

# Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention

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*The human vitamin D receptor (VDR) is a key nuclear receptor that binds nutritionally derived ligands and exerts bioeffects that contribute to bone mineral homeostasis, detoxification of exogenous and endogenous compounds, cancer prevention, and mammalian hair cycling. Liganded VDR modulates gene expression via heterodimerization with the retinoid X receptor and recruitment of coactivators or corepressors. VDR interacts with the corepressor hairless (Hr) to control hair cycling, an action independent of the endocrine VDR ligand, 1,25-dihydroxyvitamin D<sub>3</sub>. We report novel dietary ligands for VDR including curcumin,  $\gamma$ -tocotrienol, and essential fatty acid derivatives that likely play a role in the bioactions of VDR.*

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## INTRODUCTION

Vitamin D<sub>3</sub>, acquired either from dietary sources or via ultraviolet irradiation of 7-dehydrocholesterol in the epidermis, is metabolized to its hormonal form, 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>], with the final step catalyzed by the 1 $\alpha$ -OHase (CYP27B1) expressed predominantly in kidney. Circulating 1,25(OH)<sub>2</sub>D<sub>3</sub>, bound by DBP, can be delivered systemically to vitamin D target cells that retain the hormone through expression of the nuclear vitamin D receptor (VDR). Intestinal epithelial cells and osteoblasts represent primary sites of VDR expression, where the receptor mediates the actions of 1,25(OH)<sub>2</sub>D<sub>3</sub> to promote intestinal calcium and phosphate absorption, and to remodel skeletal mineral, respectively. In this fashion, 1,25(OH)<sub>2</sub>D<sub>3</sub> elicits its two major functions of preventing rickets in children and osteomalacia in adults, as well as strengthening bone via remodeling. Thus, although vitamin D has no direct role in bone calcification *per se*, it is responsible for supplying adequate amounts of calcium and phosphorus minerals

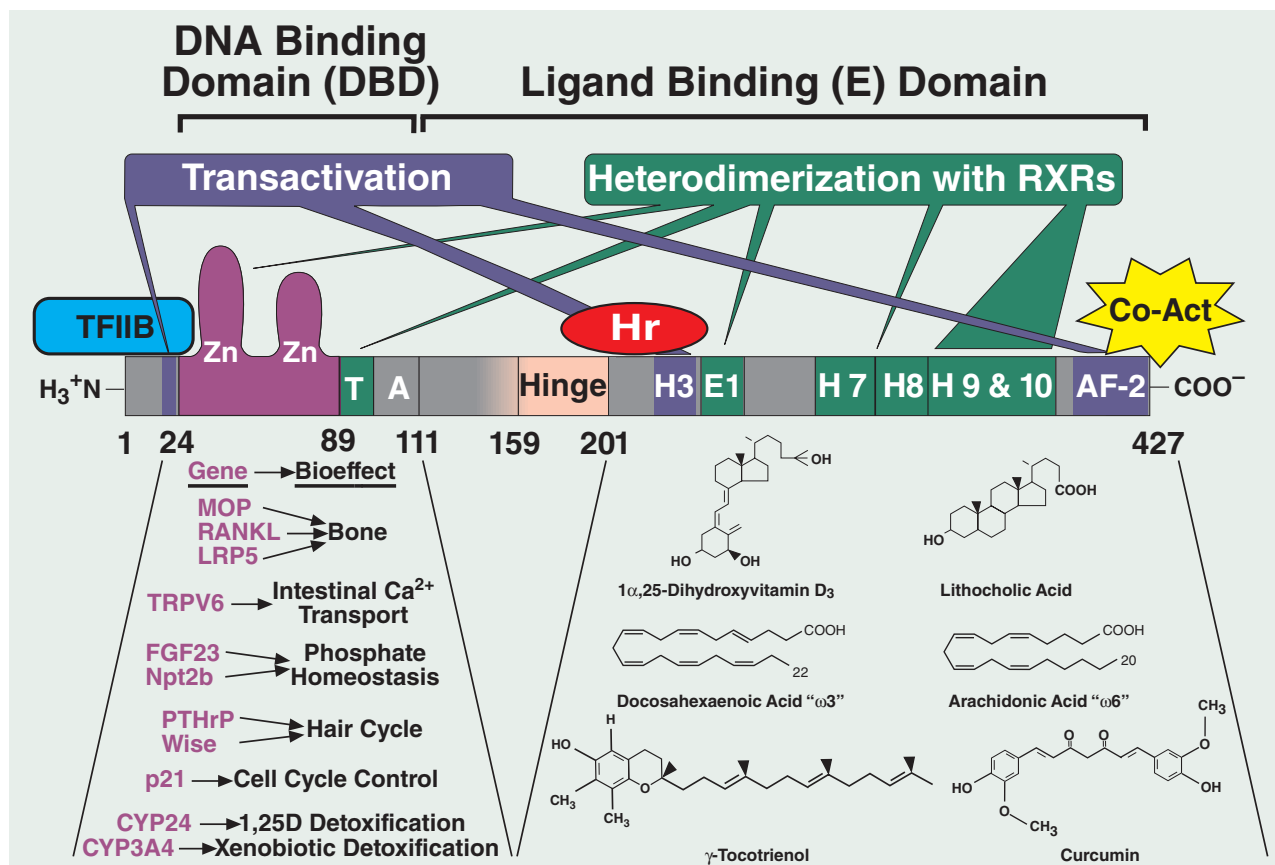
and for sculpting bone hydroxyapatite crystals in a fashion that maximizes the functioning of the skeleton.

Extrarenal 1,25(OH)<sub>2</sub>D<sub>3</sub> can also be produced locally in a number of cell types that express VDR, notably skin, cells of the immune system, colon, pancreas, and the vasculature. The significance of local extraosseous effects of 1,25(OH)<sub>2</sub>D<sub>3</sub>-VDR is not defined fully, but it appears that vitamin D, likely cooperating with other regulators, exerts immunoregulation, antimicrobial defense, xenobiotic detoxification, anti-cancer actions, control of insulin secretion and, possibly, cardiovascular benefits. The present review suggests pathways through which the panorama of 1,25(OH)<sub>2</sub>D<sub>3</sub> and VDR effects has expanded from ensuring strong bones to reducing the risk of autoimmune as well as certain microbial disorders, cancers (especially those of epithelial cells), cardiovascular disease, and perhaps even one of the ravages of aging. The key to these insights lies in the functions of VDR, one of the more evolutionarily ancient members in the family of 48 nuclear receptors encoded in the human genome. VDR is closely related to both the pregnane X receptor

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**Figure 1 Functional domains in human VDR along with the corresponding DNA (gene), protein and lipophilic ligand partners.** Highlighted at the left is the human VDR DNA binding domain which, in cooperation with the zinc fingers in the RXR heteropartner, mediates direct association with the target genes listed at the lower left, leading to the indicated physiological effects. Region E1 is one of high conservation among nuclear receptors and overlaps helices 4–5. Below the ligand binding domain (at the right) are illustrated select VDR ligands, including several novel ligands discussed in the text.

(PXR) and the farnesoid X receptor (FXR), which are respective detoxification and bile acid-sensing receptors; therefore, it is perhaps not so surprising, in retrospect, that Makashima et al.<sup>1</sup> discovered that VDR binds the carcinogenic bile acid, lithocholic acid (LCA), with low affinity compared to 1,25(OH)<sub>2</sub>D<sub>3</sub>, and it detoxifies this bile acid via CYP3A4 induction to diminish the risk of colon cancer. This also accounts for the fact that VDR is highly expressed in colon, where it appears to be the resident detoxification nuclear receptor.

An introductory overview of human VDR, the focus of the current communication, is depicted in Figure 1. Various domains of the 427 amino acid VDR are highlighted on a linear schematic of the protein (Figure 1), with the two major functional units being the N-terminal zinc finger DNA binding domain, and the C-terminal ligand binding domain. The x-ray crystallographic structure of the ligand binding domain has been determined,<sup>2</sup> with the 12  $\alpha$ -helical sandwich-like structure presenting VDR surfaces for heterodimerization with the retinoid X receptor (RXR) [helices (H) #7, 9 and

10, etc.] as well as for transactivation via interaction with coactivators (Co-Act). Coactivator interfaces in VDR consist of helices H3 and H12 (the latter constituting the AF-2 or activation function-2 domain), plus a region immediately N-terminal of the zinc fingers (residues 18–22). Human VDR also complexes with basal transcription factors such as TFIIB (near the N-terminus of VDR), as well as with transcriptional corepressors such as the *hairless* (Hr) gene product, which associates with the VDR hinge and H3. The ligand binding domain of human VDR has been co-crystallized with 1,25(OH)<sub>2</sub>D<sub>3</sub> occupying the hydrophobic pocket,<sup>2</sup> and lithocholic acid has been proposed to associate compatibly with VDR, as determined by molecular modeling.<sup>3</sup> As discussed below, we have identified several additional nutritional lipids as candidate low-affinity VDR ligands that may function locally in high concentrations. Figure 1 illustrates that these novel putative VDR ligands include  $\omega$ 3- and  $\omega$ 6-essential polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA) and arachidonic acid, respectively, the vitamin E derivative  $\gamma$ -tocotrienol, and

