Hormone-dependent Translocation of Vitamin D Receptors Is Linked to Transactivation*

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Vitamin D receptor (VDR) acts as a transcription factor mediating genomic actions of calcitriol. Our earlier studies suggested that calcitriol induces translocation of cytoplasmic VDR, but the physiologic relevance of this finding remained uncertain. Previous studies demonstrated that the activation function 2 domain (AF-2) plays an essential role in VDR transactivation. To elucidate hormone-dependent VDR translocation and its role, we constructed green fluorescent protein (GFP) chimeras with full-length VDR (VDR-GFP), AF-2-truncated VDR (AF-2del-VDR-GFP), and ligand-binding domain (LBD)-truncated VDR (LBDdel-VDR-GFP). COS-7 cells were transiently transfected with these constructs. Western blot analysis, fluorescent microscopy, and transactivation assays showed that the generated chimeras are expressed and fluoresce and that VDR-GFP is transcriptionally active. After hormone treatment, cytoplasmic VDR-GFP translocated to the nucleus in a concentration-, time-, temperature-, and analog-specific manner. Hormone dose-response relationships for translocation and for transactivation were similar. Truncation of LBD and truncation of AF-2 each abolished hormone-dependent translocation and transactivation. Our data confirm a hormone-dependent VDR translocation, demonstrate that an intact AF-2 domain is required for this translocation, and indicate that translocation is part of the receptor activation process.

The vitamin D receptor (VDR) is a member of the steroid/thyroid superfamily of transcription factors that mediates gene expression in a calcitriol-dependent fashion. The hormonally active form of vitamin D, calcitriol, is the principal regulator of calcium homeostasis and also regulates hormone secretion, immune functions, cell proliferation, and differentiation (1). After ligand binding, VDR undergoes an activation process. The mechanism and the regulatory steps involved in this activation process are under intense investigation. An understanding of these activation steps offers possibilities for selective pharmacological modulation of steroid hormone actions.

Recent studies showed that a segment of VDR close to the C terminus of the ligand-binding domain (LBD), called the activation function 2 domain (AF-2) plays a primary role in conducting the hormonal signal to the VDR-dependent genes (18, 19). Other steroid hormone receptors have homologous AF-2 domains (20, 21). AF-2 could be involved in many steps during the receptor activation process, such as hormone binding (22, 23), dimerization (6), and a repositioning of the C-terminal helix of the ligand-binding domain to form an interaction surface with coregulators (2, 3, 24) and with proteins of the transcriptional machinery (5). The role of AF-2 domain in receptor trafficking and steroid hormone actions has been investigated.

In the present study, we developed VDR-GFP chimeras to study subcellular distribution of VDR, to clarify the existence of a hormone-dependent translocation of VDR, and to investigate the possibility that translocation is part of the activation process. A GFP chimera with a truncated AF-2 region of VDR was used to investigate the role of hormone-dependent translocation in VDR function. We report here that a subset of VDR is translocating from the cytoplasm into the nucleus in a hormone analog-, dose-, time-, and temperature-dependent fashion. This translocation requires an intact AF-2 region. The close correlation between hormone-induced translocation and transactivation further supports a model for VDR activation that includes hormone-dependent translocation.

**Experimental Procedures**

Plasmid Construction—The GFP gene was amplified by polymerase chain reaction using the pQBI25 plasmid (Quantum Biotechnologies, Montreal, Quebec, Canada) as template with Pwo proofreading DNA polymerase (Roche Molecular Biochemicals). HindIII and KpnI restriction sites were inserted into the pQBI25 plasmid before the GFP gene. The wild-type VDR and the two truncated VDR sequences were also amplified by PCR, using the pAV-hVDR plasmid as template (gift from...
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Dr. J. Wesley Pike, University of Cincinnati College of Medicine, Cincinnati, OH. The generated amplicons (wild-type containing amino acids 1–427, AF-2 truncated containing amino acids 1–421, and LBD truncated containing amino acids 1–294) were inserted into the modified pQB125 plasmid between the HindIII and KpnI sites. The DNA sequence of the recombinant plasmids according to the manufacturer’s instructions (Life Technologies, Inc.). In another experiment, each flask of cells was transfected with 30 μg of plasmid or with one of the three expression plasmids (0.05 μg each). Cells were harvested, washed in PBS, resuspended in high salt buffer containing 300 mM KCl, 1.5 mM EDTA, 10 mM Tris, 10 mM sodium molybdate, 1 mM dithiotreitol (Life Technologies, Inc.), and 1 unit of lyophilized lysis buffer (Promega) and then sonicated. Lysates were subjected to centrifugation at 40,000 × g for 1 h, and the resultant supernatants were desalted and concentrated using Centricon-30 concentrators (Amicon, Beverly, MA).

