Reshaping the way we view vitamin D signalling and the role of vitamin D in health

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Although the biological requirement for vitamin D can be met by epidermal exposure to UV light, there are a number of conditions where this production does not occur or is not sufficient to meet biological needs. When this happens, vitamin D must be consumed and is a nutrient. However, two distinct observations have caused researchers to rethink certain dogma in vitamin D biology. First, it appears that in addition to the hormonally active form of 1,25 dihydroxyvitamin D ($1,25(OH)_2D$), circulating levels of 25 hydroxyvitamin D have a critical importance for optimal human health. This and other data suggest that extra-renal production of $1,25(OH)_2D$ contributes to Ca homeostasis and cancer prevention. Second, in addition to its role in the transcriptional activation of genes through the vitamin D receptor there is now compelling evidence that $1,25(OH)_2D$ has a second molecular mode of action; the rapid activation of second-messenger and kinase pathways. The purpose of this second mode of action is only now being explored. The present review will discuss how these two areas are reshaping our understanding of vitamin D metabolism and action.

Vitamin D: Calcium homeostasis: Cancer prevention: Cell signalling pathways

Introduction: vitamin D is a hormone and a nutrient

Some 20 years ago, I (J.C.F.) remember sitting in a research seminar as a graduate student. Dr Hector DeLuca was the speaker and he was talking about his research on the mechanism of vitamin D action. Something he said at the outset of his seminar made a big impression on me and stuck deeply in my mind. He said, 'Vitamin D is a hormone and not a nutrient.' Perhaps he was overstating for effect. Still, I remember having the distinct feeling that he was shutting off a line of research for the nutrition community. However, we are now at a point where we can recognise both the truth and the limits of that statement.

It takes no stretch of the imagination to see that vitamin D works biologically as a steroid hormone. Like androgens and oestrogens, it has a cholesterol backbone. In addition, in response to various physiological signals, it can be metabolically activated (first in the liver, then in the kidney) and the active metabolite, 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) acts as an endocrine signal on other tissues (for example, classically bone, intestine, and kidney) (Holick, 2003). Once in the target cell, $1,25(OH)_2D$ activates a member of the steroid hormone receptor superfamily, the vitamin D receptor (VDR), and ligand activation of this receptor leads to the transcriptional activation of vitamin D-responsive genes (Haussler *et al*. 1998). This system is critically important for the modulation of Ca homeostasis and is exemplified by the adaptations that occur in response to changes in dietary Ca intake (Fig. 1).

Because vitamin D can be produced from 7-dehydrocholesterol in the skin, it is also understandable how the dietary intake of vitamin D came to be considered irrelevant. As long as the skin is regularly exposed to UV light in the 290 to 315 nm range (MacLaughlin *et al*. 1982), vitamin D status will be high and the vitamin D endocrine system will regulate Ca homeostasis. For example, for lifeguards in the middle of the summer, there is no nutritional requirement and vitamin D is best viewed as a hormone precursor produced in the skin. However, there is a growing recognition that many individuals and specific subgroups of the population do not make enough vitamin D in the skin to meet their physiological requirements (Holick, 2003). Low skin production of vitamin D (and low vitamin D status) occurs in the elderly and dark-skinned individuals (particularly if

