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Vitamin D Target Proteins: Function and Regulation

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Abstract Recent findings have indicated that calbindin-D_{28k}, the first known target of vitamin D action, is present in osteoblasts and protects against TNF and glucocorticoid induced apoptosis of osteoblastic cells. Cytokine mediated destruction of pancreatic β cells, a cause of insulin dependent diabetes, is also inhibited by calbindin-D_{28k}. In calbindin-D_{28k} transfected pancreatic β cells free radical formation by cytokines is inhibited by calbindin. Thus, besides its role as a facilitator of calcium diffusion, calbindin has a major role in protecting against cellular degeneration in different cell types. Besides calbindin, the other known pronounced effect of $1,25(OH)_2D_3$ in intestine and kidney is increased synthesis of $25(OH)D_3$ 24-hydroxylase (24(OH)ase) which is involved in the catabolism of $1,25(OH)_2D_3$. We have noted that CCAAT enhancer binding protein β (C/EBPβ) is induced by $1,25(OH)_2D_3$ in kidney and osteoblastic cells and can enhance the transcriptional response of 24(OH)ase to $1,25(OH)_2D_3$. These studies establish C/EBPβ as a novel $1,25(OH)_2D_3$ target gene and indicate a role for C/EBPβ in 24(OH)ase transcription. These studies extend our previous studies related to factors that affect vitamin D receptor (VDR) mediated 24(OH)ase transcription (YY1, TFIIB, CBP) and the effect of signaling pathways on 24(OH)ase transcription and cofactor recruitment. J. Cell. Biochem. 88: 238–244, 2003. © 2002 Wiley-Liss, Inc.

Key words: calbindin; apoptosis; $25(OH)D_3$ 24-hydroxylase; vitamin D receptor; transcription; phosphorylation

1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), similar to other steroid hormones, is known to act by binding stereospecifically to the vitamin D receptor (VDR). The liganded VDR heterodimerizes with RXR and then interacts with the vitamin D response element in the promoter of target genes [Darwish and DeLuca, 1993; DeLuca and Zierold, 1998]. The mechanisms involved in VDR mediated transcription following the binding of the VDR–RXR heterodimer to DNA are only now beginning to be defined [Rachez and Freedman, 2000]. TFIIB, several TAFs as well as proteins known as p160 coactivators (SRC-1/NcoA1, GRIP-1/TIF-2, and ACTR/pCIP) which have histone acetylase activity and the coactivator complex DRIP (VDR interacting proteins) which acts through the recruitment of RNA polymerase, have recently been reported to be involved in VDR mediated transcriptional activity. Thus, in recent years studies related to the identification of coactivators of VDR mediated transcription have been a major focus of vitamin D research. However, in order to obtain a more complete understanding of vitamin D action, studies related to the physiological significance of target proteins in different systems are also of importance and need to be a major focus. This article focuses on research from our laboratory which combines studies related to the functional significance of major targets of $1,25(OH)_2D_3$ action with studies concerning the basic molecular mechanism of $1,25(OH)_2D_3$ action in order to provide new insight into the role of $1,25(OH)_2D_3$ in tissues involved in maintaining calcium homeostasis as well as in "nonclassical" $1,25(OH)_2D_3$ target tissues.

MAJOR TARGETS OF $1,25(OH)_2D_3$

Calbindin

Increased synthesis of the calcium binding protein calbindin in two major target tissues involved in maintaining calcium homeostasis, intestine and kidney, is one of the most pronounced effects of $1,25(OH)_2D_3$ known. The two major subclasses of calbindin have previously

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been described: calbindin-D_{9k} (a 9,000 M_r protein present in mammalian intestine and in mouse kidney) and calbindin-D_{28k} (a 28,000 M_r protein present in avian intestine, mammalian and avian kidney, mammalian and avian pancreas, and mammalian to molluskan brain) [Christakos et al., 1997]. Studies related to the distribution of calbindin and its colocalization with the VDR have resulted in key advances in our understanding of the diversity of the vitamin D endocrine system (e.g., calbindin and VDR are colocalized not only in the absorptive cells of the intestine, in the distal tubule of the kidney and in osteoblasts but also in β cells of the pancreas).

