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New Insights Into the Mechanisms of Vitamin D Action

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Abstract The biologically active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is a secosteroid whose genomic mechanism of action is similar to that of other steroid hormones and is mediated by stereospecific interaction of $1,25(OH)_2D_3$ with the vitamin D receptor (VDR) which heterodimerizes with the retinoid X receptor (RXR). After interaction with the vitamin D response element (VDRE) in the promoter of target genes, transcription proceeds through the interaction of VDR with coactivators and with the transcription machinery. The identification of the steps involved in this process has been a major focus of recent research in the field. However, the functional significance of target proteins as well as the functional significance of proteins involved in the transport and metabolism of vitamin D is also of major importance. Within the past few years much new information has been obtained from studies using knockout and transgenic mice. New insight has been obtained using this technology related to the physiological significance of the vitamin D binding protein (DBP), used to transport vitamin D metabolites, as well as the physiological significance of target proteins including 25-hydroxyvitamin D_3 24-hydroxylase (24(OH)ase), 25-hydroxyvitamin D_3-1α-hydroxylase (1α-(OH)ase), VDR, and osteopontin. The crystal structure of the DBP and the ligand binding domain of the VDR have recently been reported, explaining, in part, the unique properties of these proteins. In addition novel $1,25(OH)_2D_3$ target genes have been identified including the epithelial calcium channel, present in the proximal intestine and in the distal nephron. Thus in recent years a number of exciting discoveries have been made that have enhanced our understanding of mechanisms involved in the pleiotropic actions of $1,25(OH)_2D_3$. J. Cell. Biochem. 88: 695–705, 2003.

Key words: vitamin D receptor; vitamin D binding protein; $25(OH)D_3$ 24-hydroxylase; $25(OH)D_3$-1α-hydroxylase; transcription

INTRODUCTION

The actions of $1,25(OH)_2D_3$ include the maintenance of calcium homeostasis and effects on numerous other cell systems, including effects on the immune system and on the growth and differentiation of cancer cells. In recent years new insight has been obtained related to vitamin D metabolism and transport as well as the physiological significance of known target proteins. The elucidation of the crystal structure of the DBP and the crystal structure of the ligand-binding domain of the VDR also represent recent important contributions. In addition, novel target genes have been identified and novel regulators of vitamin D action have been discovered. In this review we will discuss new developments in these areas that have changed our understanding of the mechanisms of vitamin D action.

VITAMIN D METABOLISM

The metabolism of vitamin D to its active form is well known. Vitamin D is transported in the blood by the vitamin D binding protein (DBP) to the liver where it is hydroxylated at C-25 resulting in the formation of 25-hydroxyvitamin D_3 ($25(OH)D_3$). $25(OH)D_3$ is transported by the DBP to the kidney. In the proximal convoluted and straight tubules of the kidney $25(OH)D_3$ is hydroxylated at the 1α position resulting in the formation of the active form of vitamin D [reviewed in Christakos, 2002]. In recent studies mice deficient in DBP were generated by

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targeted mutagenesis [Safadi et al., 1999]. Studies using these mice resulted in new insight related to DBP's biological function in vitamin D metabolism and action. As expected, $DBP^{-/-}$ mice had markedly lower total serum levels of 25(OH)D and $1,25(OH)_2D$ than $DBP^{+/+}$ mice. However, surprisingly, levels of serum calcium and PTH were normal in the $DBP^{-/-}$ mice, suggesting that the low serum levels of vitamin D metabolites in the absence of DBP can exist in equilibrium with adequate intracellular concentrations of $1,25(OH)_2D_3$. Studies in $DBP^{-/-}$ and $DBP^{+/+}$ mice fed a vitamin D deficient diet indicated that DBP was able to prolong the serum half life of $25(OH)D_3$ and vitamin D. DBP did not create a buffer for $1,25(OH)_2D_3$, as might have been expected, since mice deficient in DBP were less susceptible to hypercalcemia and were resistant to vitamin D toxicity. It was suggested that the protection against toxicity may reflect more rapid urinary excretion of $25(OH)D_3$ and a more rapid conversion to inactive polar metabolites in the $DBP^{-/-}$ mice. The crystal structure of the human DBP has recently been reported [Verboven et al., 2002]. The vitamin D binding site is a cleft located at the surface of the molecule and partly in contact with the surrounding solvent. This contrasts with the $1,25(OH)_2D_3$ binding site to the VDR, which is a closed structure in the inner structure of the receptor. $1,25(OH)_2D_3$ can make the same hydrogen bonds with DBP as $25(OH)D_3$ but the axial 1-hydroxyl causes steric hindrance that may explain lower DBP binding affinity observed for $1,25(OH)_2D_3$. The studies with the DBP null mice as well as the DBP crystal structure represent important recent contributions.