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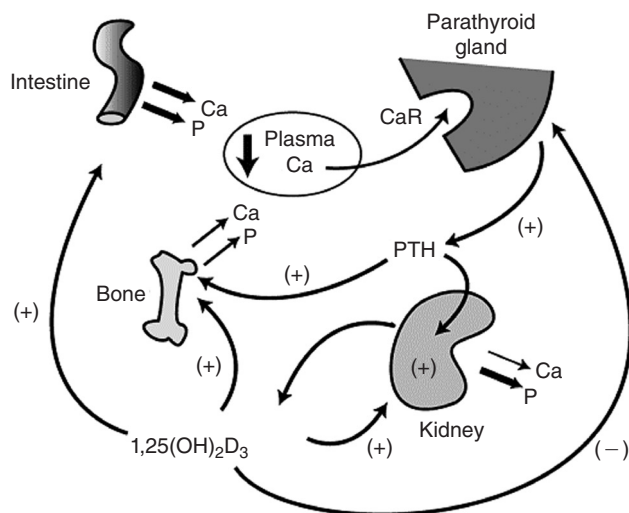
Fig. 1. Regulation of the vitamin D-parathyroid hormone (PTH) axis by dietary Ca. In this scenario changes in dietary Ca intake are reflected as a change in serum ionised Ca. These changes are detected by the Ca-sensing receptor (CaR) and signal to the parathyroid gland that PTH production and release should increase (when dietary Ca and serum Ca are low) or decrease (when dietary Ca and serum Ca are high). PTH is a regulator of the renal 1α-hydroxylase that is responsible for increased production of 1,25 dihydroxyvitamin D_3 (1,25(OH)$_2$D$_3$). An increase in PTH and 1,25(OH)$_2$D$_3$ leads to increased bone resorption, reduced renal Ca and P excretion, and improved intestinal Ca and P absorption efficiency. Once produced in the kidney, 1,25(OH)$_2$D$_3$ is a strong negative regulator of PTH production. Under high dietary Ca these hormonal and physiological changes are reversed.

they are homebound), in individuals who use strong sun-blocks in the summer and who avoid the sun, and individuals who live in the upper or lower parts of the northern or southern hemispheres during the winter. For these individuals, special efforts must be made to ensure vitamin D adequacy. This includes dietary supplementation.

High 25 hydroxyvitamin D influences both calcium homeostasis and cancer risk

While the preceding discussion highlights the nutritional importance of vitamin D, it implies that the biological importance of vitamin D depends upon an individuals ability to produce 1,25(OH)$_2$D in the kidney and suggests that adequate health is dependent upon high circulating levels of that metabolite. This is intentional, as most of the science on the mechanism of vitamin D action points to 1,25(OH)$_2$D as the critical active metabolite of vitamin D. As Fig. 1 shows, the importance of increased renal 1,25(OH)$_2$D production for maintaining Ca homeostasis can be clearly demonstrated under periods of low dietary Ca intake (Dawson-Hughes *et al*. 1993). Unfortunately, scientists who have attempted to correlate serum vitamin D metabolites to functional outcomes of Ca homeostasis do not always see a strong relationship between serum 1,25(OH)$_2$D and these outcomes in apparently normal, healthy subjects. In fact several recent studies have shown

that it is the serum level of 25 hydroxyvitamin D (25(OH)D), the precursor of 1,25(OH)$_2$D, that most closely correlates with several favourable outcomes for bone health. For example, although animal studies clearly demonstrate that 1,25(OH)$_2$D is an important regulator of intestinal Ca absorption (Bronner *et al*. 1986; Song *et al*. 2003*b*), several studies have found that the efficiency of Ca absorption was positively correlated to serum 25(OH)D when levels vary within the normal range (50·1 to 86·5 nmol/l) (Barger-Lux *et al*. 1995; Heaney *et al*. 1997, 2003; Devine *et al*. 2002). Similarly, even though 1,25(OH)$_2$D is known to suppress parathyroid hormone (PTH) gene transcription (Demay *et al*. 1992), Thomas *et al*. (1998) and Chapuy *et al*. (1997) have shown that it is serum 25(OH)D levels that are associated with lower serum PTH levels (peak suppression at 80 nmol/ml). This protection extends to protection from fractures; Trivedi *et al*. (2003) showed that the supplementation of elderly men and women with 2500 µg (100 000 IU) vitamin D_3 every 4 months for 5 years significantly increased serum 25(OH)D and reduced the age-associated relative risk of fracture.

The importance of a high 25(OH)D status also appears to be important for a non-traditional action of vitamin D as well; cancer chemoprevention. Schwartz & Hulka (1990) hypothesised that vitamin D deficiency is the underlying factor for increased prostate cancer risk due to advancing age, Black race, and northern latitudes; factors associated with decreased synthesis of vitamin D in the skin (Holick, 1997). This group later confirmed that low prostate cancer rates were associated with high UV radiation exposure in the USA (Hanchette & Schwartz, 1992). Several groups have attempted to establish a direct relationship between high vitamin D status and prostate cancer risk. For example, Ahonen *et al*. (2000) found a 70 % increased prostate cancer risk in men with 25(OH)D levels below the median, especially in younger men (<52 years) who entered the study with low serum 25(OH)D (adjusted odds ratio 3·5).

