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Nongenomic Regulation of Extracellular Matrix Events by Vitamin D Metabolites

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Abstract Vitamin D metabolites appear to regulate chondrocytes and osteoblasts via a combination of genomic and nongenomic mechanisms. Specificity of the nongenomic response to either 1,25-$(OH)_2D_3$ or 24,25-$(OH)_2D_3$ may be conferred by the chemical composition of the target membrane and its fluid mosaic structure, by the presence of specific membrane receptors, or by the interaction with classic vitamin D receptors. Nongenomic effects have been shown to include changes in membrane fluidity, fatty acid acylation and reacylation, arachidonic acid metabolism and prostaglandin production, calcium ion flux, and protein kinase C activity. Chondrocytes metabolize 25-$(OH)D_3$ to 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$; production of these metabolites is regulated by both growth factors and hormones and is dependent on the state of cell maturation. 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ may interact directly with extracellular matrix vesicles to regulate their function in the matrix, including protease activity, resulting in matrix modification and calcification. Isolated matrix vesicles, produced by growth zone chondrocytes, can activate latent transforming growth factor-β when incubated with exogenous 1,25-$(OH)_2D_3$. These observations suggest that nongenomic regulation of matrix vesicle structure and function may be a mechanism by which mesenchymal cells, like osteoblasts and chondrocytes, may modulate events in the extracellular matrix at sites distant from the cell surface.

Key words: 1,25-$(OH)_2D_3$, 24,25-$(OH)_2D_3$, matrix vesicles, nongenomic regulation, extracellular matrix, alkaline phosphatase, phospholipase A_2, protein kinase C

It is well known that vitamin D metabolites regulate bone formation, growth and remodelling, but recently, cartilage has also been found to be sensitive to these hormones. 1,25-$(OH)_2D_3$ has been shown to regulate the terminal differentiation of hypertrophic cartilage. In states of 1,25-$(OH)_2D_3$ deficiency, such as rickets, the hypertrophic cell zone greatly increases in size. Restoration of 1,25-$(OH)_2D_3$ results in mineralization of the extracellular matrix and resolution of the rachitic state. Infusion of calcium also heals the rickets [Weinstein et al., 1984; Holtrop et al., 1986] and this has led to the hypothesis that the primary function of 1,25-$(OH)_2D_3$ is to maintain the concentration of extracellular Ca ions.

Local injection of 24,25-$(OH)_2D_3$ into the upper tibial growth plate of rachitic rats can heal rickets as well [Atkin et al., 1985; Lidor et al., 1987]. It is unlikely that this is due to hydroxylation of 24,25-$(OH)_2D_3$ to 1,24,25-$(OH)_3D_3$ and subsequent action of 1,24,25-$(OH)_3D_3$ on Ca release, since local injection of 1,25-$(OH)_2D_3$ is relatively ineffective [Lidor et al., 1987]. Rather, it is more likely that 24,25-$(OH)_2D_3$ acts directly on chondrocytes in the upper growth plate to promote their differentiation along the endochondral lineage.

These observations indicate that both vitamin D metabolites have independent actions in cartilage that are related to the maturation state of the cell. In vitro studies in our laboratory, described below, also support this contention. Since receptors for both metabolites have been identified in cartilage [Corvol et al., 1980; Balmain et al., 1993; Fine et al., 1985], it is probable that many of the effects are via classical vitamin D receptor pathways, involving changes in gene transcription and messenger RNA stabilization

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[Kyeyune-Nyombi et al., 1991; Gerstenfeld et al., 1990; Schwartz et al., 1989; Sylvia et al., 1993]. This is clearly the case for 1,25-$(OH)_2D_3$, since experiments using chick limb bud mesenchymal cells [Boskey et al., 1992a], chick sternal chondrocytes [Gerstenfeld et al., 1990], and chick epiphyseal chondrocytes [Hale et al., 1986] all show 1,25-$(OH)_2D_3$-dependent synthesis of proteins such as alkaline phosphatase and osteocalcin. While less is known concerning the action of 24,25-$(OH)_2D_3$, its effects on various markers of chondrocytic differentiation strongly suggest that genomic regulatory mechanisms are involved. This is supported by studies using inhibitors of gene transcription and translation [Sylvia et al., 1993].

