MINIREVIEW

The Vitamin D Receptor—Structure and Transcriptional Activation (44131)

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Abstract. **The vitamin D receptor (VDR) is an integral part of the body's calcium regulatory system. In this review, recent advances in the understanding of VDR structure and function are discussed. Both direct mutagenesis studies on the VDR and structural studies of related receptors have been reviewed. Recent insights into DNA binding and ligand binding by the VDR are discussed, along with implications for gene regulation by the VDR. The potential role of co-activators and co-repressors in VDR-regulated transcription are discussed, and avenues of future research proposed.**

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The vitamin D receptor (VDR) protein is currently a major focus of research in the field of bone and calcium endocrinology. This protein, a member of the superfamily of steroid/thyroid hormone receptors (1), acts to mediate the vitamin D endocrine system through binding of the active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$), and subsequently increasing or decreasing the transcription of target genes. This review will concentrate on the recent advances in understanding of the structure of this protein and the mechanism by which it exerts its effects on transcription.

The VDR plays a critical role in the maintenance of serum calcium levels in the body. Serum calcium concentration is one of the most tightly regulated variables in animals due to its impact on nerve and muscle function; reduction of more than a few percent from its normal value of 2.5 m*M* can lead to hypocalcemic tetany and death (2). In times of lowered serum calcium levels, the VDR mediates upregulation of calcium-binding proteins in intestine and kidney, which are believed to mediate absorption of calcium from the gut, and reabsorption of calcium from the urine in kidney (3). The VDR also plays a role in regulation of bone, the body's major store of calcium; in times of lowered serum calcium, this leads to increased bone mobilization to maintain serum calcium levels. The critical role of the VDR in this aspect of physiology is underlined by the syndrome of vitamin D–resistant rickets type II, a rare homozygous recessive disorder in children resulting from inheritance of two defective copies of the VDR gene. The affected individuals have severe rickets associated with extremely high levels of 1,25-$(OH)_2D_3$. Sequence analysis of the VDR from affected individuals indicates that in almost all cases the mutation present results in either premature truncation of the VDR, a point mutation in the DNA-binding domain or no expression at all (4, 5).

The concentration of the VDR has been shown to be relatively high in target tissues, such as bone, kidney, and especially intestine, a rich source of VDR where the protein has been measured at levels of 3000 to 6000 fmol/mg protein (6). The receptor has also been shown to be present in many other tissues, including activated immune cells such as T cells, where it may play a role in modulating the levels of cytokines such as interleukin-2 (IL-2) (7). In contrast to some of the other steroid receptors, such as the glucocorticoid receptor, which has been shown to be associated with a large complex of proteins in the cytoplasm prior to hormone binding (8), studies with radiolabeled 1,25-$(OH)_2D_3$

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have shown that the receptor is located exclusively in the nucleus of target cells (9). Little information is available concerning whether there are any proteins associated with the VDR prior to DNA binding. There are some data suggesting that all the nuclear receptors contain a binding site for calreticulin in the DNA-binding domain (10). The physiological significance of this is as yet unknown.

The VDR has been cloned from several species (chick, quail, mouse, rat, and human) (11–15) and shows considerable sequence similarity between species. The rat receptor, currently being studied in our laboratory, is a 55-kD protein made up of 423 amino acids. In analogy with other members of the superfamily, the protein can be divided into several domains: a truncated A/B domain at the N terminus, a DNA-binding domain termed the C domain between amino acids 20 and 115 that mediates binding of the receptor to its response element, a D region or hinge domain located approximately between amino acids 115 and 220, and the carboxy-terminal EF domain located between amino acids 220 and 423 that binds ligand and upregulates gene transcription (1).