In previous studies, it had been proposed that a major role for calbindin in the intestine and kidney is to ferry calcium across the cell to the basolateral membrane where calcium is actively transported out of the cell via the plasma membrane calcium pump. In recent studies using pancreatic islets from calbindin-D_{28k} null/mutant mice and wild type mice as well as β cell lines overexpressing calbindin, we found the first clear evidence for a role of calbindin-D_{28k} in the modulation of depolarization-stimulated insulin release and that calbindin controls insulin release by regulation of intracellular calcium [Sooy et al., 1999]. Besides its role as a facilitator of calcium diffusion and as a modulator of insulin release, we found that calbindin-D_{28k} has a major role in different cell types in protecting against apoptotic cell death. Calcium is thought to play a critical role in apoptotic cell death [Khan et al., 1996; Guo et al., 1997; Christakos et al., 2000] (Fig. 1). Calcium influx, abnormal endoplasmic reticulum (ER) calcium regulation, and the release of calcium from ER stores have been linked to apoptosis. Sustained elevations in intracellular calcium result in damage to the mitochondria. Calcium dependent proteases and calcium activated endonuclease are also involved in apoptosis. Consistent with this, calbindin-D_{28k}, which buffers calcium, can block apoptosis induced by different proapoptotic pathways. For example, we found that overexpression of calbindin-D_{28k} in neural cells which express mutant presenilin-1 (PS-1), which is causally linked to familial Alzheimer's disease, attenuates the increase in intracellular calcium and prevents the impairment of mitochondrial function observed in the presence of PS-1 and in the absence of calbindin [Guo et al., 1998]. Thus, one mechanism whereby calbindin

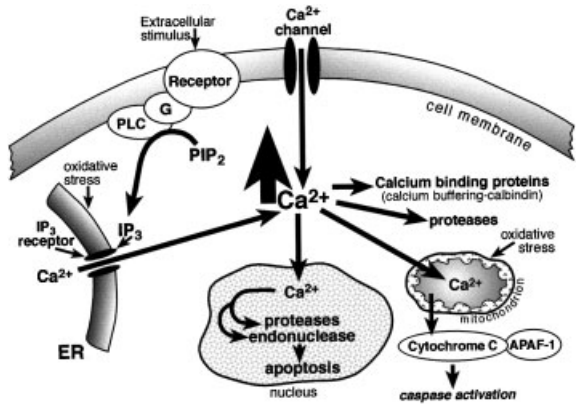

Fig. 1. Activation of programmed cell death by calcium. A major role for calbindin-D_{28k}, besides acting as a facilitator of calcium diffusion, is to protect against apoptotic cell death in different cell types. Recent studies from our lab indicate that calbindin protects against cell death by different mechanisms that include calbindin's ability to buffer calcium. (Abbreviations: ER, endoplasmic reticulum; PLC, phospholipase C; IP_3, inositol 1,4,5 triphosphate; PIP_2, phosphatidylinositol 4,5 bisphosphate; APAF-1, apoptosis protease activating factor). Reprinted with permission from Christakos et al. [2000].

may protect against apoptotic cell death would be by preventing calcium mediated mitochondrial damage and subsequent release of cytochrome c, which has been suggested to be an apoptotic trigger. In collaboration with Dr. Robert Nissenson, we have shown that calbindin, by buffering intracellular calcium, was able to protect HEK 293 kidney cells from parathyroid hormone (PTH) induced apoptosis that was mediated by a phospholipase C dependent increase in intracellular calcium [Turner et al., 2000]. In more recent studies, we have shown that cytokine mediated destruction of pancreatic β cells, a cause of insulin dependent diabetes, can be inhibited by calbindin-D_{28k} [Rabinovich et al., 2001]. Studies were done transfecting the pancreatic β cell lines βTC-3, βHC-13, and RIN-m5F cells with the calbindin-D_{28k} gene. In the calbindin transfected cells, the stimulatory effect of cytokines on lipid hydroperoxide, nitric oxide, and peroxynitrite production were significantly decreased. Thus calbindin-D_{28k}, by inhibiting free radical formation, can protect pancreatic islet β-cells from autoimmune destruction in type 1 diabetes. In addition, in recent studies, we found that calbindin can also protect against apoptosis of osteoblastic cells [Bellido et al., 2000]. Stable transfection of calbindin in osteoblastic MC3T3-E1 cells was found to block tumor necrosis factor (TNF) induced

