



## Mechanisms involved in vitamin D mediated intestinal calcium absorption and in non-classical actions of vitamin D<sup>☆</sup>

Sylvia Christakos<sup>\*</sup>, Puneet Dhawan, Dare Ajibade, Bryan S. Benn, Jingjing Feng, Sneha S. Joshi

Department of Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, 185 South Orange Ave., Newark, NJ 07103, United States

### ARTICLE INFO

#### Article history:

Received 23 October 2009

Received in revised form 12 February 2010

Accepted 1 March 2010

#### Keywords:

TRPV6

Calbindin

1,25-Dihydroxyvitamin D<sub>3</sub>

Cathelicidin

IL-17

### ABSTRACT

Recent studies in our laboratory using calbindin-D<sub>9k</sub> null mutant mice as well as mice lacking the 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) inducible epithelial calcium channel TRPV6 provide evidence for calbindin-D<sub>9k</sub> and TRPV6 independent regulation of active intestinal calcium absorption. These findings suggest that in the knock out (KO) mice there is compensation by another calcium channel or protein and that other novel factors are involved in 1,25(OH)<sub>2</sub>D<sub>3</sub> mediated active intestinal calcium absorption. In addition, 1,25(OH)<sub>2</sub>D<sub>3</sub> mediated paracellular transport of calcium may have contributed to the normalization of serum calcium in the null mutant mice. 1,25(OH)<sub>2</sub>D<sub>3</sub> downregulates cadherin-17 and upregulates claudin-2 and claudin-12 in the intestine, suggesting that 1,25(OH)<sub>2</sub>D<sub>3</sub>, by regulating these epithelial cell junction proteins, can route calcium through the paracellular path. With regard to non-classical actions, 1,25(OH)<sub>2</sub>D<sub>3</sub> has been reported to inhibit the proliferation of a number of malignant cells and to regulate adaptive as well as innate immunity. This article will review new developments related to the function and regulation of vitamin D target proteins in classical and non-classical vitamin D target tissues that have provided novel insight into mechanisms of vitamin D action.

© 2010 Elsevier Ltd. All rights reserved.

### 1. Introduction

Studies in vitamin D receptor (VDR) knockout (KO) mice have indicated that the major role of 1,25(OH)<sub>2</sub>D<sub>3</sub> is intestinal calcium transport [1,2]. However, the exact mechanisms involved in 1,25(OH)<sub>2</sub>D<sub>3</sub> stimulation of intestinal calcium absorption remain to be defined. It has been proposed that the process of transcellular calcium transport involves apical entry of calcium via the apical calcium channel, transient receptor potential vanilloid type 6 (TRPV6), translocation of calcium through the interior of the enterocyte (it has been suggested that the calcium binding protein, calbindin-D<sub>9k</sub> acts to facilitate calcium diffusion) and basolateral extrusion of calcium by the intestinal plasma membrane pump PMCA 1b [3]. Previous studies provided indirect evidence for a role of calbindin-D<sub>9k</sub> and TRPV6 in intestinal calcium absorption. Calbindin-D<sub>9k</sub> and TRPV6 are colocalized in the duodenum and jejunum and are similarly regulated [both are induced at weaning (the time of onset of active intestinal calcium transport), under conditions of low dietary calcium and after 1,25(OH)<sub>2</sub>D<sub>3</sub> injection] [4]. In addition, TRPV6 mRNA and calbindin-D<sub>9k</sub> are induced by 1,25(OH)<sub>2</sub>D<sub>3</sub> in the intestine

prior to the peak of intestinal calcium absorption [4]. However, the exact *in vivo* function of TRPV6 and calbindin-D<sub>9k</sub> has been a matter of debate. The generation of TRPV6 and calbindin-D<sub>9k</sub> KO mice made possible for the first time *in vivo* studies of the role of TRPV6 and calbindin-D<sub>9k</sub> in 1,25(OH)<sub>2</sub>D<sub>3</sub> regulated intestinal calcium absorption [5,6].

With regard to non-classical actions of 1,25(OH)<sub>2</sub>D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub> has been reported to inhibit the proliferation of a number of malignant cells including breast cancer cells and to have immunosuppressive effects. The molecular mechanisms of 1,25(OH)<sub>2</sub>D<sub>3</sub> action are only now beginning to be defined. This article focuses on research from our laboratory related to an understanding of the function of vitamin D target proteins and the mechanisms of 1,25(OH)<sub>2</sub>D<sub>3</sub> action in classical and non-classical vitamin D target tissues.