Knowledge of the structural and functional properties of the VDR protein has increased dramatically in recent years, in parallel with advances in the understanding of the steroid/thyroid hormone receptor superfamily of proteins. Information concerning the VDR has come mainly in the form of site-directed mutagenesis experiments wherein the mutated receptor is transfected into mammalian cells and the functional consequences examined (16). In contrast, the structures of the domains of other receptors have been determined recently, including the DNA-binding domains of the thyroid hormone and retinoid X receptor (RXR) co-crystallized on DNA (17), and the crystal structures of the ligand-binding domains of the retinoic acid receptor (RAR) (18), RXR (19), and thyroid hormone receptor (TR) (20). No structural data are yet available concerning the VDR, but the other receptor domains may serve as models on which to base hypotheses about the VDR and its properties.

Structural Determinants—DNA Binding

The VDR has been shown to bind with high affinity to a specific sequence of DNA termed a response element. One of the first response elements isolated for the VDR was that of the rat osteocalcin gene (21). The VDR requires RXR to bind to this and other response elements at physiological concentrations of receptor protein. This became clear both from *in vitro* gel retardation assay experiments and from experiments involving receptor expression in yeast (22–24). The sequence of the VDRE has been shown to consist in idealized form of two direct repeats of the sequence AGGTCA separated by three nonspecified base pairs. Work with other receptors as well as the VDR led to the hypothesis that the half site sequence AGGTCA is also a response element for other nuclear receptors including the TR, RAR, and RXR, and that the variable which determines receptor specificity is the number of nucleotides separating the half sites (25). The RXR has been shown to have a preference for the two half sites separated by a single nucleotide, termed a DR-1 response element, while the VDR is a DR-3, the TR a DR-4, and the RAR a DR-5. Interestingly, all the naturally occurring vitamin D response elements, or VDREs, isolated from genes upregulated by the VDR have fallen into the DR-3 category. The exception to this may be the negatively regulated genes, of which only two have been shown to date, the parathyroid hormone (PTH) gene and the interleukin-2 (IL-2) gene. Recent work indicates that the PTH VDRE may in fact consist of the canonical two half sites, leaving only the IL-2 gene as an exception to this rule (26).

From analogous studies with other heterodimeric receptors, it appears that the RXR binds the 5′ half site and the VDR the 3′ half site of the response element (27, 28). High-affinity binding of the receptor heterodimer to the response element has been shown to be considerably increased by 1,25-$(OH)_2D_3$ at salt concentrations in the physiological range (i.e., 100–150 m*M*) (Jehan-Kimmel C, Jehan F, DeLuca HF, unpublished results). Interestingly, for many of the receptors that heterodimerize with RXR, the natural ligand of RXR, 9-*cis* retinoic acid, does not enhance DNA binding. Further, RXR does not bind its ligand while in a complex with another receptor (29). In addition, at least one group has reported that the VDR-RXR complex is disrupted by addition of 9-*cis* retinoic acid (30). This contrasts with work in transfected cells indicating that both 1,25-$(OH)_2D_3$ and 9-*cis* retinoic acid enhance reporter gene expression from a vitamin D–responsive promoter (31). This conflict may be resolved with the recent discovery of receptor co-activators and co-repressors, which, it has been postulated, may modify the conformation of the receptors on their response elements sufficiently to enable binding of both ligands to both receptors resulting in enhanced transactivation (32, 33).

There is as yet no direct experimental evidence on the structure of the VDR DNA-binding domain, but structures have been obtained using both NMR and x-ray crystallography for the DNA-binding domains of other receptors, including the RXR. The RXR structure determined in solution by NMR indicated that the DNA-binding domain consists of two α-helices oriented at approximately right angles to one another (34). The recognition helix, so-called because mutagenesis studies have indicated it to be critical for recognition of the response element, was proposed to fit into the major groove of the DNA and interact specifically with the DNA. This has been confirmed, at least for the canonical response element sequence, by x-ray crystallography (16).

