β-Apo-13-carotenone Regulates Retinoid X Receptor Transcriptional Activity through Tetramerization of the Receptor*

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**Background:** β-Apo-13-carotenone, a naturally occurring apocarotenoid, functions as an antagonist of the retinoid X receptor (RXR).

**Results:** β-Apo-13-carotenone inhibits transactivation of RXRα but does not interfere with coactivator binding to the receptor like the known antagonist UVI3003.

**Conclusion:** β-Apo-13-carotenone induces the formation of a transcriptionally silent RXR tetramer.

**Significance:** β-Apo-13-carotenone is a naturally occurring rexinoid with a novel mechanism of antagonism.

Retinoid X receptor (RXRs) is activated by 9-cis-retinoic acid (9cRA) and regulates transcription as a homodimer or as a heterodimer with other nuclear receptors. We have previously demonstrated that β-apo-13-carotenone, an eccentric cleavage product of β-carotene, antagonizes the activation of RXRα by 9cRA in mammalian cells overexpressing this receptor. However, the molecular mechanism of β-apo-13-carotenone’s modulation on the transcriptional activity of RXRα is not understood and is the subject of this report. We performed transactivation assays using full-length RXRα and reporter gene constructs (RXRE-Luc) transfected into COS-7 cells, and luciferase activity was examined. β-Apo-13-carotenone was compared with the RXRα antagonist UVI3003. The results showed that both β-apo-13-carotenone and UVI3003 shifted the dose-dependent RXRα activation by 9cRA. In contrast, the results of assays using a hybrid Gal4-DBD-RXRαLBD receptor reporter cell assay that detects 9cRA-induced coactivator binding to the ligand binding domain demonstrated that UVI3003 significantly inhibited 9cRA-induced coactivator binding to RXRαLBD, but β-apo-13-carotenone did not. However, both β-apo-13-carotenone and UVI3003 inhibited 9cRA induction of caspase 9 gene expression in the mammary carcinoma cell line MCF-7. To resolve this apparent contradiction, we investigated the effect of β-apo-13-carotenone on the oligomeric state of purified recombinant RXRαLBD. β-Apo-13-carotenone induces tetramerization of the RXRαLBD, although UVI3003 had no effect on the oligomeric state. These observations suggest that β-apo-13-carotenone regulates RXRα transcriptional activity by inducing the formation of the “transcriptionally silent” RXRα tetramer.

Retinoid X receptors (RXRα, RXRβ, and RXRγ) are members of the nuclear receptor family and play a central role in nuclear receptor-regulated signaling pathways. RXRs are involved in biological processes, including cell growth and differentiation, metabolism, morphogenesis, and embryonic development (1–7). The active form of RXR is a dimer or heterodimer (8, 9). Besides the RXR homodimer, RXR also forms heterodimers with other nuclear receptor family members, including retinoic acid receptors, the vitamin D receptor, peroxisome proliferator-activated receptors, the farnesoid X receptor, and the liver X receptors (10, 11). RXR naturally forms into tetramers that are transcriptionally inactive (12).

RXRs are primarily made up of two modular domains as follows: a central DNA binding domain (DBD) and a carboxyl-terminal ligand binding domain (LBD). In addition to its role in binding of ligands, the LBD contains dimerization motifs and an activation function 2 (AF-2) domain (13, 14). Ligand-free RXR represses transcription of target genes through interaction with corepressor proteins. Ligand binding induces a conformational change of the AF-2 helix that releases corepressor protein and allows recruiting of coactivator complexes. Numerous compounds synthesized as antagonists, such as UVI3003, target the AF-2 helix (13).

