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Minireview: Vitamin D Receptor: New Assignments for an Already Busy Receptor

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With its discovery in 1920, the molecule vitamin D achieved prominence as a nutritionally essential vitamin important for calcium homeostasis, particularly in the intestine and bone. Then in 1932, the elucidation of vitamin D's chemical structure revealed that this vitamin was in fact a steroid. But it was not until the late 1960s that it was appreciated that the steroid vitamin D was a precursor of a new steroid hormone, 1α,25(OH)$_2$-vitamin D$_3$ [1α,25(OH)$_2$D$_3$], that is produced by the kidney acting as an endocrine gland. The discovery in 1969 of the nuclear vitamin D receptor (VDR) for 1α,25(OH)$_2$D$_3$ initiated a two-decade-long proliferation of reports that collectively described the broad sphere of influence of the vitamin D endocrine system that is defined by the presence of the VDR in over 30 tissue/organs of man. The new genomic frontiers defined by the cellular presence of the VDR include the immune system's B and T lymphocytes, hair follicle, muscle, adipose tissue, bone marrow, and cancer cells. Unexpectedly in the mid 1980s, a new world of 1α,25(OH)$_2$D$_3$-mediated rapid responses (RR) was discovered. These were responses that occurred too rapidly (minutes to an hour) to be explained as the simple consequence of the nuclear VDR regulating gene transcription. Some RR examples include the rapid intestinal absorption of calcium (transcaltachia), secretion of insulin by pancreatic β-cells, opening of voltage-gated Ca^{2+} and Cl^- channels in osteoblasts, and the rapid migration of endothelial cells. The question then arose as to whether there was a second receptor, apart from the nuclear VDR, which responded to the presence of 1α,25(OH)$_2$D$_3$ to generate RR? After some false starts, it now appears that the classic VDR, long known to reside in the cell nucleus, in some cells is also associated with caveolae present in the plasma membrane. Furthermore, the chemical properties of the conformationally flexible 1α,25(OH)$_2$D$_3$ allow it to generate different shaped ligands for the VDR that are selective either for genomic or for RR. This minireview summarizes a proposed conformational ensemble model of the VDR that provides insight into how different ligand shapes of 1α,25(OH)$_2$D$_3$ acting through the VDR in different cellular locations can selectively mediate both genomic and RR. (*Endocrinology* 147: 5542–5548, 2006)

STEROID HORMONES ARE able to serve as chemical messengers in a wide number of species and target tissues to transmit signals that result in both genomic and rapid responses (RR). The ability of steroid hormones, including 1α,25(OH)$_2$-vitamin D$_3$ [1α,25(OH)$_2$D$_3$], to generate biological responses is defined by the presence of their cognate receptors in selected target organs and tissues. Although it is clear that steroid hormones' genomic responses are mediated by formation of a ligand-receptor complex with the cognate receptor of the superfamily of steroid hormone nuclear receptors (1), new evidence indicates for all steroid hormones that their RR are mediated by a variety of receptor types located near or associated with the plasma membrane or its caveolae components (2). The time required for onset of the RR is system dependent and can vary from seconds (*e.g.* opening ion channels) to 10–60 min (*e.g.* activation of phosphatidylinositol-3′-kinase or endothelial nitric oxide synthase). This contrasts with genomic responses, which generally take a few hours to days to become fully manifest and which can be blocked by inhibitors of transcription and translation.

1α,25(OH)$_2$D$_3$, which is a conformationally flexible molecule, is known to stimulate a wide array of RR; some examples include the rapid intestinal absorption of Ca^{2+} (transcaltachia), secretion of insulin by pancreatic β-cells, opening of voltage-gated Ca^{2+} and Cl^- channels in osteoblasts, and the rapid migration of endothelial cells (2, 3). Intriguingly, one shape of 1α,25(OH)$_2$D$_3$ is used for genomic responses, and a different shape serves as an agonist of RR (4). The question then arose as to whether there was a second receptor, apart from the nuclear vitamin D receptor (VDR), which responded to the presence of a different shaped 1α,25(OH)$_2$D$_3$ to generate RR? The purpose of this minireview is to evaluate the VDR with respect to its involvement in genomic responses and then to review evidence that the same VDR is also involved in mediation of RR.

It has been generally assumed for receptor-ligand interactions that the ligand is frozen in a single conformation dictated by both the structural constraints of the ligand and the three-dimensional architecture of the peptide chains that create the ligand binding domain (LBD) of the receptor(s). The ligands for the thyroid receptor (T_3), the retinoic acid receptor (retinoic acid), and the VDR are all conformationally flexible, and the x-ray crystallographic structure for each receptor indicates that only one definitive conformer was present in each LBD (2). This clearly demonstrates that steroid receptors can capture one ligand conformation from a very large population of flexible conformers.

Figure 1, A–C, summarizes the three structural features of 1α,25(OH)$_2$D$_3$ that empower its exceptional flexibility and as a consequence define its ability to generate a multitude of

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Abbreviations: 1α,25(OH)$_2$D$_3$, 1α,25(OH)$_2$-vitamin D$_3$; DBP, vitamin D binding protein; JN, 1α,25(OH)$_2$-lumisterol; KO, knockout; LBD, ligand binding domain; RR, rapid response(s); VDR, vitamin D receptor.

