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Review

Retinoid X receptors: X-ploring their (patho)physiological functions

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Abstract

Retinoid X receptor (RXR) belongs to a family of ligand-activated transcription factors that regulate many aspects of metazoan life. A class of nuclear receptors requires RXR as heterodimerization partner for their function. This places RXR in the crossroad of multiple distinct biological pathways. This and the fact that the debate on the endogenous ligand requirement for RXR is not yet settled make RXR still an enigmatic transcription factor. Here, we review some of the biology of RXR. We place RXR into the evolution of nuclear receptors, review structural details and ligands of the receptor. Then processes regulated by RXR are discussed focusing on the developmental roles deduced from studies on knockout animals and metabolic roles in diseases such as diabetes and atherosclerosis deduced from pharmacological studies. Finally, aspects of RXR's involvement in myeloid differentiation and apoptosis are summarized along with issues on RXR's suitability as a therapeutic target.
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Keywords: nuclear receptor; RXR; transcription; retinoid; rexinoid

Abbreviations: RXR, retinoid X receptor; RA, retinoic acid; RAR, retinoic acid receptor; PPAR, peroxisome proliferator-activated receptor; LXR, liver X receptor; PXR, pregnane X receptor; FXR, farnesoid X receptor; DBD, DNA-binding domain; LBD, ligand-binding domain; HRE, hormone response element

Introduction

Vitamin A and its derivatives, retinoids, have profound effects in development, differentiation, homeostasis and various aspects of metabolism. The discovery of retinoid receptors substantially contributed to understanding how these small, lipophilic molecules, most importantly retinoic acid (RA), exert their pleiotropic effects.[1,2] Retinoid receptors belong to the family of nuclear hormone receptors, which includes steroid hormone, thyroid hormone and vitamin D receptors, various orphan receptors and also receptors activated by intermediary metabolites: for example, peroxisome proliferator-activated receptor (PPAR) by fatty acids, liver X receptor (LXR) by cholesterol metabolites, farnesoid X receptor (FXR) by bile acids and pregnane X receptor (PXR) by xenobiotics.[3,4] Members of this family function as ligand-activated transcription factors with a conserved domain structure: (1) DNA-binding domain (DBD) for anchoring the protein to specific DNA sequences, hormone response elements (HREs), (2) ligand-binding domain (LBD) for binding of small lipophilic molecules and (3) transactivation domain for activating the basal transcriptional machinery.[5,6] After the identification of all-*trans* retinoic acid (ATRA) receptor, another receptor termed retinoid X receptor (RXR) was discovered, which was capable of mediating retinoid signaling pathways.[7] RXR was also independently identified as a 'coregulator' necessary for efficient binding of retinoic acid receptors (RARs) to their response elements.[8–10] Most importantly, RXR was shown to form heterodimers with many other nuclear receptors,[11–14] making it unique among the members of the nuclear receptor family. The two families of retinoid receptors (RARs and RXRs) now consist of three isotypes, α, β and γ, encoded by separate genes and giving rise to numerous alternatively spliced variants.[6,15] RARs can be activated by ATRA and 9-*cis* RA, while RXRs can be activated only by 9-*cis* RA.[16,17] There are natural and synthetic compounds with selectivity for RXR[18–20] termed rexinoids.[21] RXR is unique among nuclear receptors because it forms many heterodimers, and therefore ligand activation has potentially pleiotropic effects on numerous biological pathways, but this mode of action also has the potential to independently regulate processes.

There is still no consensus about the role of RXRs. Is there really a separate RXR-mediated signaling pathway or does it serve only as a partner for other nuclear receptors and its sole role is only to modulate their actions? There have been several attempts to identify new retinoid/rexinoid-regulated processes, but these ultimately led to the identification of new partners for RXR, Nurr1 and LXR, respectively.[22,23] One of the key issues of the field is whether separable RXR responses exist, more specifically whether RXR homodimers can function as biologically relevant transcription units. RXR is unique in its ability to form both homo- and heterodimers. Unliganded RXRs are believed to preferentially form tetramers[24] that dissociate upon ligand binding,[25] leading to altered dimerization surface and formation of RXR homodimers or heterodimers in the presence of partners.[26,27]

Recently, RXR homodimers have been examined: first by the generation of mutants that showed increased homodimerization capacity over heterodimerization[28] and then by demonstrating that homodimers could activate PPAR target genes *in vivo*.[29] The other line of evidence comes from *in vivo* experiments. In rodent models of type 2 diabetes, rexinoids showed similar effects to thiazolidinedions (TZDs), a class of insulin sensitizers that act as PPARγ agonists.[21,30,31] However, later a clear difference could be detected in the tissue-specific gene expression patterns induced by the two drugs when compared.[32,33] Studies on RXR homodimers and observations in RXR agonist-treated animals revealed that RXR-specific signaling might exist and may serve as target for new therapeutic approaches. It would be critical to understand how the potentially pleiotropic rexinoids work and if there are processes regulated only by RXR, and to understand the therapeutic potential of this class of drugs. The aim of this review is to provide an overview of RXR's biology and highlight those RXR- and/or rexinoid-regulated processes that have pathophysiological importance and therapeutic potential. We must note in the outset that although numerous laboratories contributed to the body of research here, the work of the Chambon and the Evans laboratories set the pace in this field.

Evolution of nuclear receptors

Since the initial cloning of the glucocorticoid receptor (GR) in 1984,[34–36] hundreds of nuclear receptors have been isolated, cloned, sequenced and studied from a variety of organisms. The completion of genome projects gave us more definitive answers regarding the number of nuclear receptors in the different species. For example, in the worm *Caenorhabditis elegans*, 270 putative nuclear receptor genes exist, and the complete genome of *Drosophila melanogaster* contains 21 nuclear receptors, while 48 and 49 receptors have been identified in the human and mouse genomes, respectively.[37,38] The nucleic acid and the deduced protein sequences derived from various species show a high degree of conservation. The phylogeny of nuclear receptors has been studied by several authors and it has been established that nuclear receptors appeared very early during metazoan evolution and are present in all metazoan phyla.[39–41] No nuclear receptors have been found in fungi, plants or unicellular eukaryotes so far. Sequence alignment and phylogenetic analysis led to a comprehensive classification of nuclear receptors. The majority of nuclear receptors can be grouped into six subfamilies[42]:

1. A large subfamily includes the well-characterized TR, RAR, VDR and PPARs, receptors that form heterodimers with RXR as well as orphan receptors like ROR, Rev-erb, CAR, etc.
2. This group contains RXRs and the related chicken ovalbumin upstream promoter-transcription factor (COUP-TF), HNF4. Several receptors function as homodimers; RXR forms heterodimers with Group 1 and 4 receptors.
3. This is the 'classical' steroid receptor subfamily with estrogen receptor (ER), GR, progesterone receptor, androgen receptor, etc.
4. A smaller group includes NGFIB, Nurr1 and Nor1.
5. Another small group has receptors related to the *Drosophila* Fushi Tarazu Factor-1 (FTF-F1) and the mammalian steroidogenic factor receptors.
6. This group contains germ cell nuclear factor, which does not fit well into any other groups.

The conserved domain structures and the distribution in the various subfamilies suggest that nuclear receptors underwent a rapid evolution during early metazoan development. It is presumed that nuclear receptors emerged explosively after two waves of gene duplication events: the first wave during the emergence of metazoans, leading to the formation of members in the six subfamilies, and the second wave later, mainly in vertebrates, leading to the divergence of the paralogue receptors (e.g. RARα, β, γ, RXRα, β, γ). Group 3 steroid receptors were reported to be absent in invertebrates and to be the most recently emerged family. However, recently an ER orthologue was discovered in the mollusk, *Aplysia californica*.[43] It was also suggested that steroid receptors were derived from an ancient gene before the appearance of the bilaterally symmetric animals and this gene was lost in arthropods and nematodes. Cnidarians (coelenterates), which include jelly fish and sea anemones, were reported as the earliest metazoans in which nuclear receptors emerged.[44] Interestingly, these organisms express Group 2 and 5 receptors: RXR, COUP-TF homologue and an FTF-F1 homologue. Very recently, a nuclear receptor was identified in a sponge, *Suberites domuncula*, which belongs to Group 2, indicating that nuclear receptors were present at the base of metazoan evolution and RXR homologues might be indeed the most ancient members of the family.[45] From an evolutionary point of view, RXR seems to be an ancestral nuclear receptor from which many of the other receptor families emerged. In Figure 1, we show the phylogenetic tree of RXRs constructed from full-length protein sequences. It is interesting to note that the LBDs of the RXRs share a high degree of similarity from jellyfish to humans, all showing binding specificity for 9-*cis* RA as ligand.[44] An intriguing observation is that the early RXR homologue in jellyfish was capable of monomeric DNA binding. Jellyfish RXR binds 9-*cis* RA and probably induces transcription also. The more advanced arthropods including the insects have no RXR *per se* but express ultraspiracle (USP), a homologue of the RXR.[46,47] Although the amino-acid sequence of USP is different from that of RXR and USP does not bind 9-*cis* RA, its crystal structure shows similar structural features in the two receptors.[48,49] This model suggests that RXR's ligand binding appeared very early in metazoan development and there was a secondary loss mainly in arthropods. RXR signaling appeared well before RARs emerged, suggesting that the RXR signal pathway is independent from RAR and that autonomous RXR signaling (independent of RAR and other partners) may still persist.