Again, while there is no direct evidence on the structure of the VDR-RXR complex bound to DNA, the crystal structure of the TR-RXR DNA binding domains complexed on a DR-4 response element was determined in 1995 (16). Protein-DNA contacts were observed between the DNA and lysine/arginine residues in the recognition helix of the TR. Extensive protein-protein contacts were observed between

the two DNA-binding domains. Based on these, the VDR was hypothesized to have specific amino acid contacts between its asparagine residue 14 and RXR residues glutamine 49 and arginine 52. In addition, VDR residues lysine 68 and glutamate 69 were modeled to form salt bridges with RXR residues aspartate 39 and arginine 38, respectively. These interactions were thought to account for the optimal spacing of three residues between the direct repeats of the vitamin D response element (DR-3), while interactions between other residues in the DNA-binding domains of the TR and RAR were thought to account for their preferences for the DR-4 and DR-5 spacings.

Structural Determinants—Ligand Binding

As mentioned above, the crystal structures of the ligand-binding domains of the RXRα (without ligand) (17), the RARγ (with ligand) (18), and the TRα-1 (with ligand) (19) have been published recently. All three ligand-binding domains share a common motif of 12 α-helices, with a very small content of β sheet. Critical to the function of the receptors is the C-terminal portion of the protein, removal of which results in marked loss of ligand-binding affinity and in the ability of the protein to affect transcription. The most C-terminal α-helices (11 and 12) of the RAR and TR undergo a large conformational change in response to ligand binding, folding up around the ligand in what the authors of the RAR paper term a "mouse trap" action to complete the ligand-binding hydrophobic pocket (18). This action serves to bring the amino acid residues on helices 11 and 12 into position to interact with other transcription factors. The VDR shows similarity to the RAR and TR in this region and may be expected to undergo a similar conformational change upon ligand binding, although the precise details of the structure and the determinants of ligand binding remain to be determined.

The wild-type VDR binds its ligand, 1,25-$(OH)_2D_3$, with extremely high affinity, in the range of 10^{-10} *M* (35). Both the 1α and 25-hydroxyl groups have been shown to be critical for high-affinity binding; loss of either results in approximately a 500-fold decrease in affinity for the receptor (36). The exact amino acid residues in the receptor that contact the ligand remain unknown, although some work has been done recently on affinity labeling of the binding site (37). However, most of the work done on the ligand-binding domain of the VDR has been limited to determining its extent through deletion analysis. In this way it was determined that removal of the amino-terminal 116 amino acids of the receptor protein did not affect ligand binding, while removal of 160 amino acids resulted in loss of ligand affinity (38). Working from the other end, removal of the carboxy-terminal 20 amino acids of the VDR resulted in a 10-fold decrease in affinity for ligand, while removal of more than this number resulted in complete loss of ligand binding (38). Thus a core domain of approximately 300 amino acids was shown to be required for the protein to bind ligand with wild-type affinity. This is in accord with the crystal structures of the ligand-binding domains, which were all expressed in bacteria as proteins of approximately 250 to 300 amino acids. Recent work has expanded upon this somewhat: deletion mapping of the ligand-binding domain has narrowed the critical region down to between amino acids 232 and 382 (39). It should be noted that in these experiments no attempt was made to determine the affinity of the truncated proteins for ligand, so the binding constants could have been reduced compared with the wild-type protein. The reason for the apparent conflict between the two deletion analysis papers is unknown.

Site-directed mutagenesis of the VDR ligand-binding domain has been performed recently with several of the cysteine residues in the human protein (40). Alteration of cysteine 288 to glycine resulted in severe attenuation of ligand binding at room temperature, while the same mutation at cysteine 337 resulted in a smaller decrease in affinity. The exact significance of this result is uncertain, although a contact between cysteine 237 and carbon 13 of retinoic acid was noted in the binding of retinoic acid to the RARγ (18). Mutagenesis data from our laboratory indicate that, when the tryptophan residue 282 in the rat VDR is mutated to phenylalanine, ligand binding is greatly reduced (Strugnell SA, DeLuca HF, unpublished results). This is consistent with the observation that a tryptophan residue in the RAR is in close proximity to the β-ionone ring of retinoic acid when ligand is bound to the receptor (18). As well, it is consistent with the observed decrease in tryptophan fluorescence of the VDR ligand-binding domain upon binding of ligand, similar to that seen for the RXR and RAR ligand-binding domains (41, 42). In addition, NMR data from this laboratory indicate that a proton resonance with a chemical shift of 11.7 ppm undergoes a shift to 12.2 ppm when ligand binds to the protein (Strugnell S, DeLuca HF, unpublished results). A possible source of this resonance is a histidine residue, which is consistent with crystal structure data indicating the presence of a histidine side chain in close proximity to the ligand of the TR when ligand binds (19).

