VDR-Alien: a novel, DNA-selective vitamin D₃ receptor-corepressor partnership

PATSIE POLLY,*† MICHAELA HERDICK,*† UDO MOEHREN,‡ ARIA BANIAHMAD,‡ THORSTEN HEINZEL,† AND CARSTEN CARLBERG*,‡
*Institut für Physiologische Chemie I, Heinrich-Heine-Universität, D-40001 Düsseldorf, Germany; †Genetisches Institut der Justus-Liebig-Universität, D-35392 Giessen, Germany; and ‡Georg-Speyer-Haus, D-60596 Frankfurt, Germany

ABSTRACT The vitamin D receptor (VDR) is a transcription factor that transmits incoming 1,25-dihydroxyvitamin D₃ (1α,25(OH)₂D₃) signaling via combined contact with coactivator proteins and specific DNA binding sites (VDREs), which ultimately results in activation of transcription. In contrast, the mechanisms of transcriptional repression via the VDR are less well understood. This study documents VDR-dependent transcriptional repression largely via histone deacetylase (HDAC) activity. Direct, ligand-sensitive protein-protein interaction of the VDR with the nuclear receptor corepressor (NCoR) and a novel corepressor, called Alien, was demonstrated to be comparable but independent of the VDR AF-2 trans-activation domain. Functional assays indicated that Alien, but not NCoR, displays selectivity for different VDRE structures for transferring these repressive effects into gene regulatory activities. Moreover, superrepression via Alien was found to be affected only in part by HDAC inhibitors such as trichostatin A. Finally, for a dissociation of VDR-Alien complexes in vitro and in vivo, higher ligand concentrations were needed than for a dissociation of VDR-NCoR complexes. This suggests that Alien and NCoR are using different interfaces for interaction with the VDR and different pathways for mediating superrepression, which in turn characterizes Alien as a representative of a new class of corepressors. Taken together, association of the VDR with corepressor proteins provides a further level of transcriptional regulation, which is emerging as a complex network of protein-protein interaction-mediated control.—Polly, P., Herdick, M., Moehren, U., Baniahmad, A., Heinzel, T., Carlberg, C. VDR-Alien: a novel, DNA-selective vitamin D₃ receptor-corepressor partnership. FASEB J. 14, 1455–1463 (2000)

Key Words: gene regulation • repression • NCoR

The genomic effects of vitamin D₃, which is the biologically active form of 1α,25-dihydroxyvitamin D₃ (1α,25(OH)₂D₃), are principally mediated through the vitamin D receptor (VDR) (1), which is a member of the nuclear receptor transcription factor superfamily (2). VDR binds as a dimer to specific sequences in promoter regions of 1α,25(OH)₂D₃ target genes, commonly referred to as 1α,25(OH)₂D₃ response elements (VDREs) (3). The heterodimer partner for the VDR is the retinoid X receptor (RXR) (4), which is the nuclear receptor for 9-cis retinoic acid (5). Simple VDREs consist of two hexameric nuclear receptor binding sites, and VDR-RXR heterodimers can preferentially bind to directly repeated core binding site arrangements with three spacing nucleotides (DR3-type VDREs) or to inverted palindromic structures with nine intervening nucleotides (IP9-type VDREs) (4). Moreover, complex VDREs are known that are formed by more than two hexameric core binding sites and may contain additional binding sites for members of other transcription factor families (6).

The nuclear receptors for 3,5,3′-triiodothyronine (T₃), T₃R, and all-trans retinoic acid (RAR) display, in most cases, conserved functions that are characteristic for the nuclear receptor superfamily (3). A high degree of homology is present between family members in their DNA binding domain (DBD) of 66–70 amino acids (aa), whereas a lesser degree of primary structure homology is apparent in the carboxy-terminal ligand binding domain (LBD) of 250–300 aa. The DBD and the LBD are connected by a flexible hinge region of 30–50 aa (7). Crystal structure analysis of six presently characterized nuclear receptor LBDs has demonstrated a conserved spatial structure formed by 11–12 α-helices (8, 9). The LBD has diverse functions, as it is not only involved in ligand binding, but also in interaction with other nuclear receptors for the formation of homo- and heterodimeric complexes and in contact with nuclear mediator proteins, such as coactivators and corepressors, for modulation of transcriptional activities. Contact points for coactivators have been mapped in the activation function 2 (AF-2) domain of helix 12 and in helix 3, whereas interaction regions for
corepressors have been suggested to be within helix 1 (CoR-box) (10, 11) and helices 10 and 11 (12).