Ligand-free RXR tends to associate into homotetramers both in solution and when bound to DNA. However, RXR tetramers rapidly dissociate into active dimers upon binding of an agonist such as 9-cis-retinoic acid (9cRA). RXR heterodimers bind in regulatory regions of their target genes by associating with response elements. RXR homodimers bind to a retinoid DNA-response element (RXRE). Activation of DNA-bound dimers by ligands promotes the recruitment of transcriptional coactivators to the promoters of target genes and enhances transcription rate. In vitro studies have indicated that full-length RXR self-associates into tetramers, and the LBD alone is sufficient to mediate tetramer formation with 3–5 nM affinity between the dimers (12, 15). Studies have substantiated the existence in vivo of an RXR tetramer (12, 15). It also has been shown that the RXR tetramer is transcriptionally silent based on the correla-

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§ The abbreviations used are: RXR, retinoid X receptor; 9cRA, 9-cis-retinoic acid; LBD, ligand binding domain; DBD, DNA binding domain; RXRE, retinoic acid response element; hRXRα, human RXRα; AF-2, activation function 2; Ni-NTA, nickel-nitrilotriacetic acid; atRA, all-trans-retinoic acid.
tion between the transcriptional activity of RXR mutants and their ability to form tetramers (16).

The vitamin A metabolite 9cRA is a ligand of RXR (17). Binding of 9cRA as an agonist induces the dissociation of the tetramer into dimer, which is the first step for RXR activation (18, 19). Carotenoids are polyisoprenoids that are biosynthesized in plants, fungi, and bacteria. Approximately 50–60 carotenoids that contain at least one unsubstituted β-ionone ring and the correct number and position of methyl groups in the polyene chain exhibit provitamin A activity (20, 21). Dietary provitamin A, β-carotene, can be metabolized in mammals through two pathways (22). β-Carotene oxidase 1 (BCO1) catalyzes the cleavage of the 15,15′ double bond resulting in two retinaldehyde molecules, and the eccentric cleavage takes place at double bonds other than the central 15,15′ double bond to produce β-apocarotenoids with different chain lengths. β-Apo-carotenoids have been detected in foods (23) and the blood of both humans (24) and animals (25). Recently, β-apo-8′-carotenal was detected in plasma after ingestion of β-carotene by a healthy human subject (24). Our previous studies demonstrated that β-apo-13-carotenone functioned as an antagonist in transactivation assays using full-length RXRs (26) and the retinoic acid receptors α, β, and γ (27). We have reported that β-apo-13-carotenone competes for 9cRA binding to RXRα with an affinity (7–8 nM) identical to 9cRA itself (27). However, the molecular mechanism of β-apo-13-carotenone’s modulation of transcriptional activity is not understood yet. This study focused on the mechanism by which β-apo-13-carotenone antagonizes 9cRA-induced activation of RXRα. Our results show that β-apo-13-carotenone induces formation of the RXRα transcriptionally silent tetramer but does not inhibit coactivator recruitment to the isolated LBD.

**EXPERIMENTAL PROCEDURES**

**Materials**—COS-7 African green monkey kidney cells and MCF-7 ((Michigan Cancer Foundation-7 (a human mammary cancer cell line)) mammary carcinoma cells from ATCC (Rockville, MD) were cultured in DMEM supplemented with 10% FBS. Cells were maintained at 37°C with 10% CO2. 9-cis-Retinoic acid and UV13003 were purchased from Santa Cruz Biotechnology.

**Human RXRoLBD Reporter Cell Assay**—Reporter cells expressing human RXRoLBD fused to the GAL-4 DBD (Indigo Biosciences, State College PA) were treated according to the manufacturer’s protocol. Reporter cells were incubated with 0, 0.32, 1.6, 8, 40, 200, 1000, and 5000 nm 9cRA for 24 h at 37°C in the presence or absence of fixed concentrations of either β-apo-13-carotenone or UV13003. Luminescence was detected with Glomax96 luminometer (Promega).

**Quantitative Real Time PCR**—MCF-7 breast cancer cells were cultured in 6-well plates and starved for 24 h in serum-free DMEM. MCF-7 cells were then treated with ligands in serum-free medium for 4 h. Total RNA was isolated using NucleoSpin RNA II (Macherey-Nagel). Two micrograms of RNA was reverse-transcribed into cDNA using the high capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real time PCR analysis was performed in quadruplicate with TaqMan chemistry and probed for caspase 9 (Hs00154260-m1) (Applied Biosystems). Eukaryotic 18S rRNA (43337607) was used as a housekeeping gene. The comparative Ct method (ΔΔCt) was used to analyze results.