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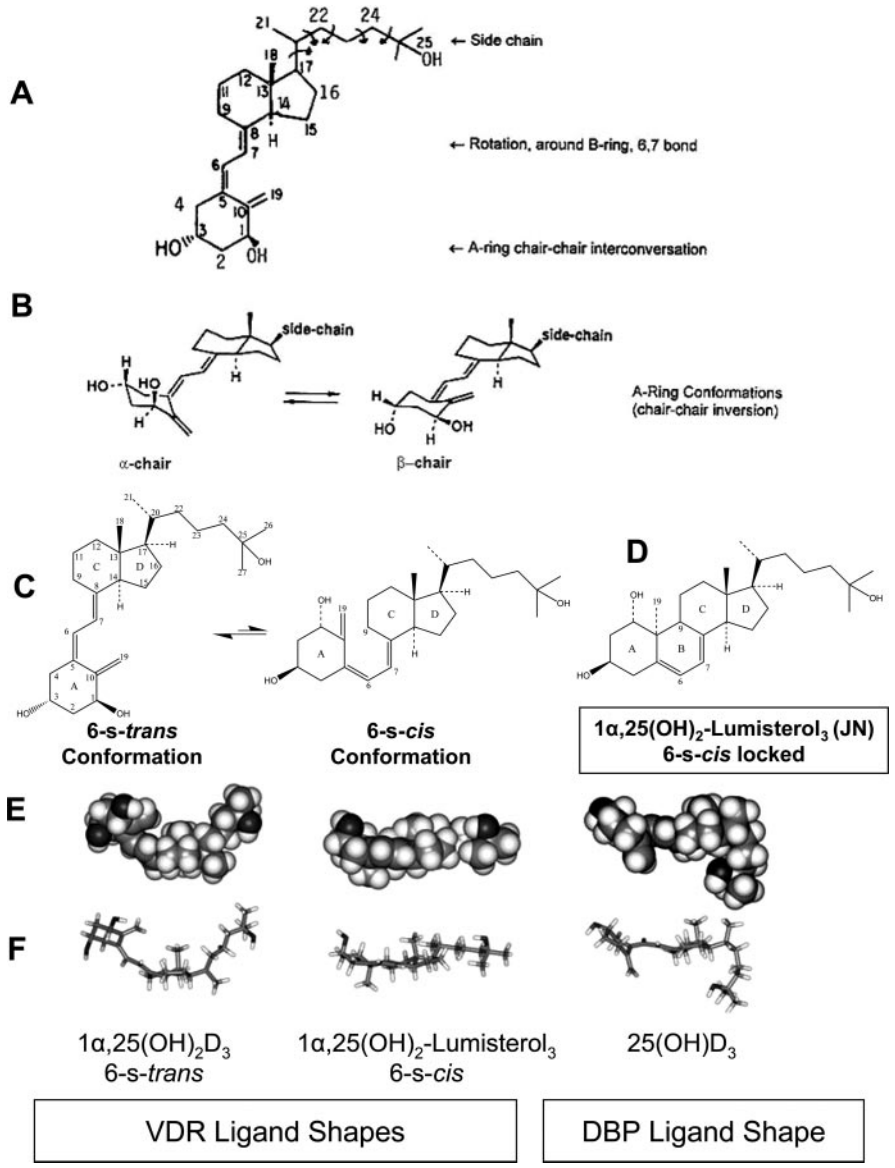

FIG. 1. The complex world of structural aspects of $1\alpha,25(OH)_2D_3$. A, Structure of $1\alpha,25(OH)_2D_3$ and identification of the three structural aspects of vitamin D seco-steroids that contribute to the conformational flexibility of this class of molecules. The side chain, because of the five single carbon-carbon bonds (indicated by the *five arrows*), is very conformationally flexible. B, The cyclohexane-like A-ring is free to interchange rapidly, millions of times per second, between a pair of chair-chair conformers effectively equilibrating the key 1α- and 3β-hydroxyls between equatorial and axial orientations. Thus, when the 1α-hydroxyl is axial, the 3β-hydroxyl is equatorial, and vice versa. C, Rotational freedom about the 6–7 carbon-carbon single bond of the seco B-ring allows conformations ranging from the open and extended 6-s-*trans* conformation, used by the VDR for genomic responses, to the more steroid-like 6-s-*cis* form of the hormone believed to be used by the VDR for RR. The *inset box* provides the structure of the 6-s-*cis* locked JN, which is of equivalent potency to $1\alpha,25(OH)_2D_3$ in mediating RR. D, Structure of JN, which is chemically locked in a permanent 6-s-*cis* shape and has potency equivalent to $1\alpha,25(OH)_2D_3$ in mediating RR (see Table 2 and text). E and F, Space-filling (E) and stick (F) representations illustrating the clear differences in the shapes of the three optimal ligands for the nuclear localized VDR (*left*), the membrane-caveolae localized VDR (*middle*), and the plasma DBP (*right*). The nuclear VDR ligand [$1\alpha,25(OH)_2D_3$] is in a bowl-shaped twisted 6-s-*trans* conformation with the A-ring and side chain both 30° above the plane of the C/D-ring. The relatively planar 6-s-*cis* locked JN is a ligand for the membrane VDR. The DBP ligand [$25(OH)D_3$] is in a twisted 6-s-*trans* orientation with the A-ring C10–C19 exocyclic alkene 30° below the plane of the C/D-ring and the side chain almost 90° below the C/D-ring plane. Each of the illustrated molecules has its 25-OH pointed to the *right* and the A-ring to the *left*. The shapes of the nuclear VDR (29) and DBP (34) ligands were learned from the separate determination of the x-ray structure of each protein, whereas the shape of the membrane VDR ligand was determined by computer modeling.

different agonist shapes for the VDR and other putative receptors. Figure 1, E and F, illustrates the shape of the optimal ligand for VDR-mediated nuclear responses and for RR as well for a second protein of the vitamin D endocrine system, the plasma transport protein, or vitamin D binding protein (DBP), the x-ray structure of which has also been elucidated. It is clear that the VDR, DBP, and the receptor for $1\alpha,25(OH)_2D_3$-mediated RR each have a characteristic ligand shape. As a consequence, it may be possible to chemically synthesize analogs of $1\alpha,25(OH)_2D_3$ that are selective for genomic responses or RR.