Many coactivators and corepressors, generally grouped as families, have been identified, characterized, and studied in the context of transcriptional activation or repression (13). The nuclear corepressor (NCoR) (11) and silencing mediator for retinoid and thyroid hormone receptors (SMRT) (10) were the ‘founding’ members of the corepressor family, which has grown to include variants of these like RIP13a, RIP13Δ1, and SMRTe (14, 15), others such as SUNCoR (16), and the most recent member, Alien (17). Interaction of corepressors with nuclear receptors has been described, especially for T₃R and RAR, but also for orphan members of the nuclear receptor superfamily such as RevErbα (18). Several mechanisms are postulated to be operating to mediate repression. Identification of several histone deacetylases (HDACs) has provided a molecular link between histone deacetylation, corepressor activity and transcriptional regulation (13, 19). In addition, corepressor interaction with components of the basal transcriptional machinery, which represent alternative pathways to HDAC-nuclear receptor interaction, has also been suggested (20, 21).

Several reports indicate a repressive function involving the VDR that includes direct repression mediated by VDR contacts with DNA sequences within target genes (22–24), competitive VDR-squelching activities resulting in heterodimer formation with other transcription factors (25, 26), VDR-mediated repression of thyroid and retinoid signaling, and hence another form of receptor squelching (27–29), and the recent report describing inhibition of Smad3-mediated potentiation of VDR function by Smad7 (30). It has previously been demonstrated that the VDR can interact with SMRT, NcoR, and the NcoR variant RIP13Δ1 (31, 32), but compared with other nuclear receptors, VDR-corepressor interaction is less well understood.

In this report, VDR-mediated repression and corepressor-mediated superrepression were studied in solution and on VDREs. VDR was confirmed to interact effectively with NCoR and was demonstrated for the first time to interact with the novel corepressor Alien. VDR-Alien interaction was found to be comparable to T₃R-Alien interaction. VDR-Alien complexes showed DNA selectivity by mediating repression only through DR3-type VDREs and not through IP9-type VDREs.

MATERIALS AND METHODS

DNA constructs

VDRE-driven reporter gene constructs

One copy of the complex DR6/DR3-type VDRE from the human osteocalcin promoter (33, 34), four copies of the simple DR3-type VDRE from the rat atrial natriuretic factor (ANF) gene promoter (35), and four copies of the simple IP9-type VDRE from the mouse c-fos promoter (36) were fused with the thymidine kinase (tk) minimal promoter driving the luciferase reporter gene; the core sequences of the VDREs are given in Fig. 1 and Fig. 3.

GAL4 fusion constructs

The DBD of the yeast transcription factor GAL4 (aa 1–147) was fused with the cDNA of the LBD of human VDR (aa 109–427), with the cDNA of the LBD of human T₃Rβ1 (aa 165–461) (11), and with the cDNA of the first repression domain of NCoR (aa 1–393) (11). For the mammalian one-hybrid assays, the luciferase reporter gene was driven by three copies of the GAL4 binding site fused to the tk promoter (11).

Yeast two-hybrid expression constructs

The fusion protein construct pLexA-VDR80–427 was created by subcloning a region of the cDNA of human VDR spanning from aa 80–427 (hinge region and LBD) into the pGilda vector (Clontech, Heidelberg, Germany) containing the LexA-DBD. pG4–5h-Alien (17) and pG4–5NCoR2240–2453 (11) were constructed by fusing the full-length cDNA of Alien (305 aa) or a region of the cDNA coding for the nuclear receptor interaction domain of NCoR (spanning from aa 2240–2453, respectively), to the activation domain of pG4–5. The β-galactosidase reporter gene vector used was pSH18-34 (17).