How does a high serum 25 hydroxyvitamin D level offer protection?

The observations regarding 25(OH)D-mediated effects on bone and cancer endpoints might be explained in a number of ways. First, they might be artefacts of the simple fact that 1,25(OH)$_2$D is unstable and that it is hard to measure long-term exposure to this metabolite. The half-life of 25(OH)D in the serum is about 2 weeks, whereas the half-life of 1,25(OH)$_2$D is less than 6 h (Haddad & Rojanasathit, 1976; Mason *et al*. 1980). As a result, a peak in serum 1,25(OH)$_2$D could initiate a change in intestinal Ca absorption but that peak could be gone by the time intestinal Ca absorption is elevated (i.e. reducing the correlation between the two parameters). Another possibility is that high levels of 25(OH)D may bind directly to the VDR leading to activation of gene transcription. Brumbaugh & Haussler (1973) have shown that even though 25(OH)D has a low affinity for the VDR (500–1000-fold lower affinity than 1,25(OH)$_2$D), high levels of 25(OH)D can displace 1,25(OH)$_2$D from the receptor. Based upon this hypothesis, 25(OH)D, whose serum levels are 1000 times greater than serum 1,25(OH)$_2$D levels, may be a direct, physiologically

relevant activator of gene transcription through activation of the VDR. While this hypothesis fails to take into account the impact of the serum vitamin D-binding protein (i.e. 25(OH)D has >600-fold higher affinity and thus may be less likely to be transferred to tissues), this effect certainly accounts for the ability of vitamin D and 25(OH)D to reverse the phenotype of genetic pseudovitamin D-deficient rickets caused by mutations in the 1α-hydroxylase gene (Glorieux & St-Arnaud, 1997). Still, massive amounts of vitamin D (>20 000 IU or 500 μg/d) and 25(OH)D (>100 μg/d) are needed for this effect. This makes it improbable that the relatively moderate increases in 25(OH)D that suppress PTH or provide protection against cancer are working through this pathway (i.e. 2–3-fold changes).

Extra-renal production of 1,25 dihydroxyvitamin D is critical for optimal health

A final hypothesis to explain the beneficial effects of high serum 25(OH)D levels is that there may be local production of $1,25(OH)_2D$ in a wide variety of tissues, i.e. $1,25(OH)_2D$ is working as an autocrine or a paracrine regulator rather than an endocrine regulator. Although textbooks teach that the production of $1,25(OH)_2D$ is exclusively renal, there has been evidence to support extra-renal production for some time. Dusso *et al*. (1988) and Jongen *et al*. (1984) both found that supplementing anephric subjects with 25(OH)D increased serum $1,25(OH)_2D$ levels from very low levels to low normal ranges. Consistent with these observations, Zehnder *et al*. (2001) found extra-renal expression of the 1α-hydroxylase protein by immunohisto-chemistry in skin, lymph nodes, pancreatic islets, brain, placenta, and in colonic epithelial cells.

Recently, several papers have provided data that support a role for the local production of $1,25(OH)_2D$ in prostate cancer prevention. Hsu *et al*. (2001) examined eighteen cell lines of normal prostatic epithelial cells, eight cell lines of cells from patients with benign prostatic hypertrophy cells, fifteen adenocarcinoma cell lines, and four established prostate cancer cell lines (LNCaP, PC-3, DU145 and MDA-PCa 2b). They found that normal prostate epithelial cells displayed a high level of 1α-hydroxylase activity ($2\cdot20 \pm 0\cdot61$ pmol/mg protein per h). This activity was impaired in benign prostatic hypertrophy cells ($1\cdot48 \pm 0\cdot18$ pmol/mg protein per h), primary cultures of prostate cancer cells ($0\cdot32 \pm 0\cdot15$ pmol/mg protein per h) and prostate cancer cell lines ($0\cdot17 \pm 0\cdot16$ pmol/mg protein per h). They subsequently showed that the percentage of growth inhibition in response to treatment of cells with 25(OH)D was dependent upon the 1α-hydroxylase activity. As a result, while the prostate cancer cell line LNCaP was responsive to $1,25(OH)_2D$ treatment, their lack of 1α-hydroxylase activity caused them to be unresponsive to 25(OH)D treatment. Whitlatch *et al*. (2002) later demonstrated that the anti-proliferative effects of $25(OH)D_3$ could be restored in LNCaP cells transfected with a transgene conferring 1α-hydroxylase activity.