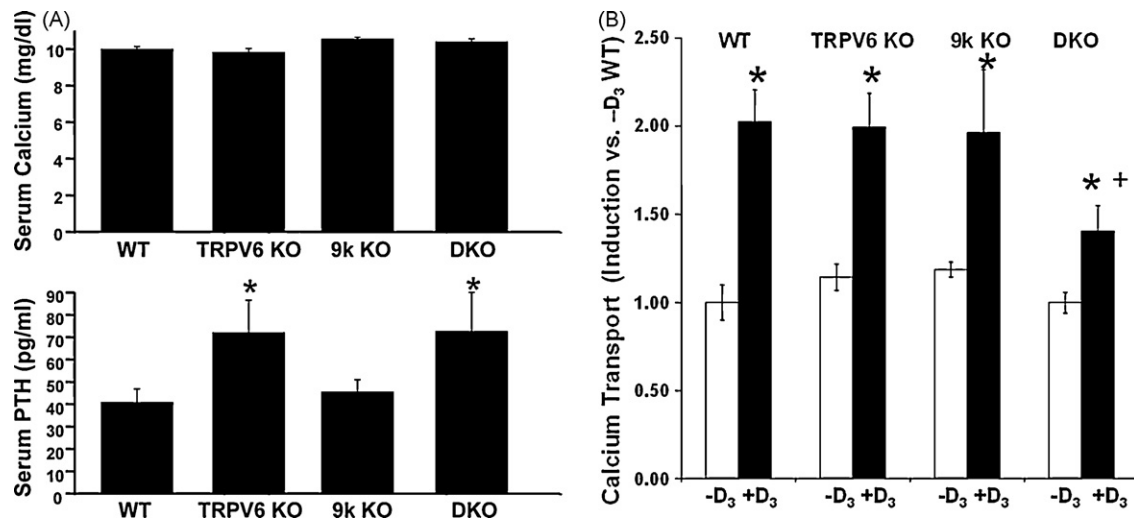
### 2. Materials and methods

TRPV6 KO mice and calbindin-D<sub>9k</sub> KO mice were generated as previously described by Bianco et al. [5] and Lee et al. [6], respectively. TRPV6/calbindin-D<sub>9k</sub> double KO (DKO) mice were generated in our laboratory by breeding TRPV6 KO females with calbindin-D<sub>9k</sub> KO male mice for generation of double heterozygote mice which were subsequently bred to obtain TRPV6/calbindin-D<sub>9k</sub> DKO mice. Intestinal calcium transport was determined by the everted gut sac

<sup>☆</sup> Special issue selected article from the 14th Vitamin D Workshop held at Brugge, Belgium on October 4–8, 2009.

<sup>\*</sup> Corresponding author. Tel.: +1 973 972 4033; fax: +1 973 972 5594.

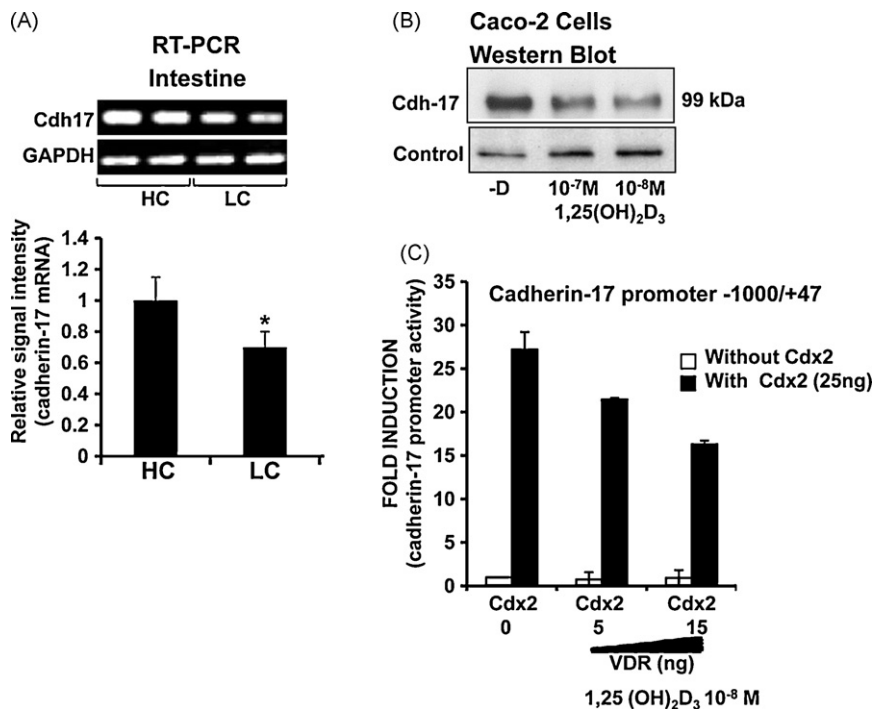
E-mail address: [christak@umdnj.edu](mailto:christak@umdnj.edu) (S. Christakos).



