

# MINIREVIEW

## Rapid Actions of $1\alpha$ -25-Dihydroxyvitamin $D_3$ Physiologic Role (43803)

DANIEL T. BARAN\*†‡<sup>1</sup> and ANN MARIE SORENSEN\*

Departments of Orthopedics,\* Medicine,† and Cell Biology,‡ University of Massachusetts Medical Center, Worcester, Massachusetts 01655

---

**Abstract.** The steroid hormone,  $1\alpha,25$ -dihydroxyvitamin  $D_3$  ( $1\alpha,25$ -[OH] $_2D_3$ ), has been shown to exert actions on cells within seconds. The rapidity of these effects has led to the speculation that they reflect nongenomic actions of the hormone which involve interaction with membrane receptors. Moreover, these rapid effects appear to modulate the actions of the hormone on gene transcription, suggesting that the physiologic role of the rapid actions may involve the regulation of hormone induced gene activation.

[P.S.E.B.M. 1994, Vol 207]

---

**1**  $\alpha,25$ -Dihydroxyvitamin  $D_3$  ( $1\alpha,25$ -[OH] $_2D_3$ ) is the most potent vitamin D metabolite. Traditionally, it was thought to play a role in the regulation of plasma  $Ca^{2+}$  by its actions on intestine, bone, and kidney (1). However, the hormone has recently been demonstrated to exert its effects not only on the regulation of plasma  $Ca^{2+}$ , but also on other processes, such as alteration of intracellular  $Ca^{2+}$ , pH, and phospholipid levels, and modulation of cell differentiation and proliferation (2).

The mechanism(s) by which the hormone alters cell growth and differentiation are largely unknown. Specific receptors (VDR) for this seco-steroid have been found in numerous organs and cell lines (2). The binding of  $1\alpha,25$ -[OH] $_2D_3$  to the VDR receptor, as well as the subsequent binding of the hormone-receptor

complex to selected DNA sequences, was thought to be the mechanism explaining all of the hormone's actions. However, since our initial observations that the hormone rapidly increases cytosolic  $Ca^{2+}$  levels in hepatocytes (3), osteoblasts and a variety of other cell types (4) have been shown to rapidly respond (seconds to minutes) to  $1\alpha,25$ -[OH] $_2D_3$ . The rapidity of these responses has led to speculation that not all actions of  $1\alpha,25$ -[OH] $_2D_3$  are mediated by genome activation (5).

The VDR for  $1\alpha,25$ -[OH] $_2D_3$  is a member of a nuclear transacting receptor superfamily and shares amino acid sequence homology with thyroid hormone receptor, other steroid hormone receptors, and the viral oncogene *erb A* product (6). The VDR is mainly cytoplasmic in the absence of  $1\alpha,25$ -[OH] $_2D_3$ , but after addition of the hormone, there is a rapid reorganization with intranuclear accumulation within 1–3 min (7). This intranuclear accumulation appears to be dependent upon interaction with microtubules (8). It appears that the  $1\alpha,25$ -[OH] $_2D_3$  occupied receptor undergoes a covalent modification within the nucleus by reacting with a kinase, which results in phosphorylation of the receptor (9) prior to its binding to double-stranded DNA (10). It is assumed that the occupied receptor phosphoprotein is the form of the vitamin D receptor that interacts with the vitamin D-responsive DNA el-

---

<sup>1</sup> To whom requests for reprints should be addressed at Department of Orthopedics, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655.

---

Received March 28, 1994. [P.S.E.B.M. 1994, Vol 207]  
Accepted June 9, 1994

---

0037-9727/94/2072-0175\$10.50/0  
Copyright © 1994 by the Society for Experimental Biology and Medicine

---

ements of target genes (2). Certain amino acid alterations within the DNA-binding domain of the receptor have no effect on DNA binding *in vitro*, but reduce the capacity of these mutants to activate transcription. This suggests that binding of the vitamin D-receptor complex to chromatin is not the only factor regulating transcription. Observations that the rapid actions of  $1\alpha,25\text{-(OH)}_2\text{D}_3$  are able to modulate certain of the hormone's genomic effects has led to the hypothesis that the rapid nongenomic actions play a role in the physiologic actions of  $1\alpha,25\text{-(OH)}_2\text{D}_3$ .

