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Ameloblasts and odontoblasts, target-cells for 1,25-dihydroxyvitamin D3: a review

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ABSTRACT The basic features on the vitamin D endocrine system, synthesis of the main metabolite 1,25 - dihydroxyvitamin D_3 (1,25) and its genomic action mediated via the vitamin D receptor (VDR), are reviewed. Calbindin-D9k, calbindin-D28k and osteocalcin are presented as the most-extensively investigated vitamin D-dependent calcium-binding proteins. The action of 1,25 on the basic process of proliferation and differentiation is introduced. Then, the basis of the systemic theory of vitamin D action on teeth (clinical and experimental data and the dissimilar distribution of VDR and of potential vitamin D-dependent proteins in dental cells) are exposed. Finally, the data obtained with calbindin-D9k, calbindin-D28k, osteocalcin and VDR, which supports the theory that ameloblasts and odontoblasts are target-cells for 1,25 is presented. As a perspective, a cross-survey of the 1,25 and tooth-related literature is proposed which may indicate potential target-genes for 1,25 in teeth as done previously for calbindins-D.

KEY WORDS: *tooth, bone, vitamin D, calbindin-D, vitamin D receptor*

Introduction

Since the early studies on the impact of vitamin D on tooth development (Mellanby, 1928), evidence has been accumulated concerning the role of vitamin D on teeth, in close relation with the decisive steps of the basic and clinical knowledge on this steroid: determination of the active hormonal metabolites (principally 1,25-dihydroxyvitamin D_3) and their anabolic and catabolic pathways (for review see Kumar, 1986), identification of the hormonal receptor (VDR) and of vitamin D-controlled genes (for review see Lowe *et al.*, 1992), and finally understanding of the molecular basis of clinical disorders related to the vitamin D-endocrine system (for review see Glorieux *et al.*, 1991).

Basic features of the vitamin D endocrine system

1,25 $(OH)_2$ vitamin D_3 and vitamin D receptor

Vitamin D is a secosteroid which can be obtained from the diet or endogenously produced in the skin in response to UV irradiation (Bikle and Pillai, 1993). Vitamin D and its metabolites circulate in the blood bound to a vitamin D binding globulin DBP (Walters, 1992). The major metabolite of vitamin D_3 is hydroxylated in the liver in position 25 and then in the kidney and other target-organs and cells in position 1alpha (for review see Kumar, 1986; De Luca *et al.*, 1990). The actions of 1,25-dihydroxyvitamin D_3 [1,25] are mediated (for review see Haussler, 1986; Pike, 1991; Lowe *et al.*, 1992) by a nuclear receptor [VDR]. Apart from the genomic effects of 1,25, this steroid is able to generate biological actions via Ca^{++}, protein kinase C- and cAMP-dependent protein kinase-pathways (Lowe *et al.*, 1992; Walters, 1992). In the nuclei, VDRs bind to DNA sequences called vitamin D responsive elements [VDRE] located in the promoter region of target-genes and control their transcription (Lowe *et al.*, 1992). VDRs may function as homodimers but also as heterodimers (Cheskis and Freedman, 1994) with RARs, RXRs and, as described more recently, with T3R (Shräder *et al.*, 1994). Therefore, the signalling pathways of 1,25 for the control of gene expression may include other hormonal metabolites (9 cis- and trans-retinoic acids, T3). VDREs were initially discovered in *osteocalcin* and *osteopontin* genes, which were first identified in mineralized tissues (for review see Pike, 1991; Lowe *et al.*, 1992). They were then found in other genes related to calcium homeostasis: *25-hydroxyvitamin D3 24-hydroxylase* gene (Ohyama *et al.*, 1994).

Calbindins-D and osteocalcin

Many investigations have focused their attention on intestine, bone and kidney in order to understand the physiological functions of vitamin D in the metabolism of calcium and phosphate (Suda *et al.*, 1991). Among the proteins that are controlled by 1,25, three calcium-binding proteins, calbindin-D9k and calbindin-D28k, as

Abbreviations used in this paper: 1,25, 1,25 dihydroxyvitamin D3; VDR, vitamin D; DBP, vitamin D binding globulin; VDRE, vitamin D responsive element; RAR, retinoic acid receptor; RxR, retinoic X receptor; T3R, thyroid hormone receptor; T3, 3,5,3'-triiodothyronine.