Recently, new evidence has suggested that at least some of vitamin D's effects in cartilage, as well as in bone, are via nongenomic mechanisms. A nongenomic action of the hormone is one that involves neither new gene transcription or protein synthesis. While many rapid effects of vitamin D metabolites may be nongenomic, time course of action is not an a priori proof, as new protein synthesis may occur even in very short time periods. Examples of nongenomic actions include changes in membrane fluidity [Swain et al., 1993], turnover of phospholipids [Swain et al., 1992; Schwartz et al., 1990], changes in Ca flux [Schwartz et al., 1991; Farach-Carson et al., 1991; Langston et al., 1990], and activation of protein kinase C [Sylvia et al., 1993]. However, even these actions may be downstream from a vitamin D-dependent nuclear event. Thus, the only proofs for nongenomic mechanisms are those which examine response in the absence of DNA, RNA, or protein synthesis.

This paper discusses a new model for examining nongenomic effects of two vitamin D metabolites: 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$. We summarize data gathered over the past 5 years that definitively show that both hormones exert at least some of their effects by such mechanisms. We also discuss the role of specific membrane receptors for vitamin D in the nongenomic process. Finally, we demonstrate how nongenomic regulatory mechanisms are used by cells, under physiologically relevant conditions, to modulate events in the extracellular matrix.

EXPERIMENTAL MODEL

We have used rat costochondral chondrocyte cultures to examine the mechanisms of vitamin D metabolite action. In this model, chondrocytes are derived from two distinct zones of costochondral cartilage, the resting zone (reserve zone) and the growth zone (prehypertrophic and upper hypertrophic zones), that enable us to study cells at two different stages of maturation. The chondrocytes derived from these two zones retain their differential phenotype through four passages in culture. These differences include: basal calcium flux [Schwartz et al., 1991; Langston et al., 1990], vitamin D metabolite production [Schwartz et al., 1992a], prostaglandin production [Schwartz et al., 1992b], and response to growth factors [Yang et al., 1991; Schwartz et al., 1993] and hormones [Boyan et al., 1988a; Schwartz et al., 1988]. Particularly striking is the differential responsiveness of these two cell types to vitamin D metabolites. In general, growth zone chondrocytes respond primarily to 1,25-$(OH)_2D_3$, whereas resting zone chondrocytes respond primarily to 24,25-$(OH)_2D_3$ [Boyan et al., 1988a; Schwartz et al., 1988, 1989, 1990].

Both types of chondrocytes produce extracellular matrix vesicles in culture; however, the phospholipid composition [Boyan et al., 1988b] and enzyme activity [Boyan et al., 1988a; Schwartz and Boyan, 1988] of these matrix vesicles depends on the cell of origin. Not only do matrix vesicles produced by growth zone chondrocytes differ from those produced by resting zone chondrocytes, but each type of matrix vesicle differs from the plasma membrane of the cell from which it was derived. Not surprisingly, the basal membrane fluidity of matrix vesicles is distinct from that of the plasma membrane [Swain et al., 1993], reflecting differences in structure and chemical composition.

Because matrix vesicles are external to the cells in the extracellular matrix, they can be isolated from cultures without disrupting the cell membranes. Following a brief trypsin digestion, matrix vesicles can be collected by differential centrifugation. They are right-side out since no homogenization is necessary and they are free of DNA, RNA, ribosomes, or contamination from intracellular membranes such as Golgi or endoplasmic reticulum.

The advantages of such a system for examining direct, nongenomic effects of steroid hormones are obvious. One can examine the genomic response of cells to 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ by incubating cultures with hormone and then studying isolated matrix vesicles in comparison with plasma membranes pre-

pared by differential centrifugation of cellular homogenates. By varying time course and using transcription and translation inhibitors, one can begin to sort out those responses which require genomic events. One can also isolate matrix vesicles and plasma membranes from naive cultures and incubate the membrane fractions with hormone in vitro, thereby obtaining definitive evidence of a nongenomic response.

Our early experiments demonstrate the power of the model [Schwartz et al., 1988]. When growth zone chondrocytes were incubated with 1,25-$(OH)_2D_3$, alkaline phosphatase specific activity was increased, whereas 24,25-$(OH)_2D_3$ had no effect. The 1,25-$(OH)_2D_3$-dependent response was found to be targeted to the matrix vesicles since there was no increase in plasma membrane alkaline phosphatase specific activity. When examining cell layer alkaline phosphatase specific activity in resting zone chondrocyte cultures exposed to 1,25-$(OH)_2D_3$ or 24,25-$(OH)_2D_3$, no apparent effect of either hormone was observed. However, matrix vesicles isolated from the 24,25-$(OH)_2D_3$-treated cultures did exhibit a dose-dependent increase in enzyme activity, whereas isolated plasma membranes did not. This indicated that the effects of 24,25-$(OH)_2D_3$ were only targeted to the matrix vesicles produced by resting zone chondrocytes and that it was necessary to isolate them to detect the hormone-specific effect.

