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Role of Ligand in Retinoid Signaling. 9-*cis*-Retinoic Acid Modulates the Oligomeric State of the Retinoid X Receptor†

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ABSTRACT: Many of the effects of retinoids on cells are mediated by the transcription factors known as retinoid nuclear receptors, but the mechanisms by which retinoids regulate the activity of the receptors are not known. It was previously shown that the retinoid X receptor (RXR) forms tetramers with a high affinity. In the present work it is demonstrated that binding of 9-*cis*-retinoic acid to RXR leads to rapid dissociation of receptor tetramers. In addition, fluorescence anisotropy studies indicate that ligand-binding results in a significant conformational change such that holo-RXR is more compactly folded as compared to the apo-protein. These findings suggest that the initial event in signaling by 9-*cis*-retinoic acid is a change in the oligomeric state of RXR. The data also imply that tetramer formation is a regulatory feature of the pathway by which RXR mediates the effects of retinoids on gene transcription.

Retinoids comprise a class of small hydrophobic compounds that are derived from vitamin A. They play key roles in the processes of cellular proliferation, differentiation, and apoptosis [for review see Gudas (1994)], and they are also potent inhibitors of carcinogenesis and are currently being explored as preventive and therapeutic agents in several types of cancer (Moon *et al.*, 1994; Hong & Itri, 1994). Many of the effects of retinoids on cellular function are exerted by two isomers of retinoic acid, 9-*cis*-retinoic acid (9cRA) and *all-trans*-retinoic acid (tRA), and are mediated by nuclear receptors that function as ligand-activated transcription factors (Mangelsdorf *et al.*, 1994). These proteins belong to a superfamily of hormone nuclear receptors that also includes the vitamin D receptor, the thyroid hormone receptor, the peroxisome proliferator-activated receptor, and a number of orphan receptors (Giguère, 1994; Glass, 1994). Two classes of retinoid receptors have been identified: the retinoid X receptors (RXRs), which bind 9cRA exclusively, and the retinoic acid receptors (RARs), which bind both 9cRA and tRA [for reviews see Giguère (1994) and Glass (1994)]. Both RARs and RXRs can bind to cognate DNA as homodimers, and RXRs can also form heterodimers with other members of the receptor superfamily, usually resulting in tighter interactions with DNA (Leid *et al.*, 1992; Yu *et al.*, 1991; Marks *et al.*, 1992). Hence, RXR is a promiscuous binding partner that communicates between various signaling pathways.

It is well documented that ligands are important for activation of gene transcription by retinoid receptors [e.g., Giguère (1994) and Glass (1994)], but little information is currently available regarding the exact role of ligand-binding for receptor function. It was reported that 9cRA strengthens RXR homodimer binding to DNA (Zhang *et al.*, 1992; Leid, 1994). However, these studies were conducted using protein obtained by *in vitro* translation, and the results could not be

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confirmed using protein obtained by expression in *Escherichia coli*, or in the insect Sf9 cells, or by using RXR derived from the mammalian COS-1 cells (Chen *et al.*, 1994). Hence, the role of the ligand in retinoid signaling remains elusive.

In an earlier report we examined the oligomeric state of RXR in solution (Kersten *et al.*, 1995). Electrophoreses of the receptor under nondenaturing conditions gave rise to three distinct protein bands. Analyses of the mobilities of these bands by Ferguson plots revealed that their molecular weights corresponded to receptor monomers, dimers, and tetramers. The presence of RXR tetramers was also indicated by covalent cross-linking of the receptor followed by analyses by SDS−PAGE (Kersten *et al.*, 1995). These findings demonstrated that, in the absence of a DNA template, RXR exists in solution predominantly as a tetramer. Here we provide evidence that binding of ligand induces dissociation of receptor tetramers. These observations shed a new light on the mechanism of regulation of RXR function and provide a basis from which the role of 9cRA in retinoid signaling mediated by RXR may be understood.