apoptosis as determined by cell viability and nuclear morphology. Our findings indicate that calbindin is capable of binding to caspase 3, a key mediator of apoptosis, and inhibiting its activity and that this inhibition results in an inhibition of TNFα induced apoptosis in MC3T3-E1 osteoblastic cells. This study demonstrated that although part of the antiapoptotic properties of calbindin may result from its ability to buffer calcium, other mechanisms, such as inhibition of caspase activity, may also play a significant role in the prevention of apoptosis by calbindin. In addition to protection against TNF induced apoptosis, we have found (in collaboration with T. Bellido, University of Arkansas) that calbindin can also protect against glucocorticoid induced bone cell apoptosis (Fig. 2). A further understanding of the mechanisms involved will have important implications for the therapeutic intervention of glucocorticoid induced osteoporosis.

In summary, although calbindin was first thought to act in the cell only as a facilitator of calcium diffusion, it is now clear that a major role for this protein is to protect against cellular destruction. A further understanding of the mechanisms whereby calbindin-D_{28k} attenuates apoptosis will have important implications for the prevention of degeneration in bone cells as well as other cells (including neurons and pancreatic β cells) and, therefore, could prove important for the therapeutic intervention of many diseases including osteoporosis.

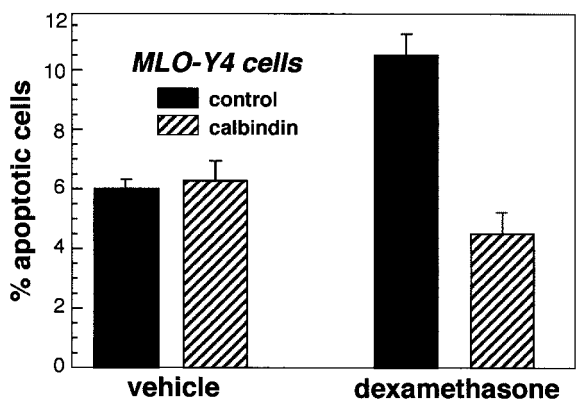

Fig. 2. Calbindin-D_{28k} protects against dexamethasone induced cell death. Forty eight hours after transfection MLO-Y4 cells were treated with 10^{-6} M dexamethasone for 6 h and then assessed for apoptosis morphologically. The percent apoptotic cells were significantly different ($P < 0.05$) compared to vehicle only for the dexamethasone treated control cells.

25(OH)D_3 24-Hydroxylase (24(OH)ase)