**mRXRoLBD Expression in E. coli and Protein Purification**—N-His-tagged mouse RXRoLBD (pET15b) was transformed into BL21(DE3). The E. coli culture was grown at 37°C to A600 of 0.6. After induction with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside, cells were incubated for another 2–4 h at 25°C. Cells were harvested and lysed in lysis buffer (20 mM Tris, 500 mM NaCl, 5 mM imidazole, and 3 mM DTT, pH 8.0). The supernatant was loaded onto a HisPur Ni-NTA affinity column followed by extensive washing with 20 mM imidazole in lysis buffer. His-mRXRoLBD was eluted with 500 mM imidazole in lysis buffer. The concentrated protein peak fraction was then applied to HiLoad Superdex 200 gel filtration column for isolation of mRXRoLBD dimer and tetramer. The gel filtration column was calibrated with protein standards of 13.7, 25, 43, 67, 158, 232, and 440 kDa and blue dextran 2000 to confirm the molecular weights of the mRXRoLBD dimer and tetramer. Protein concentration was determined with the Bradford reagent (Bio-Rad) with bovine serum albumin as a standard. The purity of protein was assessed by SDS-PAGE and Coomassie Blue staining.

**Gel Filtration Chromatography for Detection of mRXRoLBD Dimer and Tetramer**—Purified mRXRoLBD dimer (50 μM in monomer concentration) was incubated with β-apo-13-carotenone or UV13003 in various concentrations on ice for 3 h or overnight. Tween 40 was added to increase ligand solubility in the aqueous buffer. In other experiments, mRXRoLBD tetramer (50 μM in monomer concentration) was first saturated with 100 μM β-apo-13-carotenone and then was incubated with 9cRA. After ligand treatment, mRXRoLBD was subjected to gel filtration chromatography on a Superdex 200 HR column controlled by an AKTA FPLC system (GE Healthcare). The running buffer contained 20 mM Tris, 150 mM NaCl, pH 7.5, at 4°C. Protein chromatograms were monitored at 280 nm. As above, this gel filtration column was also calibrated with proteins of known molecular weight to confirm the retention volumes of the mRXRoLBD dimers and tetramers.
β-Apo-13-carotenone as an RXR Antagonist

FIGURE 1. Antagonist effects of β-apo-13-carotenone and UVI3003 on transactivation of full-length RXRα. COS-7 cells were co-transfected with full-length hRXRα, pRL-tk-luc(Renilla), and RXRE-luc(renilla). Twenty four hours after transfection, cells were treated with 9cRA at concentrations of 0, 0.064, 0.32, 1.6, 8, 40, 200, 1000, 5000, 25,000, 50,000 nM in the presence or absence of 200 nM β-apo-13-carotenone or UVI3003 for another 24 h in a 37 °C incubator, 9cRA alone, blue; 9cRA plus β-apo-13-carotenone at 200 nM, red; and 9cRA plus UVI3003 at 200 nM, green. Luciferase activity is shown on the y axis.

RESULTS

β-Apo-13-carotenone and UVI3003 Antagonize 9cRA-induced Transactivation of Full-length RXRα—To investigate the effect of β-apo-13-carotenone and UVI3003 on the functional role of RXRα, an RXRE-luciferase receptor/reporter transactivation assay was performed. Full-length hRXRα was transiently cotransfected in COS-7 cells with two reporter plasmids, a firefly luciferase reporter containing RXRE from CRBP-II and Renilla luciferase as an internal control. In transfected cells, 9cRA induced luciferase activity in a dose-dependent manner over a concentration range of 5 × 10^{-15} M (50 μM) to 3.2 × 10^{-10} M (0.32 nM), as shown in Fig. 1. To determine the antagonist function of β-apo-13-carotenone and UVI3003, cells were treated with 9cRA in the presence of β-apo-13-carotenone or UVI3003 at a constant concentration of 200 nM. We observed a shift in the 9cRA dose-response curve induced by both β-apo-13-carotenone and the known antagonist UVI3003. β-Apo-13-carotenone alone did not induce the activation of RXRα (data not shown). This suggests that β-apo-13-carotenone antagonizes 9cRA activation of full-length hRXRα with a similar efficiency as the known antagonist UVI3003.

