Interaction between Retinoic Acid and Vitamin D Signaling Pathways*

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Magdalena Schrader‡, Igor Bendik§, Michael Becker-André§, and Carsten Carlberg‡‡

From the ‡Clinique de Dermatologie, Hôpital Cantonal Universitaire, CH-1211 Genève 14, Switzerland, the §Cancer Center, La Jolla Cancer Research Foundation, La Jolla, California 92037, and the ‡Glaxo Institute for Molecular Biology, CH-1228 Plan-les-Ouates, Switzerland

The nuclear signaling pathways for retinoids and vitamin D differ in the specificity of the respective receptors for response elements. Two pathways for the action of both retinoic acid receptors (RARs) and vitamin D receptors (VDRs) have been identified, one being retinoid X receptor (RXR)-dependent and the other being RXR-independent. Moreover, RXRs were found to function as homodimers. In several steps we converted the retinoid specific response element of the human retinoid acid receptor β promoter into the vitamin D/retinoic acid response element of the human osteocalcin promoter. We found that VDR homodimers only bind to the motif RGGTGA. The extended osteocalcin element also contains an imperfect direct repeat based on the motif RGGTGA spaced by three nucleotides, which is bound by RXR homodimers and activated by 9-cis-retinoic acid. The responsiveness of the osteocalcin element in all-trans-retinoic acid is mediated neither by RAR homodimers nor by RAR-RXR heterodimers. However, a VDR-RAR heterodimer binds to the osteocalcin response element and mediates activation by all-trans-retinoic acid. This heterodimer also binds to pure retinoid response elements, but it does not mediate activation by vitamin D alone. In combination with all-trans-retinoic acid, however, vitamin D enhances VDR-RAR heterodimer-mediated gene expression. This finding suggests a direct interaction between nuclear signaling by retinoic acid and vitamin D increasing the combinatorial possibilities for gene regulation by the nuclear receptors involved.

The biologically active metabolites of vitamin A (retinol) and vitamin D, all-trans-retinoic acid (RA) and 1,25-dihydroxyvitamin D₃ (VD), have profound effects on cellular differentiation, embryonic development and calcium homeostasis (1-4). They exert their effects by binding as ligands to their respective intracellular receptors, RARs and VDRs, which are members of the large family of ligand-activated transcription factors termed the nuclear receptor family (5, 6). Studies of estrogen and glucocorticoid receptors indicated that the classical steroid hormone receptors form homodimers and modulate transcription of their target genes via cis-acting palindromic response elements (7, 8). The concept that each hormone binds to a specific receptor homodimer was found to be oversimplified for RARs and VDRs. Both receptors form heterodimeric complexes with RXRs to bind with high affinity to their response elements (9-14). Thus, for transcriptional activation by all-trans-RA and -VD, RXR-dependent pathways were postulated. However, recent evidence suggested that nuclear signaling of all-trans-RA and -VD is also transmitted through RXR-independent pathways (15, 17) presumably mediated by RAR and VDR homodimers. The specific ligand for RXRs was found to be a stereoisomer of all-trans-RA, 9-cis-RA (18, 19). The notion that homodimers of RXRs also bind to DNA (20) constituted a third retinoid signaling pathway. All three RXR-dependent pathways are transcriptionally activated by 9-cis-RA (18, 17, 20). Analogously, RAR-dependent pathways are induced by all-trans-RA (17, 19), and VDR-dependent pathways are induced by VD (15). Interestingly, 9-cis-RA was also found to bind RARs (18, 19) but seems to act as a specific antagonist of their RXR-independent action. In this way 9-cis-RA would not only positively coordinate the action of nuclear signaling of VD and all-trans-RA, it would also negatively regulate the activity of RAR homodimers.

Unlike for steroid hormone receptors, the natural response elements for RARs, RXRs, and VDRs include directly repeated rather than palindromic half-sites based on the hexamer RGGTCA. It was suggested that the spacing between the half-sites directs the binding specificity of RXR, VDR, and RAR. This rule (22, 23) predicts that directly repeated motifs with spacings of one, three, and five base pairs are RXR, VDR, and RAR binding sites, respectively. Accordingly, the RA response element of the P2 promoter of the human RARβ gene is a direct repeat of two RGGTCA motifs separated by five nucleotides (24). This very strong response element is bound by RAR homodimers, RXR homodimers, and RAR-RXR heterodimers and, therefore, responsive to all three retinoid signaling pathways.