In vitro translation/mammalian constructs

The cDNAs for human VDRwt (37), human VDR138stop (38), human RXRα (5), and chicken T₃Rα (39) were subcloned into the SV40 promoter-driven pSG5 expression vector (Stratagene, Heidelberg, Germany). The cDNA for human Alien (17) was subcloned into the pT7βSal vector and the RSV promoter-driven pABΔgal expression vector. The cDNA of mouse NCoR (11) was subcloned into the CMV promoter-driven CMX expression vector and the cDNA of the carboxy-terminal region of mouse NCoR (aa 1629–2453) was subcloned into the vector pBIIKS (Stratagene) (11).

GST fusion protein constructs

The cDNA of human Alien (17) and two cDNA fragments of mouse NCoR (spanning from aa 1954–2215 and aa 2218–2453, respectively) (11) were subcloned into the GST fusion vector pGEX (Amersham-Pharmacia, Freiburg, Germany).

Transfection and luciferase assays

Cos-7 SV40-transformed African green monkey kidney cells were seeded into 6-well plates (10⁵ cells/ml) and grown overnight in phenol red-free DMEM supplemented with 10% charcoal-treated fetal bovine serum (FBS). Liposomes were formed by incubating 1 μg of the reporter plasmid, 1 μg of each pSG5-based receptor expression vectors for VDR and RXR, 1 μg of the expression vectors for Alien or NCoR, and 1 μg of the reference plasmid pCH110 (Amersham-Pharmacia) with 15 μg Ni-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP, Roche Diagnostics, Mannheim, Germany) for 15 min at room temperature in a total volume of 100 μl. After dilution with 900 μl phenol red-free DMEM, the liposomes were added to the cells. Phenol red-free DMEM supplemented with 30% charcoal-treated FBS
expression vectors for the respective GAL4 fusion proteins and a GAL4 binding site-driven luciferase reporter gene construct were used in transfections. For both types of assays, the cells were lysed 16 h after onset of stimulation using the reporter gene lysis buffer (Roche Diagnostics) and the constant light signal luciferase reporter gene assay was performed as recommended by the supplier (Roche Diagnostics). The luciferase activities were normalized with respect to β-galactosidase activity and induction or repression factors were calculated as the ratio of luciferase activity of ligand-stimulated cells to that of solvent controls.

Yeast two-hybrid assays

Yeast two-hybrid assays were performed as described previously (12, 40). The EGY48 yeast strain was transformed with the pLexA-VDR_{40-427} as the bait, pJG4–5-Alien and pJG4–5-NCoR as the activator vectors and pSH18–34 as the reporter vector. Solvent or 1α,25(OH)\_2D\_3 (at a final concentration of 500 nM) was added to the assay to test ligand-mediated dissociation effects. β-Galactosidase reporter gene activity was determined 16 h after onset of stimulation. The intensity of solvent-treated or liganded VDR-corepressor interaction was expressed as Miller units.

In vitro protein translation

In vitro translated VDR, VDR_{413Stop}, and T\_3R proteins were generated by transcribing their linearized pSG5-based cDNA expression vectors with T\_7 RNA polymerase and translating these RNAs in vitro using rabbit reticulocyte lysate as recommended by the supplier (Promega, Mannheim, Germany). In vitro translated Alien and NCoR proteins were generated from the respective expression plasmids using the TNT rabbit reticulocyte system as recommended by the supplier (Promega). [35S]-labeled in vitro translated proteins were routinely checked for equal loading by electrophoresis through a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel, subsequent gel drying, exposure to a Fuji MP2040S PhosphorImaging screen, and quantification with the use of a Fuji FLA2000 reader (Tokyo, Japan) using Image Gauge software (Raytest, Sprockhövel, Germany).