β-Apo-13-carotenone and UVI3003 Inhibit 9cRA Induction of an Endogenous RXRα-responsive Gene—It was previously reported that caspase 9 is one of the direct target genes for RAR-RXR heterodimers (28–30). Thus, we asked whether β-apo-13-carotenone and UVI3003 would inhibit the 9cRA-induced transcription of the endogenous gene caspase 9. For these experiments, MCF-7 cells were serum-starved for 24 h and followed by incubation with 9cRA, β-apo-13-carotenone, or UVI3003 at concentrations of 200 nM for 4 h. As shown in Fig. 2, 9cRA up-regulated the expression of mRNA for caspase 9 3–4 fold. Both β-apo-13-carotenone or UVI3003 inhibited 9cRA-induced gene expression. These data support the results shown above that β-apo-13-carotenone and UVI3003 antagonize 9cRA-induced transactivation of RXRα in transactivation assays.

UVI3003 Inhibits 9cRA-induced Coactivator Binding to the RXRα Ligand Binding Domain but β-Apo-13-carotenone Does Not—To further characterize the mechanisms of β-apo-13-carotenone and UVI3003 as antagonists for RXRα, we used cells that stably express a fusion protein containing the Gal4-LBD linked to the ligand binding domain (LBD) of RXRα. The luciferase reporter gene utilized in these assays contains the Gal4 upstream activation sequence linked to the luciferase reporter gene. 9cRA activated the transcription; however, β-apo-13-carotenone alone did not activate the RXRα reporter assay (Fig. 3A). Although we tested cotreatment with β-apo-13-carotenone at several different concentrations (5, 10, 100, 200, 500, and 1000 nM) with 9cRA, no marked shift of the 9cRA dose-response curve was observed (Fig. 3B). In contrast, 200 or 500 nM UVI3003 prominently shifted the 9cRA dose-response curve, as shown in Fig. 3C. In this assay the activation of RXRα does not require the formation of RXRα-LBD dimer. 9cRA binding to the ligand binding domain of RXRα provokes a conformational change of the AF-2 motif that produces a suitable binding surface for recruitment of coactivators. Previous structural studies have shown that the binding of antagonist UVI3003 to LBD of RXRα disturbs the conformation of helix 12 (H12) and leads to inhibition of coactivator recruitment (31). Strikingly, in the experiments reported here using the hybrid receptor, β-apo-13-carotenone had no effect on coactivator binding to the RXRα-LBD.

β-Apo-13-carotenone Regulates RXRα through Tetramerization of the Receptor—To further elucidate the mechanism underlying regulation of the RXRα transcriptional activity by

