LBD does not allow for ligand binding. Crystallography relies heavily on utilizing recombinant proteins, and occasionally, the structures of proteins purified from overexpressing bacteria reveal the presence of bacterial components bound to the receptor. Although such information has to be taken with caution, these fortuitous observations can lead to novel and often unexpected insights. For

example, the recently determined crystal structure of the orphan receptor LRH-1 revealed the presence of bound phospholipids, providing a hint about the possible nature of its endogenous ligands (*59*). Additionally, the crystal structure of a constitutively active RXR mutant showed that it was associated with a 16- or 18-carbon fatty acid, tentatively identified as palmitic or oleic acid (*60*). While these fatty acids associate with RXR with low affinities and are unlikely to comprise *bona fide* ligands, the observations underscore the promiscuous nature of ligand binding by this receptor. Undoubtedly, structural information will continue to inform investigations into the nature of ligands for orphan receptors.

Considering Binding Affinities. Binding affinities of nuclear receptors toward their ligands vary widely. For example, ER, RAR, and VDR bind their respective ligands, 17β-estradiol, RA, and vitamin D_3, with subnanomolar affinities. RXR associates with 9cRA, and PPARs bind various long chain fatty acids with K_d values on the order of 10−50 nM. Other receptors, such as PXR, respond to ligands at micromolar concentrations. Notably, it has been reported that some receptors that are activated by more than one ligand exhibit disparate affinities for them. For example, in contrast to its tight association with vitamin D_3, VDR can be activated by bile acids at concentrations in the micromolar range (*61*). Similarly, RXR binds 9cRA with a nanomolar affinity but responds to DHA at micromolar concentrations. Intuitively, it may be argued that whether a ligand can be considered to be relevant for the biological functions of a receptor will depend on its abundance in cells. For example, in retina, which is highly enriched in DHA, this compound may potentially activate RXR, but DHA is unlikely to play a role in RXR biology in other tissues. In the same vein, due to their high concentrations in the gut, bile acids may be involved in activation of VDR in this organ, but it is difficult to envision that they activate VDR in other tissues, at least under normal circumstances. These considerations also suggest that, although PXR can be activated by high concentrations of steroid hormones, the low levels of these hormones in cells render them unlikely candidates for endogenous ligands for this receptor. Comparison between receptor−ligand affinities and the physiological concentrations of candidate ligands may thus serve as a guideline for initial determination of the potential relevance of specific ligands for receptor function. However, as discussed below, other parameters may bypass these considerations.

Consulting with Lipid Binding Proteins. The cooperation of some nuclear receptors with particular iLBPs that deliver ligands to them enables a new strategy in the search for orphan receptors' ligands. Hence, as these iLBPs mobilize to the nucleus in a selective response to compounds that activate their cognate receptors, the criteria for assigning a ligand to a particular receptor should include not only the fact that the ligand activates the receptor but also the fact that it induces the nuclear translocation of the respective binding protein. This powerful tool for dissecting relevant from irrelevant ligands can be applied to receptors with known cognate iLBP, a list that currently includes three orphan promiscuous receptors: PPARα, PPARγ, and PPARβ/δ, which cooperate with iLBPs FABP3, FABP4, and FABP5, respectively (*19*, *23*, *25*, *26*). It is unknown at present whether the transcriptional activities of other receptors also rely on proteins that shuttle ligands to the nucleus. However, considering that the details of the biological roles of all but three of the nine known FABP isotypes remain to be elucidated, and that other proteins that bind hydrophobic compounds, such as acyl binding proteins and sterol carrier protein, are known to exist, it is possible that some of these function in this fashion.

It should be noted that the involvement of iLBPs in receptor function may circumvent strict binding affinity considerations. This is exemplified by the recent reports that RA activates not only its classical receptor RAR but also the orphan PPARβ/δ, and that both of these receptors cooperate with iLBPs (*23*, *62*). The K_d that characterizes the association of RA with PPARβ/δ is ∼20 nM, reflecting an ∼2 order of magnitude lower affinity for RA as compared to RAR. Binding affinity consideration would thus suggest that RA will predominantly signal through RAR in any cell that contains the two receptors at similar levels. However, as the partitioning of RA between its two receptors is regulated not by the receptors themselves but by their cognate iLBPs, CRABP-II and FABP5, RA preferentially activates PPARβ/δ in cells that express a high FABP5/CRABP-II ratio despite similar RAR and PPARβ/δ expression levels, and the much lower affinity of the latter (*23*). These observations further emphasize the need for information about the mechanisms by which ligands reach their cognate receptors.