These data provide strong support for the role of autocrine or paracrine regulation in prostate cancer prevention. This model has been generalised, integrated with the more traditional renal mode of $1,25(OH)_2D$ production, and is presented in Fig. 2. Still, it must be noted that there has

not yet been a report of 1α-hydroxylase expression in the vitamin D-responsive, Ca-transporting cells of the small intestine. Similarly, it has not been demonstrated that the loss of 1α-hydroxylase activity in the prostate leads to increased prostate cancer or that the loss in the intestine leads to reduced efficiency of intestinal Ca absorption. With the development of global and conditional 1α-hydroxylase knockout mice (St Arnaud *et al*. 2003), the ability to test these questions directly (as well as the quantitative assessment of 25(OH)D as a direct activator of the VDR) is within reach.

The two modes of 1,25 dihydroxyvitamin D action

The bulk of the research on $1,25(OH)_2D$ action has focused on the activation or repression of gene transcription that is mediated through the VDR. This and other data comprise a significant body of evidence supporting a critical role for the VDR in the Ca-regulating actions of $1,25(OH)_2D$ (see Fig. 3 for a current model of the mechanism of vitamin D-mediated gene activation). For example, mutations in the VDR cause genetic rickets (vitamin D-resistant rickets or type II rickets) and alopecia in affected individuals. Also, serum PTH levels are dramatically elevated and intestinal Ca absorption is lower in nuclear VDR (nVDR) knockout mice compared with wild-type littermates (van Cromphaut
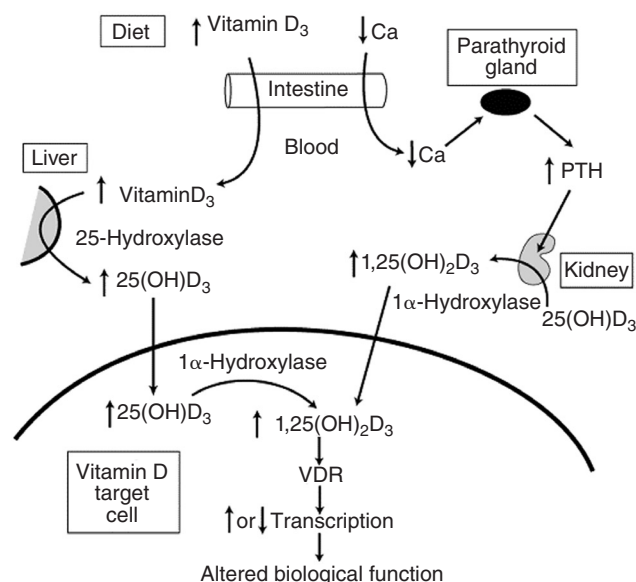