In addition, phospholipase A_2 specific activity was assayed in isolated membrane fractions. 1,25-$(OH)_2D_3$ stimulated phospholipase A_2 specific activity in matrix vesicles isolated from growth zone chondrocyte cultures, but had no effect on the plasma membrane enzyme activity; in contrast, enzyme activity in both membrane fractions from resting zone chondrocyte cultures was unaffected. 24,25-$(OH)_2D_3$ inhibited phospholipase A_2 specific activity in matrix vesicles isolated from resting zone chondrocyte cultures, but had no effect on plasma membrane enzyme activity; enzyme activity in both of the membrane fractions from growth zone cultures was unaffected. Once again, to observe the 24,25-$(OH)_2D_3$-dependent effect on resting zone cultures, it was necessary to examine isolated matrix vesicles.

These data suggested that either the chondrocytes were making new matrix vesicles in response to hormone or that the hormones were acting directly on the matrix vesicles in the matrix. To test this, matrix vesicles were isolated from cultures of growth zone and resting zone chondrocytes, which had no prior exposure to hormone, and incubated with either 1,25-$(OH)_2D_3$ or 24,25-$(OH)_2D_3$. 1,25-$(OH)_2D_3$ stimulated both alkaline phosphatase and phospholipase A_2 specific activities in matrix vesicles isolated from growth zone chondrocytes, but had no effect on either enzyme in matrix vesicles isolated from resting zone cell cultures. In contrast, 24,25-$(OH)_2D_3$ stimulated alkaline phosphatase and inhibited phospholipase A_2 specific activities in matrix vesicles from resting zone cell cultures, but had no effect on either enzyme in matrix vesicles isolated from growth zone chondrocyte cultures.

These experiments clearly demonstrated that vitamin D metabolites were able to mediate specific effects in the absence of genomic machinery. Moreover, the direct effects were essentially identical to those observed in the intact cultures. Thus, it was clearly possible that even in vivo 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ might operate, at least in part, through nongenomic pathways.

NONGENOMIC MECHANISMS OF VITAMIN D METABOLITE ACTION

Membrane Receptors for Steroid Hormones

The experiments described above indicated that the direct effects of vitamin D metabolites were likely to occur on the membrane, since both of the enzymes examined are known to be membrane bound. The fact that the two matrix vesicle populations differ in both phospholipid composition and membrane enzyme activities suggested the possibility that the action of the metabolites reflected a specific interaction of the hormone with the membrane itself. It remains unclear whether specific receptors are involved.

The present data concerning nongenomic effects of vitamin D metabolites support three potential hypotheses, summarized in Figure 1: (1) a specific protein receptor does not exist and specificity is conferred by changes in the fluid mosaic microstructure of the membrane [Sheetz, 1993]; (2) specific membrane receptors do exist that are unique and distinct from the classical vitamin D receptor [Zhou et al., 1992; Nemere and Norman, 1994]; or (3) the classical vitamin D receptor interacts with the hormone as it passes through the membrane resulting in subsequent nongenomic actions [Kim et al., 1994]. Whether any of these hypotheses is correct remains to be determined. It is probable that they