GST pull-down assays

Bacterial overexpression of GST-Alien, GST-NCoR_{1954–2215} and GST-NCoR_{2218–2453} was facilitated in the Escherichia coli BL21(DE3)pLysS strain (Stratagene). GST-Alien fusion protein expression was performed with isopropyl-β-D-thiogalactopyranoside (IPTG, 1.25 mM) for 30°C and GST-NCoR_{1954–2215} and GST-NCoR_{2218–2453} expression with IPTG (1.25 mM) for 37°C. The fusion proteins were checked for equal loading by Coomassie brilliant blue staining. GST pull-down assays were performed by coinubcation of GST-Alien, GST-NCoR_{1954–2215}, and GST-NCoR_{2218–2453} fusion proteins with in vitro translated [35S]-labeled VDRwt, [35S]-labeled VDR_{413Stop}, or [35S]-labeled T3R. Nuclear receptors were preincubated for 20 min at room temperature with solvent (ethanol or DMSO) or the respective ligands (10 μM or graded concentrations) prior to addition of a 50% GST-Alien-, GST-NCoR_{1954–2215}, or GST-NCoR_{2218–2453}-Sepharose bead slurry in PPI buffer (20 mM HEPES, pH 7.9, 200 mM KCl, 1 mM EDTA, 4 mM MgCl\_2, 1 mM dithiothreitol, 0.1% Nonidet P-40 and 10% glycerol). All GST fusion-Sepharose slurries were preblocked in PPI buffer containing bovine serum albumin (1 mg/μl) prior to use in pull-down assays. VDR or T3R proteins that were not bound to GST fusion proteins were washed away with PPI buffer. In vitro
translated VDR or T3R used (10% of input) and bound to GST-Alien, GST-NCoR_{1954–2215}, or GST-NCoR_{2218–2453} was detected by electrophoresis through a 10% SDS-polyacrylamide gel, subsequent gel drying, exposure to a Fuji MP2040S PhosphorImaging screen, and quantified with the use of a Fuji FLA2000 reader using Image Gauge software.

**Immunoprecipitation assay**

Immunoprecipitation assays were performed with in vitro translated, [35S]-labeled Alien or [35S]-labeled NCoR with an anti-VDR polyclonal antibody (directed against the carboxy-terminal region of the VDR; Santa Cruz, Heidelberg, Germany). In vitro translated [35S]-labeled Alien and [35S]-labeled NCoR (input 100%), in vitro translated VDR, and either ethanol solvent or 1α,25(OH)_{2}D_{3} (10 μM) were incubated with anti-VDR antibody for 20 min at room temperature, followed by an overnight incubation at 4°C in IP buffer (20 mM HEPES, pH 7.9, 200 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1% Nonidet P-40, and 10% glycerol) containing a mixture of protease inhibitors (Roche Diagnostics). After this incubation, 50 μl of Protein Sepharose A beads (Amersham-Pharamacia) was added to the reaction mixture and further incubated at 4°C with constant rotation. Alien or NCoR proteins that were not bound to VDR-coupled anti-VDR were vigorously washed away with a series of NET-N buffer (150 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, 0.5% Nonidet P-40, 10% glycerol, and protease inhibitors), IP buffer and NET buffer (150 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, and 10% glycerol) washes. [35S]-Corepressor-VDR-anti-VDR-antibody interaction was detected by electrophoresis through a 10% SDS-polyacrylamide gel, subsequent gel drying, exposure to a Fuji MP2040S PhosphorImaging screen, and quantification with the use of a Fuji FLA2000 reader and Image Gauge software.