**Table 1 Genes directly modulated in their expression by 1,25(OH)<sub>2</sub>D<sub>3</sub> and possibly other VDR ligands.**

Gene	Bioeffect	Type	Location	5'-Half	Spacer	3'-Half
rOC <sup>50</sup>	Bone	Positive	−456	GGGTGA	atg	AGGACA
mOC <sup>51</sup>	Bone	Negative	−444	GGGCAA	atg	AGGACA
mOP <sup>52</sup>	Bone	Positive	−757	GGTTCA	cga	GGTTCA
mLRP5 <sup>9</sup>	Bone	Positive	+656	GGGTCA	ctg	GGGTCA
mLRP5 <sup>7</sup>	Bone	Positive	+19 kb	GGGTCA	tgc	AGGTTC
rRUNX2 <sup>53</sup>	Bone	Negative	−78	AGTACT	gtg	AGGTCA
mRANKL*	Bone	Positive	−22.7 kb	TGACCT	cctttg	GGGTCA
mRANKL <sup>54</sup>	Bone	Positive	−76 kb	GAGTCA	ccg	AGTTGT
mRANKL <sup>54</sup>	Bone	Positive	−76 kb	GGTTGC	ctg	AGTTCA
cPTH <sup>55</sup>	Mineral homeostasis	Negative	−60	GGGTCA	gga	GGGTGT
hTRPV6 <sup>6</sup>	Intestinal Ca <sup>2+</sup> transport	Positive	−1270	AGGTCA	ttt	AGTTCA
hTRPV6 <sup>6</sup>	Intestinal Ca <sup>2+</sup> transport	Positive	−2100	GGGTCA	gtg	GGTTCG
hTRPV6 <sup>6</sup>	Intestinal Ca <sup>2+</sup> transport	Positive	−2155	AGGTCT	tgg	GGTTCA
hNpt2c <sup>9</sup>	Renal phosphate reabsorption	Positive	−556	AGGTCA	gag	GGTTCA
rCYP24 <sup>56</sup>	1,25D Detoxification	Positive	−151	AGGTGA	gtg	AGGGCG
rCYP24 <sup>57</sup>	1,25D Detoxification	Positive	−238	GGTTCA	gcg	GGTGCG
hCYP3A4 <sup>20,58</sup>	Xenobiotic detoxification	Positive	−169	TGAACT	caaagg	AGGTCA
hCYP3A4 <sup>1</sup>	Xenobiotic detoxification	Positive	−7.7 kb	GGGTCA	gca	AGTTCA
hp21 <sup>59</sup>	Cell cycle control	Positive	−765	AGGGAG	att	GGTTCA
hFOXO1 <sup>60</sup>	Cell cycle control	Positive	−2856	GGGTCA	cca	AGGTGA
rPTHrP <sup>61</sup>	Hair cycle	Negative	−805	AGGTTA	ctc	AGTGAA
hWise*	Hair cycle	Negative	−6214	AGGACA	gca	GGGACA

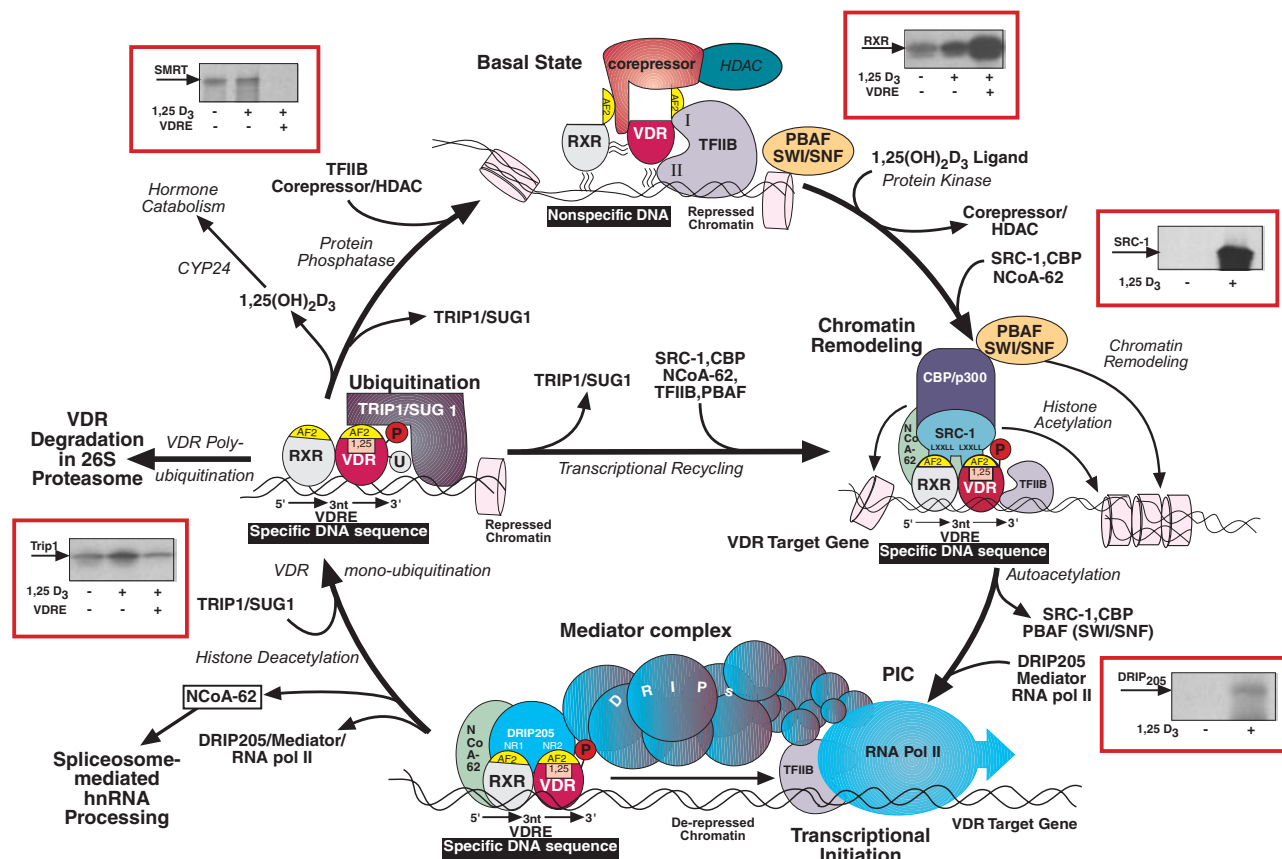
\* Genes for which VDREs are identified in this report.

curcumin, which is a turmeric-derived polyphenol found in curry.

The liganding of VDR triggers tight association between VDR and its heterodimeric partner, RXR, and only this liganded VDR-RXR heterodimer is able to penetrate the deep groove of DNA and recognize vitamin D responsive elements (VDREs) in the DNA sequence of vitamin D-regulated genes.<sup>4,5</sup> Figure 1 provides a partial list of VDR-RXR target genes recognized by the combined zinc fingers of the two receptors and their T-box and A-box C-terminal extensions. These VDR-RXR controlled genes encode proteins that determine bone growth and remodeling, intestinal calcium absorption, phosphate homeostasis, the mammalian hair cycle, cell proliferation, and lipid detoxification. Table 1 constitutes a more comprehensive list of vitamin D target genes, specifically those that are known to be under primary control of VDR-RXR via characterized VDREs within their DNA sequences. VDREs possess either a direct repeat of two half-elements with a spacer of three nucleotides (DR3) or an everted repeat of two half-elements with a spacer of six nucleotides (ER6) motif, with DR3s being the most common. Interestingly, PXR, VDR's closest relative in the nuclear receptor superfamily, also employs both DR3 and ER6 responsive element motifs, and heterodimerizes with RXR. With considerable variation in several “wobble” base pairs, the generic sequence of VDR-RXR half elements is AGGTCA, with RXR occupying the 5' half-element and VDR the 3' half-element. VDREs also occur in two functional flavors, positive for

induction and negative for repression of the regulated gene in question. The molecular nature of the difference is not understood, but appears to involve replacements in key nucleotides in either half-element. For example, rat osteocalcin (rOC) is positively controlled (induced) by 1,25(OH)<sub>2</sub>D<sub>3</sub>, but changing a T to a C at position #4 of the 5' half-element, such as occurs naturally in mouse osteocalcin (mOC), renders the latter a negatively modulated (repressed) gene (see Table 1 and Figure 3).