Concentrated supernatants were harvested for 5 min at 95 °C in Tris-glycine sample buffer (Novex, San Diego, CA) with 2.5% β-mercaptoethanol at 110 °C. For controls, purified GFP (CLONTECH Laboratories, Palo Alto, CA) and a high salt extract from VDR-overexpressing 293 cells (25) were used. Samples containing 5 μg of protein were loaded onto polyacrylamide gels (Novex, San Diego, CA) and after electrophoretic fractionation, proteins were electrotransferred to nitrocellulose membranes. Membranes were blocked with 5% milk and then with avidin/biotin blocking kit reagents (Vector Laboratories, Burlingame, CA) and then subjected to Western blot analysis using anti-VDR (a gift from Dr. Hector F. DeLuca, University of Wisconsin, Madison, WI) or polyclonal anti-GFP (CLONTECH Laboratories) antibodies. Secondary antibodies were from Sigma and Kirkegaard & Perry Laboratories (Gaithersburg, MD). Horseradish peroxidase-labeled streptavidin was from Amersham Pharmacia Biotech. Blots were developed with enhanced chemiluminescence kit reagents and protocol (Amersham Pharmacia Biotech). Microscopy—Cells—Confocal images were taken of each fluorescing cell at the highest circumference of the nuclei. 60–80 cells were evaluated for each experimental condition. Cells that showed clear morphological changes due to protein overexpression were excluded from the statistical analysis. Fluorescence intensities and area measurement values were obtained from nuclei, cytoplasm, and an area in the background using the Area Measurement function of the laser scanning microscope software (Carl Zeiss Inc.). Integrated fluorescence values were calculated by multiplying mean brightness with the respective area values. The ratios of nuclear integrated fluorescence and whole cell integrated fluorescence were corrected for background brightness of the same picture. Data are presented as mean ± 1 S.E.

RESULTS

Cloning and Expression of Fusion Proteins—To develop a physiologically relevant model for studying intracellular trafficking of VDR, we generated a series of VDR-GFP chimeras with a highly fluorescent GFP variant fused in frame to the C terminus of the human VDR. A Gly-Ala linker was used between the VDR and GFP to provide a flexible link between the two proteins. In addition to the chimera containing the wild-type VDR (VDR-GFP), two other chimeras were generated, one with a truncated AF-2 region (AF-2del-VDR-GFP), and another with a large deletion within the ligand-binding region (LBDdel-VDR-GFP). In the AF-2del-VDR-GFP, six amino acids were deleted at the C terminus of VDR. This deletion severely compromises hormone-dependent transactivation capacity (28, 29).

In the LBDdel-VDR-GFP, we generated the same mutation that was identified in cells from one of our patients with hereditary vitamin D-dependent rickets (30). Previous studies showed that the resulting truncated protein does not bind hormone and does not activate VDR target genes (31).

Fusion protein expression was monitored by both microscopy and Western blot analysis. Microscopy showed the presence of fluorescent proteins as early as 4 h after transfection, and a maximal expression after 24 h. To verify whether the predicted size proteins were expressed, we prepared high salt protein extracts 48 h after transfection with 15-μg plasmids for each flask of cells. Using Western blot analysis, fusion proteins were detected with both the anti-GFP and the anti-VDR antibodies (Fig. 1). We detected immunoreactivity with both antibodies for 1.4-kDa bands in the VDR-GFP extract (Fig. 1, A and B, lane 3), a 76-kDa band in the AF-2del-VDR-GFP extract (Fig. 1, A and B, lane 4), and a 61-kDa band in the LBDdel-VDR-GFP extract (Fig. 1, A and B, lane 5), which were the expected molecular masses for the chimeric proteins. No significant degradation products, incorrectly expressed proteins, 27-kDa GFP (compare with purified GFP on Fig. 1A, lane 2), or 50-kDa GFP (compare with extract from VDR overexpressing cells on Fig. 1B, lane 2) was detected in the extracts from cells expressing chimeric proteins. However, in samples prepared after transfection with more plasmids or with longer posttranscription time, degradation products were detectable (data not shown). Based on these data, in subsequent experiments care was taken to minimize overexpression and protein degradation by using relatively low plasmid concentrations and by minimizing the time for expression.
VDR-GFP Is Transcriptionally Competent—Transcriptional activation capacities of the full-length and truncated VDR-GFP fusion proteins were determined by a luciferase reporter assay in COS-7 cells as described under “Experimental Procedures.” When VDR-GFP-transfected cells were stimulated by 10 nM calcitriol, the reporter gene was activated by 13-fold (Fig. 2). No activation above the endogenous level was detected after 10 nM calcitriol treatment in cells that were transfected with AF-2del-VDR-GFP or LBDdel-VDR-GFP. No activation was detected after exposure to Δ4 in cells transfected by either full-length or truncated VDR-GFP constructs (Fig. 2). These data demonstrated that the transiently expressed VDR-GFP is transcriptionally active in COS-7 cells. Furthermore, VDR-GFP transcriptional activity is calcitriol analog-specific and is also contingent upon an intact ligand-binding domain.