Fig. 2. Model for local production and autocrine action of 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). Traditionally, consumption of a low-Ca diet leads to increased renal production of $1,25(OH)_2D_3$ and endocrine signalling leading to activation of the vitamin D receptor (VDR)-mediated gene transcription in vitamin D target tissues (for additional details, see Fig. 1). Alternately, the presence of extra-renal 1α-hydroxylase activity in several non-traditional vitamin D target tissues suggests that autocrine signalling may occur within cells due to elevated serum 25 hydroxyvitamin D_3 ($25(OH)D_3$) levels that result from feeding a diet with high dietary vitamin D content or after high exposure to UV light in the proper wavelength. Although not shown, locally produced $1,25(OH)_2D_3$ could also be released and act locally as a paracrine signal. PTH, parathyroid hormone.
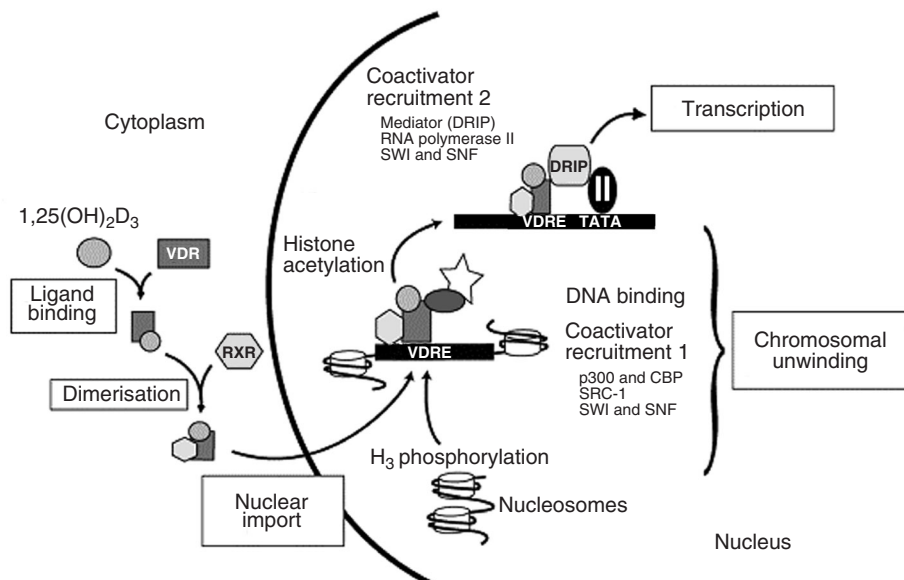
Fig. 3. Regulation of gene transcription through the nuclear vitamin D receptor (VDR). This figure is a summary of our current knowledge regarding vitamin D-activated gene transcription. The process requires multiple steps that are initiated by ligand binding to the VDR, i.e. dimerisation of VDR to the retinoid X receptor (RXR), nuclear import of the VDR-RXR complex, co-activator-mediated chromosomal unwinding, and co-activator-mediated activation of the basal transcription unit containing RNA polymerase II. DRIP, mediator D complex; SWI and SNF, ATP-dependent chromatin remodelling complex; VDRE, vitamin D response element; TATA, binding site in gene promoters for RNA polymerase II; CBP, cAMP-response element-binding protein-binding protein; SRC-1, steroid receptor co-activator-1.

et al. 2001; Song *et al.* 2003*a*). The VDR is also critical for the growth-inhibitory properties of 1,25(OH)$_2$D; prostate cancer cells with little or no VDR are not growth inhibited in response to 1,25(OH)$_2$D treatment (Miller *et al.* 1995; Hedlund *et al.* 1996*a*,*b*; Zhuang *et al.* 1997).

While research on VDR-mediated gene transcription continues to be fruitful, there is now compelling evidence for the existence of rapid activation of signal-transduction pathways by 1,25(OH)$_2$D within various cell types (Nemere & Farach-Carson, 1998; Sitrin *et al.* 1999). Fig. 4 summarises the kinase and second-messenger pathways that have been shown to be activated through rapid 1,25(OH)$_2$D-mediated signalling. This form of signalling has been more closely associated with the mechanism of action of peptide hormones and growth factors yet is being recognised as important for the action of other steroid hormones as well (Harvey *et al.* 2002). Nonetheless, the discovery of a second mechanism for vitamin D action raises several interesting questions: i.e. 'why do we need two distinct vitamin D signalling pathways?', 'are there independent biological processes regulated by each pathway?', and 'how does vitamin D signal the cell to rapidly initiate signal-transduction pathways?'