Asking the Cell. The most straightforward approach for identifying relevant endogenous ligands may be to conduct investigations into ligands that are associated with receptors in cells and tissues. Various versions of such a strategy have been used in the past. For example, information available in 1992 suggested that RXR can be activated by a RA derivative, but the nature of the derivative was unknown. To identify the compound, Levin et al. (*63*) reasoned that nuclei of cells enriched with RXR will selectively bind compounds with a high affinity for the receptor, and thus effectively "trap" the ligand. Thus, they transfected cells with RXR, treated them with radiolabeled RA, isolated nuclei, extracted lipopholic compounds into an organic solvent, and analyzed the mixture by HPLC. The analysis revealed that the trapped ligand coeluted with the 9-*cis* isomer of RA. In a somewhat different approach, Heyman et al. (*64*) treated cells with RA, and fractionated lipid extracts of treated cells using an HPLC protocol that allows for separation of various retinoids. Fractions were individually collected and tested using reporter gene assays for the presence of ligand that can activate RXR. Subsequently, HPLC, chemical derivatization, and gas chromatography−mass spectroscopy analyses identified the species present in the fraction that displayed the most potent activity to be 9-*cis*-RA. While these studies were aided by the preliminary observations that an RXR ligand may be a retinoid, identification of a completely unknown compound is more complex. However, recent advances in analytical techniques, especially in lipidomics and mass spectroscopy methodologies, should enable the identification of ligands that are associated with receptors isolated from their native environments.

Conclusions

Approximately half of the members of the nuclear receptor superfamily remain classified as orphan receptors, denoting that the nature of the ligands that activate them in vivo is

unknown. The pleiotropic activities of nuclear receptors and their involvement in multiple biological functions underscore the importance of identifying their ligands. Such information will considerably enhance our understanding of the biological roles of these receptors and may potentially be useful in developing strategies for utilizing various receptors as therapeutic targets. The identification of ligands for orphan receptors that display promiscuous ligand selectivities is particularly difficult because of the need to dissect between compounds that may bind to receptors in vitro and activate them in the context of cultured cell models, and agents that function as true endogenous activators. Future studies may utilize various approaches to arrive at clear identification of *bona fide* ligands. These may include solutions of new three-dimensional structures of receptors and receptor−ligand complexes, raising search stringencies by considering the ligand selectivities of accessory proteins that closely cooperate with receptors, such as cognate iLBPs, and direct determination of the structures of ligands that are associated with receptors when isolated from tissues.