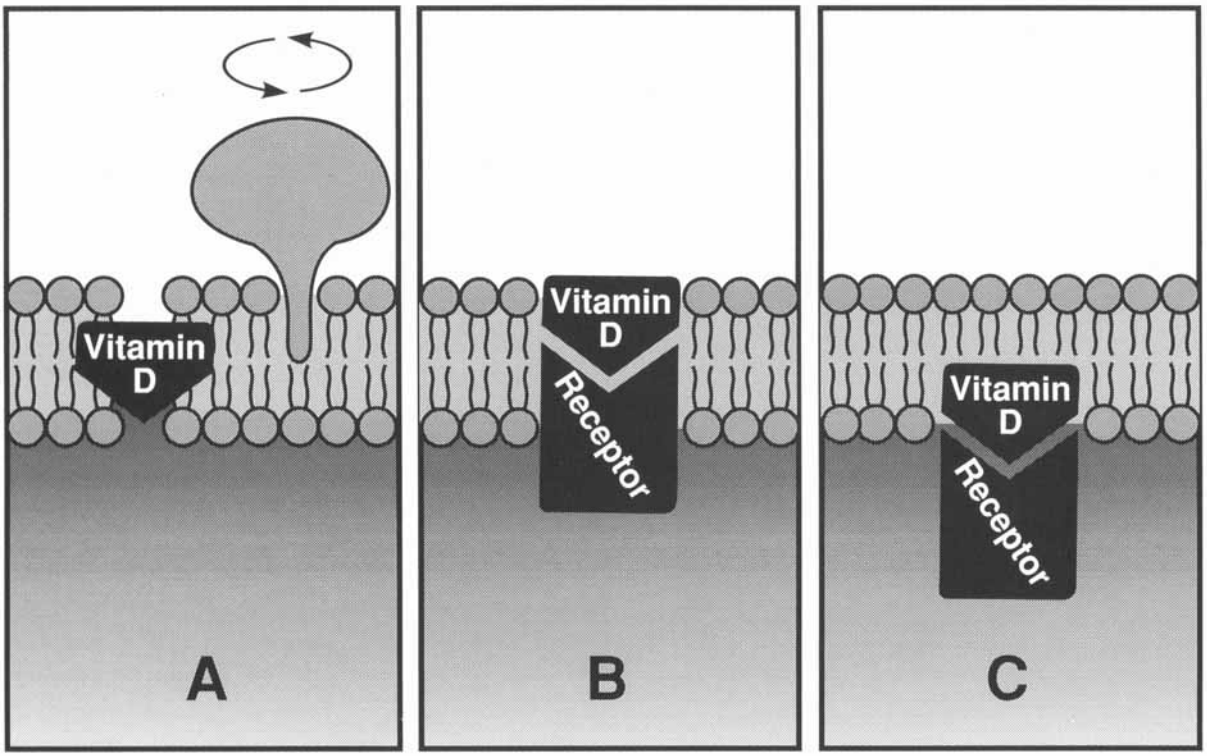

Fig. 1. Model showing how vitamin D metabolites may interact with cellular and matrix vesicle membranes. **A:** Vitamin D metabolite intercalates into the phospholipid bilayer, altering membrane fluidity, resulting in altered activity of membrane bound enzymes. **B:** Vitamin D metabolite interacts with specific membrane receptors. **C:** Vitamin D metabolite diffuses through membrane bilayer and interacts with the classic vitamin D receptor on the inner surface of the membrane.

are not mutually exclusive however, and may all play a role depending on the membrane and type of cell involved.

Membrane Fluidity

Our studies indicate that 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ have distinct effects on membrane fluidity that are cell maturation-dependent, membrane-specific, and metabolite-specific [Swain et al., 1993]. 1,25-$(OH)_2D_3$ rapidly increases fluidity of both plasma membranes and matrix vesicle membranes from growth zone chondrocyte cultures, but does not alter the fluidity of these membranes in resting zone cells. By contrast, 24,25-$(OH)_2D_3$ increases the fluidity of plasma membranes and matrix vesicle membranes from resting zone cell cultures but has no affect on those from growth zone chondrocyte cultures.

Changes in membrane fluidity, noted in these experiments, may be due to the physical presence of vitamin D metabolites in the membrane, as has been shown for cholesterol and cholesterol derivatives. Moreover, 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ differ from each other in charge density, resulting in a differential distribution in the membrane bilayer. Together with the differences in membrane composition, specificity may be conferred through physico-chemical interactions. In fact, the activity of membrane-bound enzymes has been shown to be sensitive to changes in membrane fluidity in a variety of systems and it is reasonable to assume that such could be the case in our model as well. The high levels of nonspecific binding reported by other laboratories for intestinal membrane 1,25-$(OH)_2D_3$ receptors [Norman et al., 1993; Nemere and Norman, 1994; Kim et al., 1994], strongly suggest that these lipophilic hormones rapidly incorporate into the membrane. However, to date, no one has actually measured the half-life of either metabolite in either membrane compartment.

Arachidonic Acid Turnover

In addition to the physical presence of vitamin D metabolites in the membrane, changes in fluidity may result from changes in fatty acid residues, both in their length and in their degree of saturation. Others have shown that such is the

case for intestinal brush border phospholipids in response to 1,25-$(OH)_2D_3$ [Matsumoto et al., 1981; Rasmussen et al., 1982]. To examine whether fatty acid metabolism plays a role in the chondrocyte model, we measured [^{14}C]-arachidonic acid incorporation and release. Within one minute of exposure to hormone, both acylation and reacylation are affected and the response is cell maturation dependent and metabolite specific [Schwartz et al., 1990; Swain et al., 1992].