The response element in the promoter of the human bone osteoblast-specific protein osteocalcin has been reported to be located at positions -510 to -488 (25) and -513 to -493 (26) mediating VD and all-trans-RA action (27). This response element contains two directly repeated copies of the half-site motif GGTTCA separated by six spacing nucleotides. Apparently, the spacing number is not in accordance with the spacing rule. Additional sequences downstream, however, with a third imperfect repeat separated by three nucleotides were associated with VD responsiveness (28). Recently, it was reported that this extended osteocalcin response element (-510 to -485) is bound and activated by VDR homodimers but not by VDR-RXR heterodimers (15).

Here, we show the critical sequence parameters for the
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switch in specificity from retinoid to VD signaling pathways. We converted the RARβ RA response element in several steps into the osteocalcin VD/RA response element. We found that RGGTGA motifs with five or six intervening nucleotides were bound by VDR homodimers. Neither RAR-RXR heterodimers nor RAR homodimers bound to such response elements. Moreover, only heterodimers of VDRs and RARs conferred responsiveness to all-trans-RA of the osteocalcin element. The interaction between VDRs and RARs suggests, in addition to the RXR-dependent action of VDRs, an alternative cross-talk between the nuclear signaling of VD and retinoids. Therefore, our data provide further combinatorial possibilities for the regulation of VD- and retinoid-responsive genes.

MATERIALS AND METHODS

Cell Culture—The human breast cancer cell line, MCF-7, was grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). The Drosophila cell line, SL-3 (29), was grown at room temperature in Schneider’s medium (Life Technologies, Inc.) supplemented with 15% FCS.

DNA Constructs—The two 42-mer oligonucleotides containing the RARβ gene promoter RA response element (24)

5' ctagtgctGGTAGGTTAAGGGTACCCAGAAGTTCACTGC tgtt
3' acgaGCCATCCCAAGTG67TCTAAGTGAGCtgagcagatc

were synthesized on a Pharmacia Gene Assembler Plus apparatus, purified, phosphorylated, and annealed to yield the indicated double-stranded DNA fragment. This fragment was subcloned into the XbaI site of pBLCAT2 (30) upstream of the tk promoter driving the expression of the chloramphenicol acetyltransferase (CAT) gene. All other response elements (see Fig. 1 for their core sequences) had the same flanking sequences and were synthesized and cloned in the same way. TASS and tDOC cDNAs subcloned into pSG5 (Stratagene) were kindly provided by J. F. Grippo (Roche Nutley).

Transfection and CAT Assays—For MCF-7 cells, 2 × 10^5 cells were seeded into 6-well plates and grown overnight in phenol red-free RPMI supplemented with 10% charcoal-treated FCS. For SL-3 cells, 5 × 10^5 cells/well plate were grown overnight in Schneider’s medium supplemented with 15% charcoal-treated FCS.

Liposomes were formed by incubating 1 μg of the reporter plasmid and 1 μg of the reference plasmid pCH110 (Pharmacia LKB Biotechnology Inc.) with 30 μg of DOTAP (Boehringer Mannheim) for 15 min at room temperature in a total volume of 100 μl. For the transfection of SL-3 cells, 0.25 μg of the reporter plasmid and 1 pg of the reference plasmid pCH110 were added, and the mixture was further incubated for 5 min at 37 °C. Five μg of each RNA were mixed with 175 μl of rabbit reticulocyte lysate, 100 units of RNasin and 20 μM complete amino acid mixture (all from Promega Corp.) in a total volume of 200 μl and incubated at 37 °C for 90 min. The response element probes were prepared by double digestion of the respective plasmid DNA with HindIII and BamHI, purified by gel electrophoresis, and labeled by a fill-in reaction using [α-32P]dCTP and T7 DNA polymerase (Pharmacia). Five μl of in vitro translated receptors were preincubated for 10 min at room temperature in a total volume of 20 μl of binding buffer (10 mM Hepes pH 7.9, 80 mM KCl, 1 mM dithiothreitol, 0.2 μg/μl poly(dI/dC), and 5% glycerol). About 1 ng of labeled probe (25,000 cpm) was added, and the incubation was continued for 30 min. The protein-DNA complexes were resolved on a 5% non-denaturing polyacrylamide gel (at room temperature) in 0.5 × TBE (45 μM Tris borate, 1 μM EDTA, pH 8.3) ( prerun for 2 h). The dried gel was exposed to a Kodak XAR-5 film.