Structure of RXR

Similar to other nuclear receptors, RXRs have a variable N-terminal domain (A/B domain), a highly conserved DBD, a nonconserved hinge and a moderately conserved C-terminus including the LBD. The DBD consists of two

cysteine-rich zinc-finger motifs through which nuclear receptors bind to specific DNA sequences, HREs PuG(G/T)TCA(X)$_n$PuG(G/T)TCA. These consensus nucleotide sequences form direct, indirect or inverted repeats, which consist of two half sites separated by a short spacer.[15,50] A highly conserved part in the first zinc-finger between the last two cysteines is the P-box, which determines the sequence specificity of the receptor–DNA binding.[51,52] Another conserved part in the second zinc-finger is the D-box, which dictates the half site spacing.

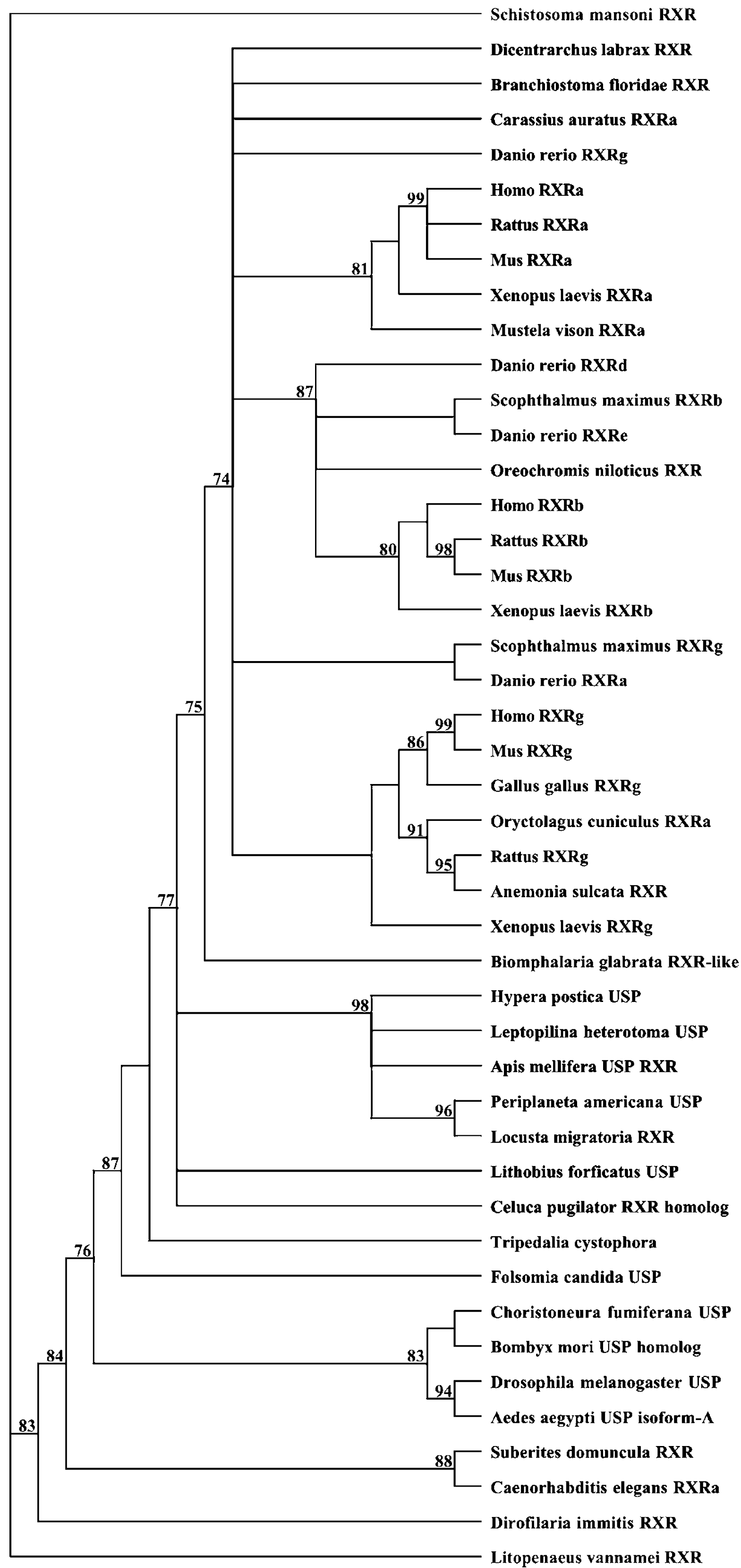
Transcriptional activation is mediated by the LBD, which contains four more-or-less overlapping surfaces: a ligand-binding pocket for binding of small, lipophilic molecules, a transactivation domain (AF2 or helix 12), a cofactor binding surface and a dimerization surface. The RXR LBD shares a common overall structure with other nuclear receptors: a three-layered alpha-helical sandwich (Figure 2). In the lower part of the structure, a nonpolar cavity, the ligand-binding pocket, is located. This pocket is sealed by a two-stranded α-sheet and the C-terminal helix 12. In some nuclear receptors (e.g. PPAR, LXR, PXR) the ligand-binding pocket is relatively large and is not fully occupied by the known ligands. In contrast to these, nuclear receptors with high-affinity ligands, like RXR, have a tighter fit between ligand and receptor. The ligand-binding pocket is much smaller (generally 400–500 Å^3), and 9-*cis* RA, for example, occupies most of the volume of the cavity. Binding of a ligand to the apoenzyme leads to conformational changes: it alters the ability of the receptor to multimerize, it also alters the surfaces that determine homo- or heterodimerization and it alters the receptor's cofactor-binding surface. These changes (mainly due to the rearrangement of helices 10/11 and 12) cause a ligand-induced switch from the receptor's repressor activity to an activation state (Figure 2).

RXR is a promiscuous dimerization partner for Group 1 receptors, but it is also capable of inducing transcription also as a homodimer. The dimerization interface of the nuclear receptors is determined mainly by residues of helix 10 with minor contributions from helix 9 and the loop between helices 8 and 9.[53,54] The steroid receptors that homodimerize harbor a symmetric interface, while Group 1 receptors that form heterodimers with RXR have an asymmetric interface for the association. In the case of RXR, the dimerization interface is energetically stable in both asymmetric and symmetric configurations. Formation of RXR homodimers leads to the appearance of a new dimerization surface (helices 11 and 12) that allows the dimers to form tetramers.[26,27] Although tetramers' DNA-binding capacity is preserved, helix 12 cannot participate in dimerization and form cofactor-binding surfaces; therefore, RXRs are most likely sequestered in tetramers.[24,55,56] When the ligand is present, it induces conformational changes effecting helix 12, leading to the loss of tetramerization interface and dissociation of the tetramers, and subsequently, RXRs form homodimers or heterodimers depending on the availability of partners.[26,27] This is a particularly attractive model, which suggests that ligands have a unique and distinct function when compared to other receptors. The heterodimerization interface is somewhat larger than the homodimerization interface (550 *versus* 500 Å^2), and this means that heterodimer formation is preferable when a partner is present.[53,54] Recently, a Tyr402 mutation in RXR's helix 9 has been reported to diminish RXR's ability to form heterodimers while exhibiting enhanced homodimerization.[28] This mutant is the first of its kind that separates RXR's homo- and heterodimer functions. It is important to note that ligand binding in the ligand-binding cavity can also lead to conformational changes (mainly of helix 7) that affect the dimerization surface and the conformation of the partner's helix 12, which results in the activation of the partner. This phenomenon may explain the permissiveness or non-permissiveness of the various RXR heterodimers. In the case of some receptors (e.g. PPARs, LXR, FXR), the length of helix 12 allows the positioning of these residues, most likely making the heterodimer 'permissive' for activation from the RXR side. In other receptors like RAR, TR and VDR, this repositioning cannot occur and therefore these heterodimers might become 'nonpermissive' (i.e. cannot be activated from the RXR side).[53,54] It is also notable that other much weaker dimerization surfaces exist in the DBD establishing a weak response element-specific interface.