EXPERIMENTAL PROCEDURES

Fluorescent probes were purchased from Molecular Probes. 9cRA acid was a gift from Hoffman La Roche (Nutley, NJ). Fluorescence measurements were performed using a SPEX Fluorolog 2 spectrofluorometer equipped with Glan-Thompson polarizers.

Protein. RXRα lacking the N-terminal A/B domain (RXRαΔAB) was synthesized by overexpression in *E. coli* essentially as described (Chen *et al.*, 1994). Protein was dialyzed against buffer A (10 mM Hepes pH 8.0, 0.1 mM EDTA, 0.4 mM DTT, 400 mM KCl, 5% glycerol) and stored at −20 °C in 50% glycerol. Protein concentrations were determined by a Bradford assay (Bio-Rad) using BSA as a standard.

Ligand-Binding. Binding of ligand was monitored by the decrease in the intrinsic fluorescence of RXRαΔAB (λ_{ex} = 280 nm, λ_{em} = 340 nm) upon titration with ligand. RXR (1 μM) in buffer A containing 100 mM KCl was titrated with 9cRA or tRA from a concentrated solution in ethanol. Titration curves were corrected for "inner filtering effects" as described by Cogan *et al.* (1976). The dissociation constant and the number of binding sites were calculated by fitting the corrected titration curves to an equation derived from simple binding theory (Norris *et al.*, 1994). Nonlinear least squares regressions were carried out using the software Origin (MicroCal, Inc.).

Nondenaturing Gel Electrophoresis. RXRαΔAB (400 μM) was electrophoresed on 6% polyacrylamide gels under nondenaturing conditions in the absence or in the presence of 2-fold molar excess of 9cRA or tRA (running gel, pH 8.8; stacking gel, pH 6.8). Electrophoresis was carried out for 18 h while cooling with circulating tap water. Protein bands were visualized by Coomassie Blue staining.

Chemical Cross-Linking of RXRαΔAB. A 12 μL amount of 61 μM RXRαΔAB in buffer A containing 50% glycerol was mixed with 3 μL of 1 M Tris, pH 9, and 11 μL of buffer A. 9cRA was added from a concentrated solution in ethanol to a ligand/protein molar ratio of 1. Cross-linking was carried out by addition of 3 μL of 25 mM disuccinimidyl glutarate (DSG) in DMSO. Mixtures were incubated for 20 min at room temperature, and the reaction was terminated by the addition of 1 μL of 1 M glycine, pH 7.5. Samples were resolved by SDS−PAGE on 7.5% acrylamide gels cooled with circulating tap water, and protein bands were visualized by staining with Coomassie Blue.

Labeling of RXRαΔAB with a Fluorescein. RXRαΔAB (10−15 μM) in buffer A was covalently labeled with bromoethylfluorescein in dimethylformamide. The mixture was incubated overnight at 4 °C and dialyzed extensively against buffer A to remove unreacted probe. The stoichiometry of labeling in different preparations was 0.5−2 mol of probe per mole of RXRαΔAB.

Concentration-Dependence of the Fluorescence Anisotropy of Labeled RXRαΔAB. Labeled RXRαΔAB was diluted to the appropriate concentrations in buffer A containing 100 mM KCl, incubated overnight at 4 °C, and equilibrated at 22 °C for 30−90 min. To study the effect of 9cRA, a 10-fold excess of ligand/protein was included in the mixtures. Since the K_d for formation of RXR−9cRA complexes is on the order of nM (see Results), a 10-fold excess of 9cRA ensured that over 90% of the protein was saturated with ligand at all of the concentrations used. The values of the fluorescence anisotropy (λ_{ex} = 491 nm, λ_{em} = 516 nm) at each point were measured four times to obtain a mean.