**Fig. 1.** Serum calcium and PTH and 1,25(OH)<sub>2</sub>D<sub>3</sub> stimulated duodenal calcium transport in WT and null mutant mice. (A) Each value for serum calcium or PTH represents the mean  $\pm$  SEM for male mice ( $n = 7$ – $23$  mice per group; \*,  $p < 0.05$  compared to WT). (B) Calcium transport was measured using everted intestinal sacs from the duodenum of 12-week-old mice made 1,25(OH)<sub>2</sub>D<sub>3</sub> deplete by feeding a 0.8% strontium diet for 7 days. Mice were then injected three times with 1,25(OH)<sub>2</sub>D<sub>3</sub> (+D) or vehicle (-D) 48, 24 and 6 h before termination (i.p., 100 ng/100 g body weight/injection). Values represent the mean  $\pm$  SEM ( $n = 6$ – $16$ /group; \*,  $p < 0.05$  for 1,25(OH)<sub>2</sub>D<sub>3</sub> treated (+D) compared with the respective deficient (-D) mice; +,  $p < 0.05$  compared with WT +D).

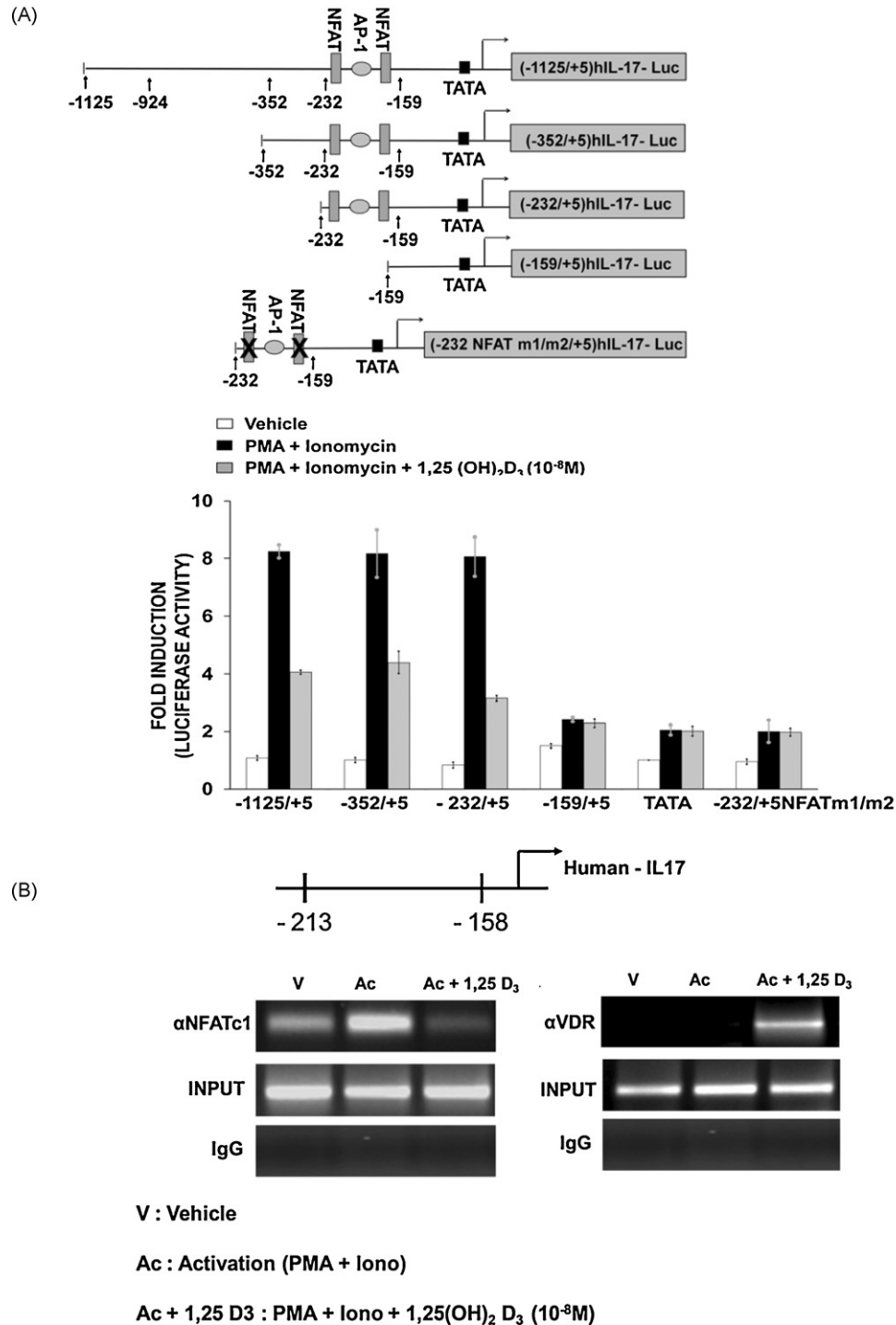
assay [7]. Serum concentrations of calcium were determined using Sigma diagnostic reagents. Serum intact PTH levels were measured using the two site immunoradiometric assay (Immunotopics, San Clemente, CA). For transcription assays using the IL-17 promoter, the Jurkat human T cells line (from ATCC) was used. The human IL-17 promoter (-1124/+5) and deletion constructs were obtained from Sarah Gaffen (U. of Pittsburgh). For transcription assays using