Subcellular Distribution of VDR-GFP in the Absence and Presence of the Ligand—For microscopy, VDR-GFP was transiently expressed in various types of cultured cells as described under “Experimental Procedures.” VDR-GFP fluorescence was easily detectable in every type of cell tested, and the subcellular distribution was also similar regardless of cell type. Without hormone treatment, fluorescence from unliganded VDR-GFP was detected both in the cytoplasm and in the nucleus (Figs. 3, A, C, and E, and 4A). The cytoplasmic distribution was homogeneous in most cells, but in a subpopulation of cells VDR-GFP concentrated in the perinuclear region (Fig. 4A). Within the nucleus, unliganded VDR-GFP distributed homogeneously, with the exclusion of the nucleoli. Significant cell-to-cell differences in the nuclear/cytoplasmic brightness ratios were apparent within the same cell population. Whereas some cells contained the receptors predominantly in the cytoplasm, most cells had the same brightness in the cytoplasm and in the nucleus, and occasionally the nucleus appeared moderately brighter than the cytoplasm. Experiments with higher plasmid concentration for transfection or with longer time after transfection showed that overexpressing cells often had large fluorescent clumps in the cytoplasm and within the nucleus and that fluorescence increased disproportionately in the nucleus.

When the VDR-GFP expressing cells were treated with 100 nM calcitriol for 30 min at 37 °C, the fluorescent signal from the cytoplasm shifted into the nucleus (Fig. 3, B, D, and F). After hormone treatment, the cytoplasm became dark, and the nuclear fluorescence intensity significantly increased. This hormone-dependent translocation was apparent in every cell line that we studied. Within the nucleus after hormone treatment, the fluorescence distribution became more heterogeneous; in some cells, small foci were detected, but the nucleoli remained void of receptors (Fig. 3).

Real-time Imaging of Ligand-dependent VDR-GFP Translocation—To demonstrate that the shift in fluorescence intensity from the cytoplasm into the nucleus after hormone treatment represents true translocation of receptors, real-time imaging was carried out on single COS-7 cells by confocal microscopy during hormone treatment. Care was taken to maintain constant 37 °C temperature and to minimize photodamage. Viability of cells after imaging was tested by monitoring membrane integrity and subsequent cell division. Translocation was detected as early as 5 min after hormone treatment and completed within 25–30 min (Fig. 4). First, receptors moved from the periphery of the cell into the perinuclear region (Fig. 4, B and C). During this time, receptors along the plasma membrane became visible (Fig. 4D, at Pi). These receptors moved from the plasma membrane into the nucleus at later time points (Fig. 4, G and H). Perinuclear vacuoli, characteristic of this cell type, remained dark during translocation. After 5 min with calcitriol, a gradual increase in nuclear fluorescence paralleled a decrease in the cytoplasm (Fig. 4, C–H). After 30 min, cytoplasmic fluorescence was minimal (Fig. 4H). No additional translocation was detected when cells were followed up to 2 h. After removal of hormone, cytoplasmic fluorescence was restored within 30 min (data not shown). Similar hormone-dependent translocations were recorded in melanoma cells (data
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Cytoplasm-to-Nuclear Translocation of VDR Is Dependent on Hormone Dose Dependence of Cytoplasm-to-Nucleus Translocation—To characterize concentration dependence of VDR-GFP translocation, images were taken from fixed cells before and after hormone exposure to graded doses of hormones. Fig. 6 shows that a small shift of fluorescence from the cytoplasm into the nucleus is detectable with 100 pM of calcitriol or 10 pM of KH1060. Half-maximal translocation was at 5 nM or 10 pM of calcitriol or KH1060, respectively (Fig. 6). When hormone-dose dependence of translocation is compared with hormone dose dependence of transactivation, the similarities of these two functions become clear. Half-maximally effective dose for calcitriol to induce transcription was also 5 nM, and for KH1060, it was 10 pM. These data show that KH1060 is more potent than calcitriol in inducing both translocation and transactivation by the full-length VDR-GFP and suggest that translocation is part of the receptor activation process.