Ligand binding is only a part of the nuclear receptor functions. Several coregulators have been identified that regulate unliganded and liganded nuclear receptor functions. A protein complex regulates nuclear receptor actions, which comprise chromatin remodeling enzymes, corepressors or coactivators depending on the state of the receptor. Both coactivators and corepressors contain interaction domains with a motif of LxxLL in coactivators[57,58] and LxxxIxxxI/L in corepressors.[59–61] From structural studies, we know that the coactivator peptide binds to helices 3, 4 and 12.[58,62,63] Helix 12 position is critical in the interaction. In a liganded receptor, it is placed in the active position (a charged clamp is formed on the surface), which favors coactivator binding and transactivation. In an unliganded receptor, helix 12 is placed into an adjacent groove of the LBD, forming an alternative binding surface, which favors corepressor binding and repression (Figure 2). This phenomenon has been termed as ligand-induced switch and reviewed in Nagy and Schwabe.[64]

Unliganded RXR monomer is likely to be rare because helix 12 extends away facilitating di- or tetramerization. Ligand binding induces conformational changes where helix 12 is repositioned onto the body of the LBD that generates an active conformation capable of coactivator binding. In the presence of a partial agonist ligand, helix 12 is bound in an antagonist conformation,[53] which allows corepressor binding. Although the structure of RXR with a full antagonist has not been solved, it is very likely that as in the case of other receptors,

Figure 1 Phylogenetic tree of the selected RXR and USP proteins. Full protein sequences of various RXRs and USPs were chosen to show the relationship among the metazoan RXR molecules. Protein sequence and phylogenetic analysis was performed with MacVector 7.2.2 (Accelrys). In all, 45 primary amino-acid sequences from GeneBank were used for the alignment utilizing Blosum matrix. An unrooted tree based on neighbor-joining phylogeny was calculated and no correction was applied to the distance matrix. A total of 1000 bootstrap replicates were calculated and the resulting values, higher than 70% are indicated as percentages on the branches of the tree


Schistosoma mansoni RXR
Dicentrarchus labrax RXR
Branchiostoma floridae RXR
Carassius auratus RXRa
Danio rerio RXRg
99
Homo RXRa
Rattus RXRa
Mus RXRa
81
Xenopus laevis RXRa
Mustela vison RXRa
Danio rerio RXRd
87
Scophthalmus maximus RXRb
Danio rerio RXRe
Oreochromis niloticus RXR
74
Homo RXRb
80
98
Rattus RXRb
Mus RXRb
Xenopus laevis RXRb
Scophthalmus maximus RXRg
Danio rerio RXRa
75
99
Homo RXRg
86
Mus RXRg
Gallus gallus RXRg
91
Oryctolagus cuniculus RXRa
95
Rattus RXRg
Anemonia sulcata RXR
77
Xenopus laevis RXRg
Biomphalaria glabrata RXR-like
98
Hypera postica USP
Leptopilina heterotoma USP
Apis mellifera USP RXR
96
Periplaneta americana USP
Locusta migratoria RXR
87
Lithobius forficatus USP
Celuca pugilator RXR homolog
76
Tripedalia cystophora
Folsomia candida USP
Choristoneura fumiferana USP
Bombyx mori USP homolog
84
83
94
Drosophila melanogaster USP
Aedes aegypti USP isoform-A
83
88
Suberites domuncula RXR
Caenorhabditis elegans RXRa
Dirofilaria immitis RXR
Litopenaeus vannamei RXR

RXR is also in an antagonist conformation because the ligand-binding pocket cannot be closed with helix 12. Similarly, deletion of helix 12 results in an antagonist conformation with increased corepressor binding by both the RXR and its partner.[65] In permissive heterodimers as PPAR, the binding of a PPAR activator results in conformational change, PPAR's helix 12 is repositioned and a strong coactivator binding site is generated.[66] This leads to the binding of coactivators to the PPAR. Liganding of the RXR side is likely to be different. Conformational changes after binding of an RXR agonist may cause changes in the PPAR structure by docking its helix 12 to the coactivator binding site and contributing to the formation of an agonist conformation. In nonpermissive heterodimers, corepressor binding is more complex. These receptors are believed to unmask RXR's corepressor binding surface leading to corepressor binding that stabilizes this conformation. Binding of an RXR agonist alone is not sufficient for corepressor release.[65,67] Several key mechanisms of the ligand-induced switch in heterodimers and the role of RXR in it need to be identified or further refined.

Function of RXR

It has been shown that vitamin A and its biologically active derivatives have important roles in vertebrate development, cellular differentiation and homeostasis. In 1990, it was discovered by Mangelsdorf *et al*. that one of the orphan receptors represented a novel retinoid-responsive transcription factor,[7] which was called RXR. The fact that RAR is more similar to the thyroid hormone receptor (TR) than it is to RXR suggests that these two groups of retinoid receptors are implicated in the regulation of distinct pathways and do not represent redundant retinoid signaling pathways. The homology of the three RXR subtypes, termed RXRα, β and γ, indicates that these receptors regulate common target sequences and respond to common ligands.[68] The expression pattern of the subtypes is rather different. RXRα shows abundant expression in the liver, kidney, spleen, placenta, epidermis and a variety of visceral tissues; RXRβ is expressed widely and can be found in almost every tissue (like RARα); RXRγ expression is mainly restricted to the muscle and brain.[68] These and other studies about differentiation and metabolic processes suggest that RXRs play critical roles in a wide range of developmental processes, from embryo implantation to organogenesis, as well as in the regulation of adult physiology and metabolic processes.

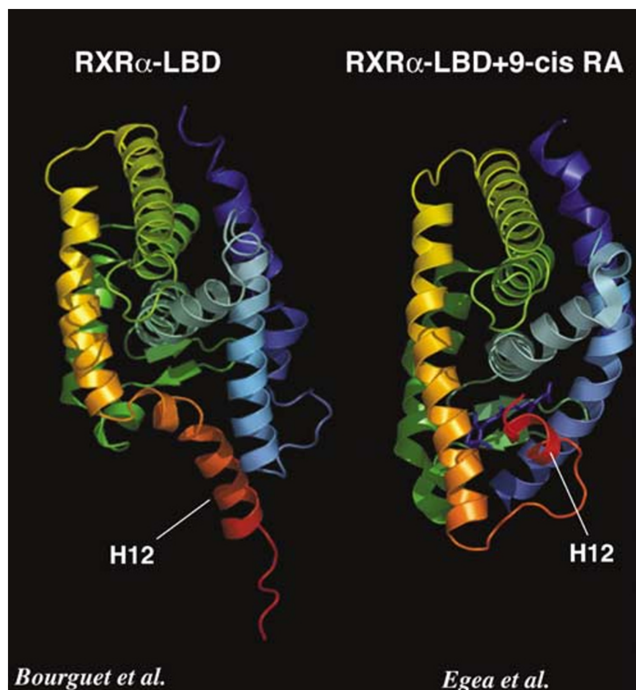
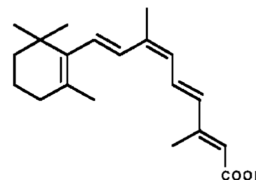


Figure 2 Structures of the unliganded and liganded RXRα's LBD. The LBD shows a three-layered helical structure. Helix 12 (H12) is colored red. In the unliganded LBD (left), H12 stands away and upon ligand binding it is repositioned onto the body of the LBD (right). The structures of the human unliganded RXRα (PDB code: 1LBD)[204] and the bound 9-*cis* RA (PDB code: 1FBY)[205] are shown

Ligands

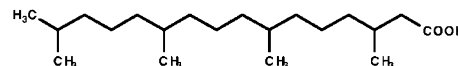
One of the most enigmatic and controversial areas of RXR research is the documentation of the presence of endogenous ligands activating RXR *in vivo*. Several molecules have been described as potential endogenous ligands for RXR (Figure 3): 9-*cis* RA,[16] phytanic acid[69] and docosahexaenoic acid,[70] but none of these has been proved to be the *bona fide* endogenous ligand so far. Perlmann and co-workers created a ligand detector mouse line.[71] Transgenic lines were constructed using Gal-DBD RXR-LBD fusion constructs and a reporter gene containing Gal binding sites and a β-galactosidase reporter gene. X-gal staining of mouse embryos revealed sites of ligand production. Specific regions of the

9-*cis* retinoic acid



Docosahexaenoic acid

Phytanic acid



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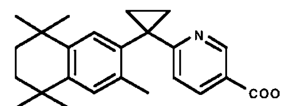

Figure 3 Structure of natural and synthetic RXR activators. 9-*cis* RA, phytanic acid and docosahexaenoic acid have been identified as possible natural RXR activators. The most widely used synthetic agonist is the LG100268

spinal cord lit up, suggesting that endogenous ligand production took place. Using a similar system with green fluorescent protein as a reporter, Luria *et al.*[72] have confirmed these results recently. They also obtained high RXR activation in the spinal cord, in the brain and in the olfactory epithelia.