the cathelicidin promoter, A549 human lung epithelial cells were used. The human cathelicidin antimicrobial peptide gene promoter was obtained from Drs. P. Koefler and A. Gombert. For transcription assays using the human cadherin-17 promoter (-1000/+48 from Eric Fearon, U. of Michigan Medical School) COS-7 cells were used. Transcription assays were performed by standard protocols [8,9]. C/EBP $\alpha$  expression vector was a gift of Simon Williams (Texas



**Fig. 2.** Inhibition of cadherin-17 under low dietary calcium conditions and by 1,25(OH)<sub>2</sub>D<sub>3</sub>. (A) Two-month-old mice were fed a high calcium (HC, 1%) calcium or low (LC, 0.02%) calcium diet from 4 weeks of age. RNA was prepared from the duodenum, reverse transcribed and subjected to PCR with primers to amplify the appropriate DNA fragment of cadherin-17 (20). \* $p < 0.05$  compared to HC. Note a decrease in duodenal cadherin-17 under low dietary calcium conditions (a 4-fold induction in 1,25(OH)<sub>2</sub>D<sub>3</sub> serum levels were noted in the mice fed the LC diet). (B) Cadherin-17 is inhibited in Caco-2 cells by 1,25(OH)<sub>2</sub>D<sub>3</sub>. Representative Western blot of cadherin-17 expression in Caco-2 cells treated with vehicle (-D) or 1,25(OH)<sub>2</sub>D<sub>3</sub> for 24 h. (C) Cdx2 induced transcription of cadherin-17 is inhibited by 1,25(OH)<sub>2</sub>D<sub>3</sub>. COS-7 cells were transfected with Cdx2 expression vector (15 ng) in the presence or absence of VDR. Cells were treated with vehicle or 1,25(OH)<sub>2</sub>D<sub>3</sub> (10<sup>-8</sup> M) for 24 h and luciferase activity was determined. The data were normalized to values for pRL-TK Renilla luciferase as an internal control. Cadherin-17 promoter activity is represented as fold induction (mean  $\pm$  SE,  $n = 3$  observations) and quantified by comparison to basal levels.

### Non classical actions of $1,25(\text{OH})_2\text{D}_3$ ; $1,25(\text{OH})_2\text{D}_3$ is a transcriptional modulator of IL-17