This review will focus on the rapid actions of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  in one of the hormone's target cells, osteoblasts, and attempt to define the mechanism of these rapid actions as well as their physiologic significance.

### Rapid Actions of $1\alpha,25$ -Dihydroxyvitamin $\text{D}_3$ on Osteoblasts

Early studies focused on the effects of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  as a stimulator of  $^{45}\text{Ca}$  accumulation in rat clonal osteoblast-like cell line, ROS 17/2 (11). Exposure of the ROS 17/2 cells to  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$ , 2 pM, for 15 min increased  $^{45}\text{Ca}$  accumulation in the cells by 10%–20%. Actinomycin did not alter these early actions of the hormone on  $^{45}\text{Ca}$  accumulation, suggesting that the mechanism(s) for the stimulating effects was independent of *de novo* protein synthesis.

A drawback of this study was that it evaluated  $^{45}\text{Ca}$  accumulation which is an insensitive indicator of intracellular calcium changes. The development of fluorescent  $\text{Ca}^{2+}$  indicators made possible the more precise quantitation of changes in intracellular calcium. The first generation fluorescent  $\text{Ca}^{2+}$  indicator, acetoxymethyl Quin 2, was employed by Lieberherr to quantify changes in cellular calcium in mouse osteoblasts (12).  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$ , 10–100 pM, increased intracellular calcium from 135 nM to 190–200 nM within 30 sec. This increase was shown to depend upon the presence of extracellular calcium, since the increments were abolished by EGTA and calcium channel blockers suggesting a direct action of the hormone on membrane calcium channels. These effects were not particularly specific since both  $24,25$ -dihydroxyvitamin  $\text{D}_3$ , 100 pM–10 nM, and  $25$ -hydroxyvitamin  $\text{D}_3$ , 10 and 100 nM, increased intracellular calcium. The effects of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  on mouse osteoblasts were also demonstrated by Oshima *et al.* (13). These effects occur within seconds, at concentrations as low as 2 pM, and also appear to depend upon the presence of extracellular calcium.  $24,25$ -Dihydroxyvitamin  $\text{D}_3$  was without effect.

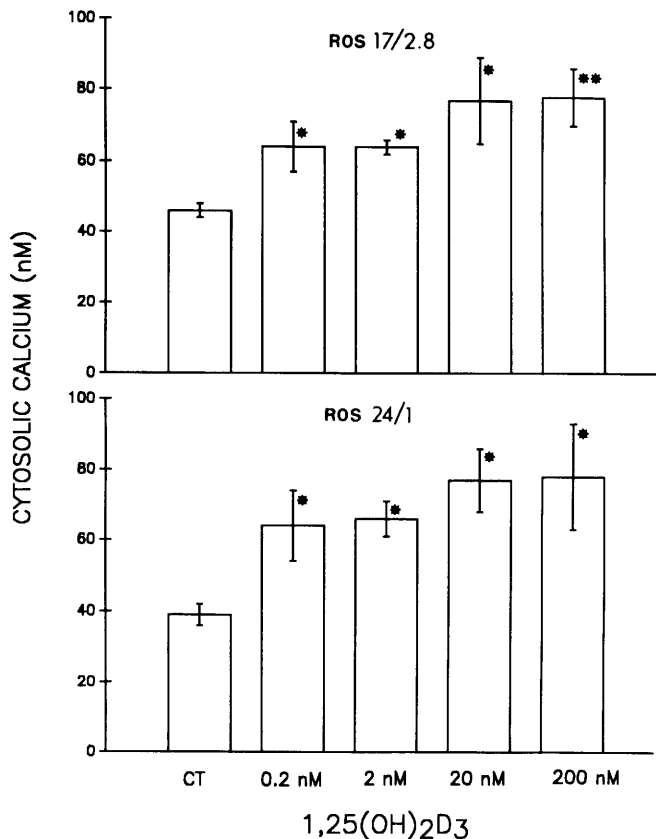
The presence of voltage-gated  $\text{Ca}^{2+}$  channels in the rat osteosarcoma cell line, ROS 17/2.8 has been