An additional recent novel finding is that megalin, a member of the LDL receptor superfamily which is present on the apical surface of renal proximal tubular cells as well as in neuroepithelium, is essential for the reabsorption of complexes of $25(OH)D_3$ and DBP into proximal tubular cells [Nykjaer et al., 1999]. These findings were unexpected and first found using megalin knock out mice. In these mice there was abnormal urinary calcium excretion of $25(OH)D_3$ that resulted in vitamin D deficiency and bone disease. These results indicated for the first time that megalin is essential to deliver $25(OH)D_3$ for the generation of $1,25(OH)_2D_3$.

$25(OH)D_3$ is converted to $1,25(OH)_2D_3$ by the renal cytochrome P450 enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (1-α-(OH)ase). In recent studies, vitamin D 1-α-hydroxylase knock out mice were generated [Dardenne et al., 2001; Panda et al., 2001]. These animals had rachitic abnormalities typically observed in pseudovitamin D-deficiency rickets (PDDR). Mice were hypocalcemic, hypophosphatemic and had hyperparathyroidism. Osteomalacia was observed in young adult mutant mice. These mice will be a useful model of PDDR in which various therapeutic interventions can be tested. Although the proximal straight tubules of the kidney are the site of expression of 1-α-(OH)ase, it has been suggested that 1-α-(OH)ase is present in other cell types including macrophages and that local production of $1,25(OH)_2D_3$ could play a role in the differentiation or function of extra renal tissues. Although reproductive and immune dysfunction have been noted in the 1-α-(OH)ase KO mouse [Panda et al., 2001], further studies are needed to test the hypotheses, that have been a matter of debate, concerning the physiological roles of 1-α-(OH)ase in extra renal tissues.

In the kidney $25(OH)D_3$ can be hydroxylated at C-24 resulting in the formation of $24,25(OH)_2D_3$. 24-Hydroxylase is capable of hydroxylating the 24 position of $1,25(OH)_2D_3$ as well as $25(OH)D_3$ and it had been suggested that the preferred substrate of 24(OH)ase in vivo may be $1,25(OH)_2D_3$ [Shinki et al., 1992]. Studies using mice with a targeted inactivating mutation of the 24(OH)ase gene (24(OH)ase null mutant mice) provided the first direct in vivo evidence for a role for 24(OH)ase in the catabolism of $1,25(OH)_2D_3$ [St-Arnaud et al., 2000]. 24(OH)ase deficient mice, in response to $1,25(OH)_2D_3$ treatment, were unable to clear $1,25(OH)_2D_3$ from the bloodstream. Whether $24,25(OH)_2D_3$ is an active metabolite of vitamin D with effects on bone had previously been a matter of debate. Intramembranous bone formation was impaired in the 24(OH)ase null mutant mice. However crossing 24(OH)ase deficient mice to vitamin D receptor (VDR) ablated mice totally rescued the bone phenotype, indicating that elevated $1,25(OH)_2D_3$ levels in the 24(OH)ase null mutant mice, acting through VDR, and not the absence of $24,25(OH)_2D_3$ was the cause of the defect.

Thus, in summary, generation of mice deficient in DBP, in 1-α-(OH)ase and in 24(OH)ase have provided new insight into vitamin D metabolism and action. The role of DBP is to

maintain stable serum stores in an environment of decreased vitamin D availability. DBP does not create a buffer for $1,25(OH)_2D_3$, as might have been expected, since $DBP^{-/-}$ mice were less susceptible to hypercalcemia and toxicity induced by vitamin D overload. The 1-α-(OH)ase null mutant mouse model has provided in vivo evidence for the importance of this enzyme in the skeletal system and has suggested the possible importance of this enzyme in the immune and reproductive systems as well. In addition, studies using the 24(OH)ase KO mouse have provided the first in vivo evidence for the role of 24(OH)ase in the catabolism of $1,25(OH)_2D_3$ and have indicated, although it had been controversial, that $24,25(OH)_2D_3$ is a relatively inactive metabolite.