Prenatal roles

To characterize the mechanisms of how RXRs are implicated in regulatory pathways, several attempts have been made to generate genetically modified animals with mutations in the RXR genes. In Figure 4, we summarized the phenotypic alterations in the various knockouts. Defect in the expression of RXRα has many more severe consequences than the loss of RXRβ and γ. Sucov *et al.*[73] and Kastner *et al.*[74] published a targeted loss-of-function mutation in the RXRα gene in the mouse germ line, which resulted in embryonic lethality between E13.5 and E16.5 in homozygous embryos. The major defect that led to lethality was the hypoplasia of the ventricular chambers of the heart manifested in the extremely thin ventricular wall and defects in ventricular septation. They also found ocular abnormalities and growth deficiency in the heterozygous mice. Deletion of one RXRα allele in mouse embryos revealed an intermediate phenotype with trabecular and papillary muscle defects, ventricular septal defects, conotruncal ridge defects and pulmonic stenosis, indicating a dosage effect for RXRα in maintaining normal cardiac morphogenesis.[75] Hepatic differentiation was also transiently retarded.

Using an experimental approach to monitor cardiac physiology in the living embryo, Dyson *et al.*[76] showed that expression of an atrial marker, atrial-specific myosin light chain 2 in the ventricle correlated with the thin-walled phenotype, poor trabeculation and the onset of dysfunction. Analyzing the early differentiation of embryonic ventricular cardiomyocytes, Kastner *et al.*[77] found that retinoids were required at early stages of cardiac development to prevent differentiation and support cell proliferation. RXRα $-/-$, RXRβ $-/-$, RXRα $-/-$ RXRβ $-/-$, RARα $-/-$, RARβ $-/-$ and vitamin A-deficient embryos exhibited a precocious differentiation of ventricular myocytes. A number of targeted mutations resulted in embryonic perturbation of cardiac development. This raised the possibility that this phenotype might represent a final common pathway of lethality occurring secondary to dilated cardiomyopathy. Ruiz-Lozano *et al.*[78] constructed a model where the RXRα null phenotype originated from two defects: morphogenic defects and defects in cardiac energy metabolism. The latter was caused by downregulation of intermediary metabolic enzymes and resulted in energy deprivation of the heart leading to heart failure.

To show whether RXRα is required in the developing cardiomyocytes to generate the phenotype of RXRα $-/-$ mutant, mice ventricular myocardium-specific gene targeting was used to knock out RXRα conditionally.[79] Surprisingly, such mice had normal embryonic viability without any defects observed in complete RXRα null animals. The same result was obtained using a completely different method.[80] Embryonic stem cells from RXRα null mice were introduced into wild-type recipient blastocysts to generate chimeric animals in which cardiomyocytes lack RXRα. These data suggested that RXRα $-/-$ phenotype was not cell autonomous for the cardiomyocyte lineage.

Overexpression of RXRα specifically in cardiomyocytes failed to prevent myocardial hypoplasia when introduced into RXRα null fetuses, supporting the noncell autonomous scenario. Furthermore, adult transgenic mice exhibited dilated cardiomyopathy.[81] The abnormalities observed in RXR null mice were highly similar to the effects of embryonic vitamin A deficiency. Defects in retinoid signaling, achieved either by the generation of vitamin A deficiency or by deletion of individual RA receptors (particularly RARγ), resulted in ocular and skeletal developmental abnormalities.[82] As in the case of the RXRs, there appeared to be functional redundancy among the RARs since animals with deletion of two RARs (particularly RARγ and α and to a lesser extent RARγ and β) showed a more extensive and severe phenotype than RARγ nulls. In each of these animal models, the function of the residual RARs was highly dependent on RXRα, since RXRα/RARγ double mutant animals displayed more severe ocular

Receptor	Expression pattern	Knockout phenotype	
		General	*Tissue specific*
RXRα	Liver, kidney, spleen, placenta, epidermis	Lethal E13.5-16.5 Cardiac, ocular, placenta abnormalities	Adipose tissue: Perturbed differentiation Resistance to obesity Skin: Alopecia, dermal cysts Liver: Disturbed lipid metabolism Impaired regenerative capacity Prostate: Multifocal hyperplasia
RXRβ	Widely expressed	Normal, except male infertility	
RXRγ	Muscle, brain	Normal, increased metabolic rate, TSH, T4 Failure in synaptic plasticity, learning Locomotor deficiency in RXRβ, γ double -/-	

Figure 4 Expression pattern of RXRs and defects in various RXR knockout animals. The expression patterns of the three RXR isotypes are shown and the observed defects in the phenotype of the various RXR knockouts

malformations and RXRα/RARα; RARγ double null mutants exhibited abnormalities not seen in the single mutants.[83] Detailed analysis of the inactivation of one or both RXR alleles combined with that of one or both alleles of a given RAR subtype concluded that, however, RARs and RXRs could function independently on activating their own network of target genes that they synergized in combined mutants, leading to specific phenotypes, which could not be found in single mutants, and sensitivity to the morphogenetic and teratogenic effects of retinoids depending critically on the levels of both RARs and RXRs in a particular tissue.[84] During development, RARs appear to be the most important partners for the RXRs; most of the phenotypic features of the RXR null animals are attributable to perturbations in RAR-dependent pathway. Furthermore, during development, RXRα is the primary heterodimeric partner of RXR and it functions to support the activity of all three of the RARs.[77]

Further support for the critical role of RXRα in regulating the sensitivity of embryos to RA also came from the observation that deletion of the RXRα gene protected mouse embryos from the teratogenic effects of exogenous RA.[85,86] RA exposure of the embryos caused malformations in limb development. In rodents, truncations, deletions of the long bones, digit deletions and fusions occurred. Using even a relatively high dose of RA that resulted in limb defects in 100% of wild-type embryos had no such effect in the mutant mice. Heterozygous embryos were intermediate in sensitivity to RA, suggesting the role of RXRα gene dosage in retinoid-induced teratogenesis. These observations indicated that RXRα was necessary for teratogenic limb development caused by RA exposure, but was not required for normal limb formation. This suggested that RXRα had distinct roles in additional pathways leading to RA responsiveness. This idea was supported by the fact that synthetic RAR agonists are very effective teratogenes but RXR agonists are not.[85]

A similar effect was observed in RA-induced cleft palate. In RXRα null mice, the frequency of cleft palate was lower than in wild-type animals.[86]

Mouse fetuses with targeted disruption of the RXRα gene also developed defects in the chorioallantoic placenta.[87] The labyrinthine zone of the placenta appeared histologically disorganized. Signs of necrosis, endothelial defects, blood stasis and abnormal thickening of the trophoblastic region were displayed with abnormal blood stasis, leading to thrombosis, abnormal thickness and impaired transfer of nutrients and oxygen. It was suggested that the differentiation of the placental parenchymal cells into mature trophoblasts was facilitated by RXRα and this process was impaired in mutant mice. These abnormalities were also similar to those observed in mice with vitamin A deficiency, suggesting a role of RAR/RXR heterodimer. Trophoblastic cells from mutant placentas were poorer in lipid droplets, a phenomenon that could reflect an abnormal PPARγ function.[88]

RXRα−/− RXRβ−/− double mutant mice were also generated[89] to address the issue of redundance. These animals died between 9.5 and 10.5 days of gestation, earlier than RXRα null mice and showed a wide range of abnormalities: truncation of the caudal region, abnormal body turning, dilated heart cavities, wavy aspect of the neural tube, shortened pharyngeal arches, hypoplasia of the frontonasal region and failure of neural tube closure. The cause of lethality was the lack of formation of the labyrinthine zone of the chorioallantoic placenta.