Prostaglandin Production as a Second Messenger

The rapid effect of both 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ on arachidonic acid release suggested that prostaglandin production might also be affected. This is, in fact, the case [Schwartz et al., 1992b]. 1,25-$(OH)_2D_3$ stimulates PGE_2 production in growth zone chondrocyte cultures, whereas 24,25-$(OH)_2D_3$ inhibits production by resting zone cells. Addition of indomethacin to growth zone cultures inhibits the 1,25-$(OH)_2D_3$-dependent increase in alkaline phosphatase activity suggesting that PGE_2 might serve as a second messenger for the hormone.

Calcium Ion Flux

A consequence of altered membrane fluidity and phospholipid metabolism is altered calcium ion flux. Farach-Carson et al. [1991] and Norman et al. [1993] showed that in osteoblast-like cells (ROS 17/2.8) and intestinal epithelial cells, respectively, 1,25-$(OH)_2D_3$ stimulates rapid transcaltachia. By further studies using metabolites and analogues that discriminate between genomic and nongenomic actions, they have been able to show that transcaltachia involves a nongenomic mechanism. In the chondrocyte model, rapid effects of hormone on calcium ion flux are also observed and the response is both cell maturation dependent and metabolite specific [Schwartz et al., 1991; Langston et al., 1990]. Within 1 min of exposure to hormone, 1,25-$(OH)_2D_3$ stimulates ^{45}Ca efflux from growth zone chondrocytes, whereas 24,25-$(OH)_2D_3$ inhibits ^{45}Ca efflux from resting zone chondrocytes.

Protein Kinase C

The data just described suggested to us that multiple signal transduction mechanisms might be affected by the vitamin D metabolites. Several studies, suggested that protein kinase C was regulated by 1,25-$(OH)_2D_3$ in a diverse array of cell types [deBoland and Norman, 1990; van Leeuwen et al., 1992; Wali et al., 1990]. Using the chondrocyte model, new evidence concerning the complex nature of this regulatory pathway was uncovered [Sylvia et al., 1993]. When growth zone cells are incubated with 1,25-$(OH)_2D_3$, there is a time- and dose-dependent increase in protein kinase C activity within 9 minutes of exposure to hormone. No effect of 1,25-$(OH)_2D_3$ on resting zone cells is observed. While 24,25-$(OH)_2D_3$ has no effect on protein kinase C activity in growth zone chondrocytes, it stimulates resting zone chondrocyte enzyme activity. The time course, however, differs from that seen in the growth zone cells in response to 1,25-$(OH)_2D_3$; 24,25-$(OH)_2D_3$ effects are delayed and sustained over a longer period of time. Use of actinomycin D and cycloheximide demonstrates that the 1,25-$(OH)_2D_3$ effect is nongenomic whereas the 24,25-$(OH)_2D_3$ effect requires both new gene transcription and new protein synthesis.

When isolated matrix vesicles are incubated directly with exogenous hormone, the results become more complex. 1,25-$(OH)_2D_3$ and 24,25$(OH)_2D_3$ inhibit growth zone and resting zone chondrocyte matrix vesicle protein kinase C, respectively. Thus, 1,25-$(OH)_2D_3$ and 24,25$(OH)_2D_3$ can exert nongenomic effects directly on the matrix vesicles which are not detected when the cell layer is assayed (unpublished data). Furthermore, 1,25-$(OH)_2D_3$ stimulates protein kinase C activity in isolated plasma membranes from growth zone cells and 24,25-$(OH)_2D_3$ stimulates enzyme activity in plasma membranes from resting zone cells. This apparent paradox was resolved when we learned that the predominant protein kinase C isoform in matrix vesicles is zeta, whereas the predominant isoform in plasma membranes is alpha (unpublished data). Thus, one mechanism by which chondrocytes may differentially regulate events in matrix vesicles, without comparable effect on their plasma membranes, may be through activation or inhibition of specific isoforms of protein kinase C.