Besides calbindin the other known pronounced effect of 1,25$(OH)_2D_3$ in intestine and kidney is increased synthesis of 24(OH)ase. Recent studies using 24(OH)ase knock out mice provided the first in vivo evidence for a role for 24(OH)ase in the catabolism of 1,25$(OH)_2D_3$ [St-Arnaud et al., 2000]. Acute administration of 1,25$(OH)_2D_3$ resulted in 20-fold greater levels of serum 1,25$(OH)_2D_3$ in the 24(OH)ase knock out mice compared to genotype controls. Chronic administration of 1,25$(OH)_2D_3$ also resulted in a marked elevation of serum 1,25$(OH)_2D_3$ as well as changes in kidney histology consistent with hypervitaminosis D in the knock out mice. Thus by inducing 24(OH)ase, 1,25$(OH)_2D_3$ self induces its deactivation. The cloning of the rat and human 24(OH)ase cDNA [Ohyama et al., 1991; Chen et al., 1993], the availability of the 24(OH)ase promoter [Chen and DeLuca, 1995; Kerry et al., 1996] as well as the development of the knock out mice made possible, for the first time, studies by us and others that have resulted in a better understanding of this other major target of 1,25$(OH)_2D_3$ action. Recent findings from Dr. DeLuca's lab indicated that although PTH inhibits renal 24(OH)ase mRNA, the suppression by PTH is not mediated by effects of PTH on the rat 24(OH)ase promoter. PTH was found to inhibit renal 24(OH)ase mRNA by altering its stability [Zierold et al., 2001]. The regulation of 24(OH)ase is cell type specific since PTH enhances 24(OH)ase activity, mRNA and transcription in osteoblastic cells [Armbrecht et al., 1998; Yang et al., 2001]. It has been suggested that the physiological significance of the enhancement by PTH of 24(OH)ase in osteoblastic cells may be to prevent increased intracellular 1,25$(OH)_2D_3$ levels that may adversely affect mineralization. We have found that this enhancement is due, at least in part, to upregulation of VDR [Yang et al., 2001]. cAMP early repressor (ICER), which serves as a dominant negative regulator of cAMP induced transcription in other endocrine systems, was found to be induced in osteoblastic cells by cAMP and PTH and to inhibit PTH enhancement of 1,25$(OH)_2D_3$ induced 24(OH)ase transcription [Huening et al., 2002]. Our data indicate that the mechanism of the inhibitory effect of ICER involves an inhibition of protein kinase A (PKA) induced VDR transcription and this inhibition may be mediated in part by binding of ICER to a

CRE-like sequence in the VDR promoter. These findings provide evidence for the first time that ICER has a key regulatory role in the PKA enhancement of VDR transcription and, therefore, in the cross-talk between the PKA signaling pathway and the vitamin D endocrine system. In addition, we have identified YY1 as a transcription factor involved in the repression of $1,25(OH)_2D_3$ induced 24(OH)ase transcription [Raval-Pandya et al., 2001]. Thus, YY1 may prevent activation at times that do not require 24(OH)ase to be expressed. Our results suggest that YY1 represses 24(OH)ase transcription, at least in part, by sequestering activator proteins involved in VDR mediated transcription. In addition, we have demonstrated a role for CBP in the relief of repression of 24(OH)ase transcription [Raval-Pandya et al., 2001]. Finally, we have found that phosphatase inhibitors can result in an enhancement of $1,25(OH)_2D_3$ induced 24(OH)ase transcription and that this enhancement may be due, in part, to an increased interaction between the VDR and the coactivator protein DRIP205 (VDR interacting protein) [Barletta et al., 2002] (Fig. 3). Thus we are now beginning, for the first time, to understand the mechanisms involved in the transcriptional response of the 24(OH)ase gene to $1,25(OH)_2D_3$ and the mechanisms by which $1,25(OH)_2D_3$ and PTH and other factors coordinately regulate 24(OH)ase expression and thus affect $1,25(OH)_2D_3$ metabolism and the maintenance of calcium homeostasis.

Recently Identified Targets of $1,25(OH)_2D_3$ Action

CCAAT enhancer binding protein β (C/EBP β). Using gene chip array, we found that a gene in addition to calbindin and 24(OH)ase activated by a factor greater than 50% in mouse kidney is CCAAT-enhancer binding protein β (C/EBPβ). Our findings were verified by Northern and Western blot analyses and induction of C/EBPβ by $1,25(OH)_2D_3$ was observed in primary murine osteoblasts, in UMR osteoblastic cells as well as in mouse kidney (Fig. 4A–C). The C/EBPs are a family of transcription factors that regulate genes of the acute phase response as well as genes involved in cell growth, differentiation, and cell type specific genes [Lekstrom-Himes and Xanthopoulos, 1998]. Previous studies indicated that C/EBP family members are expressed in kidney and osteoblasts and are involved in the regulation of osteocalcin expression in osteoblasts [Zador et al., 1998; McCarthy et al., 2000; Gutierrez et al., 2002]. Results of Northern analysis indicated that the first significant induction of C/EBPβ mRNA by $1,25(OH)_2D_3$ in UMR osteoblastic cells precedes the induction of 24(OH)ase mRNA (3 h compared to 6 h), consistent with a possible role of C/EBPβ as a mediator of the $1,25(OH)_2D_3$ response of the 24(OH)ase gene. Since two putative C/EBPβ sites were noted in the 24(OH)ase promoter, this possibility was tested in experiments in which COS-7 cells were transfected with the rat 24(OH)ase promoter (−1,367/+74) and the human VDR in the presence or absence of C/EBPβ. In dose–response studies, we found that suboptimal $1,25(OH)_2D_3$ induction of 24(OH)ase transcription could be enhanced a maximum of 20-fold by C/EBPβ (Fig. 4D). These findings establish C/EBPβ as a novel $1,25(OH)_2D_3$ target gene and indicate that C/EBPβ is a major transcriptional activator of the 24(OH)ase gene. These findings also establish for the first time cooperative effects and cross talk between the C/EBPβ and VDR in $1,25(OH)_2D_3$ induced transcription.