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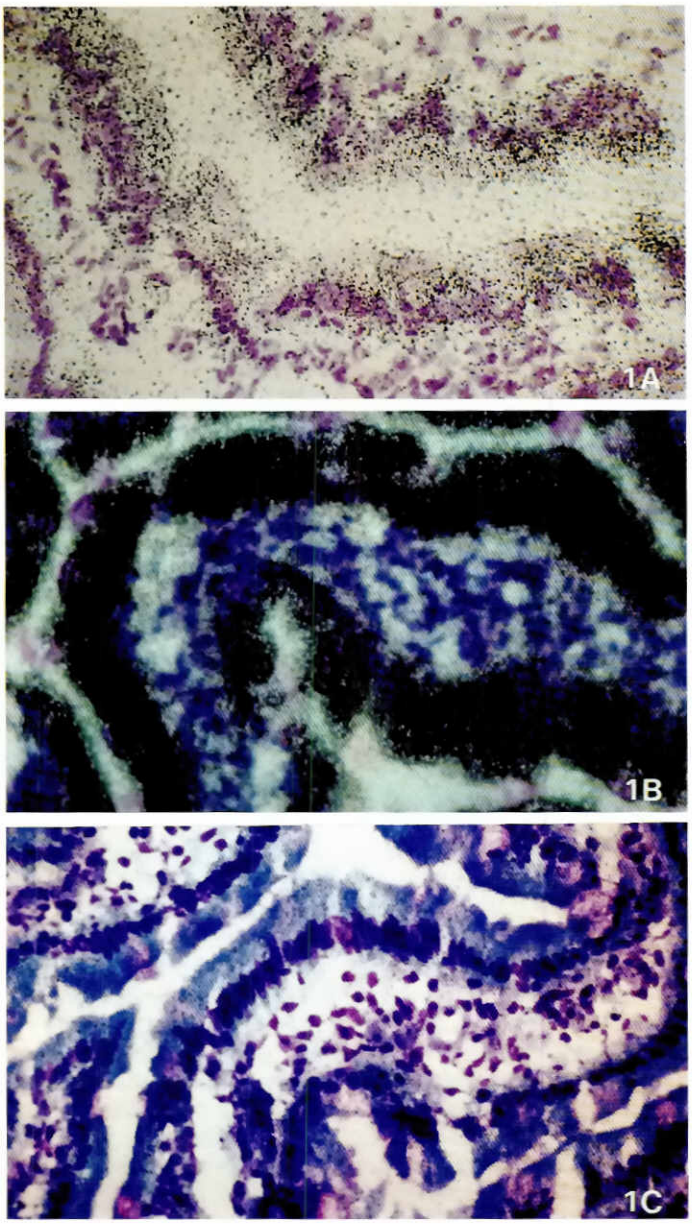

Fig. 1. Parallel expression pattern of VDR and a vitamin D-dependent calciprotein: calbindin-D_{9k} in a classical target-cell, the enterocyte. *x660.* **(A)** *In situ hybridization of VDR mRNAs in the rat duodenum. Antisense riboprobes were obtained by in vitro transcription with [S35]-UTP of VDR cDNA inserted in Gemini plasmid (C. Perret - INSERM U120). cDNA of chick VDR was provided by J.W. Pike (Ligand, San Diego, CA, USA).* **(B)** *In situ hybridization of calbindin-D_{9k} mRNAs in a serial section of rat duodenum. Antisense riboprobes were transcribed from rat calbindin-D9k cDNA cloned and sub-cloned in Gemini plasmid (C. Perret - INSERM U120).* **(C)** *In situ hybridization with the corresponding calbindin-D9k sense riboprobes in a serial section of rat duodenum.*