The author's laboratory, in collaboration with others, has

carried out an extensive structure-function study of the shape of the agonist required for optimal mediation of four separate RR; these include transcaltachia (duodenum), opening of voltage-gated Ca^{2+} and Cl^- channels (osteoblasts), insulin secretion from pancreatic β-cells, and smooth muscle cell migration (see Table 1). In these studies, the potency of $1\alpha,25(OH)_2D_3$, to initiate the indicated RR was compared with that of 6-s-*cis* locked $1\alpha,25(OH)_2$-lumisterol (JN) and a 6-s-*trans* locked $1\alpha,25(OH)$-dihydrotachysterol$_3$ (JB). In all four systems, the optimal shaped RR agonist was found to be the 6-s-*cis* locked JN, which was equipotent to that of $1\alpha,25(OH)_2D_3$. Figure 1D illustrates the structure of JN, which is chemically locked in the 6-s-*cis* shape and which is a full agonist for RR (4). As a consequence, any proposed receptor for a $1\alpha,25(OH)_2D_3$- or JN-mediated RR must clearly have a LBD that can accommodate a 6-s-*cis* ligand shape.

A timeline summarizing the important developments in our understanding of the VDR and its historical involvement in genomic responses and now, more recently, its proposed involvement in RR is presented in Table 2. The interval from its discovery in 1969 to 1984 established that the cell nucleus-localized VDR, like those for the classical ligand-occupied steroid hormone receptors (the estrogen, progesterone, androgen, glucocorticoid, and mineralocorticoid receptors), is intimately involved with selective gene transcription appropriate to the biology of each system. The VDR is known to be present in over 30 target tissues in man (5). Given the longstanding dogma that vitamin D is principally responsible for calcium homeostasis via actions in the intestine, bone, and kidney, the other 27 VDR-containing target organs collectively reflect new genomic response assignments for $1\alpha,25(OH)_2D_3$ and its VDR. Some of the new genomic frontiers include the immune system's B and T lymphocytes, the hair follicle, muscle, adipose tissue, bone marrow, and cancer cells.

The various chapters of $1\alpha,25(OH)_2D_3$-mediated RR on the VDR from 1994 to the present are also summarized in Table 2. The concept of the existence of a membrane receptor for $1\alpha,25(OH)_2D_3$ has its origins in the study of the process of $1\alpha,25(OH)_2D_3$-mediated response of transcaltachia, or the rapid hormonal stimulation of intestinal Ca^{2+} absorption in the perfused chick intestine (6). The most potent agonist is the natural hormone $1\alpha,25(OH)_2D_3$, which can stimulate the transport of calcium across the intestine within 4–5 min of its injection in the celiac artery that perfuses the duodenum (6).

The current view of this laboratory is that transcaltachia and many other $1\alpha,25(OH)_2D_3$-mediated RR are initiated by a membrane-localized receptor that binds $1\alpha,25(OH)_2D_3$ (7). Recent work in our laboratory has focused on isolation of the intestinal, kidney, or lung membrane fractions that contains caveolae. Caveolae are flask-shaped membrane invaginations that are enriched in the sphingolipids and cholesterol commonly found in both caveolae and/or lipid rafts (8). The caveolae-enriched membrane fraction is isolated from chick or rat intestine, kidney, or lung tissue by Percoll buoyant density centrifugation. We find under both *in vivo* and *in vitro* conditions that there is a saturable binding of high-specific-activity tritiated $1\alpha,25(OH)_2D_3$ in the caveolae-enriched membrane fraction. This binding activity is steroid specific for 1α-hydroxylated analogs and for $1\alpha,25(OH)_2D_3$ has a distribution constant (K_d) equal to 1.4 nM, which is identical to the nuclear VDR K_d for $1\alpha,25(OH)_2D_3$ (7). Furthermore, by confocal microscopy, we showed that green fluorescent labeled antibodies to the classical nuclear VDR colocalized with red fluorescent labeled antibodies to caveolin-1 in caveolae present in the plasma membrane of ROS-17.2,8 osteoblast cells (7).

Both the author's laboratory (7) and that of Lieberherr and Garabedian (9) have separately concluded that the RR effects of $1\alpha,25(OH)_2D_3$ require a functional VDR. We found that in VDR-knockout mice (VDR-KO), $1\alpha,25(OH)_2D_3$ was unable to stimulate the opening of voltage-gated Ca^{2+} and Cl^- channels (7, 10) and that there was a loss of 70–90% in the ability of $[^3H]1\alpha,25(OH)_2D_3$ to bind to isolated caveolae membrane fractions obtained from VDR-KO intestine, lung, and kidney tissues (7). The residual binding of $[^3H]1\alpha,25(OH)_2D_3$ present in VDR-KO tissues was found to be attributable to the presence of a truncated form of the VDR present in the strain of VDR-KO mice employed in our studies (10). Lieberherr and Garabedian found that in fibroblasts obtained from a patient with the genetic disease of vitamin D-resistant rickets, a single point mutation in the VDR (Lys45Glu) resulted in the loss of $1\alpha,25(OH)_2D_3$ RR (9).