Summary

As depicted schematically in Figure 2, vitamin D metabolites can regulate events at the membrane through a variety of nongenomic mechanisms. Regardless of whether or not specific membrane receptors exist, or whether the classical vitamin D receptor serves as a docking port for vitamin D as it diffuses through or in the

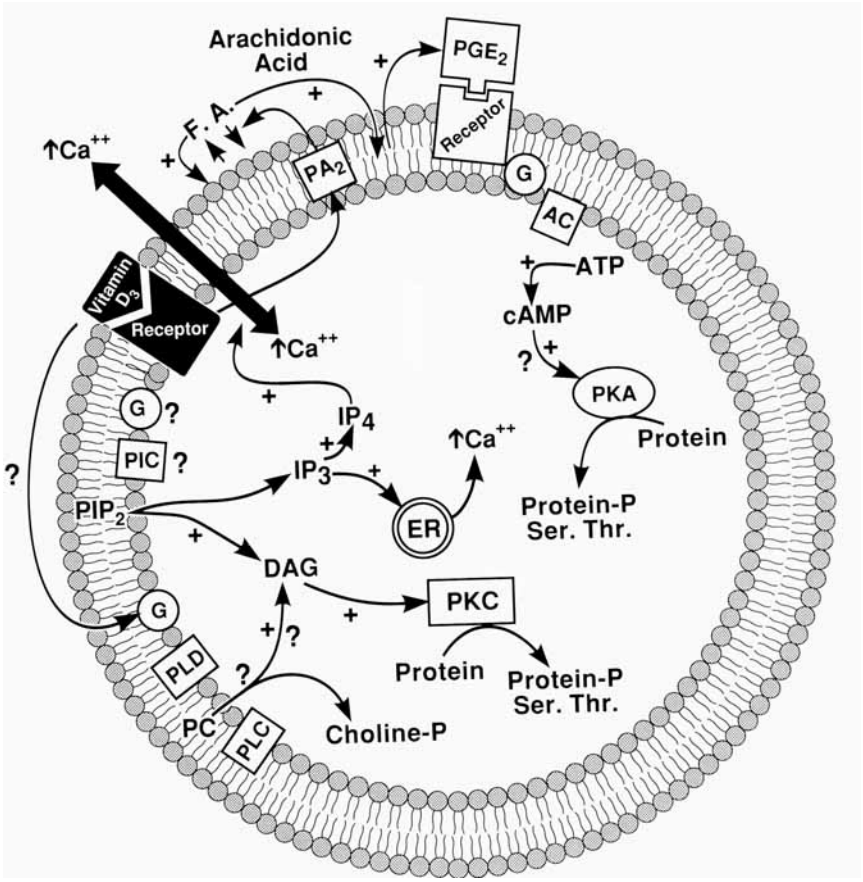

Fig. 2. Model of nongenomic transmembrane signaling by vitamin D metabolites. Vitamin D metabolites can interact with membranes as depicted in Figure 1. Upon addition of hormone to the membrane, three mechanisms are potentially activated: production of prostaglandins as second messengers; alterations in Ca flux; and changes in protein kinase C activity. Phospholipase A_2 (PA_2) is activated resulting in release of arachidonic acid as well as other fatty acids (F.A.), some of which are reacylated. Arachidonic acid is further metabolized to prostaglandin E_2 (PGE_2) which interacts with a specific receptor in the membrane. Via G proteins (G) and adenylate cyclase (AC), the local cyclic adenosine monophosphate (cAMP) concentration is increased, potentially resulting in stimulation of protein kinase A (PKA) and phosphorylation of proteins. Interaction of the vitamin D metabolite with the membrane may also activate phosphoinositide turnover. The action of phosphatidylinositol-specific phospholipase C (PIC) results in the release of phosphoinositol-4,5-bisphosphate (PIP_2) and diacylglycerol (DAG). PIP_2 may be further metabolized to inositol trisphosphate (IP_3) and inositol tetrakis-phosphate (IP_4), increasing Ca ion flux across the membrane and, through interaction with the endoplasmic reticulum (ER), alter the distribution of intracellular Ca^{+2}. Metabolism of phosphatidylcholine in the membrane by phospholipase C (PLC), releasing choline-phosphate, may also contribute to the DAG pool. Increased DAG concentrations may activate protein kinase C (PKC) with resultant phosphorylation of proteins. Phospholipase D (PLD) may play a role as well.

membrane, $1,25\text{-}(OH)_2D_3$ and $24,25\text{-}(OH)_2D_3$ modify the physical characteristics of the bilayer and change enzyme activity. One consequence of hormone action is to alter fatty acid acylation and reacylation. Released arachidonic acid may serve as a substrate for cyclooxygenase, resulting in altered prostaglandin production. Action of prostaglandin on the cell may result in changes in cAMP content. Changes in membrane fluidity, phospholipid metabolism and cAMP production may result in altered Ca ion flux and protein kinase C activity. The net consequence of this may be changes in phosphorylation of proteins required for a variety of cellular and/or matrix vesicle responses.