RESULTS

The protein used in these experiments, RXRα lacking the N-terminal A/B domain (RXRαΔAB), possesses a high affinity for 9cRA, retains its ability to properly form dimers, and displays strong positive cooperativity in dimer-binding to cognate DNA (Chen *et al.*, 1994). Purity and viability of protein preparations were ascertained by measuring the equilibrium dissociation constant (K_d) and the number of ligand-binding sites of the RXR−9cRA complex by fluorescence titrations. Due to the extensive overlap of the fluorescence emission spectra of tryptophanes and tyrosines and the absorption spectra of retinoids, the intrinsic fluorescence of many retinoid-binding proteins, including RXR, is quenched upon binding of their ligands [e.g., Chen *et al.* (1994), Cogan *et al.* (1976), Chen and Noy (1994)]. Ligand-binding by RXR can thus be monitored by constructing fluorescence titration curves (Figure 1). A steep decrease followed by a shallower, linear decrease in fluorescence was observed upon titrating the receptor with ligands. The initial phase reflected quenching of the intrinsic fluorescence of the protein due to binding of the ligand. The second phase represented the non-specific "inner filter effect" by the ligand. The data were corrected as detailed by Cogan *et al.* (1976), and the dissociation constant and the number of binding sites were calculated by fitting the corrected titration curves to an equation derived from simple binding theory (Norris *et al.*, 1994). Analyses of curves obtained by titrating RXR with 9cRA (Figure 1, closed symbols) revealed 0.6−0.8 mol of ligand-binding sites per mol of protein. The range of K_d obtained from numerous titrations using a number of RXR preparations was 5−20 nM. Since measurements at low protein concentrations were technically difficult, protein concentrations in the 0.2−1 μM range, which is significantly higher than the K_d, were used. The derived K_d should thus be considered an upper limit for the actual value. The value obtained was nevertheless within the range reported by others using different techniques (Heyman *et al.*, 1992; Levin *et*

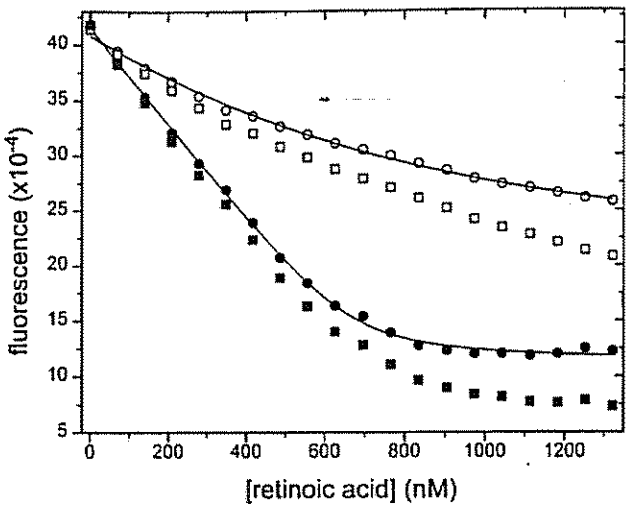

FIGURE 1: Binding of 9cRA to RXRαΔAB. RXRαΔAB (1 μM) was titrated with 9cRA (closed symbols) or with tRA (open symbols), and the intrinsic fluorescence of the protein was monitored. Raw data (squares) were corrected as described by Cogan *et al.* (1976). Equilibrium dissociation constants and number of binding sites were calculated by fitting the corrected titration curves (circles) as described under Materials and Methods.

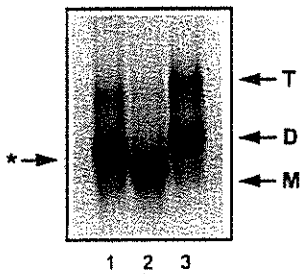

FIGURE 2: Analyses of RXR species by nondenaturing gel electrophoresis. RXRαΔAB was analyzed by nondenaturing gel electrophoresis in the presence of 1 μM tRA (lane 1), 9cRA (lane 2), or ethanol (lane 3). Binding of 9cRA resulted in the appearance of a band that moved at an intermediate position between unligated dimers and monomers (*).

al., 1992; Allegretto *et al.*, 1993; Allenby *et al.*, 1993). Titration of RXR with tRA (Figure 1, open symbols) showed that the association of this ligand with RXR was much weaker as compared with 9cRA. Data analyses yielded K_d values that were about 30-fold higher than values obtained with 9cRA. The data thus verified that the protein had a high affinity for 9cRA, that it had an almost full complement of binding sites, and that it displayed the expected selectivity toward 9cRA *vs* tRA.