FIGURE 2. β-Apo-13-carotenone and UVI3003 inhibit 9cRA-induced expression of mRNA for caspase 9. MCF-7 cells were cultured in a 6-well plate and serum-starved for 24 h before ligand treatment. Cells were incubated with 200 nM ligands (9cRA, β-apo-13-carotenone, or UVI3003) in serum-free medium for 4 h. The comparative Ct method (ΔΔCt) was used to analyze results. Data are presented as means ± S.E., n = 5. Antagonist treatment (c) significantly inhibits 9cRA-induced caspase 9 gene expression (b) at p < 0.006. Bars with different letters above are significantly different from each other at p < or = 0.05.
β-apo-13-carotenone, we investigated the dimer-tetramer equilibrium of RXRΔLBD after exposure to ligand. Mouse RXRΔLBD was expressed in *Escherichia coli* and purified to homogeneity as shown in Fig. 4. Gel filtration chromatography on calibrated columns of Superdex 200 was used to isolate the mRXRΔLBD tetramer and dimer used in the following experiments. Recombinant mouse RXRΔLBD dimer 50 μM (calculated as monomer concentration) was incubated with increasing concentrations (100, 250, and 500 μM) of β-apo-13-carotenone on ice for 3 h or overnight. Gel filtration chromatography demonstrated β-apo-13-carotenone-induced formation of RXRΔLBD tetramer (Fig. 5). Treatment with 500 μM β-apo-13-carotenone for 3 h, in the molar ratio to monomer receptor of 5:1, led to 33% tetramer formation of the RXRΔLBD, although if treatment was extended overnight, 50% tetramer RXRΔLBD formed. In contrast, the antagonist UVI3003 did not induce tetramer formation at any of the tested concentrations (Fig. 6) even if incubations were extended overnight (data not shown). Finally, β-apo-13-carotenone-saturated RXRΔLBD tetramer, in molar ratio 2:1 (β-apo-13-carotenone/monomer RXRΔLBD), was incubated with the agonist 9cRA. RXRΔLBD tetramer dissociated to dimer with the

![Graph](image1.png)

**Figure 3.** β-Apo-13-carotenone does not antagonize 9cRA-induced transactivation in cells expressing Gal4-DBD:RXRΔLBD and an upstream activation sequence-driven luciferase reporter. Cells were incubated with 9cRA or β-apo-13-carotenone at concentrations of 0, 0.32, 1.6, 8, 40, 200, 1000, and 5000 nM for 24 h at 37°C (A); cells were incubated with these same concentrations of 9cRA in the presence or absence of fixed concentrations of β-apo-13-carotenone (B) or UVI3003 (C).

![Graph](image2.png)

**Figure 4.** Purification of recombinant mRXRΔLBD. Histidine-tagged mRXRΔLBD (theoretical molecular mass for monomer = 28,821 Da) was expressed in *E. coli* strain BL21(DE3) and purified first with an Ni-NTA affinity column. Elution fraction 1 of the affinity column was then applied onto a gel filtration column of HiLoad Superdex 200 16 × 60 column to separate the mRXRΔLBD tetramer and dimer. A, SDS-PAGE of the fractions from Ni-NTA affinity column. Lane 9 was the mRXRΔLBD elution fraction collected for second step purification with gel filtration. B, separation of mRXRΔLBD tetramer and dimer with gel filtration column. mRXRΔLBD tetramer was collected between the retention volume of 61.4 ml to 68.4 ml. mRXRΔLBD dimer was collected between retention volume 72.4 and 84.4 ml. The gel filtration column was calibrated with standard proteins of known molecular weight as described in the text.
addition of agonist 9cRA. At 50 μM, in an equal molar concentration to monomer RXRαLBD, 9cRA induced ~55% of dimer, whereas higher concentrations of 9cRA almost completely converted tetramer to dimer (Fig. 7). The gel filtration chromatography results showed that β-apo-13-carotenone induced the tetramerization of RXRαLBD, which was reversed with addition of 9cRA. In contrast, the antagonist UVI3003 did not influence the tetramer-dimer equilibrium of RXRαLBD.

DISCUSSION

In this study, we characterize the activity of β-apo-13-carotenone as an antagonist to RXRα and reveal the mechanism of RXRα antagonism by β-apo-13-carotenone-induced tetramerization of the receptor. The comparison of experimental data of β-apo-13-carotenone and UVI3003 indicated that these RXRα antagonists use two distinct mechanisms. β-Apo-13-carotenone, a naturally occurring β-apocarotenoid that can be obtained from either the diet directly or from eccentric cleavage of β-carotene functioned as an antagonist of RXRα. UVI3003 is a selective antagonist of RXRα whose inhibitory effect results from an interference of its long side chains with Leu-451 of helix-12 (31). Both β-apo-13-carotenone and UVI3003 inhibited 9cRA-induced transactivation of full-length RXRα in a dual-luciferase assay, whereas higher concentrations of 9cRA overcame the inhibition by the two antagonists. Both antagonists inhibited 9cRA induction of the expression of the caspase 9 gene in MCF-7 cells.