Many VDREs are single copy and proximally positioned in the promoter region of vitamin D-regulated genes, for instance those in osteocalcin, mouse osteopontin (mOP), rat RUNX2 (rRUNX2), chicken PTH (cPTH), human sodium phosphate cotransporter 2c (hNpt2c), human p21 (hp21), and rat PTHrP (rPTHrP). However, the rat CYP24 VDRE is bipartite, as is the human CYP3A4 VDRE, with the 5' DR3 located some 7.5 kb upstream of the proximal ER6 VDRE in the latter case. These vitamin D-controlled CYP genes introduced the concepts of multiplicity and remoteness to VDREs, mechanisms apparently employed in genes more recently characterized as direct VDR-RXR docking sites by chromatin immunoprecipitation (ChIP) assays<sup>6–10</sup> such as TRPV6, LRP5, and RANKL (Table 1). Genes possessing multiple VDREs require all VDR-RXR docking sites for maximal induction by 1,25(OH)<sub>2</sub>D<sub>3</sub> and the individual VDREs appear to function synergistically to attract coactivators and basal factors for transactivation. The most attractive hypothesis is that remote VDREs are juxtapositioned with more proximal VDREs via DNA looping in chromatin,



**Figure 2 The transcriptional life cycle of VDR (clockwise from top of figure), depicting a model for the stages in transactivation of a 1,25(OH)<sub>2</sub>D<sub>3</sub>-regulated gene containing a single copy, proximal VDRE.** Insets in red boxes illustrate GST-pulldown results showing direct associations of VDR with the indicated nuclear factor, in vitro, both in the absence and presence of 1,25(OH)<sub>2</sub>D<sub>3</sub> ligand and, in some cases, a VDRE platform. Details for each step in the cycle are explained in the text.

creating a single platform that supports the transcription machine, but this conclusion has yet to be verified by chromatin conformation capture studies.

### MODEL FOR MOLECULAR SIGNALING BY 1,25(OH)<sub>2</sub>D<sub>3</sub>-VDR

As described above and in Figure 1, in the process of controlling gene transcription, VDR not only binds ligand(s) and, as a binary complex with RXR, docks on VDREs, it also associates with a number of transcriptional coregulators. Several of the coregulators are attracted to the pivotal AF-2/helix 12 domain of VDR, such as steroid receptor coactivators (SRC-1, -2, and -3) with histone acetyl transferase (HAT) activity, vitamin D-receptor interacting protein (DRIP) members of the Mediator complex such as DRIP205, and the thyroid hormone receptor interacting protein (TRIP1) that facilitates ubiquitination of nuclear receptors in preparation for degradation. In vitamin D-controlled genes with a single-copy VDRE, such as osteocalcin and osteopontin, it would be physically impossible for several coregulators

to simultaneously occupy the AF-2/helix 12 platform on the sole VDR driving the gene in question. Although a stochastic model with random AF-2 “hits” by multiple comodulators is conceivable, such a process would be inefficient; instead, we favor the cyclical combinatorial model for VDR-mediated transactivation illustrated in Figure 2. This model is based upon studies of VDR-related nuclear receptors, such as the thyroid hormone receptor (TR)<sup>11</sup> and estrogen receptor (ER),<sup>12</sup> as well as upon the in vitro data depicted in red boxes in Figure 2 showing the interaction of VDR with multiple proteins and the influence of 1,25(OH)<sub>2</sub>D<sub>3</sub> ligand and/or the VDRE on these associations.

A description of the VDR transcriptional life cycle model in Figure 2 is provided herein, utilizing the progression of time on a traditional clock as a reference. Depicted at the twelve o’clock position is the weakly associated, unliganded VDR-RXR heterodimer, sliding along DNA nonspecifically; also shown are the proposed interactions of the receptors with TFIIB and with a corepressor. The corepressor, which binds to VDR when its AF-2 is in the “inactive” configuration, acts by attracting



histone deacetylases (HDACs) that repress chromatin. Also included is PBAF, which remodels chromatin to attract the VDR-RXR heterodimer to the promoter of a controlled gene.

Illustrated at the three o'clock position are the postulated initial alterations that transpire after binding of  $1,25(\text{OH})_2\text{D}_3$  to VDR. These changes include strong heterodimerization of VDR and RXR plus one or more hormone-dependent phosphorylations of VDR (indicated by a circled "P"), along with reconfiguration of the AF-2 domains in VDR and RXR to bring their helix 12 motifs to the closed position over the ligand binding pocket. The VDR-RXR heterodimer now recognizes DNA specifically and is bound with high affinity to a VDRE adjacent to a target gene. Changes in the conformation and phosphorylation status of the VDR-RXR heterodimer serve to dissociate the corepressor/HDAC complex and promote the binding of a coactivator complex that includes not only SRC-1, but also CBP/p300, nuclear receptor coactivator-62 (NCoA-62) and a SWI/SNF chromatin remodeling complex anchored by PBAF. This coactivator complex derepresses chromatin in the vicinity of the VDR target gene by catalyzing histone acetylation and chromatin remodeling.

Illustrated at the six o'clock position is the transcriptional initiation complex. Steps that are postulated to occur between three and six o'clock are: 1) autoacetylation of SRC-1 and CBP/p300, leading to their dissociation from the VDR-RXR heterodimer, along with PBAF; 2) binding of DRIP205 to the AF-2 of VDR (and also RXR), an event that attracts other DRIPs in the Mediator complex; and 3) delivery of TFIIB to the RNA Pol II transcription machine (indicated by a rightward arrow). The complex pictured at nine o'clock is postulated to form after the transcription machine has moved away from the promoter, with DRIP205/Mediator dissociating from the VDR-RXR heterodimer to be replaced by TRIP1, a mammalian ortholog of the yeast SUG1 protein. NCoA-62 also dissociates, and may be involved in the processing of the primary heterogeneous nuclear RNA (hnRNA) transcript by the spliceosome. VDR is then subject to ubiquitination by TRIP1 and its associated ubiquitin ligation complex (an attached ubiquitin is indicated by a circled "U").

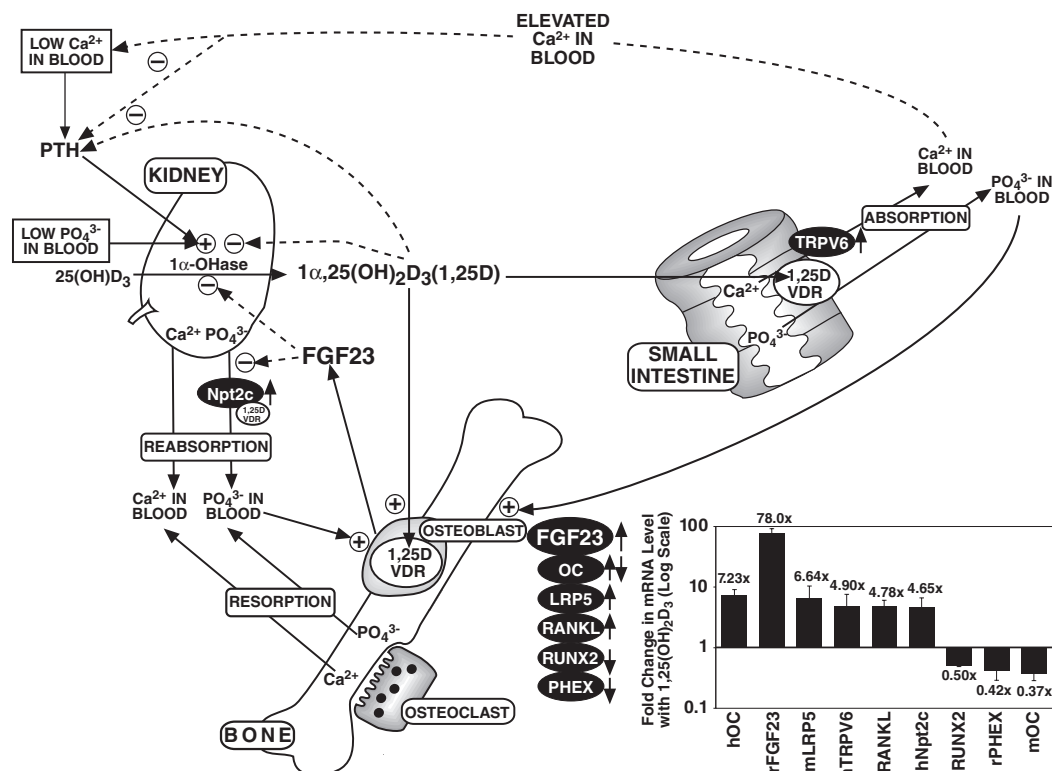
The structure illustrated at nine o'clock now has three alternative fates. The "transcriptional recycling" pathway encompasses dissociation of TRIP1, either with or without monoubiquitination of VDR, and reassociation of SRC-1, CBP/p300, NCoA-62, TFIIB, and PBAF to regenerate the complex at three o'clock for a rapid reinitiation of transcription. A second fate would be dissociation and metabolic elimination of the  $1,25(\text{OH})_2\text{D}_3$  ligand via catabolism by CYP24, leading to recovery of the unliganded complex at twelve o'clock after dissociation of

TRIP1 and reassociation of TFIIB and corepressor. A third fate (receptor degradation), which may occur after several rounds of VDR ubiquitination and amplified transcriptional initiation, would be transfer of the poly-ubiquitinated VDR to the 26S proteasome for degradation, effectively terminating the VDR transcriptional life cycle.