Transcriptional Activation by the VDR

The ability of the liganded VDR to activate transcription from vitamin D target genes has been established in a variety of assays, most often from transfection of the receptor into cultured cells followed by analysis of reporter gene activity (43, 44). Recent work has begun to map out the domains of the VDR involved in activation and the mechanisms involved. In this regard, the yeast two-hybrid system has proven to be fruitful both in mapping the interaction sites between proteins and in the cloning of novel proteins that activate or repress transcription by receptors.

A good example of the first use of the system is the investigation of the interaction between the VDR and the transcription factor TFIIB (45, 46). The carboxy terminus of the VDR was shown to interact directly with a 43 residue amino-terminal region of TFIIB. Interestingly, this interaction was absent between TFIIB and other receptors such as

the RXR or RAR. This may have some bearing on VDR function, especially as it relates to the nuclear receptor co-activators, such as SRC-1 (47), and co-repressors, such as NCoR (48). NCoR in particular binds to a conserved region of the TR and RAR, termed the CoR box, but not to the same region of the VDR even though the VDR sequence in this region is similar to that of TR and RAR (48). This may in part explain why the VDR does not seem to exhibit dominant negative silencing seen with both the TR and RAR (49, 50). As far as transcriptional activation is concerned, a recent report from the Chambon laboratory indicates that the novel co-activator termed mSUG1 binds to the VDR ligand-binding domain and enhances transactivation in the yeast two-hybrid system (32). This co-activator seems to be relatively specific for the VDR, thyroid hormone, and estrogen receptors, in contrast to many of the other activator/repressor proteins which interact with the glucocorticoid/progesterone receptors (e.g., SRC-1) or with the RARs (e.g., N-CoR and SMRT). Further research using an *in vitro* transcription system to address the effects of these proteins on VDR-mediated transcription is ongoing and should shed more light on this very interesting area of research.

Another interesting aspect of receptor-DNA binding is the question of changes in DNA structure brought about by receptor. Work with other receptors using specific probes for DNA bending have indicated that the DNA at the response element is bent by receptor (51). For the VDR, recent work indicates that the VDR-RXR heterodimer binding to DNA causes a bend of approximately 55 degrees from the horizontal (52). This is analogous to, although considerably lower in magnitude than, the bend caused by binding of the transcription factor TATA-binding protein to the TATA box present in many eukaryotic promoters. The importance of the bending of DNA to transactivation by VDR is currently unknown. Whether this bend is enhanced by binding of other proteins, such as TFIIB and the co-activators/co-repressors, to the DNA is also unknown and potentially interesting, particularly if the bending can be shown to be important in transactivation. A proposed model of the molecular mechanism of action of 1,25-$(OH)_2D_3$ on transcription is shown in the figure, taking into account all current information available (Fig. 1).