The tetramerization of RXRα induced by β-apo-13-carotenone is supported by reporter cell-based assays. The “Gal4-DBD:RXRαLBD” receptor expressed in the reporter cells will bind ligand, translocate to the nucleus, bind to the Gal4 upstream activation sequence on the reporter gene, recruit coactivator proteins, and lead to the transcription of luciferase. It is important to note that the whole process of transcription in these reporter cells does not require dimerization of RXRα. The conformational change of RXRαLBD due to ligand binding is sufficient to activate coactivator recruitment and subsequent luciferase transcription. β-Apo-13-carotenone is inactive in this assay. In contrast, full-length RXRα expressed in COS-7 cells undergoes nuclear translocation, dimer formation upon agonist binding, binding to the RXRE, coactivator recruitment,
and luciferase transcription. The distinctive difference in the mechanism between the reporter cell assay with Gal4-DBD:RXRaLBD and the transactivation assay with full-length RXRa is that dimer formation is obligatory for the latter; and β-apo-carotenone is only effective as an antagonist in this assay. These observations suggest that β-apo-13-carotenone inhibits 9cRA-induced RXRa transcription through the formation of the RXRa tetramer.

In an x-ray crystal study, atRA has been shown to bind to the transcriptionally silent tetrameric RXRa in a unique conformation (32). Previously, we showed using molecular modeling that when this bound atRA is computationally removed from the tetrameric RXR protein and redocked, it assumes the identical position as in the crystal structure (26). In addition, when β-apo-13-carotenone is built in a similar conformation to this atRA and is docked into this RXRa tetramer, it occupies the same position and has the same conformation as the bound atRA. Alternatively, when we built β-apo-13-carotenone in a conformation similar to RXRa-bound 9cRA (33) and attempted to dock this molecule into the dimeric RXRa, it assumes a very different position than the agonist ligand. Thus, we suggested that β-apo-13-carotenone should be capable of acting as an antagonist of RXRa by stabilizing the transcriptionally silent tetramer, but we had no direct biochemical evidence for that suggestion at that time.

The crystal structure study of the ligand binding domain of the RXRa suggested that a cavity corresponded to the RXR ligand-binding site and that 9cRA binding triggered a conformational modification of helix 11, which led to ligand-dependent transactivation by AF-2 (33). It has also been reported that the tetramerization domain is located in helix 11 at the RXRaLBD and tetramerization does not interfere with the function of helix 12 (34). Thus, β-apo-13-carotenone could cause tetramerization of RXRa by interacting with helix 11 and not affecting helix 12 (or coactivator binding). In contrast, inhibition of 9cRA-induced RXRa transcription by UVI3003 is due to the blockage of helix 12 as pointed out above (31).

RXRa tetramer formation induced by β-apo-13-carotenone was confirmed biochemically in this study by the observations of gel filtration chromatography with purified recombinant mouse RXRaLBD. Comparison between the β-apo-13-carotenone and UVI3003-treated dimeric RXRaLBD indicates that β-apo-13-carotenone regulates RXRa transcription through tetramerization, whereas inhibition by the antagonist UVI3003 is due to interference with helix 12. The complete dissociation of the tetramer RXRaLBD saturated with β-apo-13-carotenone to dimer by 9cRA shows that tetramerization is reversible when the agonist is in sufficient concentration. Thus, the equilibrium of RXRa dimer and tetramer could be controlled by the availability of ligands. A model of these various effects of ligands on RXRa is shown in Fig. 8.

In summary, this study revealed the mechanism of ligand-dependent regulation of RXRa transcriptional activity by the antagonist β-apo-13-carotenone. The findings imply that tetramerization of RXR and factors that modulate the oligomer state may contribute to regulation of cellular signaling. β-Apo-13-carotenone-induced tetramerization could conserve RXRa as an inactive nuclear receptor pool that can rapidly supply dimeric or monomeric RXRa upon 9cRA generation. This may also suggest a ligand-dependent modulation controlling the availability of RXRa for the heterodimerization with other nuclear receptor partners engaged in multiple signaling pathways.