confirmed by electrophysiologic studies (14). The functional properties of these channels are modulated within 1 min by the vitamin D hormone, 0.1–3.0 nM, with 2-fold increments in  $\text{Ca}^{2+}$  uptake.  $25$ -Hydroxyvitamin  $\text{D}$  was a less potent stimulator of  $\text{Ca}^{2+}$  uptake.

These observations were extended by Civitelli who observed that  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  rapidly increased intracellular calcium levels in individual osteoblast-like cells (15). The hormone also rapidly enhanced membrane phospholipase C activity resulting in the generation of inositol triphosphate, a recognized mediator of hormone-induced cellular calcium increments. As in the plasma membrane,  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  rapidly enhanced phospholipase C activity in the nuclear envelope resulting in increased inositol triphosphate levels, which in turn are able to increase nuclear calcium (16). Thus, it appeared that the rapid actions of  $1\alpha,25\text{-(OH)}_2\text{D}_3$  on cellular  $\text{Ca}^{2+}$  are mediated by alterations in the membrane phospholipid metabolism (17).

The accumulated studies suggested that vitamin D acts as a modulator of calcium channel function in mouse osteoblasts and rat and mouse osteoblast-like cells. Since current evidence suggests that the calcium ion is important in intracellular signaling processes, these studies raised the potential that the rapid actions of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  might have a physiologic role in cell function and pointed to an interaction of the hormone with a plasma membrane signaling system.

Although the rapidity of the vitamin D effects suggested a nongenomic mechanism, it was unclear whether the classic nuclear receptor was necessary for the hormone to exert these rapid effects. We were able to show that  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  increased cellular calcium in a dose-dependent fashion in a rat osteoblast-like cell line that lacked the nuclear vitamin D receptor (Fig. 1) (18). We demonstrated the absence of vitamin D receptor mRNA in ROS 24/1 cells and directly demonstrated a lack of vitamin D receptor in nuclear extracts of the ROS 24/1 that was capable of binding to VDRE of the osteocalcin gene. It was clear that at least a portion of the measured increase in cellular calcium observed after 5 min of hormone treatment in the ROS cells reflected the release from intracellular stores, since chelation of the extracellular calcium with EGTA did not abolish the  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  effect. Moreover, the  $1\beta$  epimer of  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  which does not interact with the classic receptor (19), inhibited the rapid effect of the hormone, suggesting that the epimer bound to but did not activate the signaling system mediating the rapid actions and that this signaling system differed from the classic nuclear vitamin D receptor (18). The presence of distinct nuclear and plasma membrane associated forms of the  $1\alpha,25$ -dihydroxyvitamin  $\text{D}_3$  re-

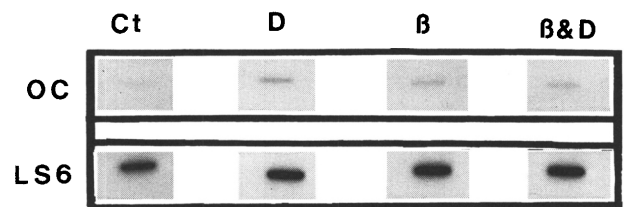


**Figure 1.** Effect of increasing concentrations of  $1\alpha,25\text{-(OH)}_2\text{D}_3$  on cellular calcium levels in ROS 17/2.8 (VDR present) and 24/1 (VDR absent) cells. Cells were loaded with Quin 2 and exposed to hormone for 5 min. \* $P < 0.05$  compared with control by Duncan's test for multiple comparisons; \*\* $P < 0.05$  compared with 0.2 and 2 nM  $1\alpha,25\text{-(OH)}_2\text{D}_3$  by Duncan's test for multiple comparisons (J Bone Mineral Res 6:1269, 1991, with permission).