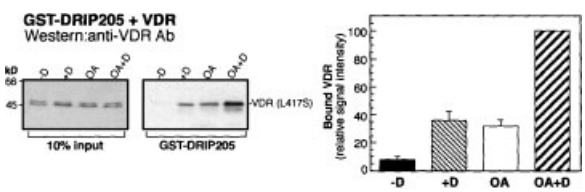

Fig. 3. Phosphorylation mediates the enhancement of VDR–DRIP205 interaction. GST pull-down assays were performed using GST–DRIP205 (527–970) fusion protein and equal amounts of VDR protein from hVDR transfected COS-7 cells treated with $1,25(OH)_2D_3$ (+D), okadaic acid (OA; 50 nM), or $1,25(OH)_2D_3$+OA (OA+D) for 24 h. Western blot analysis was performed with a monoclonal anti-VDR antibody to visualize VDR binding.

Epithelial calcium channels (ECaCs). Only recently have apical calcium channels in $1,25(OH)_2D_3$ responsive epithelia (distal nephron and proximal intestine; colocalized in the same cells as calbindin) been identified, suggesting a mechanism for calcium entry. The ECaC was first identified in rabbits [Hoenderop et al., 1999] and then identified in rats as CaT2 (also known as ECaC1) and CaT1 for the channel in rat kidney and duodenum, respectively [Peng et al., 1999, 2000]. These channels are distinct from voltage-gated calcium channels. Recent evidence indicated that *ECaC* gene expression in intestine and kidney is induced by $1,25(OH)_2D_3$ and low dietary calcium