NEW DEVELOPMENTS IN AN UNDERSTANDING OF THE ROLE OF $1,25(OH)_2D_3$ IN CLASSICAL TARGET TISSUES

Bone

Whether $1,25(OH)_2D_3$ can act directly on bone or whether the antirachitic effects of $1,25(OH)_2D_3$ are indirect resulting from an increase by $1,25(OH)_2D_3$ in intestinal absorption of calcium and phosphorous resulting in their increased incorporation into bone, had been a matter of debate. Recent studies using VDR ablated mice (VDR knock out (KO) mice) addressed this question [Li et al., 1997; Yoshizawa et al., 1997; Amling et al., 1999]. VDR KO mice are born phenotypically normal and develop secondary hyperparathyroidism, hypocalcemia, and rickets and osteomalacia after weaning. However, when VDR KO mice are fed a rescue diet (high levels of calcium, phosphorous, and lactose) serum ionized calcium and PTH levels were normalized and rickets and osteomalacia were prevented. These findings in the VDR KO mice suggest that the major effect of $1,25(OH)_2D_3$ is on intestinal calcium and phosphorous absorption, resulting in their increased availability for incorporation into bone. However, in vitro studies support a direct effect of $1,25(OH)_2D_3$ on bone. $1,25(OH)_2D_3$ stimulates the formation of bone resorbing osteoclasts. The mechanism requires cell to cell contact between osteoblast/stromal cells and hematopoetic osteoclast progenitors and involves induction by $1,25(OH)_2D_3$ in osteoblastic cells of osteoprotegerin ligand or RANK-L (osteoclast differentiating factor) [Yasuda et al., 1998; Takeda et al., 1999]. Studies using VDR KO mice, which included in vitro studies, showed that the presence of VDR in osteoblastic cells is required for the stimulation of osteoclast formation by $1,25(OH)_2D_3$ [Takeda et al., 1999]. $1,25(OH)_2D_3$ also directly stimulates the production of the bone calcium binding protein osteocalcin whose synthesis is positively associated with new bone formation [Price and Baukol, 1980]. Another calcium binding protein induced by vitamin D in osteoblasts is osteopontin. Recent studies in osteopontin KO mice have indicated that these mice are resistant to parathyroid hormone induced bone resorption, suggesting a requirement for osteopontin in bone resorption [Ihara et al., 2001]. $1,25(OH)_2D_3$ has also been shown to regulate Osf2/Cbfa1 transcription factor that regulates osteoblastic differentiation [Drissi et al., 2002]. In addition, novel analogs of $1,25(OH)_2D_3$ have been developed that exhibit selective anabolic actions in osteoblasts and result in enhanced bone formation [Peleg et al., 2002; Shevde et al., 2002]. These findings are important since they suggest that bone selective analogs of $1,25(OH)_2D_3$ may be therapeutically beneficial for the treatment of bone loss disorders. Studies using transgenic mice overexpressing the VDR in mature osteoblastic bone forming cells have noted increased bone formation, further emphasizing direct effects of $1,25(OH)_2D_3$ on bone [Gardiner et al., 2000]. Thus, although studies in VDR KO mice suggest an effect on bone secondary to an effect of $1,25(OH)_2D_3$ on intestinal calcium absorption, $1,25(OH)_2D_3$ does have direct effects on bone. Studies in the VDR KO mice may not reveal direct effects on bone due to similar actions by other factors. The effects of $1,25(OH)_2D_3$ on bone are diverse and can affect formation or resorption.

Intestine

$1,25(OH)_2D_3$ is the principal factor controlling intestinal calcium absorption. $1,25(OH)_2D_3$ interacts with the intestinal VDR and induces the production of the calcium binding protein calbindin. Calbindin is thought to act as an intracellular calcium ferry and to act as a cytosolic buffer to prevent toxic levels of calcium from accumulating in the intestinal cell during $1,25(OH)_2D_3$ mediated translocation of calcium [Raval-Pandya et al., 1998]. In VDR KO mice