well as the bone gla-protein, osteocalcin, have been the most extensively studied (for review see Lowe *et al.*, 1992). Calbindins-D are characterized by their different molecular weight, 9 kDa for calbindin-D 9k and 30 kDa for calbindin-D28k. They belong to the calcium-binding protein superfamily of parvalbumin, like calmodulin and S100 proteins (for review see Christakos *et al.*, 1989). Their tissue-specific expression pattern has been extensively investigated: they are used as a tool to map various nuclei in the nervous system (for review see Celio, 1990). They are involved in calcium-handling by acting as a calcium-shuttle, or buffer or interacting with various enzymes (for review see Christakos *et al.*, 1989). Their presence in a cell-type does not automatically imply their vitamin D-dependency. For instance, calbindin-D9k is vitamin D-dependent in duodenum, 17ß estradiol-dependent in uterus and unresponsive to both steroids in lung (Dupret *et al.*, 1992). In a classical target-cell, the enterocyte, a close relationship between *calbindin-D* and *VDR* gene expression is observed during the cell life-cycle, aligned from the crypt along the villus axis (Fig. 1, *in situ* hybridization of calbindin-D and VDR mRNAs in rat duodenum). This parallel developmental pattern illustrates the fact (Arbour *et al.*, 1993) that the amount of VDR controls the responsiveness to 1,25 (in this case control of calbindin-D9k expression). Osteocalcin is a well-known non-collagenous protein of bone matrix and is classically used as a marker of osteoblast differentiation *in vitro* (Lian *et al.*, 1992). This protein is characterized by the presence of γ-carboxyl groups on their glutamic acid residues. This addition confers the ability to link ionic calcium and hydroxyapatite to the protein. However, its precise role in the mineralization process is still unclear. The VDREs of *osteocalcin* gene have been identified in rodent and human species (for review see Lowe *et al.*, 1992).

Other actions of 1,25 $(OH)_2$ vitamin D3

The observation of VDR in many diverse cells (Stumpf, 1988) and the identification of numerous target-genes (Walters, 1992) in tissues not related to calcium homeostasis progressively showed the wider spectrum of action of this hormone. Indeed, 1,25 controls basic developmental processes. It modulates proliferation and induces differentiation in many cell-types pertaining to systems as diverse as the immune system and the skin, as well as the tissues involved in phospho-calcium metabolism, intestine and bone (Abe *et al.*, 1986; Suda *et al.*, 1991; Bickle and Pillai, 1993). These effects have been related to the control of a set of vitamin D-dependent molecules such as proto-oncogenes: c-myc and c-fos (for review see Lowe *et al.*, 1992), homeobox containing genes: *Msx II* (Hodgkinson *et al.*, 1993), various growth factors (NGF: Wion *et al.*, 1991) and their receptors, (EGF receptor: Petkovich *et al.*, 1987) and differentiation agents: (TGF-beta, Sato *et al.*, 1993). Conversely, in highly specialized cells when the cells are overtly differentiated, 1,25 controls the synthesis of peptides such as hormones (preproparathyroid hormone, prolactin, thyrotropin and calcitonin; for review see Lowe *et al.*, 1992).

The systemic theory of vitamin D action on teeth

Biological effects of vitamin D on developing teeth

Clinical investigations constantly report the existence of enamel and dentin alterations in vitamin D-deficient children and in cases of hereditary vitamin D-resistant rickets types I and II (for review see Nikiforuk and Fraser, 1979). Experimental dental features induced by hypovitaminosis (Berdal *et al.*, 1987; Limeback *et al.*, 1992) and hypervitaminosis (Pitaru *et al.*, 1982; Matsumoto *et al.*, 1990) confirmed these clinical observations. In rats raised in vitamin D-free conditions, enamel and dentin matrix as well the ameloblasts and odontoblasts ultrastructure are abnormal (Fig. 2). However, these disorders were proposed to be secondary to the systemic effects of 1,25: clinical enamel dysplasia has been