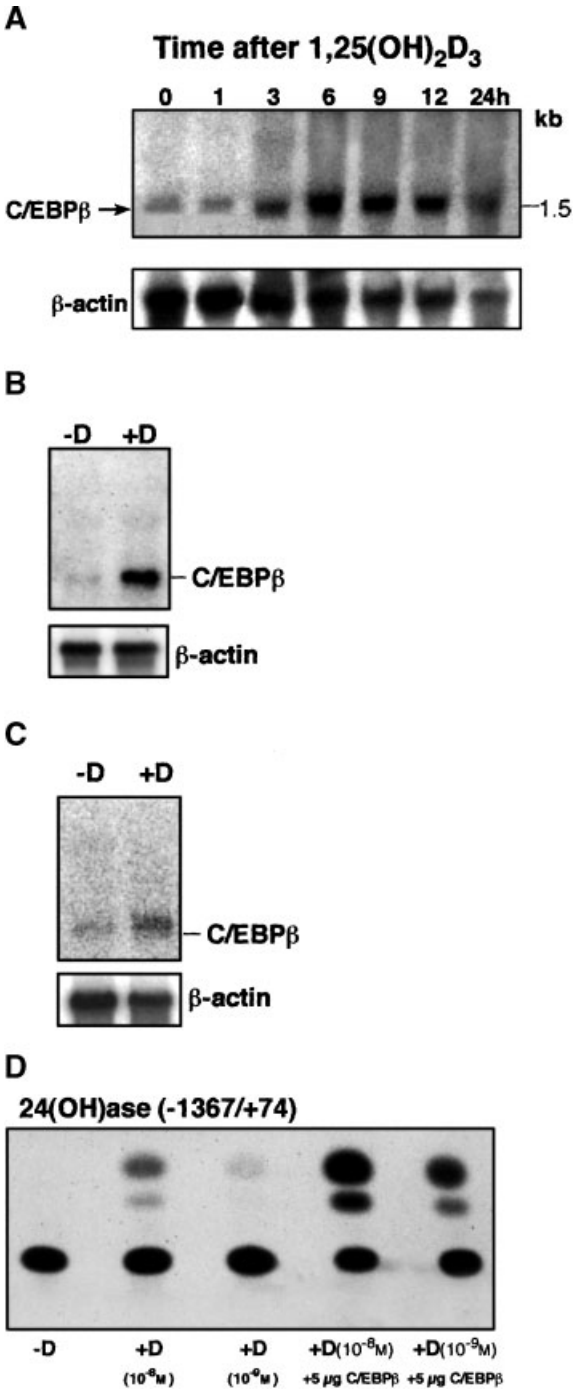

Fig. 4. C/EBP β is induced by $1,25(OH)_2D_3$ and enhances VDR mediated 24(OH)ase transcription. C/EBP β is induced by $1,25(OH)_2D_3$ (Northern blot analysis of mRNA from $1,25(OH)_2D_3$ treated UMR cells (10^{-8} M; **A**), primary murine osteoblasts (10^{-8} M, 9 h; **B**), and the kidney of $1,25(OH)_2D_3$ injected vitamin D deficient mice (**C**). **D**: C/EBP β enhances VDR mediated 24(OH)ase transcription. COS cells were cotransfected with hVDR and C/EBP β and treated with $1,25(OH)_2D_3$ for 24 h.

[Hoenderop et al., 2001; Van Cromphaut et al., 2001; Christakos and Hediger, preliminary studies]. These findings include studies in VDR knock out mice that suggest that the expression of the ECaC in intestine is strongly vitamin D dependent [Van Cromphaut et al., 2001]. Whether the ECaCs are regulated by $1,25(OH)_2D_3$ at the transcriptional or post-transcriptional level and the mechanism involved in the modulation by calcium of *ECaC* gene expression remains to be determined. In addition, an understanding of the relationship of calbindin to the ECaC in the distal nephron and in the absorptive cells in the intestine should result in new insight in our understanding of distal tubular calcium entry and the process of vitamin D dependent intestinal calcium absorption.

IN SUMMARY

In addition to its role as a facilitator of calcium diffusion, the calcium binding protein calbindin has a major role in protecting against cellular degeneration in different cell types. We found that the mechanisms involved include prevention of impaired mitochondrial function, inhibition of caspase 3 activity, and inhibition of free radical formation. A further understanding of the mechanisms whereby calbindin protects against apoptotic cell death will have important implications for the prevention of cellular degeneration. We have combined studies related to the functional significance of target proteins such as calbindin with studies related to the mechanisms involved in regulation of target genes by $1,25(OH)_2D_3$. We have demonstrated a role for YY1 in the repression of VDR mediated 24(OH)ase transcription and relief of this repression by CBP. In addition, we found that C/EBPβ and phosphatase inhibitors can result in an enhancement of $1,25(OH)_2D_3$ induced 24(OH)ase transcription. The modulation of $1,25(OH)_2D_3$ action by phosphorylation is due in part to differential preference of VDR for coactivator proteins. The identification of new target proteins as well as an increased understanding of the functional significance of target proteins combined with an increased understanding of the molecular mechanisms involved in VDR mediated transcription will be important in order to elucidate the mechanisms by which $1,25(OH)_2D_3$ mediates its multiple biological effects.

REFERENCES

Armbrecht HJ, Hodam TL, Boltz MA, Partridge NC, Brown AJ, Kumar VB. 1998. Induction of the vitamin D 24hydroxylase (CYP24) by 1,25-dihydroxyvitamin D_3 is regulated by parathyroid hormone in UMR106 osteoblastic cells. Endocrinology 139:3375–3381.