The model in Figure 2 allows liganded VDR-RXR to "juggle" the many coregulators required for reorganizing chromatin, target the VDRE in the promoter, and amplify the cycle after monoubiquitination and oscillatory recycling (from nine o'clock to three o'clock), together permitting a single VDRE to generate a significant response of transcriptional initiation when bound by liganded VDR-RXR. As stated above, when multiple and often remote VDREs are present, these may be brought into juxtaposition via DNA looping to create a kind of "cloverleaf" DNA architecture. Such an array may occur in  $1,25(\text{OH})_2\text{D}_3$ -VDR-RXR induction of genes with VDREs separated by up to 100 kb, such as TRPV6, LRP5, and RANKL. The advantage of this chromatin configuration and the utilization of multiple VDREs in a vitamin D-controlled gene is that the transcription enhancement platform possesses several liganded VDR-RXRs docked in proximity and capable of recruiting simultaneously the many coregulators required. This obviates the need to drive the cycle pictured in Figure 2 through multiple rounds. The simultaneous recruitment model has been summarized in a recent review,<sup>10</sup> and provides a mechanism to govern transcriptional activation with multiple remote VDREs that add fine tuning and combinatorial synergism to the simple cyclical model pictured in Figure 2.

### NEW INSIGHT INTO CALCIUM AND PHOSPHATE HOMEOSTASIS

As introduced above, a major function of VDR is to maintain calcium and phosphate homeostasis to ensure effective bone mineralization and remodeling. Ablation of the vitamin D receptor in mice results in alopecia and post-weaning rickets, and only the rickets can be rescued by a diet high in calcium, lactose, and phosphate.<sup>13</sup> Similarly, humans with loss of function mutations in VDR present with a phenotype of rickets and alopecia/dermal cysts, the former of which can be corrected with nightly calcium infusions.<sup>4</sup> Thus, the dominant, but not sole, action of  $1,25(\text{OH})_2\text{D}_3$ -VDR is that of promoting intestinal calcium absorption, especially when calcium is limited in the diet. Classic research revealed that in correcting hypocalcemia with  $1,25(\text{OH})_2\text{D}_3$ , the parathyroid glands, which are sensors of blood calcium concentrations, elaborate PTH that, in turn, activates the  $1\alpha$ -OHase to produce  $1,25(\text{OH})_2\text{D}_3$ .<sup>14</sup> Via VDR binding,  $1,25(\text{OH})_2\text{D}_3$  not only stimulates calcium absorption from intestine, reabsorp-



**Figure 3 The central role of 1,25(OH)<sub>2</sub>D<sub>3</sub>-bound VDR in calcium and phosphate homeostasis.** Feedback loops to control calcium, involving PTH and phosphate, including the novel phosphaturic hormone FGF23, are explained in the text. Targets of 1,25(OH)<sub>2</sub>D<sub>3</sub>-mediated gene regulation are indicated with white text on black background. The inset in the lower right displays the effect of 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment (10<sup>-7</sup> M) on the levels of each mRNA after 24 hours as monitored by real-time PCR in the following cultured cell models: MG-63 for human OC and RANKL, Caco-2 for human TRPV6 and Npt2c, UMR-106 for rat FGF23 and PHEX, and MC3T3E1 for mouse OC, RUNX2 and LRP5. Error bars are  $\pm$ SEM with  $n = 9-12$ , and all values are statistically significantly different from baseline ( $P < 0.05$ ).

**Abbreviations:** FGF23, fibroblast growth factor-23; OC, osteocalcin; LRP5, LDL receptor-related protein-5; RANKL, receptor activator of NF $\kappa$ B ligand; PHEX, phosphate-regulating gene with homology to endopeptidases on the X-chromosome; Npt2c, sodium phosphate co-transporter 2c; TRPV6, transient receptor potential vanilloid type 6.

tion from kidney, and resorption from bone, it also feedback represses PTH synthesis to create an endocrine loop for the fine control of blood calcium levels (Figure 3). However, in the process of correcting hypocalcemia, 1,25(OH)<sub>2</sub>D<sub>3</sub> also mobilizes phosphate. Initially, excess phosphate could be eliminated by the phosphaturic action of PTH, but this effect can only be transient because both calcium and 1,25(OH)<sub>2</sub>D<sub>3</sub> repress PTH, necessitating the participation of a second phosphaturic hormone (Figure 3). We propose that fibroblast growth factor-23 (FGF23) is the “second wave” phosphaturic peptide that excludes excess phosphate while 1,25(OH)<sub>2</sub>D<sub>3</sub> is chronically correcting hypocalcemia.

As illustrated in Figure 3, FGF23 is secreted primarily by osteoblasts, and the synthesis and secretion of FGF23 are under dual control by 1,25(OH)<sub>2</sub>D<sub>3</sub> and elevated blood phosphate. Once secreted by bone, FGF23 elicits phosphaturia to diminish blood phosphate, and

feedback inhibits the activity of the renal 1 $\alpha$ -OHase to close yet a second endocrine loop – with the 1,25(OH)<sub>2</sub>D<sub>3</sub>/FGF23/PO<sub>4</sub> axis superimposed physiologically on the 1,25(OH)<sub>2</sub>D<sub>3</sub>/PTH/Ca axis to facilitate integrated homeostasis of these ions and prevent bone overmineralization and ectopic calcification (Figure 3). Accordingly, FGF23-null mice have increased serum 1,25(OH)<sub>2</sub>D<sub>3</sub>, hyperphosphatemia, excessive bone mineralization, and ectopic calcification in soft tissues and they ultimately die of cardiovascular and respiratory failure.<sup>15</sup>

In characterizing this novel 1,25(OH)<sub>2</sub>D<sub>3</sub>/FGF23/PO<sub>4</sub> axis, we have identified several genes controlled by 1,25(OH)<sub>2</sub>D<sub>3</sub> for which the gene products likely play key roles in bone mineral homeostasis and bone growth. As summarized in Figure 3, besides FGF23, which is dramatically induced (78-fold) by 1,25(OH)<sub>2</sub>D<sub>3</sub> in osteoblasts, 1,25(OH)<sub>2</sub>D<sub>3</sub> also represses phosphate-regulating gene with homology to endopeptidases on the X-

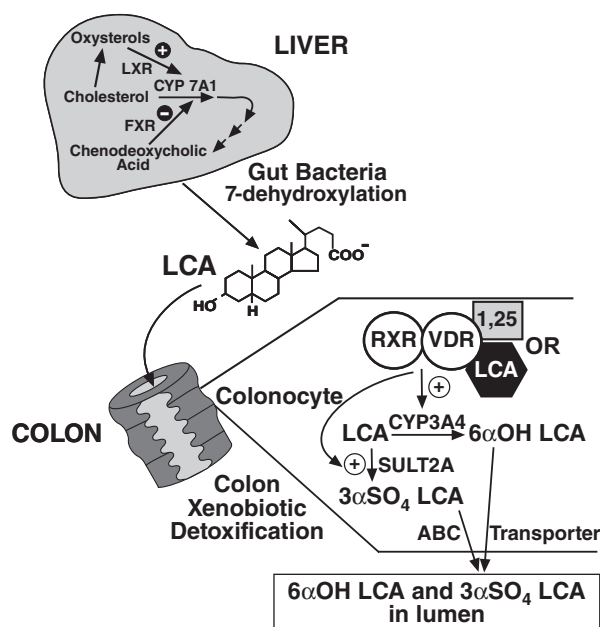
chromosome (PHEX) expression. Since PHEX is an attenuator of FGF23 in osteoblasts, this action of  $1,25(\text{OH})_2\text{D}_3$  in effect potentiates FGF23 levels. One unique feature of FGF23 and PHEX control by  $1,25(\text{OH})_2\text{D}_3$ -VDR is that the respective induction and repression responses are sensitive to inhibition by cycloheximide,<sup>16,17</sup> indicating that these genes are secondarily controlled through a mediating transfactor(s), which is (are) under primary regulation by  $1,25(\text{OH})_2\text{D}_3$ . In contrast, several other genes, including osteocalcin, LRP5, RANKL, TRPV6, Npt2c, and RUNX2, are either induced or repressed by  $1,25(\text{OH})_2\text{D}_3$ -VDR functioning in a primary fashion through VDRE binding in the gene promoters or to remote VDREs (Table 1). Notably, whereas RANKL is clearly a crucial gene in bone resorption/remodeling, a number of  $1,25(\text{OH})_2\text{D}_3$ -controlled genes can be considered anabolic to bone growth (LRP5 and RUNX2) and bone mineralization (TRPV6 and Npt2c).