To study the effect of 9cRA on the self-association of RXR, the receptor was analyzed by nondenaturing gel electrophoresis (Figure 2). In the absence of ligand, three distinct protein bands were observed, which, as previously demonstrated, reflected the presence of RXR monomers, dimers, and tetramers (Kersten *et al.*, 1995). Incubation of RXR with 9cRA lead to a dramatic decrease in the intensity of the RXR tetramer band and to the appearance of a new prominent band with a mobility which was intermediate between the mobilities of unliganded RXR monomers and dimers. TRA, which associates with RXR very weakly, did not alter the band pattern of RXR oligomers, confirming that the observed mobility changes specifically related to binding of 9cRA. These observations reveal that binding of 9cRA to RXR resulted in dissociation of protein tetramers. Since the mobility of the new band was intermediate, it could not be ascertained from the data in Figure 2 whether ligand-binding induced the dissociation of RXR tetramers to protein dimers or to monomers.

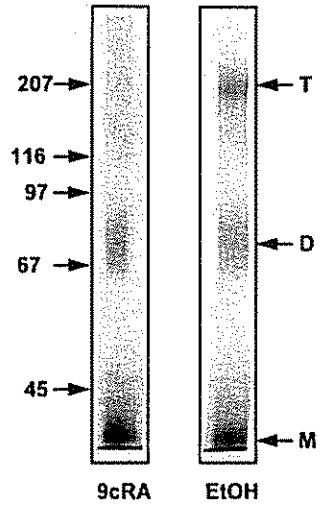

FIGURE 3: Chemical cross-linking of RXRαΔAB. RXRαΔAB was cross-linked using DSG in the absence or in the presence of 9cRA. Samples were resolved by SDS−PAGE on a 7.5% polyacrylamide gel, and protein bands were visualized by staining with Coomassie Blue.

The composition of RXRαΔAB oligomers was further investigated by chemical cross-linking of the receptor using the reagent DSG followed by SDS−PAGE analyses (Figure 3). In the absence of 9cRA, three protein bands were observed. As the molecular weight of RXRαΔAB is 40 270 (Mangelsdorf *et al.*, 1994), these bands corresponded to protein monomers, dimers, and tetramers. In the presence of ligand, the slowest-moving band, which corresponded to RXR tetramers, diminished. These data demonstrate that binding of 9cRA to RXR resulted in dissociation of receptor tetramers.

To further characterize the oligomeric state of liganded RXR, the protein was covalently labeled with the fluorescent probe fluorescein, and the concentration-dependence of the fluorescence anisotropy of the labeled protein was examined (Figure 4). Fluorescence anisotropy, in the absence of changes in the fluorescence lifetime, is a measure of the molecular size of a fluorescent molecule and provides a sensitive tool for studies of reactions leading to changes in the size of a protein complex such as oligomerization [e.g., Fernando and Royer (1992), Noy *et al.* (1992), and Kwon and Churchich (1994)]. The fluorescence lifetime of fluorescein-labeled RXR was found to be similar at high and low protein concentrations (Kersten *et al.*, 1995) and in the absence and presence of 9cRA (data not shown), indicating that the concentration-dependent changes in fluorescence anisotropy reported below indeed reflected protein oligomerization.

Both in the presence and in the absence of ligand, the fluorescence anisotropy of the labeled protein displayed a saturable increase upon raising the protein concentrations, a behavior that is indicative of protein self-association. However, the overall increase in fluorescence anisotropy was notably smaller in the presence of 9cRA than in the absence of 9cRA. This observation agrees well with the conclusion that while tetramers are the major species formed in the absence of ligand, the predominant species formed by self-association of liganded RXR is of a smaller size, i.e., dimers.