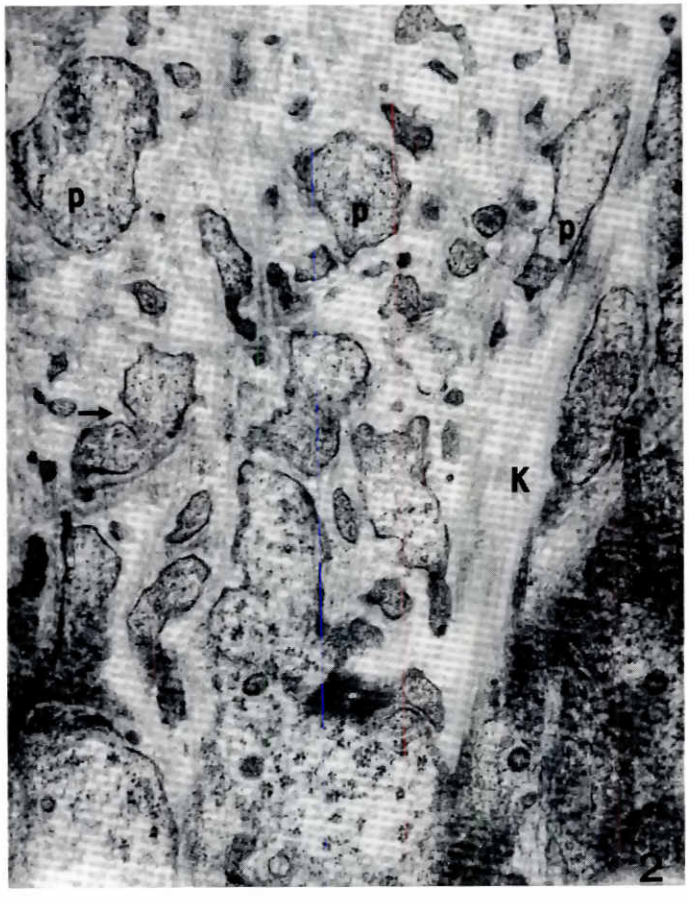

Fig. 2. Ultrastructure of the apical pole of odontoblasts in 9 day-old vitamin D-deficient rat. *x37,500. In the areas of the first cuspal row located at the cusp tip of the first rat molars, the secretory pole is disturbed. It appears to be fragmented into numerous cytoplasmic patches (Star). Von Korff fibers are present. Other collagen fibers (diameter: 40-80 nm) show a regular size throughout the widened predentin.*

described to be related to hypocalcemia and dentin hypomineralization to hypophosphatemia (Nikiforuk and Fraser, 1979). It was therefore suggested that 1,25 acts on teeth only by controlling serum calcium and phosphate. This clinical concept was supported by experimental data: 1) enamel mineralization is obtained in a chemically defined medium without any supplementation of vitamin D (Bringas *et al.*, 1987); 2) *in vitro* calcium and phosphate control enamel and dentin formation (Wöltgens *et al.*, 1987); 3) the active metabolites 1,25 and 24,25-dihydroxyvitamin D3 do not increase calcium incorporation in tooth germ *in vitro* (Bawden *et al.*, 1983, 1985).

VDR and vitamin D-dependent molecules were observed in different cell-types

Initial investigations on the vitamin D-endocrine system in teeth provided a distribution pattern which tends to confirm the systemic theory of vitamin D action. Vitamin D receptors were mapped by autoradiography (Kim *et al.*, 1983, 1985; Clark *et al.*, 1985; Stumpf, 1988) and described as present in dental cells except in ameloblasts and odontoblasts. These same cells are directly involved in matrix deposition and mineralization and contain vitamin D-dependent molecules such as immunoreactive calbindin-D9k (Taylor *et al.*, 1984) and calbindin-D28k (Celio *et al.*, 1984; Taylor, 1984; Elms and Taylor, 1987; Magloire *et al.*, 1988) for ameloblasts and osteocalcin (Helder *et al.*, 1993; Bronckers *et al.*, 1994) for odontoblasts, respectively. In view of this conflicting data, the following theory could be proposed: the organization of the promoters of vitamin D-responsive genes in bone, duodenum and kidney would result in their tissue-specific unresponsiveness in teeth, as shown for the *third component of complement (C3),* vitamin D-dependent in bone and unresponsive to the hormone in liver (Jin *et al.*, 1992) respectively. But this situation appeared quite puzzling because of the strong functional analogy between tooth (for review see Slavkin, 1991) and bone (for review see Suda *et al.*, 1991), and also between enamel organ, duodenum and kidney for ionic transfer (for review see Bawden, 1989). Indeed, dental cells and osteoblasts (for review see Table 1 and Suda *et al.*, 1991) express more than 10 common proteins which are all vitamin D-dependent in bone (for review see Lowe *et al.*, 1992). Since autoradiographic data on VDR in teeth was obtained in vitamin D-deficient animals (Kim *et al.*, 1983, 1985, in order to obtain unbound receptors), another interpretation of the absence of the VDR in ameloblasts and odontoblasts was proposed. Vitamin D-free conditions were considered to decrease the receptor quantities, specifically in ameloblasts and odontoblasts (Berdal *et al.*, 1993). Indeed, a positive autoregulation of VDR levels (also via a stabilization of the protein) is observed in rat osteosarcoma cells (Arbour *et al.*, 1993) and may control vitamin D responsiveness of the cells. Vitamin D receptors could be expressed and functional in ameloblasts and odontoblasts at a concentration not detected by autoradiographic experiments. Therefore, in order to investigate the potential VDR-mediated action of vitamin D on ameloblasts and odontoblasts, the basic concept consisted of using markers of the hormonal action: VDR as well as the widely studied vitamin D-dependent molecules, calbindin-D9k, calbindin-D28k and osteocalcin.