The general conclusion is that  $1,25(\text{OH})_2\text{D}_3$ -VDR functions in both prevention of osteoporosis through its bone mineral anabolic actions, and protection against life-threatening ectopic calcification through its role in promoting the synthesis of the secondary effector hormone, FGF23. Interesting parallels to this conclusion that  $1,25(\text{OH})_2\text{D}_3$ -VDR and FGF23 function locally at widespread target sites to promote health exist for the “sister acts” of fatty acids-PPAR $\alpha$  (peroxisome proliferator-activated receptor  $\alpha$ ) and energy homeostasis, as well as bile acids-FXR and bile acid homeostasis. Thus, just as VDR appears to use FGF23 as a governor of phosphate and calcium metabolism, PPAR $\alpha$  utilizes FGF21 as its metabolic mediator, and FXR employs FGF15/19 to exert homeostatic control of bile acid metabolism.<sup>18</sup>

### DETOXIFICATION AND CANCER PREVENTION BY NOVEL VDR LIGANDS

Although, in mammals, the predominant roles of VDR are that of prevention of rickets and hair cycle maintenance, a VDR ortholog with significant homology to human VDR is expressed in evolutionarily ancient sea lampreys, which lack both calcified tissues and hair.<sup>19</sup> Clearly VDR possesses a primitive function(s) perhaps unrelated to its modern actions on the skeleton and skin. Likely, the modern functions of VDR are the result of “evolutionary hijacking” of this nuclear receptor to allow animals leaving the ocean, where calcium is prevalent and the UV of sunlight is dampened, to acquire a mineralized skeleton for locomotion and the ability to seek calcium-containing food, as well as generate hair to protect their skin from the cancer-causing actions of UV irradiation.

Clues to the original function of VDR can be found in the cladogram of nuclear receptor relatedness, which



**Figure 4 Proposed mechanism of VDR-mediated xenobiotic detoxification of LCA.** LCA is produced from liver-derived chenodeoxycholic acid by the action of gut bacteria. LCA is not recycled in the terminal ileum, but instead travels to the colon, where it can exert tumorigenic actions on the colonocyte. The ability of VDR to bind LCA, or  $1,25(\text{OH})_2\text{D}_3$ , and activate the CYP3A4 and SULT2A genes facilitates catalysis of  $6\alpha$ -hydroxylation and  $3\alpha$ -sulfation of LCA, respectively, leading to disposal of its polar metabolites from the cell via the ABC efflux transporter.

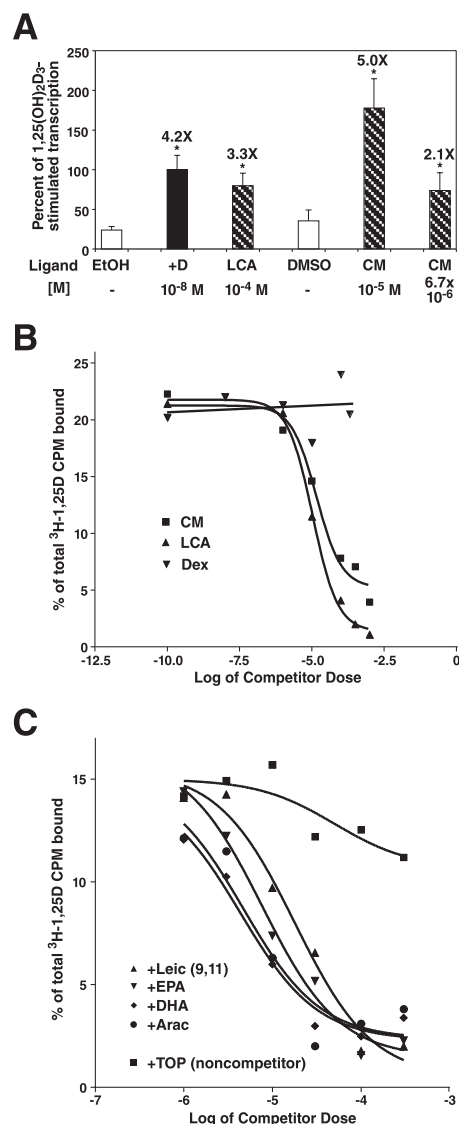
reveals that VDR is evolutionarily closest to PXR, the detoxification receptor, and is also closely related to FXR, the bile acid sensor.<sup>4</sup> Makashima et al.,<sup>1</sup> Thompson et al.,<sup>20</sup> and Jurutka et al.<sup>3</sup> have shown that VDR induces the cytochrome P450 CYP3A4 detoxification enzyme, and does so either when liganded with  $1,25(\text{OH})_2\text{D}_3$  or lithocholic acid (LCA), the toxic secondary bile acid (Figure 4). Thus, in the cholesterol-bile acid axis, which involves cholesterol metabolism to the primary bile acid, chenodeoxycholic acid (CDA) in liver under LXR (liver X receptor) and FXR reciprocal control (see Figure 4), and conversion of CDA to the secondary bile acid, LCA, by intestinal bacteria, VDR participates by detoxifying colonic LCA, which does not undergo enterohepatic circulation. In addition to inducing CYP3A4, which  $6\alpha$ -hydroxylates LCA, VDR liganded with either LCA or  $1,25(\text{OH})_2\text{D}_3$  also derepresses SULT2A,<sup>21</sup> the sulfotransferase in colon that  $3\alpha$ -sulfates LCA to expedite its extrusion by an ABC transporter and elimination in the feces (Figure 4).

Recently, Nehring et al.<sup>22</sup> showed that excess LCA administered to rats elicits the expected VDR effects of calcium transport activation proving the effectiveness

of the low-affinity LCA VDR ligand *in vivo*. Similar to our hypothesis,<sup>3,19</sup> Nehring et al.<sup>22</sup> also suggest that the modern functions of VDR evolved from its ancient role as a detoxification nuclear receptor. Interestingly, in sea lampreys, VDR is expressed predominantly in skin, an organ that might be expected to be challenged with environmental toxins and xenobiotics. Finally, the observation that VDR detoxifies colonic LCA likely explains the apparent protective effect of vitamin D against colon cancer,<sup>23</sup> particularly in individuals on a high-fat Western diet wherein bile acid production is elevated more frequently to facilitate the absorption of lipolytic digestion products.

Recently, we investigated the existence of nutritionally available lipophilic ligands that might bind and activate VDR with low affinity similar to that of LCA. Figures 5A and 5B, respectively, show that LCA activates transcription of a vitamin D-responsive reporter gene construct in intact cells comparably to 1,25(OH)<sub>2</sub>D<sub>3</sub> (albeit at ligand concentrations four orders of magnitude higher) and competes with tritiated 1,25(OH)<sub>2</sub>D<sub>3</sub> for binding to VDR. Curcumin, which is found in curry and is known to be anti-inflammatory to the degree that it reduces inflammatory bowel disease,<sup>24</sup> was then compared with LCA as a potential VDR ligand. Strikingly, the data in Figures 5A and 5B, respectively, reveal that curcumin is slightly more active than LCA in driving VDR-mediated transcription and that it binds to VDR with approximately the same affinity as LCA, based upon competition assays. The mechanism of action of curcumin (Figure 1) is not known, but we suggest that at least part of its beneficial functions are mediated by the nuclear VDR, perhaps even its ability to lower the risk of colon cancer.<sup>25</sup>

Another class of nutritionally available lipids that is critical in maintaining cell membrane fluidity and serves as precursors of the prostanooids and leukotrienes, is the essential PUFAs. DHA, for example, is an  $\omega$ -3 PUFA responsible for infant brain development and a known ligand for RXR,<sup>26</sup> the heterodimeric partner of VDR.  $\omega$ -3 PUFAs are also ligands for PPAR $\alpha$ , through which they lower VLDL and, eventually, LDL cholesterol to lessen coronary artery disease as well as reduce the incidence of metabolic syndrome.<sup>27</sup> As illustrated in Figure 5C, both  $\omega$ -3 PUFAs, such as DHA and eicosapentaenoic acid (EPA), and  $\omega$ -6 PUFAs, such as linoleic acid and arachidonic acid, compete with tritiated 1,25(OH)<sub>2</sub>D<sub>3</sub> for binding to VDR – again, with affinities for the receptor some four orders of magnitude lower than that of the 1,25(OH)<sub>2</sub>D<sub>3</sub> hormonal ligand. Nevertheless, we conclude that high local concentrations of PUFAs could occur in select cells or tissues and, if VDR is expressed, exert bioactivity including VDR-mediated anti-proliferation/pro-differentiation effects that may partially explain the chemoprotective nature of diets rich in PUFAs.