Approximately 50% of the RXRβ null mutants died before or at birth.[90] Those that survived appeared normal, except that the males were sterile. The epididymis contained very few and abnormal spermatozoa. Sertoli cells showed progressive lipid accumulation. The RXRγ null mice developed normally and were indistinguishable from the heterozygous and wild-type animals.[91] These mice had higher serum T4 levels and TSH (thyroid-stimulating hormone) levels and increased metabolic rate than wild-type animals.[92] RXRα −/− RXRγ −/− double mutants were similar to the RXRα −/− mice. Viable RXRβ −/− RXRγ −/− double and RXRα +/− RXRβ −/− RXRγ −/− triple mutant mice could be obtained.[91] They showed growth deficiency and, due to the loss of RXRβ, male sterility but reached adult age to date, indicating that a single RXRα allele was sufficient for development and morphogenesis.

Retinoids were also implicated in the processes of learning and memory.[93] RARβ deficiency eliminated hippocampal long-term potentiation and long-term depression (LTD), the most widely studied forms of synaptic plasticity. RXRγ appeared to be required only for LTD. These findings showed that these two forms of plasticity were retinoid dependent and revealed different ways of contribution of retinoid receptors. RXRβ −/− RXRγ −/− double mutants exhibited locomotor deficiencies due to a dysfunction in the dopamine signaling pathway.[94]

To address the issue of whether transactivation through RXR was essential for biological processes, a mutant mouse line was generated that expresses truncated RXRα lacking helix 12 of the LBD, which also contained the activation function domain 2 (AF-2).[95] These animals exhibited a similar range of abnormalities than the RXRα null mice, often with incomplete penetrance. The mutation was lethal; the embryos died at E14.5 and E18.5 while others did so only at birth or shortly afterwards. They also displayed defects that were not seen in RXR null littermates: agenesis of the esophageal septum, hypoplastic lungs, diaphragmatic hernia and ectopic opening of the ureter into the urogenital sinus. RXR AF-2 null/RXRγ −/− mice were indistinguishable from RXR AF-2 null mice, but RXR AF-2 null/RXRβ −/− or RXR AF-2 null/RXRβ −/−/RXRγ −/− mice were more severely affected. In these double and triple mutants nearly the full spectrum of malformations could be observed that characterized fetal vitamin A deficiency syndrome.[95] These data suggested that liganded RXRα and/or its transactivation function were required for the developmental functions of RAR/RXR heterodimers. Eliminating the other activation function domain 1 (AF-1) resulted in similar but less severe abnormalities.[96] AF-1 but not AF-2 domain was shown to be dispensable for placentation. This fact indicated that other RXR dimers (probably PPARγ/RXR) were responsible for placentagenesis.[97,98]

Postnatal roles

Conditional knockouts

In order to address the issue of specific functions of RXRs in adult animals and to bypass the embryonic lethality, conditional knockouts were generated. These models proved to be

particularly useful to address the receptor metabolic functions in adult animals.

Selective ablation of RXRα gene in adipose tissue[99] results in alteration of preadipocyte differentiation and resistance to obesity. It also impairs lipolysis in adipocytes during fasting. As PPARγ $+/-$ mice also show impaired adipogenesis, RXR can act via PPARγ/RXR heterodimer in this process.[88,100,101]

Targeted disruption of RXRα gene in the skin of adult mice results in hair loss (alopecia), hair follicle degeneration, utriculi and dermal cysts.[102,103] These observations are accompanied with epidermal interfollicular hyperplasia, keratinocyte hyperproliferation, aberrant terminal differentiation. VDR $-/-$ mice show a highly similar phenotype, indicating the role for the VDR/RXR heterodimer in hair cycling. Most interestingly, the hyperproliferation/differentiation abnormalities have not been found in VDR null mutants, suggesting other roles of RXR in skin development, which are distinct from the ones mediated by VDR/RXR.

Knocking out RXRα specifically from the liver affects many metabolic processes.[104,105] Physiological pathways regulated by LXRα, PPARα, CARβ, PXR and FXR are compromised in the absence of RXRα. Compared to LXR knockout mice, hepatic RXR null animals show further differences in lipid metabolism that cannot be explained with the impaired heterodimer function. In comparison to PPARα, hepatocyte RXRα has a unique role in lipid homeostasis.[106] The lifespan of hepatocytes from mice lacking RXRα is shorter than in wild-type animals and the regenerative capacity is also impaired.[107]

Selective disruption of RXRα from prostate epithelium results in a significant change in the profile of secretory proteins and multifocal hyperplasia, leading to preneoplastic lesions.[108]

The studies from knockout animals contributed greatly to our understanding of the biological function of RXRs in embryogenesis and adult physiology. It has also been proven unequivocally that RXR is part of the heterodimeric unit (RAR/RXR), which transduces retinoid signaling during embryonic development and in some cases of adult physiology and pharmacology (i.e. skin biology). This function at least in embryogenesis requires RXR's AF2 transactivation function. It is also clear that in adult tissues partnering with metabolite sensors such as PPARs, LXR, FXR, PXR and others extends RXR's role from cellular differentiation and morphogenesis to metabolic regulation more so than in embryonic development with the possible exception of PPARγ. The interpretation of tissue-specific knockouts is complicated by the fact that there are several partners present and their roles are not well understood. Beyond these issues some of the major questions are still unanswered. Is there an independent RXR-specific regulatory pathway in embryonic development and/or in adult tissues? Are there RXR-specific natural ligands regulating heterodimeric and/or homodimeric RXR signaling?

Pathophysiology and metabolic functions of RXR

Another intensively researched area of RXR biology is the receptor's role in physiological/pathophysiological processes, most importantly processes in which a receptor agonist or antagonist may have therapeutic potential. In the second part of this review, we will summarize our current understanding of such processes.

Role of RXRs in cardiac muscle

The development of the cardiac muscle, similar to many other tissues, is affected by vitamin A derivatives. Vitamin A deprivation is associated with the most severe congenital malformations in the neural crest and in the heart.[109] Nevertheless, exogenous sources of vitamin A have little direct effect on retinoid signaling. The vitamin A derivative able to activate RARs is RA. RA is formed from retinol by alcohol dehydrogenase and retinaldehyde dehydrogenase (RalDH) enzymes.[110,111] Deletion of the RalDH2 gene causes developmental arrest that can be rescued by administration of RA. Therefore, the tissue availability of RA primarily depends on the spatiotemporal generation of RA.[112,113]

During embryogenesis, RXRs have been implicated in differentiation processes, in the adult heart; RXRs are rather involved in the regulation of metabolic gene expression. Nuclear hormone receptors that regulate the expression of genes involved in cardiac lipid metabolism, PPARα and LXR, both require RXR as a heterodimeric partner. Since selective RXR agonists can upregulate RXR target genes in the absence of RXRγ, it was suggested that RXRα, the most abundant RXR isoform, may be the critical receptor to mediate rexinoid effects. In isolated cardiac myocytes, the selective RXR agonist, LG100268, can upregulate the expression of both PPAR and LXR target genes, such as ACS1 and ABCA1, although to a lesser extent than their PPAR- or LXR-specific counterparts (Uray *et al.*, unpublished observations). Moreover, RXR takes part in the regulation of partner nuclear receptors. The expression of LXRα can be induced by RXR agonists both in primary cardiac myocytes and *in vivo* (Uray *et al.*, unpublished observations).

Subtle changes in mRNA and protein levels of nuclear receptors may result in marked alterations of biochemical parameters. In the adult heart, the relative expression of RXRα may change in various cardiac disease states. Cardiac myocytes in culture exposed to hypoxia exhibit reduced expression of the rate-limiting fatty acid oxidation (FAO) enzyme muscle carnitine palmitoyltransferase I and reduced FAO capacity and myocardial accumulation of neutral lipids.[114] Hypoxia reduces PPARα/RXR promoter-binding activity due to reduced cellular RXRα levels. The altered metabolic phenotype of the failing heart also involves changes in the protein expression of key enzymes of free FAO, such as the medium chain acyl-coenzyme A dehydrogenase, a PPARα-regulated gene.[115] While the levels of PPARα in the failing heart are not different from those in normal myocardium, the expression of RXRα has been shown to be suppressed in the failing state.[116] Acute phase response (APR) is also characterized by reduced uptake and oxidation of fatty acids in the liver and the heart. A potent inducer of APR and sepsis, the endotoxin LPS has been shown to markedly downregulate all three RXR isoforms and a number of nuclear hormone receptors and coactivators, including PPARα, TR and PGC-1.[117]

These examples demonstrate that RXRs are not only regulators of metabolic homeostasis in the adult myocardium but their expression is also subject of regulation.