Specificity of Cytoplasm-to-Nucleus Translocation—Hormone-induced changes in receptor functions are characterized by hormone analog specificity and by a dependence on the structural integrity of the receptor protein.

To test the hormone-dependent translocation of VDR-GFP, we compared the effects of different hormone analogs on VDR-GFP translocation. Saturating doses of calcitriol induced complete receptor translocation (Fig. 7). The same degree of translocation could be achieved by saturating doses of KH1060 (Fig. 6). On the other hand, at the same concentration, D₃, a hormonally inactive analog was unable to induce any translocation of VDR-GFP (Fig. 7).

To further explore the specificity of hormone action on translocation, we monitored nuclear/cytoplasmic distribution of a LBDΔdel-VDR-GFP after hormone treatment. Before hormone treatment, the nuclear/cytoplasmic distribution of this truncated receptor was indistinguishable from the distribution of wild-type VDR-GFP. No translocation of this mutant was detected at 100 nM calcitriol (Fig. 7). These experiments prove that hormone-dependent translocation of VDR-GFP is a specific hormone action. For an effective translocation, the ligand has to be hormonally active, and the ligand-binding domain has to be intact.

Cytoplasm-to-Nuclear Translocation of VDR Is Dependent on an Intact AF-2 Region—Whereas intact nuclear localization signals are needed for hormone-independent translocation of VDR (11), we assumed that a distinct receptor domain is responsible for the hormone-dependent translocation. Because an...

Fig. 3. Hormone treatment induces VDR-GFP translocation in different cell lines. VDR-GFP was used for transfection of melanoma (A and B), 3134 (C and D), and ROS 17/2.8 (E and F) cells. 24 h after transfection, living cells were evaluated with confocal fluorescent microscopy as described under “Experimental Procedures.” Before hormone (A, C, and E), VDR-GFP was both in the cytoplasm and in the nucleus. After treatment with 100 nM calcitriol (B, D, and F), VDR-GFP translocated to the nuclei (N) in every cell line tested. Nucleoli (n) remained void of receptors. Intracellular foci (f) were detected after hormone addition, here most apparent in F. Receptors were not detected at the plasma membrane (P). Bars = 10 μm.
The intact AF-2 region of VDR is essential for hormone-dependent transactivation, we tested whether the AF-2 region is involved in the hormone-dependent translocation of VDR. COS-7 cells were transfected with AF-2del-VDR-GFP, and we measured hormone-dependent translocation by microscopy and morphometric analysis. Truncation of the AF-2 region did not affect hormone-independent translocation, but the calcitriol-induced translocation of AF-2del-VDR-GFP was completely abolished (Fig. 8). The deletion of the AF-2 region interferes not only with coactivator protein binding and transactivation but also with calcitriol binding. In contrast, the synthetic analog KH1060 is known to bind with high affinity to an AF-2 deleted VDR (3, 22). Thus, we tested AF-2del-VDR-GFP translocation after incubation with KH1060. At physiologically relevant concentrations, KH1060 failed to induce any translocation or transactivation (data not shown). However, an extremely high concentration of KH060 (100 nM) induced a slight translocation and a small increase in reporter activity in cotransfection experiments (Fig. 8). This effect further supported the idea that a connection exists between translocation and transactivation. Most importantly, these experiments suggested that beyond allowing for hormone binding, intact functioning of the AF-2 region is required for hormone-dependent translocation of VDR-GFP.