**RESULTS**

Transient transfections of the luciferase reporter gene construct driven by the complex VDRE from the human osteocalcin gene promoter (33), together with the expression vectors for VDR and RXR into Cos-7 cells, were performed to assess the ability of VDR to mediate repression (Fig. 1A). The complex VDRE was chosen because an internal AP-1 site provides this response element with a relatively high basal activity. In the absence of 1α,25(OH)_{2}D_{3}, increasing concentrations of the VDR expression vector augmented the intrinsic repression activity of the VDR, whereas overexpression of RXR was without significant effect. Moreover, increasing amounts of overexpressed VDR resulted in an increased repressing effect. This suggests that VDR-triggered repression is not only mediated by VDR-RXR heterodimers, but also by other VDR-containing protein complexes, such as VDR homodimers and VDR-RAR heterodimers. In fact, the formation of both types of dimeric complexes has previously been shown on the complex VDRE chosen here (34, 37). In the presence of the HDAC inhibitor TSA, VDR-mediated repression was found to be completely abolished. To test whether this repression was in fact mediated by the VDR, fusion proteins, each containing the DBD of GAL4 and either the LBD of VDR, the LBD of T3R or the first repression domain of NCoR, respectively, were overexpressed in HeLa cells. GAL4 binding site-driven luciferase reporter gene activity was then determined. In this mammalian one-hybrid assay, the VDR-LBD showed 28.6-fold repression, the T3R-LBD mediated 9.7-fold repression, and the repression domain I of NCoR displayed 45.1-fold repression.

VDR-corepressor interactions were assessed, in vitro, with the use of bacterially overexpressed GST fusion proteins. GST pull-down assays demonstrated that VDR can interact with the corepressor Alien in a ligand-dependent manner (Fig. 2A). The intensity of the VDR-Alien interaction appeared to be similar to the T3R-Alien interaction. A ligand-mediated dissociation of the VDR-Alien complex was seen on treatment with 1α,25(OH)_{2}D_{3} (10 μM), where VDR-Alien interaction appeared to be reduced by 50–60% in the presence of 1α,25(OH)_{2}D_{3}, which was slightly weaker than T3 mediated dissociation of T3R-Alien interaction. As a control, VDR and T3R interaction with the two established nuclear receptor interaction domains of NCoR, i.e., NCoR_{1954–2215} and NCoR_{2218–2453} was also tested in GST pull-down assays (Fig. 2B). VDR showed comparable, ligand-dependent interaction with both NCoR interaction domains. A similar range of ligand-dependent interaction with NCoR_{1954–2215} and NCoR_{2218–2453} was also found with T3R, but with NCoR_{2218–2453}, T3R displayed an interaction that was three- to fourfold more effective. The role of the VDR AF-2 domain for an interaction with corepressors was investigated using VDR_{413Stop}, an AF-2 deletion mutant of the VDR (Fig. 2C), and a range of VDR AF-2 point mutants spanning the entire VDR AF-2 domain from aa 417 to 423 (data not shown) in ligand-dependent GST pull-down assays. When assessing VDR_{413Stop}, the effect of ligand-dependent dissociation of Alien or NCoR was still present. Furthermore, when checking the VDR AF-2 point mutated series, it was shown that not one mutant demonstrated a loss of ligand-dependent dissociation from either Alien or NCoR (data not shown). Taken together, this suggested that VDR-Alien and VDR-NCoR interaction was independent of the AF-2 domain. As an additional confirmation of VDR-Alien and VDR-NCoR interaction, an antibody directed against the carboxy-terminal region of the VDR, was used in immunoprecipitation assays with in vitro translated [35S]-labeled Alien or [35S]-labeled-NCoR_{1629–2453} (Fig. 2D). In this assay, VDR-corepressor interaction also appeared to be ligand sensitive as the addition of ligand resulted in a dissociation of 50% of Alien and 26% of NCoR from the VDR-antibody complex. Moreover, an antibody directed against the amino-terminal region of the VDR was also tested and confirmed VDR-Alien...
and VDR-NCoR interaction (data not shown). Finally, yeast two-hybrid assays were performed in order to assess VDR-corepressor association in vivo (Fig. 2E). Yeast cells were transformed with expression vectors for LexA DBD-VDR80–427 fusion protein as the bait and activation domain fusion proteins containing Alien or NCoR2240–2453 as activators. β-galactosidase reporter gene assays demonstrated VDR-Alien and VDR-NCoR interaction and an ligand-dependent dissociation of these complexes.