RXRs in skeletal muscle

The discovery of rexinoids (synthetic RXR agonists) as insulin sensitizers has spurred interest in the physiological effects of these ligands and its receptors.[21] Recent studies from some of our laboratories demonstrated that skeletal muscle was a major target of rexinoid action, where it sensitized insulin-dependent glucose disposal in diabetic skeletal muscle.[32,33] The underlying mechanism of the muscle insulin sensitization involves post-translational activation of several components of insulin signaling involved in glucose uptake.[33] Such amelioration of insulin signaling is afforded via the coordinated regulation of gene expression by rexinoids in skeletal muscles. In a microarray analysis of gene expression in cultured C2C12 myotubules, a synthetic rexinoid, LG100268, most prominently regulated sets of genes involved in fatty acid and cholesterol metabolism, apoptosis and cell cycle regulation, mitochondrial energy metabolism and genes in muscle structure and contractility (Narkar *et al.*, unpublished observation). Regulation of one or more sets of these genes is responsible for the sensitization of insulin-stimulated glucose disposal in the diabetic skeletal muscle by rexinoids, as described below.

Regulation of metabolic gene expression

Insulin resistance is often associated with intramuscular saturated fatty acid accumulation, which interferes with insulin signaling and glucose uptake.[118] Rexinoids increase uptake and oxidation of saturated fatty acids in cultured skeletal muscle cells from diabetic humans.[119] Several candidate genes regulated by rexinoids have been identified, which may lead to increased FAO. Rexinoids increased the expression of FAT/CD36 (fatty acid transporter) in cultured muscle cells as well as in skeletal muscles of Zucker diabetic rats.[32,119] Increased expression of FAT/CD36 was associated with the increased uptake and oxidation of fatty acids in skeletal muscles.[120,121] Further, LG100268 robustly induced UCP3 and PDK4 expression in skeletal muscles of db/db mice (Narkar *et al.*, unpublished observation). Both of these genes were also associated with increased FAO.[122–125] While UCP3 was postulated to increase efficiency of FAO by mitochondrial cycling of fatty acid anions,[126] PDK4 preferentially promoted fat disposal over glucose.[127] Therefore, increased FAO in diabetic skeletal muscle is a potential mechanism for insulin sensitization by rexinoids.

In addition to FAO, rexinoids may also regulate the storage of fat via the induction of stearoyl-CoA desaturase (SCD1) in insulin-resistant skeletal muscle.[32] SCD1 is involved in the monounsaturation of fatty acids, which are preferentially incorporated into triglycerides for storage.[128] It was well documented that saturated fatty acids are preferentially converted to diacylglycerol (DAG) and ceramide, which interfered with the insulin signaling pathway in skeletal muscles.[129–134] Rexinoid-mediated potentiation of SCD1 expression increased the proportion of unsaturated over saturated fatty acids in skeletal muscles.[33] Thus, reduced saturated fatty acid levels could have an attenuated effect on insulin signaling in diabetic skeletal muscle.

Although rexinoids increased glucose disposal in skeletal muscles, these drugs did not affect the expression of glucose transporters (GLUT1 and 4) in this tissue.[32,119] Furthermore, to our knowledge, rexinoids have little or no effect on genes related to carbohydrate metabolism in skeletal muscles. However, rexinoids increased glycogen synthase activity in cultured skeletal muscle cells.[119] Glycogen synthesis and storage is the primary mechanism of nonoxidative glucose disposal in normal skeletal muscles.[118] Therefore, FAO seems to be the primary target of rexinoid action in diabetic skeletal muscle. Overall, rexinoids increase FAO (FAT/CD36, UCP3, PDK4) and unsaturation (SCD1) and consequently lower saturated fatty acid accumulation in skeletal muscle. This metabolic effect of rexinoids may contribute to the post-translational activation of insulin signaling components, which are inactivated by saturated fatty acids. Restored insulin signaling will increase glucose uptake in diabetic skeletal muscle. In addition, direct induction of glucogen synthase and synthesis by rexinoids may also contribute to glucose disposal in skeletal muscle.

Regulation of genes related to oxidative slow-twitch phenotype

Skeletal muscle is a heterogenous tissue broadly composed of oxidative slow-twitch and glycolytic fast-twitch fibers. As the name suggests, slow- and fast-twitch fibers rely on FAO and glycolysis, respectively, as the preferred source of energy. Furthermore, slow-twitch fibers were more insulin sensitive compared to fast-twitch fibers.[135–137] Interestingly, expression of slow-twitch fibers was decreased in type 2 diabetes, which partially contributed to attenuated FAO and insulin sensitivity.[138–140] Since rexinoids increased FAO, we further tested the effects of these drugs on fiber-type expression in skeletal muscles of db/db mice. Indeed, treatment of db/db mice with rexinoids increased the expression of slow-twitch biomarkers such as myoglobin and troponin I slow (Narkar *et al.*, unpublished observations). Therefore, the general mechanism of rexinoid action in insulin sensitization was induction of oxidative phenotype at the level of enzymes (UCP3, PDK4 and myoglobin) and contractile (troponin I slow) proteins in diabetic skeletal muscles.

Potential nuclear receptor complexes involved in skeletal muscle effects of rexinoids

As described above, rexinoids are synthetic ligands for RXR. RXR receptors can participate directly or indirectly in gene expression by forming homodimers or heterodimers with other nuclear receptors, respectively.[6] Some RXR heterodimers (e.g. PPARα and PPARγ) can be activated by rexinoids in the cultured skeletal muscle cells.[119,141–143] It was initially hypothesized that rexinoids cause insulin sensitization solely via activation of PPARγ–RXR heterodimers.[21] However, not all the effects of rexinoids in skeletal muscles involved PPARγ–RXR heterodimers. For example, TZDs decreased (PDK4, UCP3) and did not affect (myoglobin, TnIs) RXR

target genes in diabetic rodents (Narkar *et al*., unpublished observation).[144] On the other hand, activation of FAT/CD36, SCD1 and glycogen synthase might involve PPARγ–RXR heterodimers, as these genes could also be induced by TZD.[32,119] Other nuclear receptors such as PPARα and PPARδ were associated with FAO and oxidative slow-twitch fiber determination.[145–148] One may speculate that rexinoids activate PPARα/δ–RXR heterodimers in some of its effects on FAO- and slow-twich fiber-related genes. Moreover, it is important to note that both PPARα and PPARδ activation can increase insulin sensitivity.[125,149] Finally, the role of RXR homodimers cannot be ignored in the effects of rexinods. In conclusion, the mechanism of insulin sensitization and gene regulation by rexinoids might rely on activity of multiple nuclear receptor complexes in skeletal muscle and possibly other tissues.

Rexinoids and insulin resistance

Insulin resistance, the inability of insulin-sensitive tissues to respond efficiently to insulin, plays a critical role in the pathogenesis of type 2 diabetes. Insulin resistance in skeletal muscle, as manifested by a decreased ability of insulin to stimulate glucose transport, always precedes the clinical manifestation of type 2 diabetes. In addition, insulin resistance in adipose tissue results in a decrease in the antilipolytic action of insulin. The failure to adequately suppress lipolysis in adipocytes causes an increased release of fatty acids into the circulation. The elevated levels of free fatty acids (FFAs) in the circulation of insulin-resistant humans or animals results in an impairment in the ability of insulin to suppress hepatic gluconeogenesis and in increased hepatic glucose production. The β cells of the pancreas normally compensate for the increased levels of serum glucose by increasing insulin secretion. However, at a certain point, the β cells can no longer generate a sufficient amount of insulin to match the demands of the insulin-resistant tissues, and this ultimately results in the development of glucose intolerance, hyperglycemia and finally diabetes. Although there are many drugs that are currently used in the treatment of type 2 diabetes including sulfonylureas, biguanides and glucosidase inhibitors, these drugs are mainly targeted toward reducing the hyperglycemia itself and do not have their primary effects on the regulation of insulin sensitivity. TZDs are the first class of antidiabetic drugs recognized to act as insulin sensitizers.[150] The insulin-sensitizing activity of TZDs correlates well with their ability to bind to and activate PPARγ. PPARγ is expressed abundantly in adipose tissue and TZDs activate gene expression in adipocytes, altering fatty acid metabolism and lowering circulating levels of FFA.[151,152] In addition, TZDs increase the production of insulin-sensitizing adipokines such as adiponectin and suppress the circulating levels and/or the activity of insulin resistance-triggering adipokines such as TNFα and resistin. The combined effect of these alterations is an improvement in skeletal muscle insulin sensitivity.[153–155] There is increasing evidence that TZDs also have direct effects on muscle gene expression and muscle metabolism.[156–158] Although TZDs are effective insulin sensitizers, their use is frequently associated with a substantial weight gain in both rodents and humans. This increase in obesity may complicate their efficacy as antidiabetic drugs.