**DISCUSSION**

We constructed and characterized a VDR-GFP chimera to study VDR distribution and intracellular motion in living cells. With this transcriptionally active chimera, we showed that VDR partitions between the cytoplasm and nucleus. Our studies revealed that VDR-GFP translocates into the nucleus both by a hormone-independent and a hormone-dependent mechanism. Using deletion mutants we found that the AF-2 region of VDR is involved in the hormone-dependent translocation.
We decided to use a GFP chimera of VDR for these studies because other methods to visualize VDR gave controversial results about subcellular distribution and were not suitable to detect receptor motion in real time. We were also encouraged by our previous success using a GFP chimera of GR to study hormone-dependent translocation in real time (15). Our laboratory became interested in studies of VDR translocation almost 10 years ago when, using immunocytology with microwave fixation, we found that about half of the unliganded VDR resides in the cytoplasm and translocates into the nucleus after hormone addition, whereas the accepted dogma was that VDR is exclusively nuclear (10). Since then, several studies supported our findings (11, 12, 32, 33), but a direct demonstration of receptor movement was awaited to clarify the mechanisms and role of VDR translocation. Although it is relatively easy to make plasmids encoding chimeric proteins, the resulting protein must retain the functions of both proteins to be used as a model. The protein also has to be stable, as degradation products could prevent interpretation of experimental results. Here, we showed that both the GFP and the VDR parts of the chimeric protein remain functional in cultured cells; the GFP-tagged VDR remains transcriptionally active (Figs. 2 and 6),

**Fig. 6.** Dose-response curves for transactivation and translocation are similar for both calcitriol and KH1060. COS-7 cells were transfected with VDR-GFP and treated with hormones for 48 h, and then cells were harvested and transactivation assays were carried out as described under “Experimental Procedures.” Open squares represent transactivation values expressed as fold induction on the left axes. Data show that KH1060 is about 100-fold more potent than calcitriol in inducing reporter activity by VDR-GFP. Translocation was evaluated by microscopy and morphometric analysis from VDR-GFP-transfected cells before and after 30 min of incubation with graded doses of calcitriol or KH1060. Filled triangles represent nuclear fluorescence as a percentage of whole cell fluorescence on the right axes. The shift of fluorescence from the cytoplasm into the nucleus was hormone concentration-dependent. The more potent calcitriol analog KH1060 caused translocation at lower concentration than calcitriol. Error bars, 1 S.E.

**Fig. 7.** Translocation of VDR-GFP is hormone analog-dependent and contingent upon intact ligand-binding domain. COS-7 cells were transfected with VDR-GFP (open columns) and treated with vehicle, 100 nM D₃, KH060, or calcitriol. After 30 min, cells were fixed and evaluated as described under “Experimental Procedures.” D₃ caused no translocation of VDR-GFP, whereas calcitriol and KH1060 caused complete translocation of VDR-GFP. COS-7 cells were also transfected with LBDdel-VDR-GFP (solid columns) and treated with vehicle or 100 nM calcitriol at 37°C. No hormone-dependent translocation was observed. Error bars, 1 S.E.
and the GFP remains fluorescent (Figs. 3–5 and 7). Western blot analysis demonstrated that VDR-GFP is expressed and relatively stable, without significant degradation in the first 48 h after transfection (Fig. 1). These data demonstrate that VDR-GFP is suitable for studying receptor distribution and motion.

Using VDR-GFP, we clarified that unliganded VDR resides both in the cytoplasm and in the nucleus. Within the same cell population, we found great heterogeneity in the nuclear/cytoplasmic ratio of fluorescence. Frequently, the nuclei looked brighter than the cytoplasm, but because of the larger volume of the cytoplasm, statistical analysis of the integrated brightness values revealed that only about 25% of the receptors were in the nucleus without hormone. Hence, the distribution of VDR is similar to the distribution of thyroid receptor β1 (16) and mineralocorticoid receptor (13), whereas it is different from androgen receptor (14) and GR (15, 17, 34), which reside exclusively in the cytoplasm without hormone, and differs from estrogen receptor (35) and progesterone receptor (36), which are exclusively nuclear. It is possible to speculate that the presence of unliganded VDR in the nucleus has functional significance. These receptors may bind target DNA sequences and act as silencers (37). The intranuclear distribution of VDR, however, was similar to the intranuclear distribution of all other steroid hormone receptors, as the nucleoli are void of receptors. Rapid, so-called nongenomic actions of steroid hormones, including calcitriol, were suggested to be mediated by plasma membrane receptors (38, 39). In the majority of cultured cells, VDR-GFP was not localized to the plasma membrane, but occasionally small amounts of receptors were detectable in the plasma membrane of COS-7 cells (Fig. 4D, at P). The nature of this subset of VDR-GFP and its functional significance are not clear. Plasma membrane VDR-GFP could represent alternate splicing products, degradation products, or differentially processed proteins. The ability to directly observe VDR in living cells has allowed us to confirm that VDR resides both in the cytoplasm and in the nucleus without ligand binding.