PHYSIOLOGIC RELEVANCE OF NONGENOMIC REGULATION OF MATRIX VESICLES

While the cell can down-regulate undesired nongenomic effects at the plasma membrane, this is more difficult in the matrix. To control events in the matrix, the cell may modulate the rate and nature of matrix vesicles synthesized by two mechanisms. Initially, matrix vesicles are produced under genomic control. $1,25\text{-}(OH)_2D_3$ and $24,25\text{-}(OH)_2D_3$ regulate the compo-

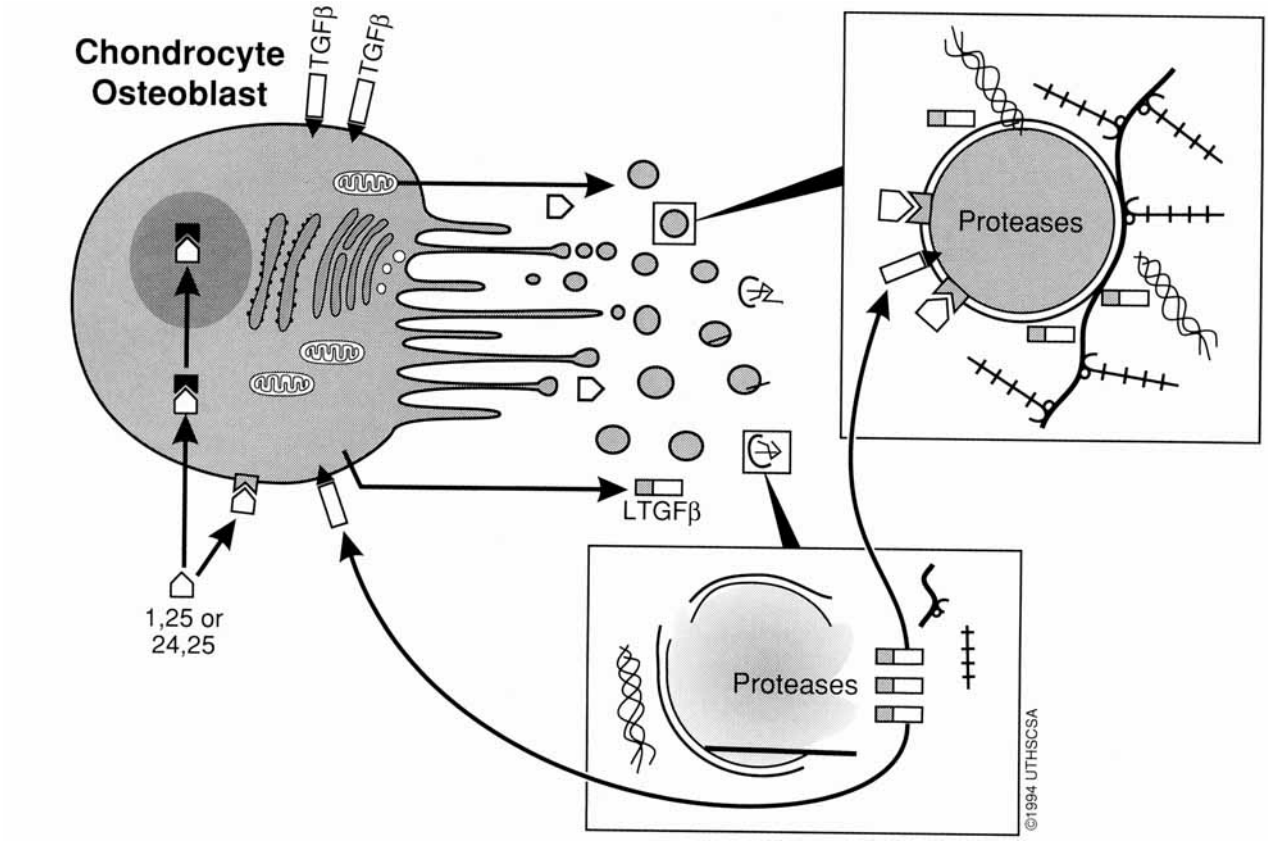

Fig. 3. Model of nongenomic regulation of extracellular matrix events by vitamin D metabolites. 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ may regulate events in the cell either via classic genomic mechanisms involving specific vitamin D receptors and their translocation into the nucleus as well as messenger RNA stabilization (for example, production of new matrix vesicles and synthesis and secretion of latent transforming growth factor-β (LTGFβ), collagen, and proteoglycan). In addition, vitamin D metabolites may interact with the cellular membrane as shown in Figure 1, initiating a series of nongenomic responses as shown in Figure 2. 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ produced by the cells are secreted into the extracellular matrix interacting directly with matrix vesicles (top inset), initiating a series of nongenomic responses. Initially, matrix vesicles are embedded in a matrix of collagen and proteoglycan aggregate, as well as other noncollagenous proteins including LTGFβ. The matrix vesicles undergo maturation, exhibiting increased alkaline phosphatase and phospholipase A_2 specific activities, and increasing in diameter as hydroxyapatite crystals form on the inner leaflet of the matrix vesicle membrane. As the integrity of the matrix vesicle membranes is lost, active proteases in the vesicles are released (bottom inset). Proteoglycans in the extracellular matrix are degraded and LTGFβ is activated. The active TGFβ acts back on the cell, resulting in an autocrine feedback loop. TGFβ may also act directly on other matrix vesicles (top inset), fine-tuning their activity.

sition of matrix vesicles through new gene transcription, protein synthesis and, finally, membrane synthesis. Once matrix vesicles are released into the extracellular matrix, the cell may regulate their maturation through secretion of vitamin D metabolites which act on the matrix vesicle through nongenomic mechanisms.

If this is the case, then it is obligatory that the cells produce vitamin D metabolites and that this production be regulated by growth factors and hormones. In fact, chondrocytes have been found to produce both [^{3}H]-1,25-$(OH)_2D_3$ and [^{3}H]-24,25-$(OH)_2D_3$ when incubated with [^{3}H]-25-$(OH)D_3$ [Schwartz et al., 1992a]. Moreover, production of vitamin D metabolites is regulated by 1,25-$(OH)_2D_3$, 24,25-$(OH)_2D_3$, TGFβ, and dexamethasone in a cell maturation-specific manner.