**Figure 5 Evaluation of novel VDR ligands.** (A) Ligand-stimulated transcription assay using a VDRE-containing reporter plasmid compared to vehicle (ethanol or DMSO) controls. +D = +1,25(OH)<sub>2</sub>D<sub>3</sub> and CM = curcumin. Fold effects (compared to vehicle control) are given above each bar, and the stars indicate a statistically significant difference ( $P < 0.05$ ) compared to vehicle controls in the student t-test; the error bars represent standard deviations. (B) Competition curves demonstrating that LCA and curcumin (CM) are able to compete directly with [<sup>3</sup>H]1,25(OH)<sub>2</sub>D<sub>3</sub> for binding to VDR. Conversely, dexamethasone (Dex) is not a VDR binding competitor and serves as a negative lipid control. (C) An assay similar to (B) demonstrates that selected PUFAs are able to compete with [<sup>3</sup>H]1,25(OH)<sub>2</sub>D<sub>3</sub> for binding to VDR, but that another lipid,  $\alpha$ -tocopherol (TOP), is a negative control.

**Abbreviations:** Leic (9,11), 9-cis, 11-trans conjugated linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Arac, arachidonic acid.

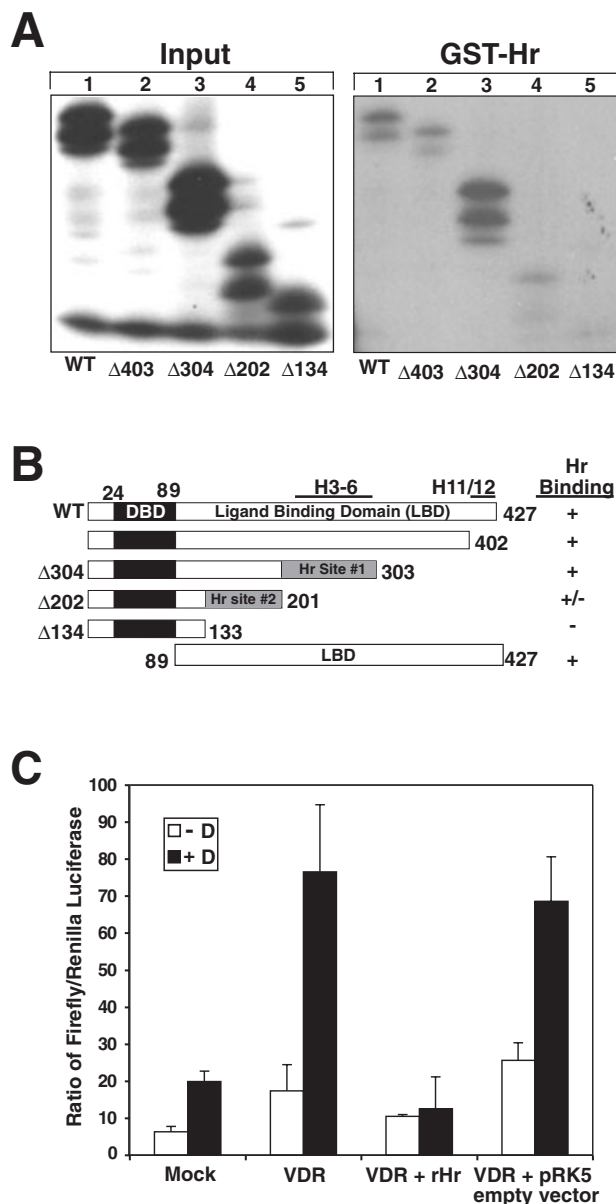


The lack of specificity among the PUFAs for VDR binding and activation<sup>10</sup> is of some concern. However, not all lipophilic compounds bind VDR with low affinity, as dexamethasone, the synthetic glucocorticoid (Figure 5B), and  $\alpha$ -tocopherol, the antioxidant vitamin E (Figure 5C), do not compete with  $1,25(\text{OH})_2\text{D}_3$  for occupation of VDR. Surprisingly, we have observed that the vitamin E metabolite,  $\gamma$ -tocotrienol (Figure 1) is a low-affinity VDR ligand that is capable of activating the receptor.<sup>28</sup> This finding reveals that, similar to the concept that vitamin D and even  $25(\text{OH})\text{D}$  are ineffective ligands for VDR, whereas metabolism to  $1,25(\text{OH})_2\text{D}_3$  generates a high-affinity hormone, the basic PUFA “core” structure could be metabolically activated to yield a higher affinity ligand(s) functioning as a cell-specific activator of VDR.

### VDR, HR, AND HAIR CYCLE SIGNALING

One tissue that is a candidate for possessing a novel VDR ligand(s) is skin. VDR knockout in mice or loss-of-function VDR mutation in humans leads to alopecia and dermal cysts, yet vitamin D deficiency does not elicit a hair cycle/skin phenotype in mice or humans. This phenotypic disparity, which contrasts with the fact that both VDR ablation and vitamin D deficiency yield rickets, indicates that VDR either functions unliganded or it utilizes a novel, non-vitamin D ligand in skin/hair follicle. Strikingly, the skin/hair-loss phenotype of VDR-null mice is mimicked when RXR $\alpha$  is conditionally ablated in skin<sup>29</sup> and when the nuclear corepressor Hr is knocked out.<sup>30</sup> Moreover, long-term absence of either VDR<sup>31</sup> or Hr<sup>30</sup> yields aged mice with wrinkled, likely hyperproliferative skin. These findings indicate that mammalian hair cycling is driven by the VDR-RXR complex, likely recruiting Hr to repress a gene or set of genes for which the product(s) are preventing the progression of the cycle from telogen (resting) to anagen (growth) phase. Whether this action of VDR requires a ligand trigger is not known, but it is curious that animals deficient in essential PUFAs freeze their hair cycles in the telogen phase, which represents the same phenotype as VDR-, RXR $\alpha$ -, or Hr-null animals. Although proof will be required, we hypothesize that a PUFA, probably a derivative thereof, occupies VDR in the hair follicle to govern the hair cycle.

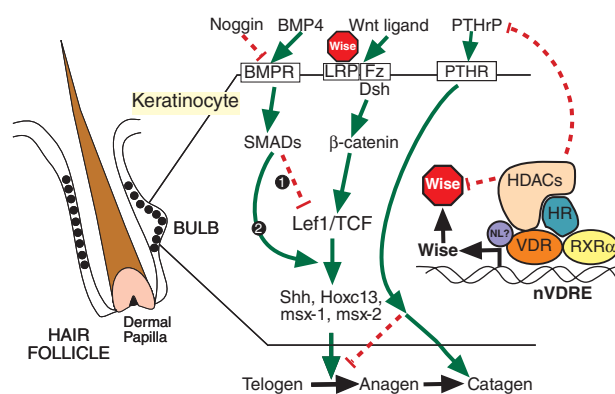
With respect to Hr, we<sup>32</sup> and others<sup>33,34</sup> have gathered evidence implicating this corepressor as a partner with VDR in signaling the mammalian hair cycle. In Figure 6A, GST-pulldown data reveal that VDR and Hr, which are co-expressed in the outer root sheath and matrix cells of the hair follicle,<sup>32</sup> interact in vitro. Based upon truncation/deletion analysis of the domains in VDR interacting with Hr using GST pulldown (Figure 6A) and co-immunoprecipitation,<sup>32</sup> we conclude that Hr is primarily associated with the helix 3–6 transactivation domain



**Figure 6 VDR interactions with mammalian hairless (Hr) protein.** (A)  $^{35}\text{S}$ -labeled VDR and its C-terminal truncation mutants:  $\Delta 403$ ,  $\Delta 304$ ,  $\Delta 202$ , and  $\Delta 134$  were incubated with the GST-Hr fusion protein to assess protein-protein interactions. (B) Schematic diagrams of the four C-terminal VDR truncation mutants,  $\Delta 403$ ,  $\Delta 304$ ,  $\Delta 202$ , and  $\Delta 134$ , as well as the N-terminal truncation mutant,  $\Delta 1-88$ , are illustrated in the context of the DNA-binding domain (DBD) and other functional/structural domains, such as several of the 12 helical domains. Hr binding by each mutant is indicated by + or – signs. (C) Hr represses  $1,25(\text{OH})_2\text{D}_3$ -stimulated transcription. Human keratinocytes (KERT-1106) were transfected with a  $1,25(\text{OH})_2\text{D}_3$ -responsive reporter plasmid, and the expression plasmids indicated below the bars. Error bars represent standard deviations, with  $n = 3$ .

(Figure 6B), likely exerting a repressive effect on VDR by competing for binding to a coactivator interaction domain in this region (Figure 1). A second, weaker Hr interaction site exists in the hinge region of VDR (Figure 1), corresponding to residue 159–201, and perhaps extending N-terminal to residue 134 (Figure 6B). Data proving that Hr is capable of repressing  $1,25(\text{OH})_2\text{D}_3$ -activated transcription of a VDR-responsive reporter transfected into intact human keratinocytes in culture, are illustrated in Figure 6C. Rat Hr, but not the pRK5 empty vector, dramatically squelches both endogenous and exogenous VDR-triggered transcription in keratinocytes. Interestingly, a number of mutations in rat<sup>35</sup> and human<sup>34</sup> Hr that confer the alopecic phenotype correspondingly abolish the ability of these loss-of-function Hr mutants to repress  $1,25(\text{OH})_2\text{D}_3$ -VDR-driven transcription. This observation indicates that nuclear receptor corepression by Hr is fundamental to its molecular role in mediating the mammalian hair cycle. Moreover, the ability of Hr to interact physically and functionally with VDR requires neither the  $1,25(\text{OH})_2\text{D}_3$  ligand nor the H-12/AF-2 region of the receptor.<sup>32</sup> In fact, patients with loss-of-function AF-2 mutations in human VDR possess normal hair coincident with hypocalcemic vitamin D-resistant rickets,<sup>36</sup> and AF-2-altered VDR is competent in rescuing the alopecic phenotype when conditionally expressed in VDR knockout mouse keratinocytes.<sup>37</sup> The overall conclusion is that VDR functions in a molecular fashion in driving the hair cycle which is fundamentally different from its role in stimulating the transcription of genes encoding proteins that execute calcium, phosphate, and skeletal homeostasis, as detailed in Figure 2.