Rexinoids are synthetic ligands for RXRs. They represent a second class of nuclear receptor ligands that have insulin-sensitizing activity. Administration of rexinoids in rodent models of type 2 diabetes (db/db and ob/ob mice and Zucker diabetic fatty rats) results in a marked decrease in both hyperglycemia and hyperinsulinemia.[21,30] Since RXRs serve as the obligate partners for PPARγ, it has been suggested that the insulin-sensitizing activity of rexinoids could be due to their 'TZD-mimetic' activity. However, there are major differences between the pharmacologic activities of rexinoids and TZDs.[32,33] TZDs can only activate the RXR/PPARγ heterodimer whereas rexinoids are capable of activating a variety of RXR-containing heterodimers and RXR homodimers. The activation of multiple receptors could have very diverse effects on metabolic regulation. Therefore, it is likely that the antidiabetic activity of rexinoids represent a novel pharmacologic mechanism of action. To address this question, we have previously performed a detailed comparison of the effects of rexinoids and TZDs on metabolic gene expression in tissues of diabetic fatty rats.[32,33] Under conditions of comparable suppression of hyperglycemia, the two compounds had clearly different tissue-specific patterns of gene expression. While TZDs had their most marked effects on gene expression in adipose tissue, the primary effects of rexinoids involved alterations in gene expression in the liver and skeletal muscle. Among the genes showing increased expression by rexinoids in skeletal muscle were SCD1 and CD36. SCD1 is a rate-limiting enzyme that catalyzes the conversion of saturated long chain fatty acyl-CoAs (LCFA-CoAs) to their unsaturated counterparts. In line with the induction of SCD1, rexinoids increased the levels of intramyocellular unsaturated LCFACoAs in db/db mice. Unsaturated LCFACoAs are preferentially incorporated into triglyceride, whereas saturated LCFACoAs form a selective pool of precursors for the biosynthesis of DAG. Since increased levels of intracellular DAG have been implicated in insulin resistance, induction of SCD1 in the muscle of rexinoid-treated animals might be linked to insulin sensitization.[129] CD36 is a long chain fatty acid transporter. Deficient CD36 expression is a primary defect responsible for insulin resistance in spontaneously hypertensive rats (SHR), whereas transgenic rescue of CD36 can ameliorate insulin resistance in SHR.[159] Thus, upregulation of CD36 might also contribute to the insulin-sensitizing activity of rexinoids.

Although insulin has many effects on skeletal muscle metabolism, the regulation of glucose transport is a critical determinant in the rate of glucose utilization. Activation of both the insulin receptor substrates (IRS)/Akt and the c-Cbl-associated protein (CAP)/c-Cbl pathways is important in regulating insulin-stimulated glucose transport.[160] To gain further insight into the molecular basis for the insulin-sensitizing activity of rexinoids, we have examined the effects of rexinoids on insulin-mediated signaling in skeletal muscle of diabetic (db/db) mice.[33] Administration of either rexinoids or TZDs could correct the deficit in insulin-promoted glucose transport in diabetic muscle. Rexinoids increased insulin-stimulated IRS1 tyrosine phosphorylation and Akt phosphorylation but had no influence on the activity of components of the

CAP/c-Cbl pathway. In contrast, TZDs are completely ineffective in affecting the IRS1/Akt pathway but dramatically increase the levels of CAP expression and insulin-stimulated c-Cbl phosphorylation. Recent evidence has shown that Ser^{307} phosphorylation of IRS1 impairs its interaction with an activated insulin receptor and thereby attenuates insulin-mediated signaling. The level of IRS1 Ser^{307} phosphorylation is modestly elevated in skeletal muscle of db/db mice and treatment with rexinoids can reverse this effect, generating a muscle in which the fraction of IRS1 available for association with insulin receptor is similar to that in nondiabetic controls. TZDs, on the other hand, show no effect on the level of IRS1 Ser^{307} phosphorylation. Collectively, these findings suggest that, in db/db mice at least, rexinoids improve insulin sensitivity via changes in skeletal muscle metabolism that are distinct from those induced by TZDs, and rexinoids therefore represent a novel class of insulin sensitizers.

The therapeutic use of rexinoids as antidiabetic agents has been contraindicated by some side effects. Although not a problem in obese and diabetic mice, administration of rexinoids to diabetic rats and to humans is frequently associated with hypertriglyceridemia.[31,161] This effect appears to be due to a rexinoid-induced suppression of lipoprotein lipase activity in both cardiac and skeletal muscles. In addition, retinoids have teratogenic potential. It is likely that some of these undesirable effects may be due to the activation of RXR receptor complexes that are unrelated to the antidiabetic activity of these drugs. By identifying those receptor complexes that are beneficial for insulin resistance and those that are responsible for the unwanted effects, it would be possible to develop modified rexinoids that preferentially activate the first class of receptors while not activating the second. Such receptors complex-selective rexinoids could be of major therapeutic significance, since they would not only be more potent but also would have fewer unwanted side effects. Having excluded PPARγ as the partner engaged by RXR to mediate the antidiabetic activity of rexinoids, we are currently exploring the possible involvement of other nuclear receptors including PPARα and LXR in this rexinoid-induced insulin-sensitizing effects. Cao *et al.*[162] have recently reported that LXR ligands suppress hyperglycemia and improve insulin sensitivity in diabetic rodents. However, unlike rexinoids, which increased insulin-stimulated Akt phosphorylation in skeletal muscle of db/db mice, LXR ligands had no effect on Akt phosphorylation in these animals (Shen *et al.*, unpublished observation). We speculate that the insulin-sensitizing effects of rexinoids might reflect the activation of the PPARα- or PPARδ-regulated pathway or possibly pathways specifically regulated by RXR homodimers.

Atherosclerosis and cholesterol metabolism

Handling of lipids by macrophages is an important metabolic process in the context of hypercholesterolemia and the development of atherosclerotic lesions.[163–165] It is critical to understand the regulatory processes associated with cholesterol and fatty acid uptake and release (efflux) of this cell type. A regulatory network has been associated with macrophage lipid metabolism in recent years. First, it has been shown that a member of the nuclear receptor superfamily can be linked to macrophage maturation and modified (oxidized) LDL uptake.[166,167] In these studies, it has been shown that oxidatively modified lipid components (9- and 13-hydroxy-octadecadienoic acid) could serve as activators of PPARγ. Later, the oxysterol receptor LXR was linked to macrophage lipid metabolism by showing that LXRα is a direct transcriptional target of PPARγ and it could induce lipid transporters such as ABCA1[168,169] and ABCG1.[170] A coordinated lipid transport is likely to be coordinated by these receptors. Linking of the two receptor systems (PPARγ and LXRα) provides an attractive but not well-understood model to explain lipid/cholesterol uptake and efflux from macrophages. LXR:RXR heterodimers were originally identified as mediators of an alternative retinoid signaling pathway,[23,171] showing that LXR:RXR heterodimer is highly permissive and can be activated from either the RXR side by retinoids or the LXR side by oxysterols.[168] These observations suggest that retinoids may also participate in the regulation of lipid homeostasis. Recently, we showed that a p450 enzyme, CYP27, which produces 27-hydroxycholesterol, an LXR activator, was directly regulated by RAR-, RXR- and PPARγ-selective agonists.[172] This enzyme is expressed in macrophages in atherosclerotic lesions.[172–174] A mutation in this enzyme leads to a human disease – cerebrotendinous xanthomatosis, a rare sterol storage disease characterized by xanthomas in tendons and also in the CNS leading to ataxia, spinal cord paresis, neurological dysfuntions, normolipidemic xanthomatosis and accelerated atherosclerosis.[175–177]