Thus, this laboratory's view is that the classical VDR normally associated with the cell nucleus and gene transcription can also be resident near to or associated with caveolae present in the plasma membrane. It has also been reported that the estrogen receptor and androgen receptor are associated with caveolae of (2).

It is apparent that for the VDR there is a perceived problem, because a 6-s-*trans* shape of $1\alpha,25(OH)_2D_3$ is obligatory for genomic responses, whereas a 6-s-*cis* shape is required for RR. This then poses the conundrum as to how a receptor with one LBD can bind ligands of quite different shapes to gen-

TABLE 1. $1\alpha,25(OH)_2D_3$ structure-function analysis of rapid responses

Response	Case studies			
	Chick duodenum	Osteoblasts	Pancreatic β-cells	Endothelial cells
Agonist	Transcaltachia (rapid Ca^{2+} absorption)	Opening Cl^- channels and secretion	Insulin secretion	Smooth muscle cell migration
$1\alpha,25(OH)_2D_3$ conformationally flexible	Yes	Yes	Yes	Yes
JN 6-s-*cis* locked	Yes	Yes	Yes	Yes
$1\alpha,25(OH)$-dihydrotachysterol$_3$ 6-s-*trans* locked	No	No	No	
(HL) $1\beta,25(OH)_2D_3$	Antagonism			Antagonism
Ref.	4	13	14	15

TABLE 2. Vitamin D receptor timeline: a historical past (genomic responses) and new assignments for the future (rapid responses)

Year	Comment	Ref.
1968–1971	Discovery of $1\alpha,25(OH)_2D_3$ as a new steroid hormone and its chemical characterization	16–18
1969	Discovery of the nuclear receptor for $1\alpha,25(OH)_2D_3$	19, 20
1973	Actinomycin D, an inhibitor of DNA-directed RNA synthesis, blocks the biological actions of both vitamin D_3 and $1\alpha,25(OH)_2D_3$	21, 22
1982	$1\alpha,25(OH)_2D_3$ unoccupied receptor partitioned between the cytosol and nucleus of the cell	23
1985–1995	Appreciation that $1\alpha,25(OH)_2D_3$ is a very conformationally flexible molecule/receptor ligand as a consequence of its eight-carbon side chain, 360° rotation about its 6,7 single carbon, and A-ring chair-chair interconversion	24, 25
1984	Discovery of $1\alpha,25(OH)_2D_3$ RR of transcaltachia	26
1984-	Appreciation that the VDR is present in over 30 target tissues of man including classical (intestine, kidney, cartilage, and bone) as well as nonclassical (pancreas β-cell, hair follicle, many cancer cells, and activated B and T lymphocytes) target organs of vitamin D through its daughter steroid hormone, $1\alpha,25(OH)_2D_3$	5, 27
1994	Postulate of a membrane receptor for $1\alpha,25(OH)_2D_3$ involved in RR	28
1997	Demonstration for $1\alpha,25(OH)_2D_3$ that a 6-s-*cis* shape is the RR agonist, whereas a 6-s-*trans* shape is the genomic responses agonist	4
2000	First x-ray structure of the VDR LBD reported	29
2003	Evidence that annexin II is not a membrane receptor for $1\alpha,25(OH)_2D_3$	30
2003	Discovery of a [^{3}H]-$1\alpha,25(OH)_2D_3$ binding protein in caveolae membrane fractions of chick intestinal cells	31
2004	VDR is present in caveolae-enriched plasma membranes and binds $1\alpha,25(OH)_2$-vitamin D_3 i*n vivo* and *in vitro*	7
2004	Putative$1\alpha,25(OH)_2D_3$ membrane binding protein (1,25D-MARRS, or membrane-associated, rapid-response steroid-binding) linked to intestinal phosphate transport is found to be identical to the protein ERp57	32
2004	RR effects of $1\alpha,25(OH)_2D_3$ requires a functional VDR	9
2004	Identification of an alternative ligand-binding pocket in the VDR and its functional importance in $1\alpha,25(OH)_2D_3$ signaling emphasizes the importance of a VDR ensemble model	2, 11
2004	Rapid modulation of osteoblast ion channel RR is abrogated in VDR-KO mice emphasizing that $1\alpha,25(OH)_2D_3$-mediated RR require the presence of a functional VDR	33
2005	Presence of a truncated form of the VDR in a strain of VDR-KO mice	10

erate two quite different biological outcomes? Yet, the x-ray structure of the VDR revealed only one LBD, that which bound the 6-s-*trans* shape (see Fig. 1E).