A working model for the participation of VDR in mammalian hair cycling is depicted in Figure 7. Hair cycling is initiated by keratinocytes in the bulb or bulge area of the hair follicle and involves at least two major parallel pathways. One pathway consists of BMP4 signaling through SMAD transcription factors, which is modulated by Noggin release from the dermal papilla. BMP4 first signals a buildup of the Lef1/TCF DNA-binding protein, which is kept silent in terms of transcriptional activation until  $\beta$ -catenin is stabilized and reaches a critical concentration that confers the DNA-tethered Lef1/TCF- $\beta$ -catenin complex with transcriptional regulatory properties. Thus, Wnt liganding of the LRP-frizzled heterodimeric receptor and transduction of the signal via Dsh and other factors to elicit accumulation of  $\beta$ -catenin via the canonical pathway that sends  $\beta$ -catenin into the nucleus represents the other major arm of the mechanism of hair cycle signaling, with SMADs from the BMP4 arm acting in a second phase to potentiate Lef1/TCF trans-regulation to induce the expression of genes (Shh, Hoxc13, msx-1, msx-2, etc.) that cause the transition of the hair cycle from telogen to anagen.



**Figure 7 An RXR $\alpha$ /VDR/Hr/HDAC supercomplex is proposed to drive the hair cycle by repressing genes encoding PTHrP and/or other tonic inhibitors such as Wise.** Wise (Wnt-modulator in surface ectoderm) is a Wnt inhibitory signaler that functions as an LRP antagonist.<sup>62</sup> VDR may be occupied by a novel ligand (NL), perhaps a PUFA derivative. nVDRE signifies a negative (repressive) VDRE. See text for a description of hair cycle signaling.

How does VDR impact hair cycle signaling? As pictured in Figure 7 (right side), we propose that VDR expressed in keratinocytes in the bulb impinges on hair cycle signaling through heterodimerizing with RXR $\alpha$  to repress genes encoding PTHrP, a known suppressor of the telogen to anagen transition, and Wise, an antagonist ligand for Wnt signaling. VDR has been shown to potentiate Wnt signaling via  $\beta$ -catenin enhancement in human keratinocytes,<sup>38</sup> and from studies of VDR knockout keratinocytes.<sup>39</sup> Based upon analysis of genes overexpressed in Hr-null mice, Thompson et al.<sup>30,40</sup> identified Wise as a candidate gene that links Hr to Wnt signaling, since the Wise gene product is antagonistic to Wnt ligands. Thompson et al.<sup>30,40</sup> have also shown that Hr normally interacts with HDACs to function as a nuclear receptor corepressor.<sup>41</sup> Therefore, as illustrated in Figure 7, we hypothesize that a VDR-RXR $\alpha$ -Hr-HDAC supercomplex, docked on negative VDREs in the Wise and PTHrP genes and likely liganded with a novel ligand such as a PUFA derivative, represses the expression of these two genes to coordinately control the hair cycle. Negative VDREs have been identified in rat PTHrP and, tentatively, in human Wise (Table 1), consistent with this hypothesis. Further research is required to test the hypothesis put forth in Figure 7, and no doubt VDR, RXR $\alpha$ , and Hr modulate the expression of numerous other genes expressed in keratinocytes, some of which could affect hair cycling. Nevertheless, Wnt signaling and  $\beta$ -catenin may represent a common denominator for VDR action in skin, bone, and large intestine. Wnt signaling is crucial to bone mineralization<sup>42</sup> and LRP5 gene variants have been associated with hypo- and hyper-

mineralization in humans.<sup>10</sup> Finally, constitutive  $\beta$ -catenin activity is a common feature of colon cancer, and we have shown<sup>38</sup> that unlike keratinocytes and osteoblasts, where VDR promotes Wnt- $\beta$ -catenin signaling, VDR attenuates  $\beta$ -catenin action in human colon cancer cells. These observations may explain how VDR acts to promulgate hair cycling and bone mineralization, yet is preventative with respect to colon cancer. In fact, if one combines the suppressive effect of VDR on  $\beta$ -catenin in colon with the ability of  $1,25(\text{OH})_2\text{D}_3$  to induce cell cycle control genes such as p21, and detoxification genes including CYP3A4 (Table 1 and Figure 4), this provides three independent mechanisms whereby  $1,25(\text{OH})_2\text{D}_3$ -VDR can reduce the risk of colon cancer.

### VITAMIN D: BONE AND BEYOND

The changing faces of vitamin D and VDR actions described in the present communication unveil a vitamin and its receptor that, for many years, have been masquerading as simple promoters of dietary calcium and phosphate absorption to ensure adequate bone mineralization. We now realize that this hormone and its nuclear receptor also mediate both the sculptured delimiting and remodeling of the skeleton as well as the prevention of ectopic calcification through their novel peptide mediator, FGF23. These latter roles of  $1,25(\text{OH})_2\text{D}_3$ -VDR can be considered as both protective against osteoporotic fractures and active in reducing the ravages of ectopic calcification that occurs with aging, especially with respect to diminishing cardiovascular calcification and mortality.<sup>43</sup> It is striking indeed that the calcemic and phosphatemic hormone,  $1,25(\text{OH})_2\text{D}_3$ , and its receptor, co-evolved mechanisms for countermanding the potential deleterious effects of calcification. These mechanisms include feedback repression of calcemic PTH in one endocrine loop, and feed-forward induction of FGF23 in yet a second endocrine loop. FGF23 acts as a check and balance on bone mineral by inhibiting skeletal calcification, retarding ectopic calcification, mostly via its phosphaturic action, and feedback repressing  $1,25(\text{OH})_2\text{D}_3$  production by the kidney. Although both  $1,25(\text{OH})_2\text{D}_3$ /PTH/Ca and  $1,25(\text{OH})_2\text{D}_3$ /FGF23/ $\text{PO}_4$  are intricate and essential axes for mineral homeostasis, they represent only the tip of the iceberg in vitamin D and VDR functions significant to health (Figure 8).

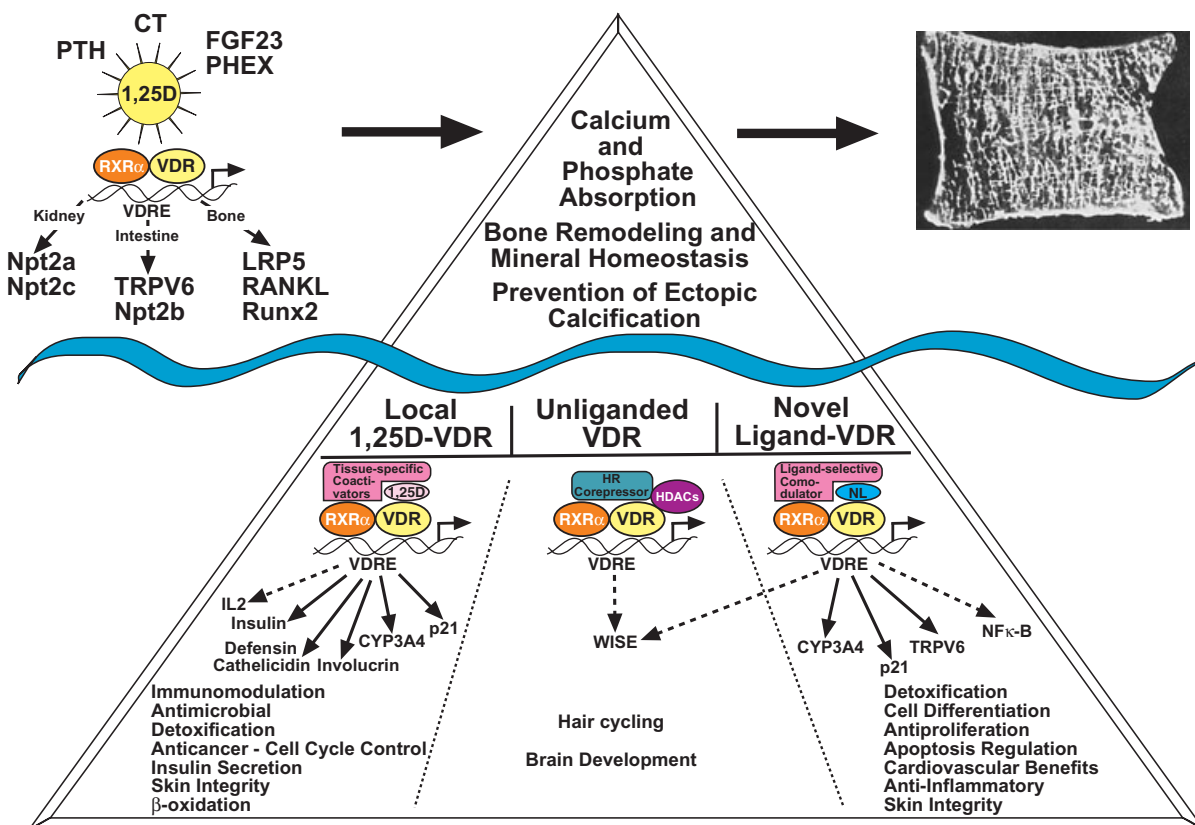
Figure 8 depicts the actions of vitamin D and VDR within the metaphoric iceberg, with the traditional bone and mineral (antirachitic/antiosteoporotic) effects, as well as newly recognized bone anabolic and counter-ectopic calcification functions, as the fraction of the iceberg above the water line. As illustrated, the bulk of VDR's actions are novel extraosseous effects that are diagrammed in the submerged portion of the iceberg. A nutri-