Matrix vesicles may have multiple functions in the matrix. Those in the lower hypertrophic cell zone of cartilage or in the osteoid of bone are probably involved in matrix calcification [Sela et al., 1992]. In addition, matrix vesicles also appear to be involved in matrix maturation, since they contain matrix-processing enzymes that degrade proteoglycans [Dean et al., 1992, 1994]. Recently, we showed that matrix vesicles can reverse the inhibition of calcification caused by proteoglycan aggregate in vitro [Boskey et al., 1992b], suggesting that the proteinases in matrix vesicles are active in a functional capacity as well.

In addition to their role in calcification, it now seems evident that matrix vesicles may play an important role in activation of growth factors present in the extracellular matrix. When chondrocytes are incubated with vitamin D metabolites, only growth zone cells incubated with 1,25-$(OH)_2D_3$ show a decrease in the amount of latent TGFβ found in the conditioned media. This decrease is further correlated with an increase in active TGFβ when isolated matrix vesicles are incubated directly with latent TGFβ and 1,25-$(OH)_2D_3$ in vitro. These observations suggest that nongenomic regulation of matrix vesicles can result in changes in local growth factor activation. This is a particularly attractive hypothesis in cartilage where activation of latent growth factor by local decreases in pH (as occurs in osteoclasts) have not been reported.

These observations have led us to propose the following scheme for nongenomic regulation of events in the extracellular matrix (Fig. 3). Chondrocytes produce matrix vesicles under hormonal and growth factor regulation. At the same time vitamin D metabolites are synthesized by the cells and secreted in response to regulatory factors like 1,25-$(OH)_2D_3$, 24,25-$(OH)_2D_3$, TGFβ, or corticosteroids [Schwartz et al., 1993]. These factors diffuse into the matrix and interact directly with the plasma membrane. In addition, they also interact with the matrix vesicle membrane, where their effects initiate a cascade of biochemical events which lead to maturation of the matrix vesicle, hydroxyapatite crystal formation, degeneration of the integrity of the matrix vesicle membrane, and eventual release of active proteases. The proteases then degrade proteoglycan aggregates in the vicinity of the matrix vesicle, facilitating extracellular matrix calcification. In addition, they may activate latent growth factors which can then act on the cell in an autocrine manner or on adjacent cells via paracrine interactions.

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