Barletta F, Freedman LP, Christakos S. 2002. Enhancement of VDR-mediated transcription by phosphorylation: Correlation with increased interaction between the VDR and DRIP205, a subunit of the VDR-interacting protein coactivator complex. Mol Endocrinol 16:301–314.

Bellido T, Huening M, Raval-Pandya M, Manolagas SC, Christakos S. 2000. Calbindin-D28k is expressed in osteoblastic cells and suppresses their apoptosis by inhibiting caspase-3 activity. J Biol Chem 275:26328–26332.

Chen KS, DeLuca HF. 1995. Cloning of the human 1 alpha, 25-hydroxyvitamin D-3 24-hydroxylase gene promoter and identification of two vitamin D-responsive elements. Biochim Biophys Acta 1263:1–9.

Chen KS, Prahl JM, DeLuca HF. 1993. Isolation and expression of human 1,25-dihydroxyvitamin D_3 24hydroxylase cDNA. Proc Natl Acad Sci USA 90:4543–4547.

Christakos S, Beck J, Hyllner SJ. 1997. Calbindin-D_{28k}. In: Feldman D, Glorieux F, Pike JW, editors. Vitamin D. San Diego: Academic Press. pp 209–221.

Christakos S, Barletta F, Huening M, Kohut J, Raval-Pandya M. 2000. Activation of programmed cell death by calcium: Protection against cell death by the calcium binding protein, calbindin-D_{28k}. In: Pochet R, Donato R, Haiech J, Heizmann C, Gerke V, editors. Calcium: The molecular basis of calcium action in biology and medicine. The Netherlands: Kluwer Academic Publishers. pp 259–275.

Darwish H, DeLuca HF. 1993. Vitamin D-regulated gene expression. Crit Rev Eukaryot Gene Expr 3:89– 116.

DeLuca HF, Zierold C. 1998. Mechanisms and functions of vitamin D. Nutr Rev 56:S4–S10.

Guo Q, Sopher BL, Furukawa K, Pham DG, Robinson N, Martin GM, Mattson MP. 1997. Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: Involvement of calcium and oxyradicals. J Neurosci 17:4212–4222.

Guo Q, Christakos S, Robinson N, Mattson MP. 1998. Calbindin-D28k blocks the proapoptotic actions of mutant presenilin1: Reduced oxidative stress and preserved mitochondrial function. Proc Natl Acad Sci USA 95: 3227–3232.

Gutierrez S, Javed A, Tennant DK, van Rees M, Montecino M, Stein GS, Stein JL, Lian JB. 2002. CCAT/enhancer-binding proteins beta and delta activate osteocalcin gene transcription and synergize with Runx2 at the C/EBP element to regulate bone-specific expression. J Biol Chem 277:1316–1323.

Hoenderop JGJ, van der Kemp AW, Hartog A, van de Graaf SF, van Os CH, Willems PH, Bindels RJ. 1999. Molecular identification of the apical Ca^{2+} channel in 1,25-dihydroxyvitamin D3-responsive epithelia. J Biol Chem 274: 8375–8378.

Hoenderop JG, Muller D, van der Kemp AW, Hartog A, Suzuki M, Ishibashi K, Imai M, Sweep F, Willems PH, van Os CH, Bindels RJ. 2001. Calcitriol controls the epithelial calcium channel in kidney. J Am Soc Nephrol 12:1342–1349.

Huening M, Yehia G, Molina CA, Christakos S. 2002. Evidence for a regulatory role of inducible cAMP early repressor in protein kinase A mediated enhancement of vitamin D receptor expression and modulation of hormone action. Mol Endocrinol 16:2052–2064.

Kerry DM, Dwivedi PP, Hahn CN, Morris HA, Omdahl JL, May BK. 1996. Transcriptional synergism between vitamin D responsive elements in the rat 25hydroxyvitamin D3 24hydroxylase (CYP24) promoter. J Biol Chem 271:29715–29721.