Ligand-driven, DNA-independent VDR-corepressor dissociation was assessed in GST pull-down assays in the presence of graded concentrations of 1α,25(OH)₂D₃ (Fig. 3A). On close examination of the dose-dependent dissociation of VDR-corepressor interaction, VDR-NCoR interaction appeared to be slightly more ligand-sensitive, i.e., more susceptible to 1α,25(OH)₂D₃-mediated dissociation with a half-maximal inhibition (IC₅₀) value of 330 nM, than VDR-Alien interaction with an IC₅₀ value of 400 nM. However, these differences in the IC₅₀ values were not found to be statistically significant. Incomplete VDR-corepressor dissociation was observed. To assess the functional consequences of DNA-dependent effects of corepressor-VDR interaction, luciferase reporter gene assays were performed from Cos-7 cells that were transfected with expression vectors for VDR, RXR, Alien, or NCoR1–2453 together with luciferase reporter gene constructs driven by the DR3-type VDRE of the rat ANF gene promoter (35) (Fig. 3B) or IP9-type VDRE of the mouse c-fos gene promoter (36) (Fig. 3C). Cells were then stimulated...
for 16 h with graded 1α,25(OH)₂D₃ concentrations. β-Galactosidase normalized luciferase reporter gene activities were expressed as fold induction and provided typical dose-response curves. The overexpression of NCoR and Alien appeared to be similar as judged by Western blotting (data not shown). On DR3-type VDREs, an overexpression of Alien as well as of NCoR reduced reporter gene activity over the whole concentration range from 0.01–100 nM, but did not significantly affect the half-maximal activation (EC₅₀) value of 4 nM (Fig. 3B). On IP9-type VDREs, an overexpression of NCoR resulted in similar effects, but shifted the EC₅₀ value from 1.4 nM to 3.2 nM (Fig. 3C). In all three dose-response curves, the overexpression of corepressors resulted in a repressive effect, even at saturating ligand concentrations. This indicates that there is also an incomplete dissociation of VDR-corepressor interaction in vivo. An overexpression of Alien on IP9-type VDREs did not provide a significant repressive effect on gene activation or a shift in ligand sensitivity.

Luciferase reporter gene assays performed with overexpressed Alien and NCoR on DR3- and IP9-type VDREs in direct comparison (Fig. 4) could confirm the selectivity of Alien to mediate ‘superrepression’ of VDR-RXR heterodimer activity only via DR3-type VDREs. Moreover, in this experimental series, the effect of TSA was demonstrated to partially relieve the NCoR-mediated superrepression. In contrast, the incomplete reduction of the repressive effect of VDR-Alien on DR3-type VDREs suggested that Alien is relatively TSA insensitive and is using, at least in part, alternate molecular pathways.

An additional difference between Alien and NCoR became obvious when their superrepressive effects on DR3-type, VDRE-bound VDR-RXR heterodimers in Cos-7 cell reporter gene assays were normalized. At a concentration of 0.3 nM 1α,25(OH)₂D₃, Alien
still displayed 70% repression whereas NCoR showed only 40% of its repression potential (Fig. 5). In contrast, at 10-fold higher ligand concentration (3 nM) for both proteins, less than 15% of their superrepressive activity remained.