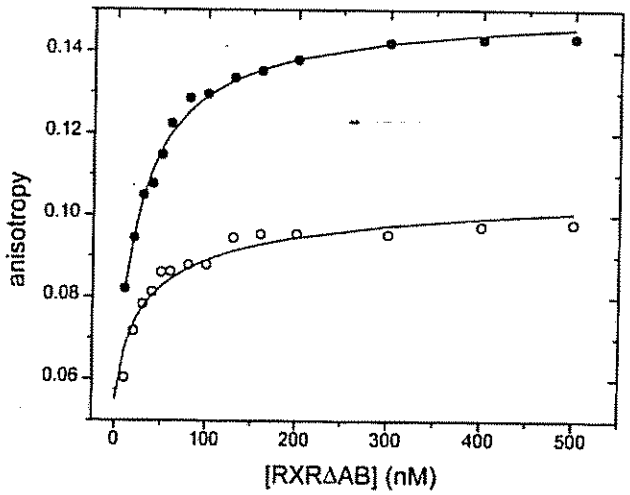

FIGURE 4: Concentration dependence of the fluorescence anisotropy of fluorescein-labeled RXRΔAB. The fluorescence anisotropy of fluorescein-labeled RXRαΔAB was measured at different receptor concentrations in the presence (open circles) or in the absence (closed circles) of 9cRA. Data points represent a mean of two independent titrations. Lines represent the fit to the data calculated using the BIOEQS program. The models used in the fitting routines contained monomers, dimers, and tetramers in the absence of 9cRA, or protein monomers and dimers in the presence of 9cRA (see text).

It is interesting to note as well that the value of the fluorescence anisotropy at the beginning of the titration curve, where the predominant protein species are monomers, was significantly lower in the presence of ligand than in the absence of ligand. In addition, the anisotropy value of liganded RXR at the end of the titration was only slightly higher than the anisotropy value of monomeric, unliganded protein. These observations are consistent with the data in Figure 2 and indicate that the molecular volume of liganded RXR dimers is intermediate between those of unliganded RXR monomers and dimers. The data hence imply that ligand-binding by RXR not only leads to dissociation of RXR tetramers but also causes a conformational change which results in a more compactly folded protein. Very similar results were obtained when RXR was labeled the fluorescent probe pyrenesulfonyl chloride, a probe with a fluorescence lifetime that is about 5-fold longer than that of fluorescein (data not shown).

The fluorescence anisotropy titration data (Figure 4) were used to calculate the free energies ($\Delta G°$) for the formation of the various oligomeric species of RXR. To do so, the experimental data were fitted using the numerically-based program BIOEQS, which allows for analysis of models containing multiple subunit equilibria (Royer & Beechem, 1992). Data from four separate titrations using two different preparations of fluorescein-labeled RXR were analyzed, and the standard deviation was 3%−5% of the mean values reported below.

The titration curves in the absence of 9cRA were analyzed using a model including protein monomers, dimers, and tetramers. The best fit for the data, achieved with limiting anisotropies of 0.074 and 0.154, yielded mean $\Delta G°_{\text{monomer}\rightleftharpoons\text{dimer}}$ and $\Delta G°_{\text{monomer}\rightleftharpoons\text{tetramer}}$ values of 9.26 and 30.0 kcal/mol, respectively. $\Delta G°_{\text{dimer}\rightleftharpoons\text{tetramer}}$ isolated from these data [see Kersten *et al.* (1995) for calculation details] was 11.5 kcal/mol.

Modeling the self-association of liganded RXR is more complex since the analysis requires consideration of a large number of species including liganded and unliganded monomers, dimers, and tetramers. We have not attempted to rigorously analyze these data. As an approximation, a model was used that was based on the assumption that the only species present in the concentration range probed were liganded monomers and dimers. This assumption seems to be justified in view of the results of the electrophoresis analyses (Figures 2 and 3). The best fit, obtained with limiting anisotropies of 0.055 and 0.1125, yielded $\Delta G°$ for formation of liganded RXR dimers of 9.68 kcal/mol.