Most importantly, however, the VDR-GFP could be observed in real time, to reveal a rapid hormone-induced translocation of cytoplasmic receptors into the nucleus. Earlier studies with immunocytoology and with a fluorescent calcitriol derivative, BODIPY-calcitriol, suggested that a time-dependent shift of VDR takes place from the cytoplasm to the nucleus. Here, with the use of VDR-GFP, we were able to record the translocation of the receptor itself in real time (Fig. 4). Morphometric analysis of digital images allowed for detailed characterization of this hormone-dependent translocation. Our experiments showed that this process, like nuclear VDR functions, is time-dependent (Figs. 4 and 5A), temperature-sensitive (Fig. 5, B and C), is hormone dose-dependent (Fig. 6), shows calcitriol analog specificity (Fig. 7), and shows dependence on an intact ligand binding domain (Fig. 7). These results demonstrate conclusively for the first time that hormone induces VDR translocation, much as hormone induces translocation of GR (15), androgen receptor (14), mineralocorticoid receptor (13), and thyroid receptor (16). Distribution of VDR-GFP within the nucleus also changed with addition of calcitriol. After hormone treatment, the intranuclear VDR-GFP pattern became less homogeneous, and small intranuclear foci developed. The availability of functional VDR-GFP should advance our ability to explore the significance of these intranuclear changes and investigate the architecture of VDR target sites in a physiologically relevant context.

The ease of tracking VDR movement with this GFP chimera
invites its use to investigate mechanisms of both hormone-independent and hormone-dependent VDR movement.

The hormone-independent translocation likely results from receptor association with proteins of the nuclear import machinery. Previous studies determined that the nuclear import of steroid receptors requires binding of their NLS sequences to the NLS receptor importin α. Two NLS sequences were identified in the VDR (11, 40). The functional importance of the NLS signal C-terminal from the second zinc finger (40) has not been explored in detail. However, a very important recent study from Haussler's laboratory (11) showed that mutations that disrupt the NLS between the two zinc fingers within the DNA-binding domain of the VDR abolish hormone-independent translocation but allow the hormone-dependent translocation to take place. Furthermore, we found significant heterogeneity in the extent of hormone-independent translocation within the same cell population, suggesting additional level of controls. In a cultured cell population, cells are in different stages of cell cycle. Differences in nuclear/cytoplasmic ratios of VDR could be the result of regulatory mechanisms related to the cell cycle, as was suggested by previous studies with GR (41) and thyroid receptor (16). Taken together, these results suggest that the hormone-independent translocation of VDR requires receptor interactions with importins and is regulated by additional factors.

We explored the mechanisms of the hormone-dependent VDR translocation as well. Previous papers (5, 42) reported that a highly conserved region at the extreme C terminus of nuclear receptors, including the vitamin D receptor, the AF-2 domain, mediates hormone-dependent transactivation and interaction with coactivators (5, 10). Recently, it became clear that hormone binding results in the repositioning of the helix 12 that contains the AF-2 domain and that this conformational change presents an interface for binding to coactivating proteins, such as CBP and SRC-1 (2, 3, 21, 22, 28, 42). Our results indicate that the AF-2 region also mediates hormone-dependent translocation of VDR (Fig. 8). We found that truncation of the AF-2 domain did not affect hormone-independent translocation but completely abolished the ability of calcitriol to initiate VDR-GFP translocation. Because truncation of the AF-2 domain interferes with calcitriol binding we also tested the ability of a synthetic calcitriol analog, KH1060, to induce translocation. Because truncation of the AF-2 domain interferes with calcitriol binding we also tested the ability of a synthetic calcitriol analog, KH1060, to induce translocation. This analog has been shown to bind the AF-2-truncated receptor with high affinity but was ineffective in inducing receptor motion. This new model permits us for the first time to search for ligands and regulatory mechanisms that differentially affect receptor motion.

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We developed the first transcriptionally active highly fluorescent VDR-GFP chimera, allowing us to easily see and study VDR movement in real time. The use of VDR-GFP yielded new insights into the mechanisms of VDR activation. Our experiments clarified that VDR translocates into the nucleus by both a hormone-independent and a hormone-dependent mechanism. Using AF-2del-VDR-GFP, we identified that the AF-2 region mediates hormone-dependent translocation. Further details of the mechanisms of hormone-dependent translocation, including the identification of transport proteins, remain to be determined. This new model permits us for the first time to search for ligands and regulatory mechanisms that differentially affect receptor motion.
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