**Fig. 3.** Inhibition of hIL-17 promoter activity by  $1,25(\text{OH})_2\text{D}_3$  and recruitment of NFATc1 and VDR by ChIP assay on the hIL-17 promoter. (A) The human T cell line Jurkat was cotransfected with the hIL-17 promoter or deletion/mutation promoter constructs and VDR expression vector. Cells were incubated with PMA and ionomycin for 8 h in the presence or absence of  $1,25(\text{OH})_2\text{D}_3$ . Note, mutation of the NFAT sites (and not the AP1 site) inhibits activation and inhibition by  $1,25(\text{OH})_2\text{D}_3$ . Using the  $-232/+5$  construct, cotransfection with NFATc1 expression vector reversed the inhibition observed with  $1,25(\text{OH})_2\text{D}_3$  (not shown). (B) For ChIP assays HUT102 cells were used which constitutively produce IL-17. HUT cells transfected with VDR were treated with vehicle, PMA + ionomycin or PMA + ionomycin +  $1,25(\text{OH})_2\text{D}_3$  for 1 h and cross-linked by 1% formaldehyde for 15 min. Cross-linked cell lysates were subjected to immunoprecipitation with NFATc1 antibody or VDR antibody. DNA precipitates were isolated and then subjected to PCR using specific primers designed to the  $-213/-158$  region of the hIL-17 promoter which contains NFAT sites. Note, ChIP assay shows that in the presence of  $1,25(\text{OH})_2\text{D}_3$  and VDR recruitment of NFATc1 was inhibited and recruitment of VDR to this site is now observed, suggesting that blocking of the NFAT site by VDR is one mechanism involved in  $1,25(\text{OH})_2\text{D}_3$ /VDR mediated inhibition of IL-17.

Tech University, Lubbock, TX). C/EBP $\alpha$ , VDR, cadherin-17 and NFAT antisera were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Chromatin immunoprecipitation assays were performed as described earlier [8,9].