RESULTS

Conversion of the RARβ RA Response Element into the Osteocalcin VD Response Element—To define the sequences that are necessary and sufficient to provide a VD response, we mutated the RA response element within the promoter of the human RARβ gene promoter (24) to convert it in several steps into the VD response element of the osteocalcin gene promoter (25, 26). The RARβ RA response element is a direct repeat of two RGGTGA motifs with five intervening nucleotides (Fig. 1A). The osteocalcin response element, contains two directly repeated GGTTGA motifs with six spacing nucleotides (Fig. 1H). Therefore, we initially mutated the binding motifs and subsequently increased the spacing distance. The T residue in the third position was converted into a G residue (B: DR5[GGTTCA]), alternatively the C residue in the fifth position was changed into a G residue (C: DR5[GGTTGA]), or both mutations were done in parallel (D: DR5[GGGTGA]). Either the spacer was mutated to resemble that of the osteocalcin element without restoring the AP-1 site (E: DR5[GGGTGA]) or simply one nucleotide was inserted (F: DR6[GGGTGA]). The response element G contains the core sequence of the osteocalcin element (positions -511 to -495), which is an extended version of element G containing an additional imperfect motif spaced by three nucleotides. The response elements G and H contain the TGACTCA binding motif for the ubiquitous transcription factor AP-1, a heterodimer of the proto-oncogene products c-Fos and c-Jun (27).

Induction of Endogenous VD and Retinoid Receptors in MCF-7 Cells—The eight response elements (Fig. 1) were cloned in combination with a tk promoter in front of a CAT expression of the chloramphenicol acetyltransferase (CAT) gene. All transcription reactions were incubated for 60 min at 37 °C, then 10 units of RQ1 RNase-free DNase I (Promega Corp.) were added, and the mixture was further incubated for 5 min at 37 °C. Five μg of each RNA were mixed with 175 μl of rabbit reticulocyte lysate, 100 units of RNasin and 20 μM complete amino acid mixture (all from Promega Corp.) in a total volume of 200 μl and incubated at 37 °C for 90 min. The response element probes were prepared by double digestion of the respective plasmid DNA with HindIII and BamHI, purified by gel electrophoresis, and labeled by a fill-in reaction using [α-32P]dCTP and T7 DNA polymerase (Pharmacia). Five μl of in vitro translated receptors were preincubated for 10 min at room temperature in a total volume of 20 μl of binding buffer (10 mM Hepes pH 7.9, 80 mM KCl, 1 mM dithiothreitol, 0.2 μg/μl poly(dI/dC), and 5% glycerol). About 1 ng of labeled probe (25,000 cpm) was added, and the incubation was continued for 30 min. The protein-DNA complexes were resolved on a 5% non-denaturing polyacrylamide gel (at room temperature) in 0.5 × TBE (45 μM Tris borate, 1 μM EDTA, pH 8.3) ( prerun for 2 h). The dried gel was exposed to a Kodak XAR-5 film.

FIG. 1. Response elements. Conversion of the human RA response element of the RARβ (hRARβ) gene (A) (24) into the VD/RA response element of the human osteocalcin (hOC) gene promoter (B) (20) by six intermediate mutations (B–G). The core sequences have identical flanking sequences and were cloned next to a tk promoter in front of a CAT reporter gene (see "Materials and Methods"). Sequences of the response element half-sites are typed in bold letters.
reporter gene. Their transactivation potential was analyzed in the human breast cancer cell line MCF-7. This cell line expresses VDRs, RARs, and RXRs endogenously (15, 17) and, therefore, can be induced by the specific ligands of these receptors, VD, all-trans-RA and 9-cis-RA. Transfected cells were stimulated with the three ligands, each alone and in the three possible combinations (Fig. 2). Short-term incubations (16 h) were performed with the ligands to diminish isomerization of the retinoids. The maximal induction was 4.9-fold for VD, 10.1-fold for all-trans-RA, 9.8-fold for 9-cis-RA, 19.8-fold for all-trans- and 9-cis-RA, and 10.2-fold for VD and all-trans-RA.