the major defect in intestinal calcium absorption is accompanied by a marked reduction in calbindin-D_{9k} [Li et al., 1997]. Calcium extrusion at the basolateral membrane of the intestine is an active process and previous studies have shown that the intestinal plasma membrane calcium pump (PMCA) and PMCA mRNA are stimulated by $1,25(OH)_2D_3$ [Wasserman et al., 1992; Cai et al., 1993]. In addition to the role of $1,25(OH)_2D_3$ on transcellular movement of calcium and on the extrusion of calcium form the intestinal cell, it has been known that the rate of calcium entry can be increased by $1,25(OH)_2D_3$. However the existence of a calcium channel at the brush border membrane responsible for this process had been controversial. Recently a calcium selective channel, that is potentially important in the control of intestinal calcium absorption, was cloned from rat duodenum [Peng et al., 1999]. This novel calcium channel may indeed play a key role in vitamin D dependent calcium entry into the enterocyte and may be the rate-limiting step in vitamin D dependent intestinal calcium absorption [Van Cromphaut et al., 2001]. This channel is distinct from voltage gated calcium channels and is part of a subfamily of channels of which the capsaicin receptor was the first identified member. The epithelial calcium channel cloned from rat duodenum, CaT1, contains 6 transmembrane domains and 4 ankyrin repeat domains [Peng et al., 1999] (Fig. 1). The presence of the 4 ankyrin repeat domains suggests that CaT1 may associate with cytoskeletal proteins supporting the microvilli at the apical pole of the absorptive cells of the intestine. An understanding of the regulation of the epithelial calcium channel as well as an understanding of the relationship of calbindin to this channel in the absorptive cells of the intestine should result in new insight in our understanding of the process of vitamin D dependent intestinal calcium absorption.

Kidney

In the kidney, the key site of the hormonal regulation of calcium is transcellular calcium transport in the distal tubule [Friedman, 1999]. Similar to transcellular intestinal calcium absorption, the effect of $1,25(OH)_2D_3$ on renal calcium transport involves calcium entry through the apical membrane, diffusion

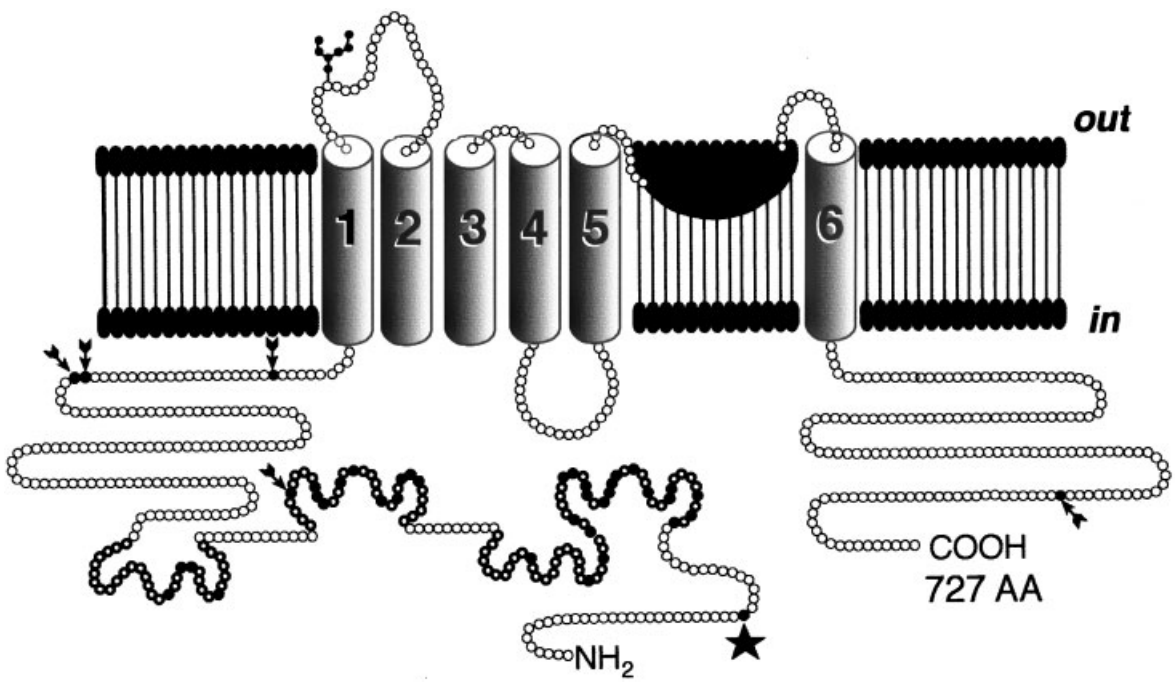

Fig. 1. CaT1 (epithelial calcium channel cloned from rat duodenum): predicted membrane topology and domain structure. CaT1 contains 6 transmembrane domains. The putative N-linked glycosylation site (branched chain) as well as putative protein kinase A (star) and C (arrows) phosphorylation sites are marked. This novel calcium channel has been suggested to play a key role in vitamin D dependent calcium entry into the enterocyte. [Reproduced with permission from Peng et al., 1999].