tional analogy can be drawn between fat-soluble vitamins A and D. The well-recognized role of vitamin A (retinaldehyde) in vision represents just a small fraction of the effects of vitamin A when one considers the pleiotropic influence of its retinoic acid metabolites on growth, development, and reproduction. Similar to retinoic acid and RAR/RXR,  $1,25(\text{OH})_2\text{D}_3$  exerts a wide array of effects beyond bone. These actions are all mediated by VDR, consistent with the broad cellular distribution of VDR expression.

Many of the extraosseous effects of VDR appear to be triggered by locally produced  $1,25(\text{OH})_2\text{D}_3$ , again similar to the scenario with retinoic acid liganding of RAR/RXR. Numerous tissues besides the kidney express the  $1\alpha$ -OHase enzyme, including cells of the immune system (e.g., T-cells), the pancreas, skin, etc. This locally produced  $1,25(\text{OH})_2\text{D}_3$  does not contribute significantly to circulating  $1,25(\text{OH})_2\text{D}_3$ , but it retains the capacity to be active in a cell- and tissue-specific manner. Examples of local  $1,25(\text{OH})_2\text{D}_3$ -VDR actions include repression of IL-2 in T-cells,<sup>14</sup> induction of defensin and cathelicidin as local antimicrobial effectors,<sup>44</sup> stimulation of involucrin synthesis in skin,<sup>45</sup> CYP3A4, and p21 induction in epithelial cells (especially in the colon),<sup>20</sup> and promotion of insulin secretion from the  $\beta$ -cells of the pancreas.<sup>46</sup> By locally stimulating the above-mentioned genes, the vitamin D/VDR system emerges, likely redundantly with other regulators, as an immunomodulator that stimulates the innate and suppresses the adaptive immune system to effect both antimicrobial and anti-autoimmune actions, detoxifies xenobiotics to be chemoprotective, controls cell proliferation and regulates apoptosis to reduce cancer, and moderates type II diabetes by promoting insulin release as well as possibly enhancing fatty acid  $\beta$ -oxidation via induction of FOXO1 (Table 1).

A second possibility obviating the need to generate  $1,25(\text{OH})_2\text{D}_3$  locally would be for VDR to function unliganded. As indicated above, VDR but not vitamin D is required to sustain the mammalian hair cycle. Thus, as depicted in Figure 8 (lower center), the Hr corepressor could function as a surrogate VDR "ligand" to suppress *Wise* or other genes that normally keep the hair cycle in check. Also, unlike the case of intestine, kidney, and bone, calbindin induction by VDR does not require vitamin D in brain.<sup>47</sup> VDR is widely expressed in the central nervous system, as is Hr, raising the possibility that unliganded VDR, along with Hr, acts in select neurons. Notably, it has been reported recently that VDR-null mice exhibit behavioral abnormalities including anxiety, etc.<sup>48</sup>

The ability of VDR to function unliganded is difficult to justify physicochemically because the tertiary structure of VDR and its functionally interactive surfaces cannot be stabilized unless the hydrophobic binding



**Figure 8** A summary of the classical bone mineral effects of  $1,25(\text{OH})_2\text{D}_3$ -VDR with its relevant gene targets as the “tip of the iceberg”, and novel “extraosseous” effects of the VDR-RXR heterodimer with candidate-regulated genes as the metaphorical submerged portion of the iceberg. Also shown at the upper left are the calcemic hormones that participate in feedback loops to maintain bone mineral homeostasis, as discussed in the text; a normally mineralized human vertebral body with its trabeculations is illustrated at the upper right. Thus, the upper portion of the figure depicts actions of  $1,25(\text{OH})_2\text{D}_3$  liganded VDR to maintain bone health, including interactions with other hormones (PTH, CT, FGF23). The lower portion of the figure summarizes the many extraosseous effects of VDR in the  $1,25(\text{OH})_2\text{D}_3$ -bound, unliganded, and novel ligand-bound states, as discussed in the text. Repressive actions of VDR are depicted as dashed arrows. We hypothesize that VDR occupied by locally generated  $1,25(\text{OH})_2\text{D}_3$  uses cell-context-specific coactivators, and that VDR occupied by a novel ligand (NL) may utilize ligand-selective comodulators.

pocket is occupied by a lipophilic ligand. We therefore suggest that VDR binds one or more naturally occurring non-vitamin D ligands to effect its extraosseous actions. As discussed above, we have identified several potential examples of non-vitamin D-related VDR ligands, including LCA,  $\gamma$ -tocotrienol, and PUFAs. Because VDR is capable of binding these lipids, albeit with low affinity, the receptor may have retained its promiscuity for ligand binding that presumably originated with its primitive detoxification function. Also, the ligand binding pocket of VDR is second only to PXR in volume among the crystallized nuclear receptor ligand binding domains, suggesting (but not proving) that it can accommodate a broad array of lipids. The question remains whether in the course of its evolution VDR co-evolved higher affinity local ligands that would explain the broad health ben-

efits of vitamin D and other lipid nutrients beyond bone. For example,  $1,25(\text{OH})_2\text{D}_3$ -VDR is anti-inflammatory and suppresses NF $\kappa$ B.<sup>49</sup> This action would be desirable for instance in preventing atherosclerosis. Is there, perhaps, a local novel VDR ligand in endothelial cells that could trigger the anti-inflammatory influence of VDR? Combined with the anti-calcification effect of FGF23, VDR would then be able to exert a two-pronged attack in preventing arteriosclerosis. Only the future will reveal the actual mechanisms for the apparent cardiovascular benefits of vitamin D/VDR. Clearly, VDR will emerge as a versatile therapeutic and preventative target once we fully understand the pleiotropic extraosseous effects of vitamin D. No doubt the faces of vitamin D and VDR will again change to reflect new frontiers in health and disease.



## CONCLUSION

The 1,25(OH)<sub>2</sub>D<sub>3</sub>-FGF23-phosphate axis is as important pathophysiologically as the 1,25(OH)<sub>2</sub>D<sub>3</sub>-PTH-calcium axis in bone and mineral homeostasis. FGF23 appears to be a novel mediator of 1,25(OH)<sub>2</sub>D<sub>3</sub>-VDR action that prevents ectopic calcification and reduces cardiovascular mortality.

1,25(OH)<sub>2</sub>D<sub>3</sub> is both anabolic and catabolic to bone, providing an explanation as to how vitamin D-VDR is associated with dynamic maintenance of the skeleton and reduced osteoporotic fractures.

The modern bone, calcium, and phosphate homeostatic functions of VDR evolved from a primitive detoxification role.

1,25(OH)<sub>2</sub>D<sub>3</sub> functions molecularly via VDR-RXR in either a cyclical progressive fashion of coactivator attraction or a “cloverleaf” model, wherein DNA in chromatin is looped out and remote VDRE enhancers are clustered to nucleate a gene transcription machine that simultaneously recruits HATs, SWI/SNFs, DRIP/Mediator, TFIIB, etc., to initiate and process mRNAs that are translated into vitamin D-induced effector proteins.

Naturally occurring, novel, low-affinity agonist VDR ligands, (e.g., lithocholic acid, curcumin, and PUFAs) may trigger VDR actions in specific tissues such as colon and skin, lowering the risk of cancer and mediating the hair cycle, respectively.

Hair cycle signaling by VDR involves neither vitamin D nor transactivation, and is apparently mediated through Hr/HDACs and the repression of Wnt, which results in the stimulation of Wnt/LRP/β-catenin.

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**Declaration of interest.** The authors have no relevant interests to declare.

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