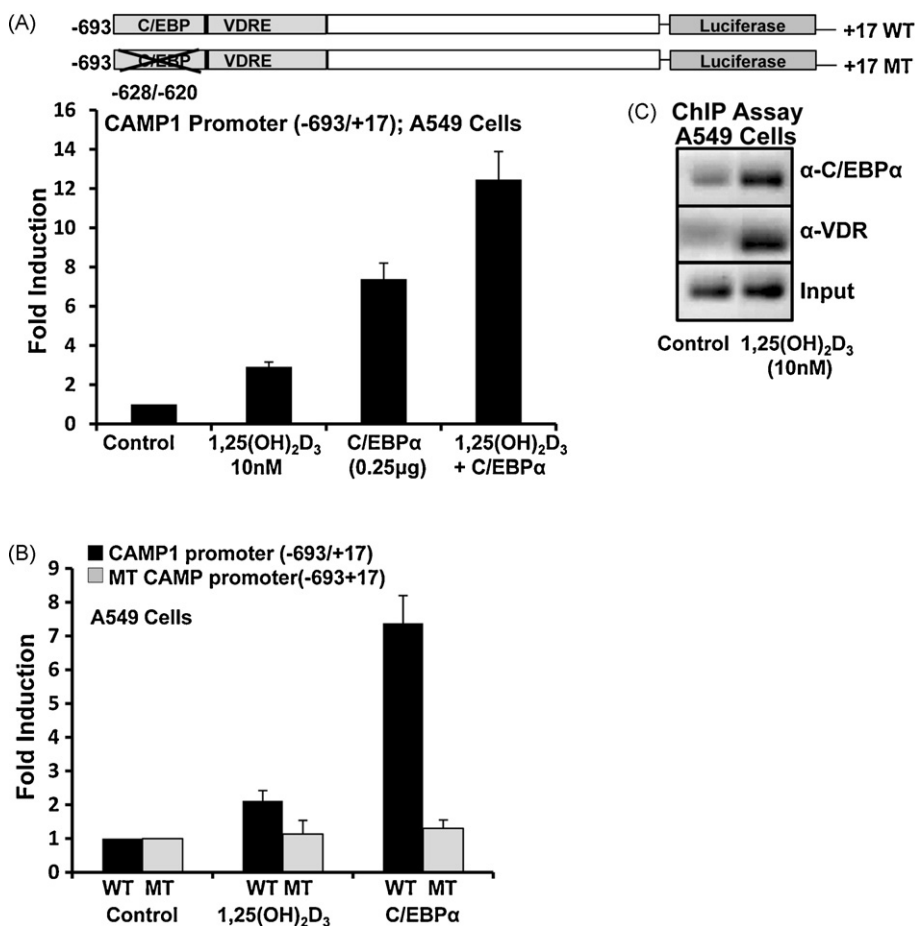
### 3. Results and discussion

#### 3.1. Intestine: intestinal calcium transport in the absence of TRPV6 and calbindin- $D_{9k}$

When fed a standard rodent chow diet, TRPV6 KO, calbindin- $D_{9k}$  KO and TRPV6/calbindin- $D_{9k}$  DKO mice were found to have serum calcium levels similar to those of WT mice (Fig. 1A, upper panel) [10]. In the TRPV6 KO and the TRPV6/calbindin- $D_{9k}$  DKO mice serum PTH levels were significantly increased compared to WT (Fig. 1A, lower panel) [10]. The increase in PTH is consistent with a 9.6% decrease in femoral bone density previously reported in the TRPV6 KO mice and suggests that TRPV6 may have an indirect role in regulating bone formation and/or mineralization [5]. Active intestinal calcium transport in these mice was assessed using the everted gut sac assay. A significant 2-fold increase in active intestinal calcium transport was observed in vitamin D deficient WT, TRPV6 KO and calbindin- $D_{9k}$  KO mice after  $1,25(\text{OH})_2\text{D}_3$  administration [no significant difference among these groups ( $p > 0.1$ )].

In the TRPV6/calbindin- $D_{9k}$  DKO mice there was a 1.4-fold induction in active calcium transport after  $1,25(\text{OH})_2\text{D}_3$  administration (Fig. 1B;  $p < 0.05$  compared to calcium transport in WT and single KO mice injected with  $1,25(\text{OH})_2\text{D}_3$ ), suggesting that the response to  $1,25(\text{OH})_2\text{D}_3$  may be more sensitive to the lack of both TRPV6 and calbindin than to the absence of either TRPV6 or calbindin alone [10]. These findings also suggest that in the KO mice there is compensation by another calcium channel or protein and that other factors involved in  $1,25(\text{OH})_2\text{D}_3$  mediated intestinal calcium absorption remain to be identified. It is possible that  $1,25(\text{OH})_2\text{D}_3$  mediated paracellular transport of calcium may have contributed to the normalization of serum calcium in the null mutant mice. Recent studies have shown that  $1,25(\text{OH})_2\text{D}_3$  can regulate tight junction and transmembrane proteins in the intestine including claudin-2, claudin-12 and cadherin-17 (the cadherin expressed in humans and mice exclusively in intestine and colon), suggesting an additional role for  $1,25(\text{OH})_2\text{D}_3$  in the transjunctional movement of calcium [11,12]. Studies in our lab have indicated a significant decrease in cadherin-17 expression in duodenum under low dietary calcium conditions and in Caco-2 cells after  $1,25(\text{OH})_2\text{D}_3$  treatment (Fig. 2). In addition,  $1,25(\text{OH})_2\text{D}_3$ /VDR can inhibit the induction of cadherin-17 transcription by the intestine specific transcription factor Cdx2 (Fig. 2). Thus, although it has been a matter of debate, these studies suggest that  $1,25(\text{OH})_2\text{D}_3$  does in fact affect

#### Non classical actions of $1,25(\text{OH})_2\text{D}_3$ ; Modulation of innate immunity by $1,25(\text{OH})_2\text{D}_3$



**Fig. 4.** Activation of the cathelicidin antimicrobial peptide (CAMP) gene promoter by  $1,25(\text{OH})_2\text{D}_3$  and C/EBP $\alpha$  and recruitment of C/EBP $\alpha$  and VDR by ChIP assay on the CAMP promoter. (A and B) Human lung epithelial cells A549 were transfected with the CAMP promoter or with the CAMP promoter with the putative C/EBP site (at -628/-620) mutated (MT CAMP promoter, B). Note, mutation of the C/EBP site attenuates the transcriptional response to C/EBP $\alpha$  as well as to  $1,25(\text{OH})_2\text{D}_3$ . (C) ChIP analysis using specific primers designed to include both the C/EBP site (-628/-620) and the adjacent VDRE (-616/-601) shows C/EBP $\alpha$  and VDR recruitment to the CAMP promoter by  $1,25(\text{OH})_2\text{D}_3$  in A549 cells.

paracellular transport of the intestinal epithelium. Other possible factors involved in intestinal calcium transport include the L-type calcium channel isoform Cav1.3 present in highest concentrations in the jejunum and ileum [13] and the calcium binding protein sorcin, which is induced by  $1,25(\text{OH})_2\text{D}_3$  [14], has been reported to bind and modulate L-type calcium channels [15] and is also present in highest concentrations in jejunum and ileum (Ajibade and Christakos, unpublished). Further studies are needed examining different regions of the intestine (not only duodenum) as well as novel  $1,25(\text{OH})_2\text{D}_3$  regulated proteins involved in both transcellular and paracellular transport in order to provide new insight into the major role of  $1,25(\text{OH})_2\text{D}_3$  in intestinal calcium absorption.