ceptor that are involved in genomic and nongenomic activation of osteoblast activity was further suggested by demonstration of a structural hierarchy of vitamin  $\text{D}_3$  analogs with regard to their efficacy as transducers of the genomic and nongenomic pathways respectively (20, 21).

### Physiologic Role

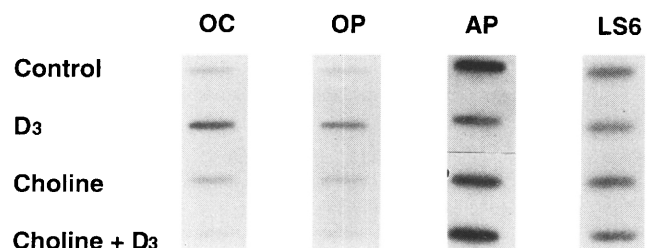
Using the  $1\beta$  epimer of  $1\alpha,25\text{-dihydroxyvitamin D}_3$ , we were able to probe the functional significance of the rapid actions of the hormone.  $1\beta,25\text{-dihydroxyvitamin D}_3$  inhibits the rapid effects of  $1\alpha,25\text{-dihydroxyvitamin D}_3$  but does not alter the binding of the vitamin D receptor complex to the vitamin D responsive element of the osteocalcin gene (22). However, the epimer inhibited  $1\alpha,25\text{-dihydroxyvitamin D}_3$ -induced increases in osteocalcin mRNA transcription at 1 hr (Fig. 2) and steady-state levels at 3 hr in ROS 17/2.8 cells. The results indicated that the rapid nongenomic actions of  $1\alpha,25\text{-dihydroxyvitamin D}_3$  have a functional role in the genomic activation of the osteocalcin gene, perhaps by modifying subtle structural



**Figure 2.** Nuclear run-on assay of osteocalcin mRNA transcription. ROS 17/2.8 cells were treated with vehicle (Ct),  $1\alpha,25\text{-(OH)}_2\text{D}_3$ , 20 nM (D),  $1\beta,25\text{-(OH)}_2\text{D}_3$ , 20 nM (B), or both (B + D) for 1 hr. Isolated nuclei were transcribed in the presence of  $^{32}\text{P}$ -UTP and radiolabeled RNA transcripts hybridized to slot blots of cloned pOC 3.4 and ribosomal clone LS-6 (J Cell Biochem 50:124, 1992, Wiley-Liss, Inc., a division of John Wiley & Sons, Inc., with permission).

and/or functional properties of the vitamin D-receptor DNA complex, or by affecting other protein DNA interactions that support OC gene transcription (22). Moreover, the hormone was shown to increase nuclear calcium levels in both intact cells and isolated nuclei from rat-osteoblast-like cells suggesting that the rapid nongenomic activation of nuclear calcium plays an important role in osteoblast activity (23). However, subsequent studies have cast doubt on the functional role of the rapid increases in cellular or nuclear calcium in the modulation of the genomic actions of the hormone.