One possible solution to the conundrum was obtained from molecular modeling of the VDR (11). Using the atomic coordinates of the x-ray structures of the VDR and computer modeling (see Fig. 2A), we have been able to identify the presence of a putative alternative LBD in the VDR that can accommodate via computer docking either the appropriate natural hormone or analogs that are known to be agonists only for RR (see Fig. 2A) (11). Figure 2A illustrates for the VDR the classical nuclear ligand pocket (*red*) and the proposed alternative ligand pocket (*blue*). Each ligand pocket is envisioned to have separate portals. The entrance to the nuclear pocket is dependent upon helix 12 being in an open configuration so that the hormone/ligand can enter into the LBD pocket and gain access to integral H-bonding residues thereby fully occupying the genomic pocket. Next, helix 12 moves to reclose the portal. The surprising discovery is that the alternative pocket and the genomic pocket overlap. Each of these pockets uses the same hydrogen-bonding partners of the VDR to stabilize the A-rings of the bound ligands; however, the remaining interactions of the C/D-ring and side chain of the ligand in the genomic or alternative pockets are entirely unique. Although the K_d of the genomic pocket is known from equilibrium binding experiments with [^{3}H]$1,25(OH)_2D_3$ to be approximately 1.4 nM (7), it is possible that the alternative pocket may have a different K_d. In general, the effective doses of $1\alpha,25(OH)_2D_3$ required to initiate RR have been found to be in the range of 1–10 nM.

The author's laboratory believes that the resolution of the conundrum stated above is to propose a receptor ensemble model that can describe how a classic steroid (nuclear) receptor could accommodate differently shaped ligands so as to result in the initiation of either rapid or genomic responses (Fig. 2B). This model posits that unbound receptor macromolecules exist in the cytoplasm as multiple, equilibrating receptor conformations that follow the laws associated with standard statistical distributions (11). Thus, a steroid hormone would sample the existing population of receptor conformations available and form a receptor-hormone complex with the receptor species that formed the best complementary fit between the two molecules; this would shift the equilibrium among the receptor species so as to favor the energetically most stable hormone-bound receptor conformation. It should be noted that $1\alpha,25(OH)_2D_3$ should be able to change its conformation much more quickly than the receptor protein, so essentially the whole ensemble of $1\alpha,25(OH)_2D_3$ conformations can sample each of the individual protein ensemble conformations. Implicit in this model is that the receptor sampling by the ligand involves gaining entrance and exploring the interior surface of the LBD to determine whether a complementary fit can be achieved. A related model to describe ligand/receptor-induced dissociation of rapid from genomic responses was included in the comprehensive analysis of nongenotropic, sex-nonspecific signaling by the estrogen and androgen receptors (12). Although this model has not yet been fully validated, it is possible to identify experiments involving site-directed mutagenesis of the VDR that may give further insight.