Khan AA, Soloski MJ, Sharp AH, Schilling G, Sabatini DM, Li SH, Ross CA, Snyder SH. 1996. Lymphocyte apoptosis: Mediation by increased type 3 inositol 1,4,5-triphosphate receptor. Science 273:503–507.

Lekstrom-Himes J, Xanthopoulos KG. 1998. Biological role of CCAAT enhancer binding protein family of transcription factors. J Biol Chem 273:28545–28548.

McCarthy TL, Ji C, Chen Y, Kim KK, Imagama M, Ito Y, Centrella M. 2000. Runt domain factor (Runx)-dependent effects on CCAATT enhancer-binding protein delta expression and activity in osteoblasts. J Biol Chem 275: 21746–21753.

Ohyama Y, Noshiro M, Okuda K. 1991. Cloning and expression of cDNA encoding 25hydroxyvitamin D_3 24 hydroxylase. FEBS Lett 278:195–198.

Peng JB, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, Brown EM, Hediger MA. 1999. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. J Biol Chem 274:22739–22746.

Peng JB, Chen XZ, Berger UV, Vassilev PM, Brown EM, Hediger MA. 2000. A rat kidney-specific calcium transporter in the distal nephron. J Biol Chem 275:28186–28194.

Rabinovich A, Suarez-Pinzon W, Sooy K, Strynadka K, Christakos S. 2001. Expression of calbindin-D_{28k} in a pancreatic islet β-cell line protects against cytokine-induced apoptosis and necrosis. Endocrinology 142: 3649–3655.

Rachez C, Freedman LP. 2000. Mechanisms of gene regulation by the vitamin D_3 receptor: A network of coactivator interactions. Gene 246:9–21.

Raval-Pandya M, Dhawan P, Barletta F, Christakos S. 2001. YY1 represses vitamin D receptor-mediated 25-hydroxyvitamin D_3 24-hydroxylase transcription: Relief of repression by CREB-binding protein. Mol Endocrinol 15:1035–1046.

Sooy K, Schermerhorn T, Noda M, Surana M, Rhoten WB, Meyer M, Fleischer N, Sharp GWG, Christakos S. 1999. Calbindin-D_{28k} controls $[Ca^{2+}]_i$ and insulin release: Evidence obtained from calbindin-D_{28k} knockout mice and β cell lines. J Biol Chem 274:34343–34349.

St-Arnaud R, Arabian A, Travers R, Barletta F, Raval-Pandya M, Chapin K, Depovere J, Mathieu C, Christakos S, Demay MB, Glorieux FH. 2000. Deficient mineralization of intramembranous bone in vitamin D-24-hydroxylase ablated mice is due to elevated 1,25dihydroxyvitamin D and not to the absence of 24,25-dihydroxyvitamin D. Endocrinology 141:2658–2666.

Turner PR, Mefford S, Christakos S, Nissenson RA. 2000. Apoptosis mediated by activation of the G protein–

coupled receptor for PTH/PTHrP. Mol Endocrinol 14: 241–254.

Van Cromphaut SJ, Dewerchin M, Hoenderop JG, Stockmans I, Van Herck E, Kato S, Bindels RJ, Collen D, Carmeliet P, Bouillon R, Carmeliet G. 2001. Duodenal calcium absorption in vitamin D receptor knockout mice: Functional and molecular aspects. Proc Natl Acad Sci USA 98:13324–13329.

Yang W, Hyllner SJ, Christakos S. 2001. Interrelationship between signal transduction pathways and $1,25(OH)_2D_3$ in UMR106 osteoblastic cells. Am J Physiol Endocrinol Metab 281:E162–E170.

Zador IZ, Hsieh CC, Papaconstantinou J. 1998. Renal CCAAT/enhancer-binding proteins in experimental diabetes mellitus. Nephron 79:312–316.

Zierold C, Mings JA, DeLuca HF. 2001. Parathyroid hormone regulates 25-hydroxyvitamin D_3-24-hydroxylase mRNA by altering its stability. Proc Natl Acad Sci USA 98:13572–13576.