In the context of atherosclerosis macrophages' cholesterol metabolism is only one question besides the other main problem: the control of the absorption and metabolism of cholesterol in the liver and its distribution to peripheral tissues. All of these processes are tightly controlled by two nuclear receptors, the LXRs and FXR.[178] Both of these receptors form permissive heterodimers with RXR, so perturbations in the level of expression and the activity of the RXRs can have dramatic effects on cholesterol metabolism. Administration of rexinoids to mice and hamsters can cause a complete block in the absorption of dietary cholesterol.[168] This effect is in part due to disruption of the production of bile acids essential for normal cholesterol absorption and also for alterations in the absorptive properties of the intestinal epithelium. The former effect is due to the activation of hepatic RXR/FXR heterodimers by the rexinoids and the consequent repression of the CYP7a1 and 7b1 hydroxylases that are critical for normal bile acid production.[179] The effect on the absorptive properties of the intestinal epithelium reflect the activation of RXR/LXR heterodimers in the enterocytes and the induction of ATP-binding cassette protein 1 (ABC-1), a transport protein that facilitates the efflux of free cholesterol from the interior of the enterocyte back into the intestinal lumen.[180] It is the combination of these distinct pharmacologic responses that leads to the functional block in cholesterol absorption. The effects of rexinoids on cholesterol absorption provide an excellent example of how rexinoids, by regulating the activity of multiple nuclear receptor heterodimers, can generate integrated control of complex metabolic pathways in ways that are not replicated by individual nuclear receptor ligands.

The ability of rexinoids to provide concerted induction and activation of both the PPAR and LXR signal transduction pathways was exploited by Auwerx and associates to demonstrate that an RXR agonist could be used to drastically reduce the development of atherosclerosis in an experimental mouse model (apoE null animals).[181] Both RXR and LXR agonists induced ABCA1 expression and stimulated ABCA1-mediated cholesterol efflux from macrophages from wild-type, but not from LXRα and β double $-/-$ mice, suggesting that activation of RXR/LXR heterodimers contributes to the beneficial effects of rexinoids on atherosclerosis.[181]

Role of RXR in myeloid cell differentiation

There is extensive evidence to suggest that retinoids and RARs play critical roles in both physiologic and pathologic processes associated with myeloid cell differentiation.[182] Relatively little is known about the role of RXR in these processes other than its role as an obligatory heterodimerization partner for RARs. The most abundant RXR in myeloid cells is RXRα.[183–186] As RXRα $-/-$ mouse is embryonic lethal, its myelopoiesis cannot be analyzed.[73,74] In a genetic study, Sunaga *et al*.[187] investigated the *in vivo* function of RXRβ by generating transgenic mice with targeted expression of a dominant-negative form of the receptor in myeloid cells. Out of 12 mice analyzed, one exhibited a severe maturation arrest at the promyelocytic stage. Three other transgenic mice showed a mild inhibition of myeloid differentiation. These results indicated that myeloid differentiation is perturbed in the transgenic mice by the dominant-negative effect of the transgenic RXR, indicating that RXR plays a role in physiologic myelopoiesis *in vivo*.

An alternative approach to the investigation of the role of the RXRs in myeloid maturation has been the use of RAR- and RXR-selective ligands. These pharmacologic studies have demonstrated that while RXR ligands have relatively modest effects on their own, they synergize with RAR ligands in activating gene expression, inhibiting clonal growth and inducing differentiation of myeloid leukemia cell lines such as HL-60 and NB4.[185,188,189]

There are relatively few studies documenting RAR-independent RXR activities in myeloid cells. In HL-60 myeloid leukemia cells, RAR-specific ligands are sufficient to induce myeloid differentiation but activation of endogenous RXRs is required for the induction of apoptosis.[185,188,190] In a different model – MPRO promyelocytes – Collins and associates utilized RXR- and RAR-specific agonists and antagonists to determine how RA overcomes the dominant-negative activity of a truncated RARα in myeloid precursor cells.[191] They observed that RXR-specific rather than RAR-specific agonists induce terminal granulocytic differentiation of these cells.

Lanotte and associates explored RXR signaling in t(15;17) (the NB4 cell line) and found evidence for a crosstalk between RXR agonist and protein kinase A (PKA) signaling pathways. The activation of RXR with selective ligands and PKA with a selective agonist (8CPT-cAMP) induces cell maturation.[192] In this study, the authors could exclude the possibility of PML-RAR:RXR heterodimer activation as well as the activation of VDR, PPAR or TR in this process, further supporting the idea of the existence of an independent RXR signaling pathway in some myeloid leukemia cell lines.

RXR and apoptosis

Retinoids can induce differentiation and apoptosis. RXRs seem to cooperate with RARs and potentiate both effects. Additionally, RXR activators have their independent effect on apoptosis induction. As discussed above, while RAR agonists induce differentiation of the myeloid cells, RXR activators triggered apoptosis.[185,188,190] An apoptosis-linked gene, tissue transglutaminase, was also induced in parallel with RXR-selective ligands, suggesting the existence of RXR-ligand dependent pathways effecting myeloid cell apoptosis.[193] A similar link between the activation of RXRs and the induction of apoptosis has been reported in the NB4 acute promyelocytic leukemia (APL) cell line.[194] Groenemeyer and co-workers suggested that there is a rexinoid signaling pathway that triggers apoptosis of immature APL cells and may correspond to a default death pathway, which is operative in the absence of ‘survival’ factors. RXR but not RAR antagonists can inhibit this rexinoid apoptosis that is distinct from that triggered by RAR agonists, which control cell maturation and postmaturation apoptosis.[195]

Although mostly myeloid cells have been analyzed, retinoids and RXR have been implicated in apoptotic processes in other tissues as well. RXRα was knocked out in the F9 murine embryonal carcinoma cell line and these cells not only failed to differentiate upon RA treatment but the process of apoptosis was also impaired.[196]

RXR-selective compounds were shown to induce apoptosis in ATRA-resistant breast cancer cell line MDA-MB-231. It was supposed that RXR activators induced RARβ through an RXR:Nurr77 heterodimer bound to RARβ promoter.[197] Recently, the subcellular localization of RXRα in this cell line has been linked to retinoid responsiveness: in retinoid-sensitive cells, RXRα is localized in the nucleoplasm, while in resistant cells, it was located in the splicing factor compartment. This intranuclear distribution of the receptor is under the control of the C-terminus.[198] Both RAR and RXR activators induced apoptosis in MCF-7 breast cancer cell line.[199]

On the contrary, only RAR but not RXR agonists induced activation and apoptosis in human peripheral blood B-lymphocytes.[200] Insulin-like growth factor binding protein (IGFBP)-3 was shown to bind RXR. IGFBP-3 and RXR ligands were additive in apoptosis induction and IGFBP-induced apoptosis was abolished in RXRα knockout cells.[201] Polyunsaturated fatty acids, including DHA, were reported to reduce proliferation and enhance apoptosis of colonocytes.[202] This effect might be mediated by RXR also. More recently, RXR-selective agonists have been reported to suppress pancreatic cancer cell proliferation, leading to apoptosis.[203]

From these one can summarize that RAR and RXR activators are capable of inhibiting proliferation, and inducing differentiation and/or apoptosis. RXR ligands enhance RAR-driven effects once and also have specific, retinoid-independent consequences. These effects are beneficial in the treatment of cancer as some trials suggest. RXR may be a suitable target of cancer therapy because retinoid-induced

side effects can be by-passed with RXR-specific agonists. Although several connections have been found between RXR and cancer, the discussion of these results is beyond the scope of this review.

Summary and perspectives

Since their initial discovery, a lot has been learnt about the enigmatic RXR nuclear receptors, but still some very basic questions remain to be answered. The family of RXR receptors may be considered to function as 'master regulators' of a number of different nuclear receptor-based signal transduction pathways.

These include cellular and organ differentiation, apoptosis and an ever increasing list of metabolic processes. Their activity is most likely controlled both by their expression level and their activation by endogenous ligands. These mechanisms allow for the fine-tuning and in certain cases amplification of signals during cellular responses to disparate signals. RXRs also present intriguing and unusual targets for pharmacologic interventions, allowing development of therapies targeting integrated pathways of biological regulation rather than one single pathway. If and how the pharmaceutical industry can capitalize on this opportunity remains to be seen.

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