Figure 3 is a summary figure that integrates the signal

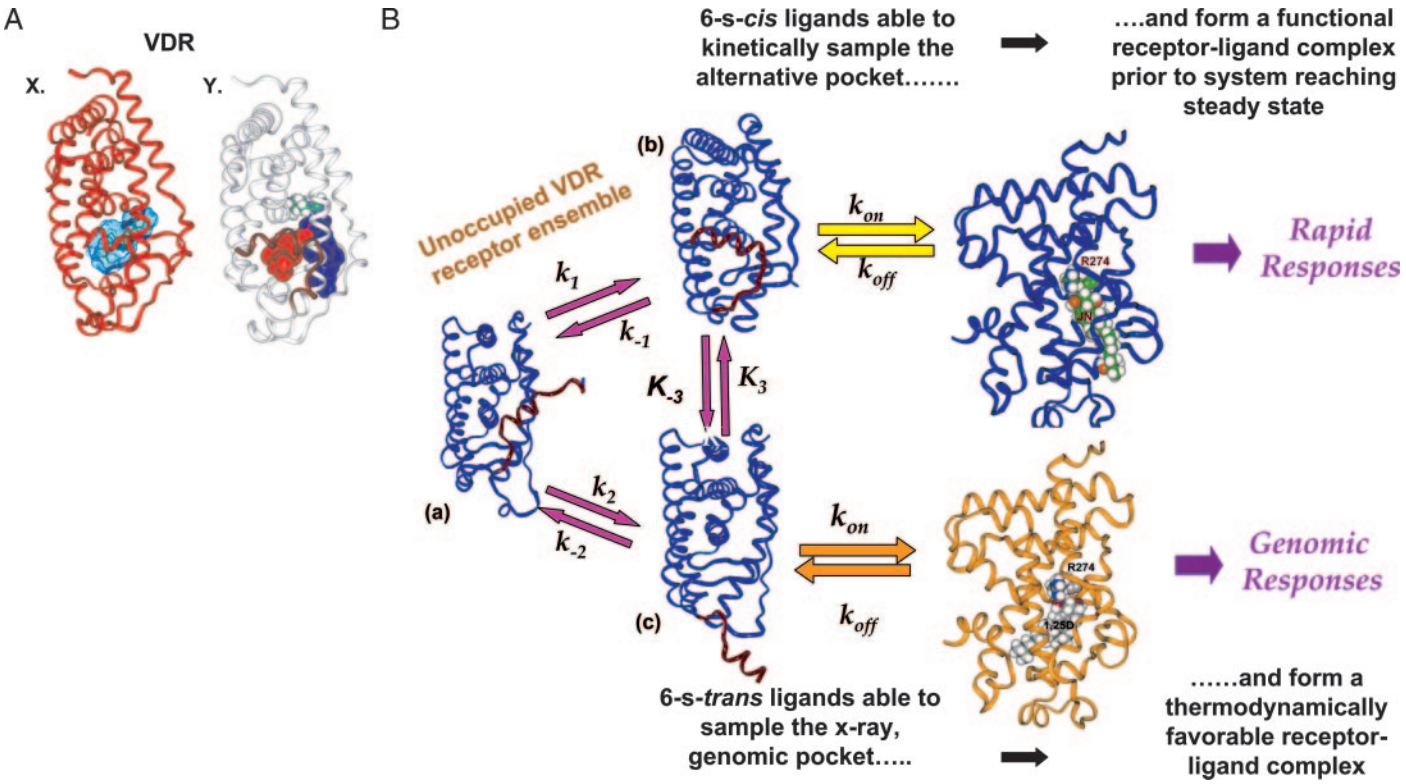

FIG. 2. Structural details of the VDR that support a conformational ensemble model. A, Details of the LBD in the ribbon structures of the nuclear VDR: X, differences in the volume and shape of the interior of the VDR LBD (*light blue*) with the structure of the 1α,25(OH)$_2$D$_3$ shown in wire-frame as revealed by x-ray crystallography of the protein plus ligand; Y, illustration for the VDR of the location of the classic genomic pocket (*red*: 1,25(OH)$_2$-vitamin D$_3$, a genomic agonist) and a putative alternative pocket (*blue*: 1,25(OH)$_2$-lumisterol, a nongenomic 6-s-*cis* locked agonist) as studied by molecular modeling. Helix 12 (H12) is colored *brown* and shown in the closed conformation. The proposed alternative pocket portal lies between the C terminus of H1 and N terminus of H3. An intriguing aspect of the two ligand binding pocket model is that the A-ring of ligands bound to either the genomic pocket or alternative pocket use the same hydrogen-bonding partners (R274, S237, and S278) to stabilize their 1α- and 3β-hydroxyls. The conserved helix 5 R274 is shown in Corey Pauling (CPK) space filling model; thus, the VDR cannot bind two ligands simultaneously. Details of the modeling are provided in Refs. 2 and 11. B, Schematic model of a receptor ensemble model to describe how a classic steroid receptor could accommodate differently shaped ligands that result in the initiation of either RR or genomic responses. The ensemble model proposes that there is a population of three different unoccupied receptor species (a, b, and c) illustrated by different positions of helix 12 (H12; colored *brown*) that are in rapid equilibrium with one another; each receptor conformer species may preferentially bind differently shaped ligands (35). In this example, transient occupancy of the alternative pocket by a ligand could/would lead to initiation of RR, whereas occupancy of the classical pocket by a ligand would lead to activation of genomic responses; both of these pockets are illustrated for the VDR in A. The alternative pocket is accessible in all three H12 conformers (a, b, and c). Occupancy of this alternative pocket is favored before achieving the steady-state equilibrium only for the receptor's natural ligands given the increased accessibility coupled with weaker van der Waals interactions compared with genomic pocket occupancy. Receptor conformer (c) is the only conformer able to accept ligands that can bind to the classical ligand binding site leading to genomic responses because in conformers a and b, H12 blocks ligand accessibility to the conserved H5 Arg residue located at the innermost part of the classic ligand binding pocket. An interesting consequence of the receptor ensemble concept may be that association of the nuclear receptor with caveolae or cell membranes may change the ligand binding preferences to favor the alternative pocket. Ligand occupancy of this genomic pocket is favored under steady-state conditions because of the increase in thermodynamic stability derived from hydrophobic interactions. It is possible that drug analogs, especially those related to the natural hormone(s), may have differential fractional occupancies of the two pockets, thus affecting the efficiency of the cellular signaling pathways mediated by either pocket.

transduction pathways that are used by the nuclear VDR and the membrane-associated VDR using 1α,25(OH)$_2$D$_3$ as a conformationally flexible agonist. This model emphasizes the complexity of overlapping and interconnecting signal transduction pathways. Although there is a solid and secure foundation describing 1α,25(OH)$_2$D$_3$-mediated genomic actions, 1α,25(OH)$_2$D$_3$-mediated RR are still in their developmental phase. One extraordinary challenge will be to identify and fully biochemically characterize the membrane-associated VDR. An equally daunting task will be to define in the various cell systems that display RR the identification of the specific signal transduction pathways that contribute to mediation of a specific rapid biological response. An additional important question is whether the membrane VDR can communicate with the nucleus of the cell to modulate gene transcription. In data not shown, but summarized in Fig. 3, there are at least five different systems where molecular biological evidence has been obtained for the process of cross-talk from 1α,25(OH)$_2$D$_3$ RR to changes in gene expression in the cell nucleus. In these studies, 6-s-*cis* locked analogs have been shown to modulate genomic responses at relatively early time intervals (2–4 h). Without a doubt, it is clear that the already busy genomic oriented VDR has new assignments in the realm of 1α,25(OH)$_2$D$_3$-mediated RR.