through the cytosol involving the vitamin D inducible calcium binding protein, calbindin, and active calcium extrusion at the basolateral membrane [Raval-Pandya et al., 1998; Sooy et al., 2000]. Similar to studies in the intestine, an apical calcium channel was also identified in the distal convoluted tubule and the distal connecting tubules (CaT2 or ECaC1) [Hoenderop et al., 1999; Peng et al., 2000] and found to be induced by $1,25(OH)_2D_3$ [Hoenderop et al., 2001]. CaT2 shares 73.4% sequence homology with the apical calcium channel localized in the intestine, CaT1 [Peng et al., 2000]. These calcium channels represent a new family of calcium selective ion channels. The identification of these channels is of interest since it will provide for the first time an understanding of how calcium enters the apical membrane of $1,25(OH)_2D_3$ responsive epithelia.

NON CLASSICAL ACTIONS OF $1,25(OH)_2D_3$

Inhibition of Proliferation of Cancer Cells

$1,25(OH)_2D_3$ has been reported to inhibit the proliferation of a number of malignant cells, including breast and prostate cancer cells [Colston and Hansen, 2002; Polek and Weigel, 2002]. Recent studies indicate that a combination of $1,25(OH)_2D_3$ or $1,25(OH)_2D_3$ analog and retinoids may have greater therapeutic potential for the treatment of breast and prostate cancer [Blutt et al., 1997; Campbell et al., 1998, 1999; Zhao et al., 1999; Wang et al., 2000, 2001]. A combination of $1,25(OH)_2D_3$ and retinoic acid lowers the threshold for killing of breast cancer cells by chemotherapeutic agents [Wang et al., 2000]. In addition, expression of the retinoic acid receptor β can sensitize prostate cancer cells to growth inhibition mediated by a combination of retinoids and vitamin D analog, suggesting that simultaneous treatment of vitamin D analog and receptor selective retinoids may have therapeutic potential for the treatment of androgen dependent and independent prostate cancer [Campbell et al., 1998].

Effects on the Immune System

$1,25(OH)_2D_3$ can affect the differentiation and function of cells in the immune system. CD4 cells of T lymphocytes have been reported to be the preferential target of $1,25(OH)_2D_3$. The two distinct functional CD4 cell types are Th1 and Th2 cells. The Th1 cells preferentially produce IL-2, IFN-γ, and TNF-α and stimulate the cellular immune system. Th2 cells preferentially secrete IL-4 and IL-10 and inhibit Th1 function. Th1 cells are the main effector cells of a number of autoimmune diseases and of organ rejection [Liblau et al., 1995]. It has been shown that $1,25(OH)_2D_3$ inhibits Th1 cells and the production of Th1 cytokines IL-2, IFN-γ, and TNF-α [Lemire and Archer, 1991]. For IL-2 and IFN-γ it has been reported that the mechanism involves VDR mediated inhibition of gene transcription [Alroy et al., 1995; Cippitelli and Santoni, 1998]. $1,25(OH)_2D_3$ has also been reported to upregulate IL-4 [Cantorna et al., 1998]. Most recently $1,25(OH)_2D_3$ has been reported to inhibit the differentiation and survival of dendritic cells, resulting in impaired alloreactive T cell activation [Penna and Adorini, 2000; Griffin et al., 2001]. Several previous studies indicated that $1,25(OH)_2D_3$ can either prevent or at least partially protect against the induction of a number of experimental autoimmune disease including experimental allergic encephalitis (the murine model for multiple sclerosis) [Lemire and Archer, 1991], experimental lupus erythematosus [Abe et al., 1990], and autoimmune thyroiditis [Fournier et al., 1990]. $1,25(OH)_2D_3$ has also been reported to prevent autoimmune diabetes in nonobese diabetic (NOD) mice [Casteels et al., 1998]. Thus, analogs of $1,25(OH)_2D_3$ with increased immunomodulatory properties and low calcemic activity may be candidates for the treatment of autoimmune diseases.