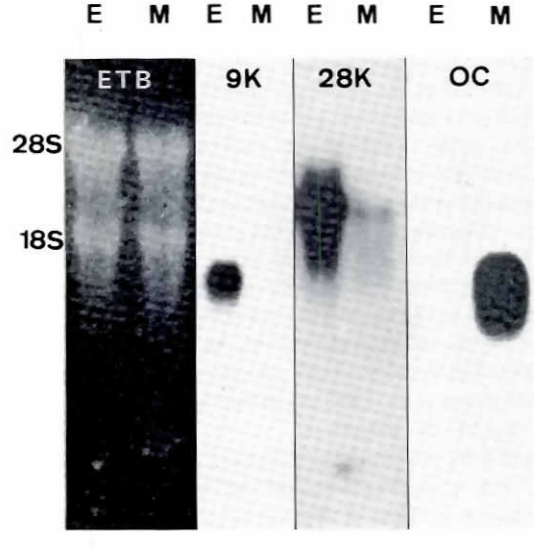

Fig. 3. Northern-blot analysis of enamel organ and dental mesenchyme mRNAs. *Total RNAs extracted from dental epithelium (E) and dental ectomesenchyme (M) are fractioned under denatured conditions and stained by ethidum bromide (ETB). Calbindin-D9k mRNAs (9k) are detected in E and not in M while both E and M contain calbindin-D28k (28k). Osteocalcin mRNAs (OC) are restricted to M.*

TABLE 1

POTENTIAL TARGET-GENES FOR 1,25 IN DEVELOPING TOOTH

Involvement in tooth germ	Expression in teeth		Control by 1,25 in other tissues	
Cell growth and differentiation				
Growth factors and receptors	*EGFr*	Davideau *et al.*, in press	breast carcinoma	Desprez *et al.*, 1991
	NGF	Mitsiadis and Byers, this issue	Fibroblast L-292	Wion *et al.*, 1991
	TGFß	D'Souza *et al.*, 1990	Bone	Finkelman *et al.*, 1991
	BMP	Bègue-Kirn *et al.*, 1992		
Proto-oncogenes	*c-fos*	Caubet and Bernaudin, 1988	Osteoblastic cells	St. Arnaud *et al.*, 1994
	c-myc	Hirning *et al.*, 1992	Keratinocytes	Hanafin *et al.*, 1994
Factors controlling transcription	*Msx-2*	McKenzie *et al.*, 1992	Bone	Hodgkinson *et al.*, 1993
	VDR	Berdal *et al.*, 1993	Bone	Arbour *et al.*, 1993
Extracellular matrix	*Fibronectin*	review: Lesot, 1986 Thesleff *et al.*, 1991	Fibroblasts	Franceschi *et al.*, 1987
	α1(I) collagen	Andujar *et al.*, 1991		Lichtler *et al.*, 1989
Hard tissue formation				
Matrix secretion	*α1(I) collagen*	Pavlin *et al.*, 1992	Bone	Lichtler *et al.*, 1989
	Osteocalcin	Bronckers *et al.*, 1994	Bone	Ozono *et al.*, 1991
	Osteopontin			Oldberg *et al.*, 1990
Ionic handling during mineralization	*Calbindins*	Berdal *et al.*, 1993	Intestine, kidney	for review: Lowe *et al.*, 1992
	Calcium pump	Borke *et al.*, 1993	Intestine	Wasserman *et al.*, 1994
	Alkaline phosphatase	Engström *et al.*, 1977	Bone	Kyeyune-Nyombi *et al.*, 1989

VDR and control of dental gene expression by 1,25(OH)$_2$ vitamin D3

Calbindin-D9k, calbindin-D28k, osteocalcin and VDR

The effects of 1,25 were analyzed in vitamin D-deficient and control rat teeth. The continuously erupting rat incisor provided a useful experimental model since biochemical analysis (RIA, Western-blotting and Northern-blotting) can be performed separately on microdissected enamel organ and dental ectomesenchyme (Berdal *et al.*, 1989, 1991c, 1993). Moreover, since the enamel presecretion, secretion and maturation stages are arranged along the incisor axis, the developmental pattern of calbindin-D9k and calbindin-D28k expression could be easily followed throughout the ameloblast life-cycle (Berdal *et al.*, 1991b,c; Hotton *et al.*, in press). Target-cells for VDR were identified by light microscopy immunolocalization in rat (Berdal *et al.*, 1993) and human developing teeth (Bailleul-Forestier *et al.*, unpublished). VDR visualization in epithelial as well as ectomesenchymal progenitor cells indicates that 1,25 may play a part in the initial stages of ameloblast and odontoblast life-cycles, as described in various other cell-types (Suda *et al.*, 1991; Bikle and Pillai, 1993). Indeed, vitamin D-deficient rat molars show major disturbances in the process of morphogenesis, histodifferentiation and terminal differentiation of ameloblasts and odontoblasts (Berdal *et al.*, 1987). 1,25 effects on cell proliferation shown in tooth germ *in vitro* (Sakakura *et al.*, 1988) may be a key mechanism in vitamin D control of early development.