A Direct Repeat of the Motif RGGTGA Mediates Inducibility by VD—Fig. 2a shows that responsiveness to VD is linked to the presence of RGGTGA motifs within the response element (D–H). Spacing of five and six nucleotides mediates VD response with similar efficiency. However, the induction is increased by an additional 25% when the spacer contains an inverted copy of the RGGTGA motif, as in the two natural sequences, G and H. This augmentation could be related to the AP-1 site formed by these sequences (27). The two segments of the osteocalcin promoter, G and H, showed nearly identical responses to VD suggesting that, in contrast to former results (28), the third motif (GGGGCA) might not be involved in activation by VDRs.

The Osteocalcin Element Is Also Responsive to All-trans-RA—As shown previously (17) the first two mutations, B and C, of the RARα RA response element decreased its responsiveness to all-trans-RA (Fig. 2b). The five VD response elements based on RGGTGA motifs (D–H) conferred induction by all-trans-RA. The response to 9-cis-RA of elements B and C was similar to that to all-trans-RA (Fig. 2e). As previously reported (15), the extended osteocalcin element (H) was also weakly induced by 9-cis-RA; however, the shorter osteocalcin element (G) and the elements D, E, and F did not respond to this ligand. The response of the eight elements to combined stimulation by VD and 9-cis-RA (Fig. 2d) appears to represent a superposition of their responsiveness to the individual ligands (Figs. 1, a and c). The relative induction of the set of response elements by a combination of all-trans- and 9-cis-RA (Fig. 2e) was very similar to that obtained with 9-cis-RA alone (Fig. 2c); only the RARα element (A) and mutant B responded strongly. The stimulation pattern with VD and all-trans-RA (Fig. 2f) was nearly identical to that obtained with all-trans-RA alone (Fig. 2b).

Homodimer Activity of VDRs, RARs, and RXRs—The presence or absence of VDRs, RARs, and/or RXRs and their specific ligands seems to determine the signaling pathway through which VD- and retinoid-regulated genes are modulated. However, detailed studies in MCF-7 cells are hampered by the fact that these, as almost all mammalian cells, express the two retinoid receptor subfamilies. We have shown previously (15, 17) that in Drosophila SL-3 cells, which are devoid of endogenous VDRs, RARs, and RXRs, the VD and retinoid pathways can be reconstituted separately by transfection with appropriate receptor expression plasmids.

For all response elements tested, transient expression of VDR in SL-3 cells (Fig. 3a) gave a response pattern very similar to that of MCF-7 cells stimulated with VD (Fig. 2a). This not only substantiated the finding that the osteocalcin response element (H) is activated by VDRs in a RXR-independent way (15) but also established this for the other...
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elements with RGGTGA motifs (D–G). Gel shift experiments with in vitro translated VDRs suggested that these types of VD response elements were bound by VDR homodimers. When RARα was expressed, induction with all-trans-RA (Fig. 3b) yielded significant CAT activity only with the RARβ RA response element (A) and mutant B, in accordance to the gel shift experiments. As reported recently (17), RAR homodimers bind to RA response elements, similar to that of the RARβ gene promoter, but apparently they do not mediate the responsiveness to all-trans-RA of the osteocalcin element. The response pattern to 9-cis-RA of SL-3 cells expressing RXRα (Fig. 3c) resembles that established for 9-cis-RA-induced MCF-7 cells (Fig. 2c). RXR homodimers transactivated the RARβ RA response element (A) and mutant B and, although less efficiently, the extended osteocalcin element (H) but not its shorter version (G). This suggested that the imperfect direct repeat, but not the RGGTGA motifs, promotes the binding of RXR homodimers. Gel shifts support this suggestion. As observed previously (15, 17), the in vitro binding of VDRs and RARs to their response elements is for some unknown reason much weaker than that of RXR homodimers or heterodimers.