TRANSCRIPTIONAL REGULATION BY $1,25(OH)_2D_3$: FACTORS INVOLVED

Vitamin D Receptor

The functional domains of the VDR are the N-terminal DNA binding domain (DBD) and the C terminal ligand binding domain (LBD). The hinge region links the two functional domains and is also immunogenic [see Haussler et al., 1998 for review]. Within the DBD two zinc atoms form zinc finger DNA binding motifs (residues 24–90 in the hVDR). The C terminal regions of hVDR in closest proximity to the $1,25(OH)_2D_3$ have been reported to extend approximately from residues 227–422. The LBD of VDR has also been shown to be involved in protein–protein interaction of VDR with RXR and other cofactors. Mutagenesis studies have indicated that amino acids 317 and 395

and amino acids 244 and 263, corresponding to portions of helices 7–10 and 3–4, respectively are important for ligand dependent heterodimerization with RXR [Whitfield et al., 1995; Jin et al., 1996]. Most recently the crystal structure of the VDR LBD bound to $1,25(OH)_2D_3$ or analogs of $1,25(OH)_2D_3$ has been published [Rochel et al., 2000; Tocchini-Valentini et al., 2001]. The hVDR LBD structure is most closely related to the holo hRARγ LBD. In the VDR, the $1,25(OH)_2D_3$ binding site is a closed pocket formed in the inner structure of the receptor. In the VDR pocket, the A-ring of $1,25(OH)_2D_3$ has a B-chair conformation. The crystal structure of the VDR has revealed the active conformation of the bound ligand and will allow, in future studies, a more complete understanding of the three dimensional contacts between VDR and RXR and between VDR and other accessory factors.

VDR Subcellular Trafficking

Although there is little question that the liganded form of VDR is localized predominantly in the nucleus, there exists some controversy regarding the subcellular localization of the unliganded VDR. Previous cell fractionation studies suggested that unliganded VDR is localized mainly in the nucleus. However, recent studies using fluorescent protein chimeras of VDR and fluorescent microscopy in living cells showed that unliganded VDR can partition between the cytoplasm and the nucleus, that $1,25(OH)_2D_3$ induces cytoplasmic translocation to the nucleus and that deletion of the activation function 2 domain (AF-2) close to the C terminus of the LBD prevents hormone induced translocation [Racz and Barsony, 1999; Prufer et al., 2000]. These findings suggest that cofactors that are known to bind to the VDR in this region participate in VDR transport. RXR was also reported to promote the nuclear accumulation of VDR and to inhibit export of VDR from the nucleus [Prufer and Barsony, 2002]. These studies have provided new findings that suggest trafficking of VDR and RXR in the regulation of $1,25(OH)_2D_3$ action.

Hereditary Vitamin D Resistant Rickets

Hereditary Vitamin D resistant rickets (HVDRR) is a rare autosomal recessive disorder characterized by early onset of rickets and organ resistance to $1,25(OH)_2D_3$. The resistance to $1,25(OH)_2D_3$ is caused by heterogeneous mutations in the VDR. A number of specific mutations have been previously characterized [reviewed in Haussler et al., 1998]. Recent studies using three HVDRR mutations in the LBD (R274L, H305Q, and F251C) revealed that treatment with analogs of $1,25(OH)_2D_3$ partially or completely restored the responsiveness of the mutated VDR, suggesting the possibility of using $1,25(OH)_2D_3$ analogs to treat selected patients with HVDRR [Gardezi et al., 2001]. Most recently, the first case of a naturally occurring mutation in the VDR (E420K) that disrupts coactivator binding to the VDR and causes HVDRR has been identified [Malloy et al., 2002]. In addition, a case of HVDRR with alopecia and normal VDR has been demonstrated and found to be due to overexpression of an hnRNPA-related nucleic acid binding protein, recently named vitamin D response element binding protein (VDRE-BP) [Adams et al., 2002]. The VDRE-BP competes with VDR-RXR for binding to the VDRE [Chen et al., 2000]. VDRE-BP resistance to $1,25(OH)_2D_3$ can be compensated for another set of proteins known as intracellular vitamin D binding proteins (IDBP) [Wu et al., 2000]. They bind 25-hydroxylated vitamin D metabolites. They are in the heat shock protein family and promote the uptake, VDR-RXR directed transactivation and preferential 1 hydroxylation of 25-hydroxylated vitamin D metabolites [Wu et al., 2002]. The VDRE-BP and the IDBPs are newly discovered, novel regulators of vitamin D action.