REFERENCES

1. Laudet, V., and Gronemeyer, H. (2002) *The Nuclear Receptor FactsBook*, Academic Press, London.
2. Wang, Z., Benoit, G., Liu, J., Prasad, S., Aarnisalo, P., Liu, X., Xu, H., Walker, N. P., and Perlmann, T. (2003) Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors, *Nature 423*, 555−560.
3. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1995) The nuclear receptor superfamily: The second decade, *Cell 83*, 835−839.
4. Berg, J. M. (1989) DNA binding specificity of steroid receptors, *Cell 57*, 1065−1068.
5. Moras, D., and Gronemeyer, H. (1998) The nuclear receptor ligand-binding domain: Structure and function, *Curr. Opin. Cell Biol. 10*, 384−391.
6. Bourguet, W., Germain, P., and Gronemeyer, H. (2000) Nuclear receptor ligand-binding domains: Three-dimensional structures, molecular interactions and pharmacological implications, *Trends Pharmacol. Sci. 21*, 381−388.
7. Gronemeyer, H., Gustafsson, J. A., and Laudet, V. (2004) Principles for modulation of the nuclear receptor superfamily, *Nat. Rev. 3*, 950−964.
8. Brelivet, Y., Kammerer, S., Rochel, N., Poch, O., and Moras, D. (2004) Signature of the oligomeric behaviour of nuclear receptors at the sequence and structural level, *EMBO Rep. 5*, 423−429.
9. Kersten, S., Reczek, P. R., and Noy, N. (1997) The tetramerization region of the retinoid X receptor is important for transcriptional activation by the receptor, *J. Biol. Chem. 272*, 29759−29768.
10. Gampe, R. T., Jr., Montana, V. G., Lambert, M. H., Wisely, G. B., Milburn, M. V., and Xu, H. E. (2000) Structural basis for autorepression of retinoid X receptor by tetramer formation and the AF-2 helix, *Genes Dev. 14*, 2229−2241.
11. Naar, A. M., Boutin, J. M., Lipkin, S. M., Yu, V. C., Holloway, J. M., Glass, C. K., and Rosenfeld, M. G. (1991) The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors, *Cell 65*, 1267−1279.
12. Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991) Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D_3 receptors, *Cell 65*, 1255−1266.
13. Kersten, S., Dong, D., Lee, W., Reczek, P. R., and Noy, N. (1998) Auto-silencing by the retinoid X receptor, *J. Mol. Biol. 284*, 21−32.
14. Kersten, S., Kelleher, D., Chambon, P., Gronemeyer, H., and Noy, N. (1995) Retinoid X receptor α forms tetramers in solution, *Proc. Natl. Acad. Sci. U.S.A. 92*, 8645−8649.
15. Thompson, P. D., Jurutka, P. W., Haussler, C. A., Whitfield, G. K., and Haussler, M. R. (1998) Heterodimeric DNA binding by the vitamin D receptor and retinoid X receptors is enhanced by 1,25-dihydroxyvitamin D_3 and inhibited by 9-cis-retinoic acid. Evidence for allosteric receptor interactions, *J. Biol. Chem. 273*, 8483−8491.
16. Dong, D., and Noy, N. (1998) Heterodimer formation by retinoid X receptor: Regulation by ligands and by the receptor's self-association properties, *Biochemistry 37*, 10691−10700.
17. Picard, D., and Yamamoto, K. R. (1987) Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor, *EMBO J. 6*, 3333−3340.
18. Feige, J. N., Gelman, L., Tudor, C., Engelborghs, Y., Wahli, W., and Desvergne, B. (2005) Fluorescence imaging reveals the nuclear behavior of peroxisome proliferator-activated receptor/retinoid X receptor heterodimers in the absence and presence of ligand, *J. Biol. Chem. 280*, 17880−17890.