$1\alpha,25\text{-dihydroxyvitamin D}_3$  rapidly increases intracellular pH in osteoblast-like cells, an effect that is dependent upon  $\text{Na}^+/\text{H}^+$  transport (24). Incubation of cells in  $\text{Na}^+$ -free medium abolishes the vitamin D-induced increase in intracellular pH and in steady-state levels of osteocalcin and osteopontin mRNA (Fig. 3), but not the increase in intracellular calcium (24). Thus, although the genomic effects of  $1\alpha,25\text{-dihydroxyvitamin D}_3$  appear to be modulated by its rapid actions, it does not appear that the rapid increases in intracellular calcium per se are critical in this process. Recent studies in human osteoblast MG63 cells have also demonstrated that  $1\beta,25\text{-dihydroxyvitamin D}_3$  is an antagonist of the nongenomic actions of  $1\alpha,25\text{-dihydroxyvitamin D}_3$  (25). Those studies were unable



**Figure 3.** Hybridization analysis. The effect of  $1\alpha,25\text{-dihydroxyvitamin D}_3$ , 20 nM, ( $\text{D}_3$ ) on osteocalcin (OC), osteopontin (OP), alkaline phosphatase (AP), and ribosomal (LS6) mRNA. RNA was from ROS 17/2.8 cells treated with  $\text{D}_3$  for 3 hr in the presence of sodium (control and  $\text{D}_3$ ) and in the absence of sodium with choline substituted to maintain osmolality (Choline and choline +  $\text{D}_3$ ). (J Cell Biochem 53:234, 1993, Wiley-Liss, Inc., a division of John Wiley & Sons, Inc., with permission).

to document an antagonistic effect of the  $1\beta$  epimer on the hormone-induced increment in cell medium osteocalcin levels; however, osteocalcin levels were measured in the medium only after 72 hr without documentation that the rapid effects were also being inhibited at that time.

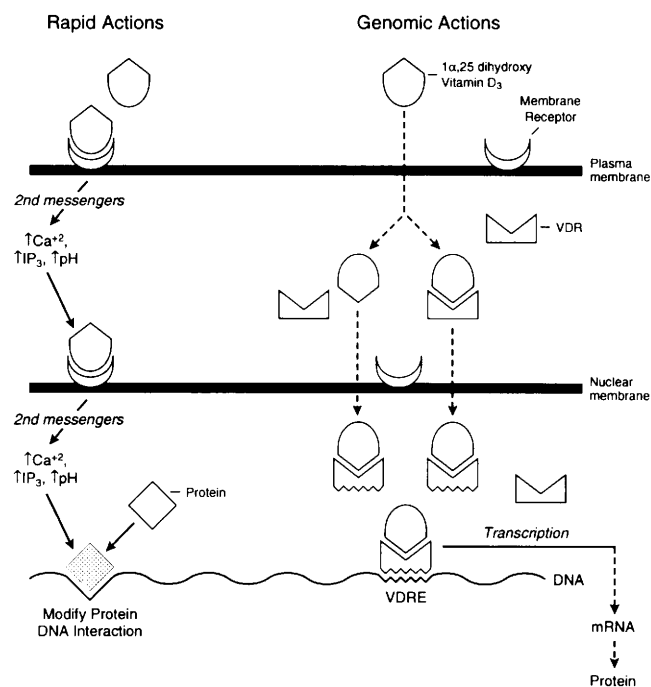
## Summary

It has been clearly demonstrated that the steroid hormone  $1\alpha,25\text{-(OH)}_2\text{D}_3$  exerts rapid, nongenomic effects in a variety of cell types. These rapid actions appear to involve interaction with a membrane signaling system that differs from the classic nuclear vitamin D receptor. Interaction with the membrane signaling system results in enhanced phospholipase C activity, opening of voltage-gated membrane  $\text{Ca}^{2+}$  channels, and increased cellular alkalization regulated by the  $\text{Na}^+/\text{H}^+$  antiport. The plasma membrane of osteoblast-like cells possesses a receptor that recognizes  $1\alpha,25\text{-(OH)}_2\text{D}_3$  and its inactive  $1\beta$  epimer (26). Activation of this rapid, nongenomic pathway may alter

protein binding to DNA (Fig. 4) or could affect interactions of DNA and the nuclear matrix. The precise mechanisms by which these rapid nongenomic actions interface with the genomic pathway is as yet not defined, but it is clear that these effects occur within seconds to minutes and appear to modulate the actions of  $1\alpha,25\text{-(OH)}_2\text{D}_3$  on gene transcription and mRNA steady-state levels in osteoblasts.