DISCUSSION

According to the present model of nuclear hormone signaling (41, 42) in the absence of ligand, class II nuclear receptors are complexed with corepressor molecules that keep chromatin condensed and therefore have to be released before transcriptional activation can occur. This suggests that a primary nuclear hormone-responding gene is in a repressed state until ligand actively destabilizes the nuclear receptor-corepressor complex. This report describes VDR as a nuclear receptor with intrinsic repression activity that, as being sensitive to TSA treatment, appears to act through the established Sin3-HDAC pathway (43–45). On natural VDREs, full-length VDR has relatively weak silencing activity compared with T3R and RAR (11, 16), but in the mammalian one-hybrid system the VDR-LBD demonstrated more potent repressive activity than T3R. On the complex VDRE of the human osteocalcin gene promoter, VDR is known to act as a homodimer as well as a heterodimer (37, 46); however, overexpression of RXR was found to be without additional effect on repression. This suggests that in addition to VDR-RXR heterodimers, other VDR-containing nuclear complexes can also serve as mediators of VDR-triggered repression. Moreover, NCoR was confirmed to directly contact the VDR with similar binding affinity with both of its nuclear receptor interaction domains. The VDR-NCoR complex dissociates with increasing 1α,25(OH)2D3 concentrations, suggesting that VDR-NCoR interaction is valid and can be compared to established T3R- or RAR-NCoR interactions (10, 11, 17).

The most significant finding of this report is the demonstration of a protein-protein interaction of VDR with the novel corepressor Alien, both in vivo and in vitro. Compared to NCoR (2453 aa and a molecular mass of 270 kDa), Alien is much smaller (305 aa and a molecular mass of 34 kDa) and shows no obvious sequence homology to known corepressor molecules (17). Alien is known to interact with T3R and ecdysone receptor but not with RAR (17). This study shows that in its functionality as a corepressor of VDR, Alien appears to be at least as potent as NCoR. Alien even appears to interact more effectively with VDR than NCoR does with VDR, as higher 1α,25(OH)2D3 concentrations are needed to dissociate the VDR-Alien complex than VDR-NCoR complexes both in vitro and in vivo. However, the most interesting characteristic of Alien is that it mediates repression only from VDR-RXR complexes that are bound to DR3-type VDREs and not from those bound to IP9-type VDREs. The protein-DNA complexes that are formed on these two types of response elements are clearly distinct. On DR3-type elements, the DBDs of VDR and RXR bind to the same side of the DNA, they contact each other and show a head-to-tail orientation; on IP9-type elements, the two DBDs bind to opposite sides of the DNA at a distance that is too wide for a direct contact of the core DBDs (1, 7). Since the LBDs of VDR and RXR...
in both types of protein-DNA complexes are assumed to interact in a similar fashion, it is more likely that a differential effect of Alien is due to its interaction with the hinge region rather than with the LBD of VDR. The conformation of VDR-RXR heterodimers on IP9-type VDREs suggests that the interaction interface of VDR with Alien may not be accessible due to steric hindrance. For interaction with nuclear receptors, NCoR requires the so-called CoR-box, a conserved aa sequence within helix 1 of the LBD, within nuclear receptors such as T3R and RAR (11). This suggests that Alien and NCoR are using different interaction interfaces within the VDR. Both in vitro GST pull-down as well as in vivo mammalian reporter gene assays suggested that, at saturating ligand concentrations, there is incomplete dissociation between VDR and corepressors. An explanation for this effect may be that there are two forms of VDR-corepressor complexes, of which only one is ligand sensitive and behaves as described in the present model of nuclear hormone signaling (41, 42). In contrast, in the ligand-insensitive complex type, the VDR molecule is blocked by the corepressor and is probably not able to participate in the activation process via coactivators in a fashion similar to what has been shown for RAR-RXR heterodimers bound to a DRI-type response element (47). Therefore, it can be hypothesized that in this corepressor-complexed form, VDR may take alternate functions such as ligand-dependent down-regulation of gene activity of, for example, the parathyroid hormone gene.

In summary, an alternate pathway for repression involving the VDR has been presented. The novel interaction between the VDR and Alien, which is representative of a new class of corepressor, presents a mechanism for repression that appears to be VDRE selective.

P.P. is the recipient of a fellowship from the Alexander von Humboldt Foundation. This work was supported by the Sonderforschungsbereich 503, project A6.

REFERENCES

influences on nuclear receptor structure and function by a DNA element. J. Biol. Chem. 273, 10338–10348


Received for publication August 27, 1999.
Accepted for publication November 11, 1999.