When cells are overtly differentiated, 1,25 up-regulates the VDR detected in the ameloblasts and odontoblasts (Berdal *et al.*, 1993). The distribution pattern of the investigated calciproteins varies depending on the cell-type. *Calbindin-D9k* and *calbindin-D28k* genes are both expressed by ameloblasts (Berdal *et al.*, 1993). The quantities of cytoplasmic proteins and mRNAs co-vary depending on the developmental stage (Berdal *et al.*, 1991b,c; Hotton *et al.*, in press). Calbindin-D28k protein and mRNAs are present in differentiated odontoblasts (Berdal *et al.*, 1993, 1994). These calciproteins show a cellular distribution very similar to that described for active transcellular calcium transport (for review see Bawden, 1989). Osteocalcin (Helder *et al.*, 1993; Bronckers *et al.*, 1994) is selectively restricted to the odontoblasts in dental ectomesenchyme. Northern-blot illustrates this selective pattern of gene expression in the enamel organ and the dental ectomesenchyme of the rat incisor (Fig. 3). These three calcium-binding proteins could therefore be used as markers of 1,25 genomic action, specifically in ameloblasts and odontoblasts. An increase in the steady-state levels of calbindins-D mRNAs in the enamel organ and dental mesenchyme induced by a single injection of 1,25 indicates that these genes are also under the control of vitamin D in teeth. This data, supported by the immunodetection of VDR, shows that these cells may be considered as target-cells for 1,25 as classically accepted for duodenum, kidney and bone cells (for review see Lowe *et al.*, 1992). This assertion is supported by the apparent decrease of immunoreactive osteocalcin in vitamin D-deficient rat molar dentin and odontoblasts (which, however, appears to contain dental constitutive proteins: dentin phosphoproteins, Berdal *et al.*, 1991a). Therefore, 1,25 would control enamel and dentin formation, at least in part, by acting on the expression of dental genes important for matrix secretion *(osteocalcin)* and mineralization *(calbindins-D)*. Further characterization of the respective role for 1,25 and calcium and the different transcriptional and/or post-transcriptional steps in the 1,25-control of gene expression, as described in other systems (for review see Lowe *et al.*, 1992), are critically needed in teeth. Other roles for vitamin D (control of the exocytosis) were also suggested by the absence of the dentin phosphoproteins in dentin and their presence in odontoblasts of vitamin D-deficient rat molars (Berdal *et al.*, 1991a).

Conclusion and perspectives

Other potential target-genes for 1,25 in developing tooth germ may be identified, as done previously for calbindins-D, by the cross-control in the literature of their expression in teeth and

vitamin D-dependency in other tissues (Table 1). However, their vitamin D-dependency should be characterized since there may exist tissue-specific differences in the responsiveness to 1,25 (Dupret *et al.*,1992; Jin *et al.*, 1992).

Furthermore, an interesting issue would be to investigate the control of 1,25 on the expression of dental-specific genes: *amelogenins* and *non-amelogenins* for ameloblasts (for review see Slavkin *et al.*, 1991; Brookes *et al.*, 1995), *dentin phosphoproteins* (MacDougall *et al.*, 1992; George *et al.*, 1994) and *sialoprotein* (Ritchie *et al.*, 1993) for odontoblasts. Investigations of the up-stream region of X *chromosome-amelogenin* gene may provide the identification of the VDRE consensus sequence while its effective function could be established in transgenic mice containing 5'-located promoter combined with a reporter gene as initiated recently for the developmentally-controlled constitutive expression of this protein (Chen *et al.*, 1994). Finally, the interactions of retinoic acids which act on similar stages of development when compared to 1,25 (Bloch-Zupan *et al.*, 1994 a,b) and 1,25 effects on tooth germ formation, may clarify the complex cross-pathway of these steroids (Cheskis and Freedman, 1994) on the control of gene expression important in the basic processes of development.

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