This work was supported in part by National Institutes of Health Grant DK39085.

### Speculated Mechanisms of the Rapid Action of $1\alpha,25$ Dihydroxyvitamin $\text{D}_3$



**Figure 4.** The rapid actions of  $1\alpha,25\text{-(OH)}_2\text{D}_3$  which include increases in cellular  $\text{Ca}^{2+}$ ,  $\text{IP}_3$  levels, and pH appear to be the result of interaction with a "receptor" located on the plasma (26) and nuclear membranes. The precise mechanism by which the rapid actions modulate the hormone-induced changes in gene transcription is unclear. Inhibition of the rapid actions of  $1\alpha,25\text{-(OH)}_2\text{D}_3$  prevents vitamin D-induced gene transcription (22, 24) without altering interaction of the hormone-VDR complex with the VDRE (22). Thus, the second messengers activated by binding to the membrane receptor may modify protein DNA interactions that are necessary to support transcription, or may alter the interaction of DNA regulatory elements and the nuclear matrix.

- DeLuca HF, Schnoes HK. Metabolism in mechanism of action of vitamin  $\text{D}_3$ . *Annu Rev Biochem* **45**:631–666, 1976.
- Minghetti PP, Norman AW.  $1\alpha,25\text{-(OH)}_2\text{D}_3$  Vitamin  $\text{D}_3$  receptors: Gene regulation and genetic circuitry. *FASEB J* **2**:3043–3053, 1988.
- Baran DT, Milne ML.  $1,25\text{-Dihydroxyvitamin D}_3$  increase hepatocyte cytosolic calcium levels. *J Clin Invest* **77**:1622–1626, 1986.
- Walters MR. Newly identified actions of the vitamin D endocrine system. *Endocr Rev* **13**:719–764, 1992.
- Duval D, Durant S, Homo-DeLarche F. Nongenomic effects of steroids: Interactions of steroid molecules with membrane structures and functions. *Biochim Biophys Acta* **737**:409–442, 1973.
- Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* **240**:889–895, 1988.
- Barsony J, Pike JW, DeLuca HF, Marx SJ. Immunocytology with microwave fixed fibroblasts show  $1\alpha,25\text{-dihydroxyvitamin D}_3$ -dependent rapid and estrogen-dependent slow reorganization of vitamin D receptors. *J Cell Biol* **111**:28355–28395, 1990.
- Barsony J, McKoy W. Molybdate increases intracellular 3',5'-guanosine cyclic monophosphate and stabilizes vitamin D receptor association with tubulin-containing filaments. *J Biol Chem* **267**:24,457–24,465, 1992.
- Brown TA, DeLuca HF. Phosphorylation of the  $1\alpha,25\text{-dihydroxyvitamin D}_3$  receptor: A primary event in  $1\alpha,25\text{-dihydroxyvitamin D}_3$  action. *J Biol Chem* **265**:10025–10029, 1990.
- Pike JW. Intracellular receptors mediate the biologic action of  $1\alpha,25\text{-dihydroxyvitamin D}_3$ . *Nutr Rev* **43**:161–168, 1985.
- Kim YS, Birge SJ, Avioli LV, Miller R. Early manifestations of vitamin D effects in rat osteogenic sarcoma cells. *Calcif Tissue Int* **41**:223–227, 1987.
- Lieberherr M. Effects of vitamin  $\text{D}_3$  metabolites on cytosolic free calcium in confluent mouse osteoblasts. *J Biol Chem* **262**:13168–13173, 1987.
- Oshima J, Watanabe M, Hirosumi J, Orimo H.  $1\alpha,25\text{-(OH)}_2\text{D}_3$  increases cytosolic  $\text{Ca}^{++}$  concentration of osteoblastic cells, clone MC3T3-E1. *Biochem Biophys Res Commun* **145**:956–960, 1987.
- Caffrey JM, Farach-Carson MC. Vitamin  $\text{D}_3$  metabolites modulate dihydropyridine-sensitive calcium currents in clonal rat osteosarcoma cells. *J Biol Chem* **264**:20265–20274, 1989.
- Civitelli R, Kim YS, Gunsten SL, Fugimori A, Huskey M, Avioli LV, Hruska KA. Nongenomic activation of the calcium message system by vitamin D metabolites in osteoblast-like cells. *Endocrinology* **127**:2253–2262, 1990.
- Sorensen AM, Baran DT.  $1\alpha,25\text{-dihydroxyvitamin D}_3$  rapidly alters phospholipid metabolism in the nuclear envelope of osteoblasts. *J Bone Miner Res* **8**:S127, 1993.
- Grosse B, Bourdeau A, Lieberherr M. Oscillations in inositol