### 3.2. Non-classical actions of $1,25(\text{OH})_2\text{D}_3$

With regard to non-classical actions of  $1,25(\text{OH})_2\text{D}_3$ , numerous studies have shown that  $1,25(\text{OH})_2\text{D}_3$  can exert inhibitory effects on the growth of a number of malignant cells, including breast cancer cells. The molecular mechanisms are now beginning to be defined. Recent studies in our lab have shown that C/EBP $\alpha$ , considered a potential tumor suppressor gene in breast cancer, is induced by  $1,25(\text{OH})_2\text{D}_3$  in MCF-7 breast cancer cells, cooperates with Brm (an ATPase that is a component of the SWI/SNF complex) and enhances VDR transcription [16]. Since levels of VDR correlate with the antiproliferative effects of  $1,25(\text{OH})_2\text{D}_3$  and since  $1,25(\text{OH})_2\text{D}_3$  and C/EBP $\alpha$  upregulate p21 (that functions as a regulator of cell cycle progression), these findings suggest mechanisms whereby  $1,25(\text{OH})_2\text{D}_3$  acts to inhibit the growth of breast cancer cells. These findings also provide evidence for C/EBP $\alpha$  as a candidate for breast cancer treatment.

Additional non-classical actions of  $1,25(\text{OH})_2\text{D}_3$  include effects on the immune system.  $1,25(\text{OH})_2\text{D}_3$  has immunosuppressive effects which are correlated with a decrease in IL-2, IFN  $\gamma$  and GM-CSF [17]. Recent studies in our lab, in collaboration with the L. Steinman lab (Stanford University) have indicated that  $1,25(\text{OH})_2\text{D}_3$  has a direct repressive effect on the expression of IL-17, a cytokine that has been reported to play a role in the pathogenesis of autoimmune inflammation [18]. The mechanism involves, at least in part, a competition of VDR with NFAT for binding to the NFAT element (Fig. 3).  $1,25(\text{OH})_2\text{D}_3$  not only regulates adaptive but also innate immunity.  $1,25(\text{OH})_2\text{D}_3$  induces the antimicrobial peptide cathelicidin with subsequent killing of bacteria including *Pseudomonas aeruginosa*, a pathogen of lung infection in cystic fibrosis [19]. Recently we found that C/EBP $\alpha$  is a potent enhancer of cathelicidin antimicrobial peptide (CAMP) gene transcription and that C/EBP $\alpha$  functionally cooperates with VDR in the regulation of CAMP transcription (Fig. 4). Thus, there is increasing evidence that C/EBP isoforms may be key mediators of  $1,25(\text{OH})_2\text{D}_3$  action in different cells. Further studies are needed examining global networks regulated by VDR to promote the host response to pathogens.

In conclusion, identification of target proteins as well as studies related to mechanisms of  $1,25(\text{OH})_2\text{D}_3$  action, including genome-wide action, will result in new insight in both classical and non-classical actions of vitamin D that may suggest therapeutic targets.

### Acknowledgements

These studies were supported by National Institutes of Health grant DK-38961-21 (to S.C.). Studies using the IL-17 promoter were done in collaboration with Sarah Gaffen and studies related to the regulation of CAMP were done in collaboration with G. Diamond, A. Gombart and P. Koeffler.