VDR can Function As a Receptor for Lithocholic Acid

Recent studies suggested that VDR mediates not only the effects of $1,25(OH)_2D_3$ but also functions as a receptor for the bile acid, lithocholic acid (LCA). Makishima et al. [2002] indicated that LCA activates VDR and induces expression of CYP3A, an enzyme that detoxifies LCA in the liver and intestine. Recent previous studies identified CYP3A as a novel target gene of $1,25(OH)_2D_3$ action [Schmiedlin-Ren et al., 1997; Thummel et al., 2001]. Thus both $1,25(OH)_2D_3$ and LCA can activate VDR and induce expression of CYP3A. Micromolar concentrations of LCA, compared to nanomolar concentrations of $1,25(OH)_2D_3$, are needed to induce transcription of CYP3A. It was suggested that VDR could be a bile acid sensor in the enteric tract where elevated concentrations of LCA are observed. These novel findings

suggest a mechanism that may explain the proposed protective effect of $1,25(OH)_2D_3$ against colon cancer and the enhanced cellular proliferation observed in the colon of VDR KO mice [Kane et al., 1996; Kallay et al., 2001].

Interaction of VDR with the Transcription Machinery

The mechanisms involved in VDR mediated transcription following the binding of the VDR-RXR heterodimer to the VDRE of target genes are only now beginning to be defined. TFIIB as well as several TATA-binding protein (TBP)-associated factors (TAFs) have recently been reported to be involved in VDR mediated transcription. TFIIB interacts with unliganded VDR and $1,25(OH)_2D_3$ disrupts the VDR-TFIIB complex, suggesting that in the presence of ligand, TFIIB is released for assembly into the preinitiation complex [Masuyama et al., 1997]. TAF_II135 potentiates VDR mediated transcriptional activity and TAF_II28 and TAF_II55 interact with VDR (at α helices H3–H5 and at α helix 8) [Mengus et al., 1997, 2000; Lavigne et al., 1999]. A mutation in the H3–H5 region was reported to abolish VDR-mediated transcription [Mengus et al., 2000].

SRC/p160 Coactivators

The p160 coactivators, that include SRC-1 (NcoA-1 or p160), GRIP-1 (TIF2, NcoA-2 or SRC-2), ACTR (pCIP, RAC3 or SRC-3) share a series of LxxLL (or NR box) motifs, possess histone acetyl transferase (HAT) activity and interact with the AF2 domain of steroid receptors, including VDR, in a ligand dependent manner. The p160 coactivators have been reported to recruit CBP (CREB binding protein), which also has HAT activity, resulting in a multisubunit complex [see McKenna et al., 1999; Freedman, 1999 for reviews]. Recent studies by Issa et al. [2001] indicated that GRIP-1 and ACTR (RAC3) could interact with VDR simultaneously and suggested that cell and promoter specific functions of VDR may be mediated through differential recruitment of coactivators.

NcoA-62/Ski-interacting protein

Ski-interacting protein (SKIP) is an AF-2 independent coactivator for VDR that lacks LxxLL motifs and HAT activity but acts by forming a ternary complex with VDR and SRC coactivators (GRIP and SRC-1) to result in synergistic effects on VDR mediated transcription [Zhang et al., 2001].

Vitamin D Receptor Interacting Protein

VDR mediated transcription is also mediated by a coactivator complex, DRIP [vitamin D receptor interacting protein, also called TRAP and ARC, depending on the transcription factor initially identified as the target (TR or AR, respectively)]. The complex does not have HAT activity but functions in part through recruitment of RNA polymerase II [Rachez et al., 1999] (Fig. 2).

Other Factors Modulating VDR Mediating Transcription

A number of promoter specific transcription factors have recently been reported to modulate VDR mediated transcription. The Ras activated Ets transcription factor was found to be important for $1,25(OH)_2D_3$ mediated 24(OH)ase transcription [Dwivedi et al., 2000]. YY1 has been identified as a transcription factor involved in the repression of VDR mediated 24(OH)ase and OC transcription [Guo et al., 1997; Raval-Pandya et al., 2001]. CCAAT/enhancer binding protein (C/EBP) β and δ, induced by $1,25(OH)_2D_3$ in osteoblasts, can enhance 24(OH)ase and OC transcription [Dhawan et al., 2002; Gutierrez et al., 2002]. In the regulation of OC transcription there is synergism between C/EBP β and Runx 2 (Cbfa 1) mediated by the interaction between Runx 2 and C/EBP β [Gutierrez et al., 2002].

Phosphorylation has also been reported to regulate VDR mediated transcription. Phosphatase inhibitors can result in enhancement of VDR mediated transcription and this enhancement may be due in part to increased interaction between VDR and DRIP205, a subunit of the DRIP coactivator complex [Barletta et al., 2002]. Increased interaction between VDR and coactivators such as DRIP205 may be a major mechanism that couples extracellular signals to vitamin D action. Thus, we are now beginning to understand the multiple factors and mechanisms involved in the transcriptional response of various target genes to $1,25(OH)_2D_3$.