- 1,4,5,-triphosphate and diacylglycerol induced by vitamin D<sub>3</sub> metabolites in confluent mouse osteoblasts. *J Bone Mineral Res* 8:1059–1069, 1993.
18. Baran DT, Sorensen AM, Shalhoub V, Owen T, Oberdorf A, Stein G, Lian J. 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> rapidly increases cytosolic calcium in clonal rat osteosarcoma cells lacking the vitamin D receptor. *J Bone Mineral Res* 6:1269–1275, 1991.
  19. Holick SA, Holick MF, MacLaughlin JA. Chemical synthesis of [ $1\beta$ -<sup>3</sup>H] 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and [ $1\alpha$ -<sup>3</sup>H] 1 $\beta$ ,25-dihydroxyvitamin D<sub>3</sub>: Biological activity of 1 $\beta$ ,25-dihydroxyvitamin D<sub>3</sub>. *Biochem Biophys Res Commun* 97:1031–1037, 1980.
  20. Farach-Carson MC, Sergeev I, Norman AW. Nongenomic actions of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> in rat osteosarcoma cells: Structure-function studies using ligand analogs. *Endocrinology* 129:1876–1884, 1991.
  21. Norman AW, Okamura WH, Farach-Carson MC, Allewaert K, Branisteanu D, Nemers I, Muralidharan KR, Bouillon R. Structure function studies of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and the vitamin D endocrine system. *J Biol Chem* 268:13811–13819, 1993.
  22. Baran DR, Sorensen AM, Shalhoub V, Owen T, Stein GS, Lian JB. The rapid nongenomic actions of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> modulate the hormone-induced increments in osteocalcin gene transcription in osteoblast-like cells. *J Cell Biochem* 50:124–129, 1992.
  23. Sorensen AM, Bowman D, Baran DT. 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> rapidly increases nuclear calcium levels in rat osteosarcoma cell. *J Cell Biochem* 52:237–242, 1993.
  24. Jenis LG, Lian JB, Stein GS, Baran DT. 1 $\alpha$ ,25-Dihydroxyvitamin D<sub>3</sub>-induced changes in intracellular pH in osteoblast-like cells modulate gene expression. *J Cell Biochem* 53:234–239, 1993.
  25. Norman AW, Bouillon R, Farach-Carson MC, Bishop JE, Zhou L-X, Nemere I, Zhao J, Muralidharan KR, Okamura WH. Demonstration that 1 $\beta$ ,25-dihydroxyvitamin D<sub>3</sub> is an antagonist of the nongenomic but not genomic biological responses and biological profile of the three A-ring diastereomers of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>. *J Biol Chem* 268:20022–20030, 1993.
  26. Baran DT, Ray R, Sorensen AM, Honeyman T, Holick MF. Binding characteristics of a membrane receptor that recognizes 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and its epimer. 1 $\beta$ ,25-dihydroxyvitamin D<sub>3</sub>. *J Cell Biochem* (in press).