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REFERENCES

1. Sakashita, A., Kizaki, M., Pakkala, S., Schiller, G., Tsuruoka, N., Tomosaki, R., Cameron, J. F., Dawson, M. I., and Koeffler, H. P. (1993) 9-cis-Retinoic acid: effects on normal and leukemic hematopoiesis in vitro. Blood 15, 1009–1016
2. Robertson, K. A., Emami, B., Mueller, L., and Collins, S. J. (1992) Multiple
β-Apo-13-carotenone as an RXR Antagonist

members of the retinoic acid receptor family are capable of mediating the glucuronidation differentiation of HL-60 cells. *Mol. Cell. Biol.* 12, 3743–3749

3. Sanz, M. J., Albertos, F., Otero, E., Juez, M., Morcillo, E. I., and Piqueras, L. (2012) Retinoid X receptor agonists impair arterial mononuclear cell recruitment through peroxisome proliferator-activated receptor-γ activation. *J. Immunol.* 189, 411–424

4. Qian, L., Zolfaghari, R., and Ross, A. C. (2010) Liver-specific cytochrome P450 CYP2C22 is a direct target of retinoic acid and a retinoic acid-metabolizing enzyme in rat liver. *J. Lipid Res.* 51, 1787–1792

5. Thaller, C., Hofmann, K., and Eichele, G. (1993) 9-cis-Retinoic acid, a potent inducer of digit pattern duplications in the chick wing bud. *Development* 118, 957–965

6. Pijnappel, W. W., Hendriks, H. F., Folkers, G. E., van den Brink, C. E., Dekker, E. J., Edeleboshc, C., van der Saag, P. T., and Durston, A. J. (1993) The retinoid ligand 4-oxo-retinoic acid is a highly active modulator of positional specification. *Nature* 366, 340–344

7. Carter, C. J., Farrar, N., Carlone, R. L., and Spencer, G. E. (2010) Developmental expression of a molluscan RXR and evidence for its novel, non-genomic role in growth cone guidance. *Dev. Biol.* 343, 124–137

8. Zhang, X. K., Lehmann, J., Hofmann, B., Dawson, M. L., Cameron, J., Graupner, G., Hermann, T., Tran, P., and Pfahl, M. (1992) Homodimer formation of retinoid X receptor by 9-cis-retinoic acid. *Nature* 358, 587–591

9. Zhang, X. K., Hoffmann, B., Tran, P. B., Graupner, G., and Pfahl, M. (1992) Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature* 355, 441–446

10. Lefebvre, P., Benomar, Y., and Staels, B. (2010) Retinoid X receptors: common hetero-dimerization partners with distinct functions. *Trends Endocrinol. Metab.* 21, 676–683

11. Evans, R. M., and Mangelsdorf, D. J. (2014) Nuclear Receptors, RXR, and the Big Bang. *Cell* 157, 255–266

12. 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

13. Dawson, M. I., and Xia, Z. (2012) The retinoid X receptors and their ligands. *Biochim. Biophys. Acta* 1821, 21–56

14. de Lera, A. R., Bourguet, W. M., Albucci, L., and Gronemeyer, H. (2007) Design of selective nuclear receptor modulators: RAR and RXR as a case study. *Nat. Rev. Drug Discov.* 6, 811–820

15. Kersten, S., Pan, L., Chambon, P., Gronemeyer, H., and Noy, N. (1995) Role of ligand in retinoid signaling. 9-cis-Retinoic acid modulates the oligomeric state of the retinoid X receptor. *Biochemistry* 34, 13717–13721

16. Kersten, S., Dong, D., Lee WY, Reczek, P. R., and Noy, N. (1998) Auto-silencing by the retinoid X receptor. *J. Mol. Biol.* 284, 21–32

17. Mangelsdorf, D. J., Borgmeyer, U., Heyman, R. A., Zhou, J. Y., Ong, E. S., Oro, A. E., Kakizuka, A., and Evans, R. M. (1992) Characterization of three RXR genes that mediate the action of 9-cis-retinoic acid. *Genes Dev.* 6, 329–344