FUTURE DIRECTIONS

In the future, novel target genes and new factors involved in VDR mediated transcription

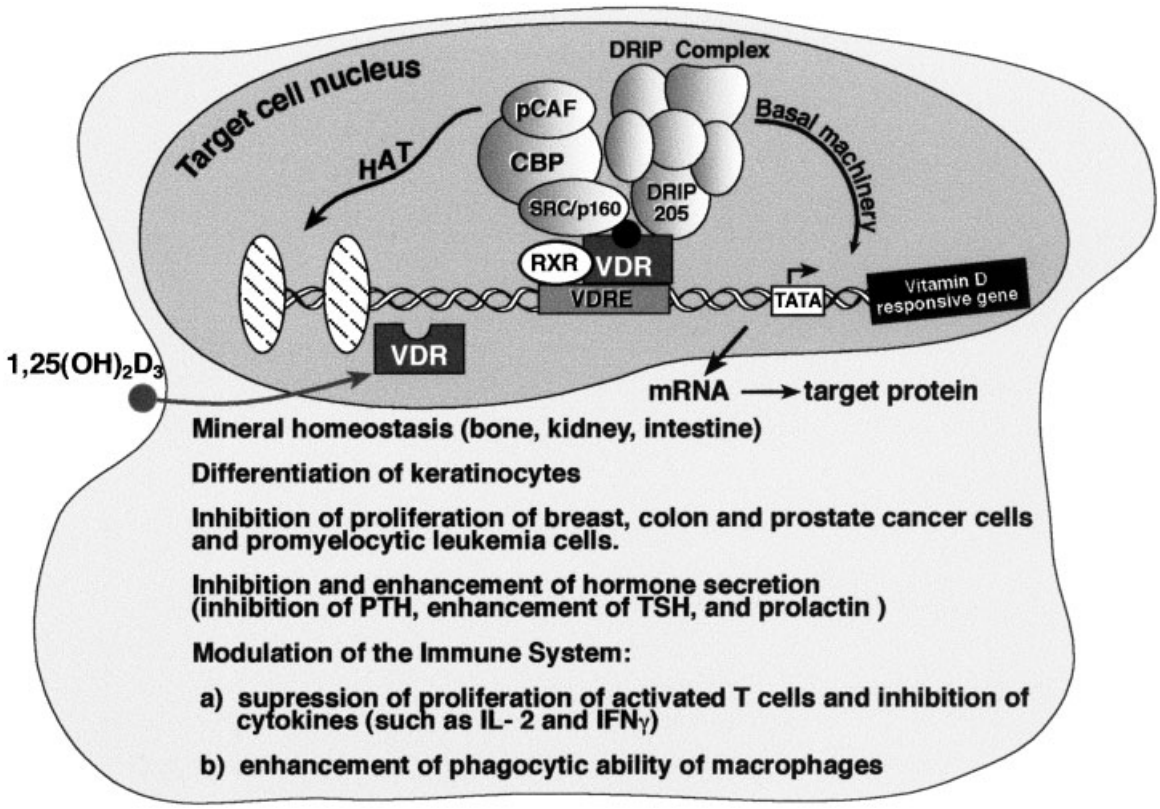

Fig. 2. Mechanism of action of $1,25(OH)_2D_3$ in target cells. VDR heterodimerizes with RXR. After interaction with the VDRE, transcription proceeds through the interaction of VDR with coactivators and with the transcription machinery. The histone acetyl transferase (HAT) activity-containing complex (SRC/p160 and CBP) may be recruited first by liganded VDR followed by binding of the DRIP complex or there could be simultaneous recruitment of these coactivators. $1,25(OH)_2D_3$ is known to maintain calcium homeostasis as well as to affect numerous other cell systems. Effects on other cell systems include modulation of the immune system and inhibition of proliferation of cancer cells.

will be identified in numerous different systems. With the elucidation of the crystal structure of VDR, an increased understanding of the structure of VDR in the presence and absence of ligand and/or protein partners will be obtained. Based on the structure of VDR synthetic analogs of $1,25(OH)_2D_3$ may be designed that would selectively modulate specific $1,25(OH)_2D_3$ responses in specific target tissues. The analogs would have therapeutic potential not only for the treatment of bone loss disorders but also for the treatment of various types of cancer and for the treatment of autoimmune disorders.

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