While the recent observations on rapid 1,25(OH)$_2$D signalling are interesting, the central importance of VDR-mediated signalling appears to leave little room for an independent effect of these pathways. To date, the area that best demonstrates the importance of the rapid activation of kinase pathways by 1,25(OH)$_2$D is the modulation of growth-zone chondrocyte biology (Boyan *et al.* 1994, 1999, 2003). Treatment of growth-zone chondrocytes with 1,25(OH)$_2$D activates phospholipase A$_2$ and causes an

increase in membrane fluidity, activates phospholipase C leading to the activation of protein kinase C and release of intracellular Ca, and indirectly activates protein kinase A through production of prostaglandin E$_2$. These changes are important for the activation of growth factors (i.e. latent transcriptional growth factor-β in matrix), proteoglycan degradation, and matrix mineralisation. The fact that these effects occur in the nucleus-free matrix vesicles released from chondrocytes, as well as in intact chondrocytes, clearly demonstrates that the events are independent of transcriptional activation. The final aspect of this system that bears noting is that chondrocytes have the ability to produce 1,25(OH)$_2$D from 25(OH)D (Schwartz *et al.* 1992). This is consistent with the idea of an autocrine or paracrine signalling pathway that was developed in the first part of the present review.

Do rapid and nuclear 1,25 dihydroxyvitamin D signalling pathways interact?

It is not essential that rapid vitamin D signalling be independent of the VDR or VDR-mediated gene activation. In fact, there are two lines of evidence that suggest the rapid activation of kinases by 1,25(OH)$_2$D and the function of the VDR are related. A model for these interactions is presented in Fig. 5.

Modulation of vitamin D receptor function by kinase activation. Several studies support the hypothesis that signal-transduction pathways are important regulators of nVDR-mediated gene expression. For example, the
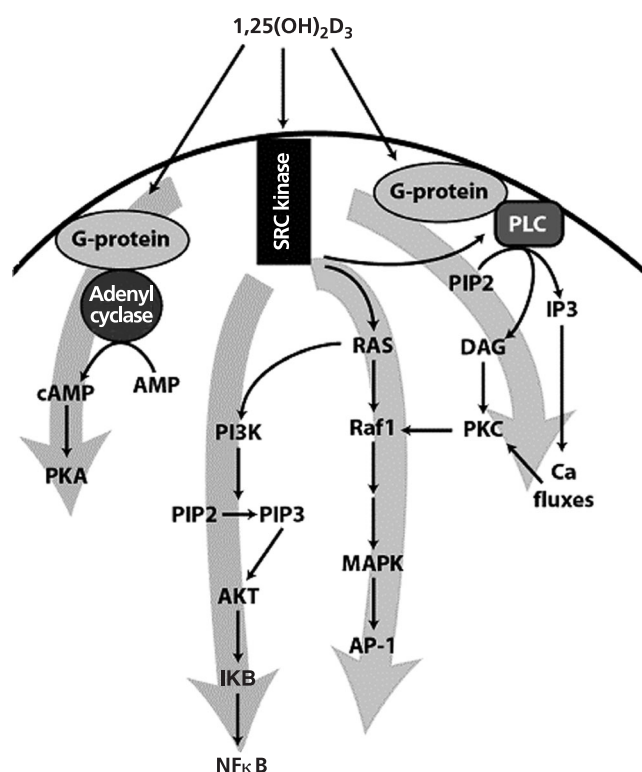
Fig. 4. Rapid activation of signal-transduction pathways by 1,25 dihydroxyvitamin D_3 (1,25$(OH)_2D_3$). Recent evidence indicates that in addition to the classical mode of action utilising the vitamin D receptor (see Fig. 3), 1,25$(OH)_2D_3$ can rapidly activate various kinase and second-messenger pathways: protein kinase A (PKA), NF-κB, mitogen-activated protein kinases (MAPK), and protein kinase C (PKC). Please note that not all of the pathways listed have been described in all cell types. SRC, receptor tyrosine kinase; P13K, phosphatidylinositol 3 kinase; PIP2, phosphatidylinositol-4,5 bisphosphate; PIP3, phosphatidylinositol-3,4,5 trispohosphate; AKT, protein kinase B; IKB, inhibitor of NFκB; Raf1, mitogen activated kinase kinase kinase; AP, activated protein; PLC, phospholipase C; DAG, diacylglycerol; IP3, inositol triphosphate.