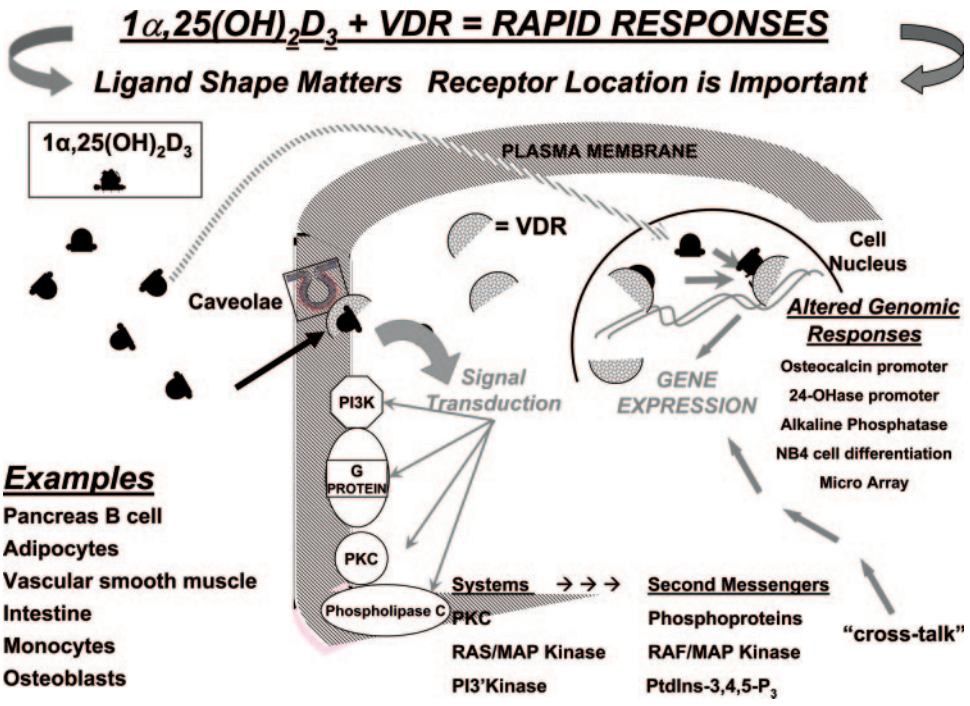

FIG. 3. Schematic model describing how the conformationally flexible $1\alpha,25(OH)_2D_3$ can interact with the VDR localized in the cell nucleus to generate genomic responses or in caveolae of the plasma membrane to generate RR. Binding of $1\alpha,25(OH)_2D_3$ to the caveolae-associated VDR may result in the activation of one or more second messenger systems, including phospholipase C (PKC), protein kinase C, G protein-coupled receptors, or phosphatidylinositol-3′-kinase (PI3K). There are a number of possible outcomes including opening of the voltage-gated calcium or chloride channels or generation of the indicated second messengers. Some of these second messengers, particularly RAF/MAPK, may engage in cross-talk with the nucleus to modulate gene expression. PtdIns-3,4,5-P_3, Phosphatidylinositol-3,4,5-trisphosphate.

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References

1. **Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM** 1995 The nuclear receptor superfamily: the second decade. Cell 83:835–839
2. **Norman AW, Mizwicki MT, Norman DPG** 2004 Steroid hormone rapid actions, membrane receptors and a conformational ensemble model. Nat Rev Drug Discov 3:27–41
3. **Norman AW** 2005 $1\alpha,25(OH)_2$-Vitamin D_3 mediated rapid and genomic responses are dependent upon critical structure-function relationships for both the ligand and receptor(s). In: Feldman D, Pike JW, Glorieux FH, eds. Vitamin D. 2nd ed. San Diego: Elsevier Academic Press; 381–407
4. **Norman AW, Okamura WH, Hammond MW, Bishop JE, Dormanen MC, Bouillon R, Van Baelen H, Ridall AL, Daane E, Khoury R, Farach-Carson MC** 1997 Comparison of 6-*s*-*cis* and 6-*s*-*trans* locked analogs of $1\alpha,25(OH)_2$-vitamin D_3 indicates that the 6-*s*-*cis* conformation is preferred for rapid nongenomic biological responses and that neither 6-s-*cis* nor 6-s-*trans* locked analogs are preferred for genomic biological responses. Mol Endocrinol 11:1518–1531
5. **Reichel H, Koeffler HP, Norman AW** 1989 The role of the vitamin D endocrine system in health and disease. New Engl J Med 320:980–991
6. **Yoshimoto Y, Nemere I, Norman AW** 1986 Hypercalcemia inhibits the "rapid" stimulatory effect on calcium transport in perfused duodena from normal chicks mediated by 1,25-dihydroxyvitamin D. Endocrinology 118:2300–2304
7. **Huhtakangas JA, Olivera CJ, Bishop JE, Zanello LP, Norman AW** 2004 The vitamin D receptor is present in caveolae-enriched plasma membranes and binds $1\alpha,25(OH)_2$-vitamin D_3 *in vivo* and *in vitro*. Mol Endocrinol 18:2660–2671
8. **Razani B, Woodman SE, Lisanti MP** 2002 Caveolae: from cell biology to animal physiology. Pharmacol Rev 54:431–467
9. **Nguyen TM, Lieberherr M, Fritsch J, Guillozo H, Alvarez ML, Fitouri Z, Jehan F, Garabedian M** 2004 The rapid effects of 1,25-$(OH)_2D_3$ require the VDR and influence 24-hydroxylate activity: studies in human skin fibroblasts bearing vitamin D receptor mutations. J Biol Chem 279:7591–7597
10. **Bula CM, Huhtakangas J, Olivera C, Bishop JE, Norman AW, Henry HL** 2005 Presence of a truncated form of the vitamin D receptor (VDR) in a strain of VDR-knockout mice. Endocrinology 146:5581–5586
11. **Mizwicki MT, Keidel D, Bula CM, Bishop JE, Zanello LP, Wurtz JM, Moras D, Norman AW** 2004 Identification of an alternative ligand-binding pocket in the nuclear vitamin D receptor and its functional importance in $1\alpha,25(OH)_2$-vitamin D_3 signaling. Proc Natl Acad Sci USA 101:12876–12881
12. **Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC** 2001 Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 104:719–730
13. **Zanello LP, Norman AW** 1997 Stimulation by $1\alpha,25(OH)_2$-vitamin D_3 of whole cell chloride currents in osteoblastic ROS 17/2.8 cells: a structure-function study. J Biol Chem 272:22617–22622
14. **Kajikawa M, Ishida H, Fujimoto S, Mukai E, Nishimura M, Fujita J, Tsuura Y, Okamoto Y, Norman AW, Seino Y** 1999 An insulinotropic effect of vitamin D analog with increasing intracellular Ca^{2+} concentration in pancreatic β-cells through nongenomic signal transduction. Endocrinology 140:4706–4712
15. **Rebsamen MC, Sun J, Norman AW, Liao JK** 2002 $1\alpha,25$-Dihydroxyvitamin D_3 induces vascular smooth muscle cell migration via activation of phosphatidylinositol 3-kinase. Circ Res 91:17–24
16. **Haussler MR, Myrtle JF, Norman AW** 1968 The association of a metabolite of vitamin D_3 with intestinal mucosa chromatin, in vivo. J Biol Chem 243:4055–4064
17. **Myrtle JF, Haussler MR, Norman AW** 1970 Evidence for the biologically active form of cholecalciferol in the intestine. J Biol Chem 245:1190–1196
18. **Norman AW, Myrtle JF, Midgett RJ, Nowicki HG, Williams V, Popjak G** 1971 1,25-Dihydroxycholecalciferol: identification of the proposed active form of vitamin D_3 in the intestine. Science 173:51–54
19. **Haussler MR, Norman AW** 1969 Chromosomal receptor for a vitamin D metabolite. Proc Natl Acad Sci USA 62:155–162
20. **Tsai HC, Norman AW** 1973 Studies on the mode of action of calciferol. VI. Effect of 1,25-dihydroxyvitamin D_3 on RNA synthesis in the intestinal mucosa. Biochem Biophys Res Commun 54:622–627
21. **Norman AW** 1965 Actinomycin D and the response to vitamin D. Science 149:184–186
22. **Tsai HC, Midgett RJ, Norman AW** 1973 Studies on calciferol metabolism.VII. The effects of actinomycin D and cycloheximide on the metabolism, tissue and subcellular localization, and action of vitamin D3. Arch Biochem Biophys 157:339–347
23. **Walters MR, Hunziker W, Norman AW** 1980 Unoccupied 1,25-dihydroxyvitamin D_3 receptors: nuclear/cytosol ratio depends on ionic strength. J Biol Chem 255:6799–6805
24. **Okamura WH, Norman AW, Wing RM** 1974 Vitamin D: concerning the