VDR-RXR and RAR-RXR Heterodimer Activity—In SL-3 cells we expressed RAR, RXR, and VDR in the three combinations of two, thus creating by free association variations of homodimeric and heterodimeric receptor species. Subsequently, we treated these cells with the responsive ligands either individually or in appropriate combinations of two. In the presence of VDR and RXRα the response pattern after stimulation with VD alone (Fig. 4a, black columns) was nearly identical to that established in the presence of VDR alone (Fig. 3a). The response pattern to 9-cis-RA alone (Fig. 4a, light gray columns) was also very similar to that established after expression of RXRα alone (Fig. 3c). The response pattern after treatment with both ligands (Fig. 4a, white columns) was essentially identical to that observed for the response elements A–C after treatment with 9-cis-RA alone and to that observed for the response elements D–G after treatment with VD alone. Only the extended osteocalcin element (H), which is responsive to each of the two ligands, showed an increased induction by combined stimulation with VD and 9-cis-RA. This induction was only additive and not synergistic, as expected for the action of VDR-RXR heterodimers (15). Since homodimers of in vitro translated RXRs migrate in gel shift experiments slightly faster than homo- and heterodimers of VDRs and RARs, RXR homodimers could be discriminated in gel shift experiments from other protein complexes (Fig. 4a). A plausible interpretation is that the response elements A–C were bound by RXR homodimers, the elements D–G by VDR homodimers, and the extended osteocalcin element by both VDR and RXR homodimers but not by VDR-RXR heterodimers. The combined expression of RARα and RXRα followed by stimulation with all-trans-RA (Fig. 4b, dark gray columns) resulted in a response pattern similar to that observed in the presence of RARα alone (Fig. 3b). Likewise, the 9-cis-RA response pattern (light gray columns) is comparable with that observed in the presence of RXRα alone (Fig. 3c).

For a detailed comparison the higher absolute stimulation rate for RAR-RXR heterodimers as compared with RAR and RXR homodimers has to be taken into account. The stimulation with both ligands (white columns) resulted in a synergistic response for element B, whereas for elements A, C, D, and E, only an additive effect was observed. As already shown in the previously mentioned experiments, the extended osteocalcin element (H) was not activated by all-trans-RA, and its weak activation by 9-cis-RA was mediated by RXR homodimers.

Fig. 3. Homodimer activity of VDR, RAR, and RXR. SL-3 cells were transfected with CAT reporter plasmids containing one of the eight different response elements (A–H, see Fig. 1) and expression vectors for VDR (a), RARα (b), or RXRα (c). Induction by VD (a), all-trans-RA (b), and 9-cis-RA (c) is expressed as percentage of that obtained with the VD/RA response element of the human osteocalcin gene promoter (a) and the human RA response element of the RARβ gene promoter (b and c), respectively. The bars indicate standard deviation. As a control, equal amounts of the respective 32P-labeled DNA fragments were shifted with in vitro translated VDR (a), RARα (b), or RXRα (c) receptor protein, respectively. The shifted protein-DNA complexes are shown below the histograms.
Fig. 4. Heterodimer activity of VDR, RAR, and RXR. SL-3 cells were transfected with CAT reporter plasmids containing one of the eight different response elements (A–H, see Fig. 1) and expression vectors for VDR and RXRα (a), RARα and RXRα (b), or VDR and RARα (c). Induction values by VD (black columns), all-trans-RA (dark gray columns), 9-cis-RA (light gray columns), and mixtures of VD and 9-cis-RA (white bars in a), all-trans-RA and 9-cis-RA (white bars in b), or VD and all-trans-RA (white bars in c) are expressed as percentage of those obtained with the VD/RA response element of the human osteocalcin gene promoter (in a) and the human RA response element of the RARP gene promoter (in b and c), respectively. The bars indicate standard deviation. As a control, equal amounts of the respective 32P-labeled DNA fragments were shifted with preincubated mixtures of in vitro translated VDR and RXRα (a), RARα and RXRα (b), or VDR and RARα (c) receptor proteins, respectively. The shifted protein-DNA complexes are shown below the histograms.