suppression of protein kinase C activity with staurosporine or H7 inhibited 1,25$(OH)_2$D-regulated 25-hydroxyvitamin D 24-hydroxylase (CYP24) gene expression in proliferating, small-intestine crypt-like, rat IEC-6 cells (Koyama *et al.* 1994) and the activation of protein kinase C with phorbol esters enhanced 1,25$(OH)_2$D-regulated CYP24 gene transcription in IEC-6 and IEC-18 cells (Armbrecht *et al.* 2001). Similar findings have been observed for 1,25$(OH)_2$D-mediated osteocalcin gene expression in the osteoblast-like ROS 17/2·8 cell (Desai *et al.* 1995), CYP24 gene induction in COS-1 cells (Dwivedi *et al.* 2002), c-myc activation in proliferating skeletal muscle (Buitrago *et al.* 2001*a*) and CYP3A4 gene regulation in proliferating Caco-2 cells (Hara *et al.* 2002). Specific cross-talk between rapid, membrane-initiated vitamin D actions and nVDR-mediated genomic actions are also supported by the observation that an antagonist of the non-genomic pathway, 1β,25$(OH)_2$D, blocks 1α,25$(OH)_2$D-mediated osteocalcin gene transcription in osteoblasts (Baran *et al.* 1992).
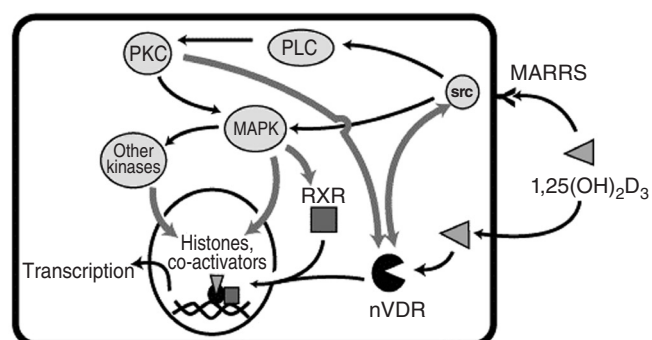

Fig. 5. A model of cross-talk between 1,25 dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) signalling pathways. The model here depicts simplified versions of the classical vitamin D receptor (VDR)-mediated transcriptional activation pathway and the rapid activation of kinases by 1,25$(OH)_2D_3$. The larger, grey arrows indicate potential points of cross-talk between the two pathways that are supported by the literature. This cross-talk includes a role of kinase pathways in modulating the transcriptional activity of the VDR as well as a non-transcriptional role for the VDR in transducing the signal to initiate kinase activity. PKC, protein kinase C; PLC, phospholipase C; src, receptor tyrosine kinase; MARRS, membrane-associated rapid response steroid-binding protein; MAPK, mitogen-activated protein kinase; RXR, retinoid X receptor; nVDR, nuclear VDR.

The mechanism for this interaction between 1,25$(OH)_2$D-induced kinase pathways and VDR-mediated gene transcription is unclear but is probably due to the modulation of protein–protein interactions that are responsible for the recruitment of co-activators necessary for the disruption of higher–order chromatin structure and transcriptional activation (see Fig. 3). For example, Paredes *et al.* (2002) showed that access to vitamin D response elements in the osteocalcin gene promoter is limited when the DNA is present in a chromosomal context. As a result, protein–protein interactions mediated by the nVDR are critical for chromosomal unwinding. That is, before transcriptional activation, the nVDR–retinoid X receptor dimer recruits cAMP-response element-binding protein-binding protein and steroid receptor co-activator-1 to make a complex with histone acetyl transferase activity that acetylates histones H_3 and H_4 and relieves the constraints imposed by chromatin structure (Chen *et al.* 1999; Freedman, 1999). Both cAMP-response element-binding protein-binding protein and steroid receptor co-activator-1 are targets of mitogen-activated protein kinase-mediated phosphorylation and this may lead to increased histone acetyl transferase activity of the complex (Rowan *et al.* 2000; Lee *et al.* 2003). In addition, after chromosomal unwinding, the nVDR–retinoid X receptor dimer recruits the mediator D complex (also known as DRIP) and utilises it to recruit and activate the basal transcription unit containing RNA polymerase II (Rachez *et al.* 1999; Chiba *et al.* 2000). Barletta *et al.* (2002) showed that the association between the nVDR and a member of the mediator complex, DRIP205, was enhanced following the treatment of cultured bone and kidney cells with the phosphatase inhibitor okadaic acid. This interaction enhanced 1,25$(OH)_2$D-mediated reporter gene

activity. These and other data suggest that 1,25(OH)$_2$D-mediated activation of various kinase pathways could lead to the phosphorylation of critical co-activators and this in turn could increase the transcriptional activation of vitamin D target genes by increasing VDR co-activator interactions. However, while this suggests that cross-talk between the two vitamin D signal pathways exists, more work is needed to solidify the connections between the rapid actions of 1,25(OH)$_2$D and the classical activation of the VDR by 1,25(OH)$_2$D.