19. Tan, N. S., Shaw, N. S., Vinckenbosch, N., Liu, P., Yasmin, R., Desvergne, B., Wahli, W., and Noy, N. (2002) Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription, *Mol. Cell. Biol. 22*, 5114−5127.
20. Budhu, A. S., and Noy, N. (2002) Direct channeling of retinoic acid between cellular retinoic acid-binding protein II and retinoic acid receptor sensitizes mammary carcinoma cells to retinoic acid-induced growth arrest, *Mol. Cell. Biol. 22*, 2632−2641.
21. Budhu, A., Gillilan, R., and Noy, N. (2001) Localization of the RAR interaction domain of cellular retinoic acid binding protein-II, *J. Mol. Biol. 305*, 939−949.
22. Manor, D., Shmidt, E. N., Budhu, A., Flesken-Nikitin, A., Zgola, M., Page, R., Nikitin, A. Y., and Noy, N. (2003) Mammary carcinoma suppression by cellular retinoic acid binding protein-II, *Cancer Res. 63*, 4426−4433.
23. Schug, T. T., Berry, D. C., Shaw, N. S., Travis, S. N., and Noy, N. (2007) Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors, *Cell 129*, 723−733.
24. Sessler, R. J., and Noy, N. (2005) A ligand-activated nuclear localization signal in cellular retinoic acid binding protein-II, *Mol. Cell 18*, 343−353.
25. Ayers, S. D., Nedrow, K. L., Gillilan, R. E., and Noy, N. (2007) Continuous Nucleocytoplasmic Shuttling Underlies Transcriptional Activation of PPARγ by FABP4, *Biochemistry 46*, 6744−6752.
26. Gillilan, R. E., Ayers, S. D., and Noy, N. (2007) Structural Basis for Activation of Fatty Acid-binding Protein 4, *J. Mol. Biol.* (in press).
27. Xu, L., Glass, C. K., and Rosenfeld, M. G. (1999) Coactivator and corepressor complexes in nuclear receptor function, *Curr. Opin. Genet. Dev. 9*, 140−147.
28. Bour, G., Lalevee, S., and Rochette-Egly, C. (2007) Protein kinases and the proteasome join in the combinatorial control of transcription by nuclear retinoic acid receptors, *Trends Cell Biol. 17*, 302−309.
29. Wu, R. C., Feng, Q., Lonard, D. M., and O'Malley, B. W. (2007) SRC-3 coactivator functional lifetime is regulated by a phospho-dependent ubiquitin time clock, *Cell 129*, 1125−1140.
30. Kretschmer, X. C., and Baldwin, W. S. (2005) CAR and PXR: Xenosensors of endocrine disrupters? *Chem.-Biol. Interact. 155*, 111−128.
31. Pascussi, J. M., Gerbal-Chaloin, S., Duret, C., Daujat-Chavanieu, M., Vilarem, M. J., and Maurel, P. (2007) The Tangle of Nuclear Receptors that Controls Xenobiotic Metabolism and Transport: Crosstalk and Consequences, *Annu. Rev. Pharmacol. Toxicol.* (in press).
32. Kliewer, S. A., Moore, J. T., Wade, L., Staudinger, J. L., Watson, M. A., Jones, S. A., McKee, D. D., Oliver, B. B., Willson, T. M., Zetterstrom, R. H., Perlmann, T., and Lehmann, J. M. (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway, *Cell 92*, 73−82.
33. Dussault, I., Yoo, H. D., Lin, M., Wang, E., Fan, M., Batta, A. K., Salen, G., Erickson, S. K., and Forman, B. M. (2003) Identification of an endogenous ligand that activates pregnane X receptor-mediated sterol clearance, *Proc. Natl. Acad. Sci. U.S.A. 100*, 833−838.
34. Watkins, R. E., Wisely, G. B., Moore, L. B., Collins, J. L., Lambert, M. H., Williams, S. P., Willson, T. M., Kliewer, S. A., and Redinbo, M. R. (2001) The human nuclear xenobiotic receptor PXR: Structural determinants of directed promiscuity, *Science 292*, 2329−2333.
35. Chrencik, J. E., Orans, J., Moore, L. B., Xue, Y., Peng, L., Collins, J. L., Wisely, G. B., Lambert, M. H., Kliewer, S. A., and Redinbo, M. R. (2005) Structural disorder in the complex of human