VDR-RAR Mediate the All-trans-RA Responsiveness of the Osteocalcin VD Response Element—Since potential isomerization of retinoids in our experimental system was kept at a minimum, responsiveness to all-trans-RA should be considered specifically mediated by the action of RARs. The experiments in Figs. 3b and 4b clearly indicated that RARs do not bind to the osteocalcin response element, neither as homodimers nor as RAR-RXR heterodimers. Therefore, we tested the third combinatorial possibility for heterodimer formation between VDRs, RARs, and RXRs, i.e. VDR-RAR heterodimers. When SL-3 cells were transfected with expression plasmids encoding VDR and RARα, we observed for the elements D–G a response to VD (Fig. 4c compared with Figs. 3a and
In addition, these elements showed a response to all-trans-RA (Fig. 3c) as well as through a pathway of coupled action of VD and all-trans-RA, since in mammalian cell systems, like MCF-7 cells (Fig. 2), one cannot discriminate between the transcriptional effects of homo- and heterodimers of VDRs, RARs, and RXRs, we have used for our studies Drosophila SL-3 cells. We have previously shown (15,17) that SL-3 cells do not contain nuclear receptors that can functionally substitute for the mammalian RXR even though they express the ultraspireacle gene product, which is closely related to RXRs (31). Also putative "accessory" factors (32) do not bind 9-cis-RA or VD and therefore cannot be responsible for the observed synergistic effects related to transcriptionally active heterodimers.

We found that activation by VDR homodimers is mediated only by response elements that are based on direct repeats of two RGGTGA motifs separated by five or six nucleotides (Fig. 3a). From the few known VD response elements only the human osteocalcin gene promoter contains this kind of direct repeat. Other VD response elements, as those in the promoters of the mouse osteopontin gene (33) or of the rat osteocalcin gene (34), consist of direct repeats with only three spacing nucleotides and are activated by VDR-RXR heterodimers (15). The extended human osteocalcin response element additionally contains an imperfect direct repeat with three intervening nucleotides and was, therefore, believed to bind the VDR-RXR heterodimers to the human homologue of this response element (Fig. 4c).