From these data, the dissociation constants characterizing the formation of protein oligomers could be calculated. In the absence of ligand, the values of $K_{d_{\text{monomer}\rightleftharpoons\text{dimer}}}$ and $K_{d_{\text{dimer}\rightleftharpoons\text{tetramer}}}$ were 130 and 2.8 nM, respectively indicating that tetramer formation is characterized by strong positive cooperativity. For liganded RXR, $K_{d_{\text{monomer}\rightleftharpoons\text{dimer}}}$ was calculated to be 63 nM. Hence, ligand-binding by RXR, while almost completely abolishing tetramer formation, does not significantly alter the free energy for formation of dimers.

DISCUSSION

The data presented above provide strong evidence that binding of 9cRA to RXR induces dissociation of protein tetramers and creates a new equilibrium in which the main protein species are RXR dimers. The data also suggest that ligand-binding induces a dramatic conformational change in RXR and results in a more compactly folded protein. These findings suggest that one function of RXR tetramers may be to sequester RXR species with high affinity for cognate DNA, i.e., dimers and monomers. Binding of ligand liberates RXR dimers, which can bind to DNA directly, and RXR monomers, which can interact with other members of the thyroid/retinoid receptor family and bind to DNA as heterodimers. Such a regulatory mechanism is somewhat reminiscent of the regulation of another hormone nuclear receptor, the glucocorticoid receptor (GR). In the absence of ligand, GR is found in the cytosol bound to the heat-shock protein hsp90. Ligand-binding results in the dissociation of the complex and in the release of GR which then translocates into the nucleus and interacts with DNA (Jensen, 1991; Picard *et al.*, 1990). RXR seems to function as its own chaperone, i.e., it sequesters dimers and monomers into tetramers, and 9cRA induces dissociation of this complex, thereby elevating the concentration of active species.

The enhanced availability of RXR dimers upon binding of 9cRA might suggest that the presence of ligand will lead to enhanced DNA-binding by this protein. Such a prediction would agree with the reported observations that 9cRA strengthened RXR binding to DNA (Zhang *et al.*, 1992; Leid, 1994). However, as discussed in the introduction, these findings could not be confirmed by others. Thus, the effects of tetramer formation and ligand-binding by RXR on the interactions of the receptor with cognate DNA need to be examined further.

An alternative hypothesis for the role of tetramerization in RXR-mediated transcriptional activation is that tetramers may bind to DNA as such, and that binding of ligand will in turn lead to redistribution of DNA-bound species. It was recently reported, in regard to this, that the complex response element of the CRBPII gene, which is composed of multiple half-sites, cooperatively binds RXR tetramers (Chen & Privalsky, 1995). However, it has not been ascertained

whether the observed cooperativity stemmed from protein–protein interactions in solution or when bound to DNA. It is still unclear, therefore, whether tetramers can bind to the DNA as such.

The findings of the present work demonstrate that 9cRA regulates the distribution of RXR species. Other mechanisms that modulate the self-association of RXR may exist. For example, post-translational modifications of RXR may affect its oligomerization state. It is interesting to note in this regard that phosphorylation of retinoid and vitamin D receptors transfected into CV1 cells was recently reported to induce transcriptional activation in a ligand-independent manner (Matkovts & Christakos, 1995). In view of the model proposed here, this effect may have stemmed from phosphorylation-dependent modulation of the self-association characteristics of the receptor.

In summary: the results detailed above lead to the identification of the initial event in signaling by 9-*cis*-retinoic acid. This event was found to be a ligand-induced change in the oligomeric state of RXR. These data lend further support to our previous postulate that tetramer formation is an important regulatory feature of the effects of retinoids on gene transcription mediated by RXRs.