Involvement of vitamin D receptor in the rapid activation of signal-transduction pathways. There is considerable controversy regarding the mechanism used by 1,25(OH)$_2$D to transduce a signal across the membrane of cells and activate kinase pathways. One line of evidence suggests that there may be a unique membrane receptor for 1,25(OH)$_2$D. This protein has been termed the membrane-associated rapid response steroid-binding protein (Farach-Carson & Nemere, 2003). This protein binds 1,25(OH)$_2$D with high affinity, is distinct from the classical VDR, and is found on the membrane of a number of cells that respond to 1,25(OH)$_2$D treatment by activating kinase pathways and stimulating Ca fluxes. However, in addition to this protein, several recent papers have indicated that the classical VDR may be involved in at least some of the rapid 1,25(OH)$_2$D signalling pathways.

Buitrago *et al.* (2000, 2001*b*) have demonstrated that in myocytes 1,25(OH)$_2$D treatment activates the membrane-associated tyrosine kinase, src kinase, and this coincides with an increased association of VDR with src kinase as well as tyrosine phosphorylation of the VDR, i.e. the VDR is a target of src kinase activation. Bettoun *et al.* (2003) found that in Caco-2 cells VDR is part of a terniary complex with the protein phosphatase PP1c and the p70 S6 kinase that is known to control the movement of cells through the G1–S transition in the cell cycle (Bettoun *et al.* 2003). Treatment of cells with 1,25(OH)$_2$D resulted in the PP1c-mediated dephosphorylation and inactivation of p70 S6 kinase. This indicates that rapid signalling requiring the VDR in a non-traditional role can lead to the growth arrest of proliferating Caco-2 cells in part through the inactivation of p70 S6 kinase. Finally, Erben *et al.* (2002) found that the 1,25(OH)$_2$D-mediated activation of Ca fluxes is blocked in osteoblasts from mice expressing a nVDR that lacks the DNA-binding domain. Although this observation conflicts with data generated by Wali *et al.* (2003) in VDR-null mice, coupled with the other data from cell systems, it suggests that the VDR may participate in at least some rapid signalling pathways.

Summary and conclusions

The purpose of the present review was to demonstrate that certain dogma that has been used to explain the biology of vitamin D action is falling under question. In the first part of the review, we discussed how in some circumstances the circulating level of 25(OH)D is a better marker of vitamin D action than serum 1,25(OH)$_2$D levels (the exception being severe dietary Ca insufficiency) even though the traditional thinking suggests that vitamin D acts in an endocrine fashion only after being converted to 1,25(OH)$_2$D in the kidney. Other data indicate that this is possible because extra-renal production of 1,25(OH)$_2$D is a critical component of the protection mediated by high serum 25(OH)D levels. This puts 1,25(OH)$_2$D in a new role as an autocrine or paracrine mediator rather than an endocrine hormone. In the second part of the review, we examined the evidence that rapid activation of signal-transduction pathways by 1,25(OH)$_2$D constitutes a new, and important mode of vitamin D action apart from the traditional activation of gene transcription mediated through the VDR. With the possibility that a paracrine role exists for 1,25(OH)$_2$D, we can begin to make physiological sense of these rapid actions for 1,25(OH)$_2$D. It will be interesting to learn whether regional fluxes in 1,25(OH)$_2$D levels due to local production could be occurring and signalling cells, even though serum levels of the hormone do not change. These data, while complicating our model of vitamin D action, provide new life to the study of the nutritional role of vitamin D and stress the importance of high vitamin D status for maximising the protective effects of vitamin D on Ca homeostasis and for cancer prevention.

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