relationship between molecular topology and biological function. Proc Natl Acad Sci USA 71:4194–4197
25. **Wing RM, Okamura WH, Pirio MR, Sine SM, Norman AW** 1974 Vitamin D_3: conformations of vitamin D_3, 1α,25-dihydroxyvitamin D_3, and dihydrotachysterol$_3$. Science 186:939–941
26. **Nemere I, Yoshimoto Y, Norman AW** 1984 Calcium transport in perfused duodena from normal chicks: enhancement within fourteen minutes of exposure to 1,25-dihydroxyvitamin D_3. Endocrinology 115:1476–1483
27. **Norman AW, Roth J, Orci L** 1982 The vitamin D endocrine system: Steroid metabolism, hormone receptors and biological response (calcium binding proteins). Endocr Rev 3:331–366
28. **Nemere I, Dormanen MC, Hammond MW, Okamura WH, Norman AW** 1994 Identification of a specific binding protein for 1α,25-dihydroxyvitamin D_3 in basal-lateral membranes of chick intestinal epithelium and relationship to transcaltachia. J Biol Chem 269:23750–23756
29. **Rochel N, Wurtz JM, Mitschler A, Klaholz B, Moras D** 2000 The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. Mol Cell 5:173–179
30. **Mizwicki MT, Bishop JE, Olivera CJ, Huhtakangas JA, Norman AW** 2003 Evidence that annexin II is not a putative membrane receptor for 1α,25(OH)$_2$-vitamin D_3. J Cell Biochem 91:852–863
31. **Norman AW, Olivera CJ, Barreto Silva FR, Bishop JE** 2002 A specific binding protein/receptor for 1α,25-dihydroxy D_3 is present in an intestinal caveolae membrane fraction. Biochem Biophys Res Commun 298:414–419
32. **Nemere I, Farach-Carson MC, Rohe B, Sterling TM, Norman AW, Boyan BD, Safford SE** 2004 Ribozyme knockdown functionally links a 1α,25(OH)$_2$D$_3$ membrane binding protein (1,25D$_3$-MARRS) and phosphate uptake in intestinal cells. Proc Natl Acad Sci USA 101:7392–7397
33. **Zanello LP, Norman AW** 2004 Rapid modulation of osteoblast ion channel responses by 1α,25(OH)$_2$-vitamin D_3 requires the presence of a functional vitamin D nuclear receptor. Proc Natl Acad Sci USA 101:1589–1594
34. **Verboven C, Rabijns A, De Maeyer M, Van Baelen H, Bouillon R, De Ranter C** 2002 A structural basis for the unique binding features of the human vitamin D-binding protein. Nat Struct Biol 9:131–136
35. **Bursavich MG, Rich DH** 2002 Designing non-peptide peptidomimetics in the 21st century: inhibitors targeting conformational ensembles. J Med Chem 45:541–558

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