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REFERENCES

Allegretto, E. A., McClurg, M. R., Lazarchik, S. B., Clemm, D. L., Kerner, S. A., Elgort, M. G., Boehm, M. F., White, S. K., Pike, J. W., & Heyman, R. A. (1993) *J. Biol. Chem. 268*, 26625–26633.

Allenby, G., Bocquel, M.-T., Saunders, M., Kazmer, S., Rosenberger, M., Lovey, A., Kastner, P., Grippo, J. F., Chambon, P., & Levin, A. A. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 30–34.

Chen, H., & Privalsky, M. L. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 422–426.

Chen, Y., & Noy, N. (1994) *Biochemistry 33*, 10658–10665.

Chen, Z.-P., Shemshedini, L., Durand, B., Noy, N., Chambon, P., & Gronemeyer, H. (1994) *J. Biol. Chem. 269*, 25770–25776.

Cogan, U., Kopelman, M., Mokady, S., & Shinitzky, M. (1976) *Eur. J. Biochem. 65*, 71–78.

Fernando, T., & Royer, C. (1992) *Biochemistry 31*, 3429–3441.

Giguère, V. (1994) *Endocrinol. Rev. 15*, 61–77.

Glass, C. K. (1994) *Endocrinol. Rev. 15*, 391–407.

Gudas, L. J. (1994) *J. Biol. Chem. 269*, 15399–15402.

Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M., & Thaller, C. (1992) *Cell 68*, 397–406.

Hong, W. K., & Itri, L. M. (1994) in *The Retinoids*, 2nd ed. (Sporn, M. B., Roberts, A. B., & Goodman, D. S., Eds.) pp 597–630, Raven Press, New York.

Jensen, E. V. (1991) in *Nuclear Hormone Receptors* (Parker, M. G., Ed.) Academic Press Inc., San Diego.

Kersten, A., Kelleher, D., Chambon, P., Gronemeyer, H., & Noy, N. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8645–8649.

Kwon, O.-S., & Churchich, J. E. (1994) *J. Biol. Chem. 266*, 266–271.

Leid, M. (1994) *J. Biol. Chem. 269*, 14175–14181.

Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J.-M., Mader, S., & Chambon, P. (1992) *Cell 68*, 377–395.

Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A., & Grippo, J. F. (1992) *Nature 355*, 359–361.

Mangelsdorf, D. J., Umesono, K., & Evans, R. M. (1994) in *The Retinoids*, 2nd ed. (Sporn, M. B., Roberts, A. B., & Goodman, D. S., Eds.) pp 319–349, Raven Press, New York.

Marks, M. S., Hallenbeck, P. H., Nagata, T., Segars, J. H., Appella, E., Nikodem, V. M., & Ozato, K. (1992) *EMBO J. 11*, 1419–1435.

Matkovts, T., & Christakos, S. (1995) *Mol. Endocrinol. 9*, 232–242.

Moon, R. C., Mehta, R. G., & Rao, K. V. N. (1994) in *The Retinoids*, 2nd ed. (Sporn, M. B., Roberts, A. B., & Goodman, D. S., Eds.) pp 573–595, Raven Press, New York.

Norris, A. W., Cheng, L., Giguère, V., Rosenberger, M., & Li, E. (1994) *Biochim. Biophys. Acta 1209*, 10–18.

Noy, N., Slosberg, E., & Scarlata, S. (1992) *Biochemistry 31*, 11118–11124.

Picard, D., Kumar, V., Chambon, P., & Yamamoto, K. R. (1990) *Cell. Regul. 1*, 291–299.

Royer, C. A., & Beechem, J. M. (1992) *Methods Enzymol. 210*, 481–504.

Yu, V., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Näär, A. M., Kim, S. Y., Boutin, J-M., Glass, C. K., & Rosenfeld, M. G. (1991) *Cell 68*, 1251–1266.

Zhang, X.-K., Lenmann, J., Hoffmann, B., Dawson, M. I., Cameron, J., Graupner, G., Hermann, T., Tran, P., & Pfahl, M. (1992) *Nature 358*, 587–595.

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