pregnane X receptor and the macrolide antibiotic rifampicin, *Mol. Endocrinol. 19*, 1125−1134.
36. Dussault, I., and Forman, B. M. (2002) The nuclear receptor PXR: A master regulator of "homeland" defense, *Crit. Rev. Eukaryotic Gene Expression 12*, 53−64.
37. Baes, M., Gulick, T., Choi, H. S., Martinoli, M. G., Simha, D., and Moore, D. D. (1994) A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements, *Mol. Cell. Biol. 14*, 1544−1552.
38. Suino, K., Peng, L., Reynolds, R., Li, Y., Cha, J. Y., Repa, J. J., Kliewer, S. A., and Xu, H. E. (2004) The nuclear xenobiotic receptor CAR: Structural determinants of constitutive activation and heterodimerization, *Mol. Cell 16*, 893−905.
39. Xu, R. X., Lambert, M. H., Wisely, B. B., Warren, E. N., Weinert, E. E., Waitt, G. M., Williams, J. D., Collins, J. L., Moore, L. B., Willson, T. M., and Moore, J. T. (2004) A structural basis for constitutive activity in the human CAR/RXRα heterodimer, *Mol. Cell 16*, 919−928.
40. Kawamoto, T., Kakizaki, S., Yoshinari, K., and Negishi, M. (2000) Estrogen activation of the nuclear orphan receptor CAR (constitutive active receptor) in induction of the mouse Cyp2b10 gene, *Mol. Endocrinol. 14*, 1897−1905.
41. Mandard, S., Muller, M., and Kersten, S. (2004) Peroxisome proliferator-activated receptor α target genes, *Cell. Mol. Life Sci. 61*, 393−416.
42. Cimini, A., Bernardo, A., Cifone, M. G., Di Marzio, L., and Di Loreto, S. (2003) TNFα downregulates PPARδ expression in oligodendrocyte progenitor cells: Implications for demyelinating diseases, *Glia 41*, 3−14.
43. Tan, N. S., Michalik, L., Noy, N., Yasmin, R., Pacot, C., Heim, M., Fluhmann, B., Desvergne, B., and Wahli, W. (2001) Critical roles of PPARβ/δ in keratinocyte response to inflammation, *Genes Dev. 15*, 3263−3277.
44. Wang, Y. X., Zhang, C. L., Yu, R. T., Cho, H. K., Nelson, M. C., Bayuga-Ocampo, C. R., Ham, J., Kang, H., and Evans, R. M. (2004) Regulation of muscle fiber type and running endurance by PPARδ, *PLoS Biol. 2*, e294.
45. Tan, N. S., Michalik, L., Desvergne, B., and Wahli, W. (2004) Peroxisome proliferator-activated receptor-β as a target for wound healing drugs, *Expert Opin. Ther. Targets 8*, 39−48.
46. Barish, G. D., Narkar, V. A., and Evans, R. M. (2006) PPARδ: A dagger in the heart of the metabolic syndrome, *J. Clin. Invest. 116*, 590−597.
47. Lehrke, M., and Lazar, M. A. (2005) The many faces of PPARγ, *Cell 123*, 993−999.
48. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., and Kliewer, S. A. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ), *J. Biol. Chem. 270*, 12953−12956.
49. Desvergne, B., and Wahli, W. (1999) Peroxisome proliferator-activated receptors: Nuclear control of metabolism, *Endocr. Rev. 20*, 649−688.
50. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) 15-Deoxy-Δ12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ, *Cell 83*, 803−812.
51. Xu, H. E., Lambert, M. H., Montana, V. G., Parks, D. J., Blanchard, S. G., Brown, P. J., Sternbach, D. D., Lehmann, J. M., Wisely, G. B., Willson, T. M., Kliewer, S. A., and Milburn, M. V. (1999) Molecular recognition of fatty acids by peroxisome proliferator-activated receptors, *Mol. Cell 3*, 397−403.
52. Fyffe, S. A., Alphey, M. S., Buetow, L., Smith, T. K., Ferguson, M. A., Sorensen, M. D., Bjorkling, F., and Hunter, W. N. (2006) Recombinant human PPARβ/δ ligand-binding domain is locked in an activated conformation by endogenous fatty acids, *J. Mol. Biol. 356*, 1005−1013.
53. Stehlin-Gaon, C., Willmann, D., Zeyer, D., Sanglier, S., Van Dorsselaer, A., Renaud, J. P., Moras, D., and Schule, R. (2003) All-trans retinoic acid is a ligand for the orphan nuclear receptor RORβ, *Nat. Struct. Biol. 10*, 820−825.
54. Lemotte, P. K., Keidel, S., and Apfel, C. M. (1996) Phytanic acid is a retinoid X receptor ligand, *Eur. J. Biochem. 236*, 328−333.
55. Kitareewan, S., Burka, L. T., Tomer, K. B., Parker, C. E., Deterding, L. J., Stevens, R. D., Forman, B. M., Mais, D. E., Heyman, R. A., McMorris, T., and Weinberger, C. (1996) Phytol metabolites are circulating dietary factors that activate the nuclear receptor RXR, *Mol. Biol. Cell 7*, 1153−1166.
56. de Urquiza, A. M., Liu, S., Sjoberg, M., Zetterstrom, R. H., Griffiths, W., Sjovall, J., and Perlmann, T. (2000) Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain, *Science 290*, 2140−2144.
57. Kersten, S., Dawson, M. I., Lewis, B. A., and Noy, N. (1996) Individual subunits of heterodimers comprised of retinoic acid and retinoid X receptors interact with their ligands independently, *Biochemistry 35*, 3816−3824.
58. Stafslien, D. K., Vedvik, K. L., De Rosier, T., and Ozers, M. S. (2007) Analysis of ligand-dependent recruitment of coactivator peptides to RXRβ in a time-resolved fluorescence resonance energy transfer assay, *Mol. Cell. Endocrinol. 264*, 82−89.
59. Ortlund, E. A., Lee, Y., Solomon, I. H., Hager, J. M., Safi, R., Choi, Y., Guan, Z., Tripathy, A., Raetz, C. R., McDonnell, D. P., Moore, D. D., and Redinbo, M. R. (2005) Modulation of human nuclear receptor LRH-1 activity by phospholipids and SHP, *Nat. Struct. Mol. Biol. 12*, 357−363.
60. Bourguet, W., Vivat, V., Wurtz, J. M., Chambon, P., Gronemeyer, H., and Moras, D. (2000) Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains, *Mol. Cell 5*, 289−298.
61. Makishima, M., Lu, T. T., Xie, W., Whitfield, G. K., Domoto, H., Evans, R. M., Haussler, M. R., and Mangelsdorf, D. J. (2002) Vitamin D receptor as an intestinal bile acid sensor, *Science 296*, 1313−1316.
62. Shaw, N., Elholm, M., and Noy, N. (2003) Retinoic acid is a high affinity selective ligand for the peroxisome proliferator-activated receptor β/δ, *J. Biol. Chem. 278*, 41589−41592.
63. Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A., et al. (1992) 9-cis-Retinoic acid stereoisomer binds and activates the nuclear receptor RXRα, *Nature 355*, 359−361.
64. Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M., and Thaller, C. (1992) 9-cis-Retinoic acid is a high affinity ligand for the retinoid X receptor, *Cell 68*, 397−406.

BI7018699