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REFERENCES

1. Tabin, C. J. (1991) Cell 66, 199-217
2. Leid, M., Kastner, P., and Chambon, P. (1992) Trends Biochem. Sci. 17, 427-433
3. Pike, J. W. (1991) Annu. Rev. Nutr. 11, 189-216
4. Norman, A. W., Nemere, I., Zhou, L.-X., Bishop, J. E., Lowe, K. E., Mayar, A. C., Collins, E. D., Taoka, T., Sargee, I., and Farsch-Carson, M. C. (1992) J. Steroid Biochem. Mol. Biol. 41, 251-260
5. Evans, R. M. (1988) Science 240, 889-890
6. O'Malley, B. W. (1990) Mol. Endocrinol. 4, 363-369
7. Green, S. and Chambon, P. (1986) Trends Genet. 4, 309-314
8. Luisi, B. F., Xu, W. W., Orwinowski, Z., Freedman, L. P., Yamamoto, K. R., and Sigier, P. B. (1991) Nature 352, 497-505
9. Yu, V. C., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Naar, A. M., Kim, S. Y., Botzin, J.-M., Glass, C. R., and Rosenfeld, M. G. (1992) Cell 67, 125-134
10. Bogge, T. H., Foh, J., Lonnoy, O., and Stunnenberg, H. G. (1992) EMBO J. 11, 1409-1418
11. Kluweer, S. A., Unoeno, K., Mangeldorff, D. J., and Evans, R. M. (1992) Nature 355, 446-449
12. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J. Y., Staib, A., Garnier, J.-M., Mader, S., and Chambon, P. (1992) Cell 68, 377-385
13. Marks, M. S., Heilender, P. L., Nagata, T., Segars, J. H., Appella, E., Nikodem, V. M., and Onko, K. (1992) EMBO J. 11, 1419-1435
14. Zhang, X.-K., Hoffman, B., Tran, P. B.-V., Gruenp, G., and Pfahl, M. (1990) Nature 350, 441-446
15. Carlini, C., Bendik, I., Wyss, A., Meier, E., Stirzenbecker, L. J., Grippi, J. F., and Hunkeler, W. (1993) Nature 361, 687-689
16. Green, S. (1990) Nature 361, 590-591
17. Schröder, M., Wyss, A., Stirzenbecker, L. J., Grippi, J. F., LeMotte, P., and Carlberg, C. (1993) Nature 361, 1231-1237
18. Heyman, R. A., Mangeldorff, D. J., Dicj, Y. A., Stein, R. R., Eichele, G., Evans, R. M., and Thaller, C. (1992) Cell 68, 397-406
19. Levin, A. A., Stirzenbecker, L. J., Kramar, S., Bozakowski, T., Huseulon, C., Allenby, G., Speck, J., Kratziesen, C., Rosenberger, M., Lovey, A., and Heyman, J.-H. (1991) EMBO J. 10, 363-369
and Grippo, J. F. (1992) Nature 355, 359-361
20. Zhang, X.-K., Lehmann, J., Hoffmann, B., Dawson, M. L., Cameron, J., 
Graupner, G., Herrmann, T., Tran, P., and Pitel, M. (1992) Nature 358, 
587-591.
21. Allenby, G., Bocquel, M.-T., Saunders, M., Kazmer, S., Speck, J., Rosen- 
berger, M., Lovey, A., Kastner, P., Grippo, J. F., Chambon, P., and Levin, 
A. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 89, 33-34.
22. Nair, A. M., Beutin, J.-M., Lipkin, S. M., Yu, V. C., Holloway, J. M., Glass, 
C. K., and Rosenfeld, M. G. (1991) Cell 68, 1267-1276.
23. Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991) 
Cell 65, 1255-1266.
24. de The, H., Vivanco-Ruiz, M. M., Tiollais, P., Stunnenberg, H., and Dejean, 
A. (1990) Nature 345, 177-180.
25. Kerner, S. A., Scott, R. A., and Pike, J. W. (1989) Proc. Natl. Acad. Sci. 
U. S. A. 86, 4455-4459.
26. Morrison, N. A., Shue, J., Fragonas, J.-C., Verkest, V., McMenemey, M. 
L., and Eisman, J. A. (1989) Science 246, 1158-1161.
27. Schule, R., Umesono, K., Mangelsdorf, D. J., Bolaso, J., Pike, J. W., and 
Evans, R. M. (1990) Cell 81, 497-504.
28. Ozono, K., Liao, J., Kerner, S. A., Scott, R. A., and Pike, J. W. (1990) J. 
Biol. Chem. 265, 21881-21886.
29. Schneider, I. (1972) J. Embryol. Exp. Morphol. 27, 353-365.
30. Luckow, H., and Schütz, G. (1987) Nucleic Acids Res. 15, 5490.
31. Oro, A. E., McKeeown, M., and Evans, R. M. (1990) Nature 347, 298-301.
32. Yao, T.-P., Segraves, W. A., Oro, A. E., McKeeown, M., and Evans, R. M. 
(1992) Cell 71, 63-72.
33. Nota, M., Vogel, R. L., Craig, A. M., Pahl, J., DeLuca, H. F., and Denhardt, 
D. T. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9965-9969.
34. Demay, M. B., Gerard, J. M., DeLuca, H. F., and Kronenberg, H. M. (1990) 
Proc. Natl. Acad. Sci. U. S. A. 87, 369-372.
35. Liao, J., Ozono, K., Sone, T., McDonnell, D. P., and Pike, J. W. (1990) 
Proc. Natl. Acad. Sci. U. S. A. 87, 9751-9755.
36. Sone, T., Ozono, K., and Pike, J. W. (1991) Mol. Endocrinol. 5, 1578-1586.
37. Ross, T. K., Moss, V. E., Pahl, J. M., and DeLuca, H. F. (1992) Proc. Natl. 
Acad. Sci. U. S. A. 89, 558-560.
38. Glass, C. K., Devary, O. V., and Rosenfeld, M. G. (1990) Cell 63, 729-738.