18. 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

19. Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speak, J., Kratzeisen, C., Rosenberger, M., and Lovey, A. (1992) 9-cis-Retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. *Nature* 355, 359–361

20. Olson, J. A., and Kinsky, N. I. (1995) Introduction: the colorful, fascinating world of the carotenoids: important physiologic modulators. *FASEB J.* 9, 1547–1550

21. Parker, R. S. (1996) Absorption, metabolism, and transport of carotenoids. *FASEB J.* 10, 542–551

22. Harrison, E. H. (2005) Mechanisms of digestion and absorption of dietary vitamin A. *Annu. Rev. Nutr.* 25, 87–103

23. Fleshman, M. K., Lester, G. E., Riedl, K. M., Kopec, R. E., Narayanasamy, S., Curley, R. W., Jr., Schwartz, S. J., and Harrison, E. H. (2011) Carotene and novel apocarotenoid concentrations in orange-fleshed *Cucumis melo* melons: determinations of β-carotene bioaccessibility and bioavailability. *J. Agric. Food Chem.* 59, 4448–4454

24. Ho, C. C., de Moura, F. F., Kim, S. H., and Clifford, A. J. (2007) Excential cleavage of β-carotene in vivo in a healthy man. *Am. J. Clin. Nutr.* 85, 770–777

25. Shmarakov, I., Fleshman, M. K., D’Ambrosio, D. N., Piantedosi, R., Riedl, K. M., Schwartz, S. J., Curley, R. W., Jr., von Litig, J., Rubin, L. P., Harrison, E. H., and Blaner, W. S. (2010) Hepatic stellate cells are an important cellular site for β-carotene conversion to retinoid. *Arch. Biochem. Biophys.* 504, 3–10

26. Eroglu, A., Hruszczewycz, D. P., Curley, R. W., Jr., and Harrison, E. H. (2010) The eccentric cleavage product of β-carotene, β-apo-13-carotenone, functions as an antagonist of RXRβ. *Arch. Biochem. Biophys.* 504, 11–16

27. Hruszczewycz, D. P., de Sena, C., Narayanasamy, S., Riedl, K. M., Kopec, R. E., Schwartz, S. J., Curley, R. W., Jr., and Harrison, E. H. (2012) Naturally occurring eccentric cleavage products of provitamin A β-carotene function as antagonists of retinoic acid receptors. *J. Biol. Chem.* 287, 15886–15895

28. Donato, L. J., and Noy, N. (2005) Suppression of mammary carcinoma growth by retinoic acid: proapoptotic genes are targets for retinoic acid receptor and cellular retinoic acid-binding protein II signaling. *Cancer Res.* 65, 8193–8199

29. Donato, L. J., Suh, J. H., and Noy, N. (2007) Suppression of mammary carcinoma cell growth by retinoid acid: the cell cycle control gene Btg2 is a direct target for retinoid acid receptor signaling. *Cancer Res.* 67, 609–615

30. Yasin, R., Kannan-Thulasiraman, P., Kagechika, H., Dawson, M. L., and Noy, N. (2010) Inhibition of mammary carcinoma cell growth by RXR is mediated by the receptor’s oligomeric switch. *J. Biol. Chem.* 397, 1121–1131

31. Nahoum, V., Pérez, E., Germain, P., Rodríguez-Barrios, F., Manzo, F., Cammerer, S., Lemaire, G., Hirsch, O., Royer, C. A., Gronemeyer, H., de Lera, A. R., and Bourguet, W. (2007) Modulators of the structural dynamics of the retinoid X receptor to reveal receptor function. *Proc. Natl. Acad. Sci. U.S.A.* 104, 17323–17328

32. Gampe, R. T., Jr., Montana, J. A., 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

33. Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H., and Moras, D. (1995) Crystal structure of the ligand-binding domain of the human nuclear receptor RXRα. *Nature* 375, 377–382

34. 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