### References

- [1] Y.C. Li, A.E. Pirro, M. Amling, G. Delling, R. Baron, R. Bronson, M.B. Demay, Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia, *Proc. Natl. Acad. Sci. U.S.A.* 94 (1997) 9831–9835.
- [2] T. Yoshizawa, Y. Handa, Y. Uematsu, S. Takeda, K. Sekine, Y. Yoshihara, T. Kawakami, K. Arioka, H. Sato, Y. Uchiyama, S. Masushige, A. Fukamizu, T. Matsumoto, S. Kato, Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning, *Nat. Genet.* 16 (1997) 391–396.
- [3] R.H. Wasserman, Vitamin D and the intestinal absorption of calcium: a view and overview, in: D. Feldman, J.W. Pike, F. Glorieux (Eds.), *Vitamin D*, Elsevier Academic Press, San Diego, CA, 2005, pp. 411–428.
- [4] Y. Song, X. Peng, A. Porta, H. Takanaga, J.-B. Peng, M.A. Hediger, J. Fleet, S. Christakos, Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by  $1,25\text{-dihydroxyvitamin D}_3$  in the intestine and kidney of mice, *Endocrinology* 144 (2003) 3885–3894.
- [5] S.D. Bianco, J.-B. Peng, H. Takanaga, Y. Suzuki, A. Crescenzi, C.H. Kos, L. Zhuang, M.R. Freeman, C.H. Gouveia, J. Wu, H. Luo, T. Mauro, E.M. Brown, M.A. Hediger, Marked disturbance of calcium homeostasis in mice with targeted disruption of the TRPV6 calcium channel gene, *J. Bone Miner. Res.* 22 (2007) 274–285.
- [6] G.S. Lee, K.Y. Lee, K.C. Choi, Y.H. Ryu, S.G. Paik, G.T. Oh, E.B. Jeung, A phenotype of a calbindin-D9k gene-knockout is compensated for by the induction of other calcium transporter genes in a mouse model, *J. Bone Miner. Res.* 22 (2007) 1968–1978.
- [7] H.J. Armbrecht, M.A. Boltz, T.L. Hodam, Differences in intestinal calcium and phosphate transport between low and high bone density mice, *Am. J. Physiol. Gastrointest. Liver Physiol.* 282 (2002) G130–G136.
- [8] Q. Shen, S. Christakos, The vitamin D receptor, Runx2 and the notch signaling pathway cooperate in the transcriptional regulation of osteopontin, *J. Biol. Chem.* 280 (2005) 40589–40598.
- [9] Y. Zhong, H.J. Armbrecht, S. Christakos, Calcitonin: a regulator of 25-hydroxyvitamin D3 1 $\alpha$  hydroxylase gene, *J. Biol. Chem.* 284 (2009) 1159–1169.
- [10] B.S. Benn, D. Ajibade, A. Porta, P. Dhawan, M. Hediger, J.-B. Peng, Y. Jiang, G.T. Oh, E.B. Jeung, L. Lieben, R. Bouillon, G. Carmeliet, S. Christakos, Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D<sub>9k</sub>, *Endocrinology* 149 (2008) 3196–3205.
- [11] G.D. Kutuzova, H.F. DeLuca, Gene expression profiles in rat intestine identify pathways for  $1,25\text{-dihydroxyvitamin D}_3$  stimulated calcium absorption and clarify its immunomodulatory properties, *Arch. Biochem. Biophys.* 432 (2004) 152–166.
- [12] H. Fujita, K. Sugimoto, S. Inatomi, T. Maeda, M. Osanai, Y. Uchiyama, T. Wada, T. Kojima, H. Yokozaki, T. Yamashita, S. Kato, N. Sawada, H. Chiba, Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent  $\text{Ca}^{2+}$  absorption between enterocytes, *Mol. Biol. Cell.* 19 (2008) 1912–1921.
- [13] E.L. Morgan, O.J. Mace, P.A. Helliwell, J. Affleck, G.L. Kellett, A role for Cav1.3 in rat intestinal calcium absorption, *Biochem. Biophys. Res. Commun.* 312 (2003) 487–493.
- [14] R.J. Wood, L. Tchack, G. Angelo, R.E. Pratt, L.A. Sonna, DNA microarray analysis of vitamin D-induced gene expression in a human colon carcinoma cell line, *Physiol. Genomics* 17 (2004) 122–129.
- [15] M.B. Meyers, T.S. Puri, A.J. Chien, T. Gao, P.H. Hsu, M.M. Hosey, G.I. Fishman, Sorcin associates with pore-forming subunit of voltage-dependent L-type  $\text{Ca}^{2+}$  channels, *J. Biol. Chem.* 273 (1998) 18930–18935.
- [16] P. Dhawan, R. Wieder, S. Christakos, CCAAT enhancer binding protein alpha is a molecular target of  $1,25\text{-dihydroxyvitamin D}_3$  in MCF-7 breast cancer cells, *J. Biol. Chem.* 284 (2009) 3086–3095.
- [17] A. Raghuvanshi, S. Joshi, S. Christakos, Vitamin D and multiple sclerosis, *J. Cell. Biochem.* 105 (2008) 338–343.
- [18] S. Joshi, S. Youssef, S. Gaffen, L. Steinman, S. Christakos, A key mechanism underlying the immunosuppressive effects of vitamin D:  $1,25\text{-dihydroxyvitamin D}_3$  is a transcriptional modulator of IL-17, *J. Bone Miner. Res.* 23 (2008) S105.
- [19] S. Yim, P. Dhawan, C. Raganath, S. Christakos, G. Diamond, Induction of cathelicidin in normal and CF bronchial epithelial cells by  $1,25\text{-dihydroxyvitamin D}_3$ , *J. Cyst. Fibros.* 6 (2007) 403–410.