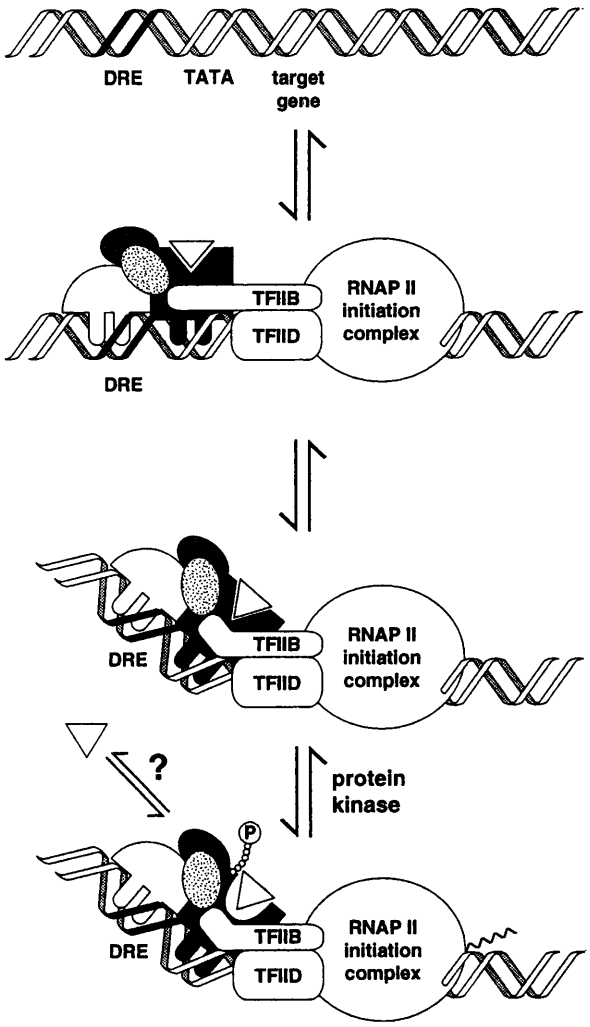

Figure 1. Although the exact sequence of events that occur during activation of a vitamin D–regulated gene is unclear, a general model can be proposed based on current evidence. Binding of 1,25-$(OH)_2D_3$ to the VDR causes a conformational change resulting in binding of the VDR to a response element (DRE) upstream of a target gene. The VDR binds in a 1:1 complex with RXR, with RXR occupying the 5′ half site and VDR occupying the 3′ half site. Other transcription factors including TFIIB interact with the VDR-RXR complex, and the DNA at the response element is bent into an altered conformation. Phosphorylation of the VDR occurs, followed by an alteration in the rat of transcription of the target gene by RNA polymerase II.

Receptor Phosphorylation and Transcriptional Activation

Rapid phosphorylation of the VDR has been shown to occur in organ culture systems upon addition of ligand (53). The phosphorylated residues have been localized to the ligand-binding domain of the protein. However, the exact functional consequences of this phosphorylation have been difficult to determine. While estrogen receptor phosphorylation by MAP kinase has been shown to have a direct and measurable effect on transcription (54), this has not yet been clearly shown for the VDR. Indeed, even the kinase or kinases responsible for the phosphorylation *in vivo* has yet to be determined. Mutagenesis studies have been performed which suggest that serine 208 can be phosphorylated by casein kinase II and that phosphorylation of this residue may cause increased transcriptional activity (55, 56). These results are based on transfection experiments in which co-expression of VDR and casein kinase II in COS-7 cells resulted in increased reporter gene expression, while mutation of serine 208 to glycine abolishes the increased transcription. Another study revealed that mutation of serine 205 (which is serine 208 in some VDR nomenclature) failed to affect transcription although alternate phosphorylation on adjacent serines was noted (56). These experiments are difficult to interpret because the degree of phosphorylation of the receptor in the cells is difficult to determine, and thus the properties of the phosphorylated versus unphosphorylated

receptor cannot be compared directly. In addition, it is unclear what effect phosphorylation at this site would have on the receptor. Is it released from a complex with an inhibitor, for example, or is its affinity for another transcription factor significantly increased? A great deal of work remains to be done in this area to clear up the role of phosphorylation on VDR function.

Summary

Although recent work has provided a clearer picture of the binding of 1,25-$(OH)_2D_3$ to VDR and the binding of VDR to the vitamin D response element resulting in altered transcriptional activity, much remains unknown. Certainly RXR is a required participant in all known transcriptional activation initiated by VDR. However, the participation of other transcription factors such as TFIIB and TFIID, and of co-activators or co-repressors remains to be established. Elucidation of the 3-dimensional structure of the VDR, the changes that occur following ligand binding, and its interaction with transcriptional machinery is required to achieve an understanding of the molecular events in transcriptional regulation of target genes by 1,25-$(OH)_2D_3$.

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