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Transcriptional Control of Vitamin D-Regulated Proteins

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Abstract Vitamin D is a physiological regulator of gene transcription associated with control of a broad spectrum of biological processes that include but is not restricted to growth, differentiation and calcium-mediated homeostatic control. Transcriptional regulation is mediated by sequence-specific interactions of a $1,25(OH)_2D_3$-vitamin D receptor-accessory factor complex with vitamin D responsive elements (VDRE) residing in the promoters of hormone responsive genes. Functioning primarily as a transcription enhancer, activity at the VDRE is controlled by diverse and integrated cellular signalling pathways acting synergistically and/or antagonistically with a series of basal regulatory elements and other hormone regulated sequences that are components of modularly organized vitamin D-responsive gene promoters. Molecular mechanisms that integrate the activities at promoter elements contributing to vitamin D-related transcriptional control include overlapping transcription factor binding domains within regulatory elements and cooperative activities at independent regulatory sequences that determine the level of vitamin D responsiveness.

Key words: differentiation, osteocalcin, osteoblast, vitamin D, responsive element, promoter elements

The regulatory role of vitamin D in controlling expression of a broad spectrum of genes is becoming increasingly evident. Such regulation by the calcitropic hormone is operative in a complex and highly integrated series of signalling pathways modulating expression of genes that include, but are not restricted to, those associated with cell growth, tissue-specific structure and function and general metabolic parameters supporting calcium-mediated homeostatic control [Minghetti and Norman, 1988; DeLuca, 1988; Suda et al., 1990; Stern, 1990]. A complete understanding of the multiple influences exerted by vitamin D on gene expression necessitates the elucidation of molecular mechanisms that impinge on the transcription of vitamin D-responsive genes as well as on post-transcriptional regulatory events. Not to be dismissed is the growing awareness of non-vitamin D receptor-regulated control by the hormone (frequently referred to as "rapid" effects; see Prospect by A.W. Norman, this issue). However, in this Prospect, we will restrict our considerations to transcriptional control of vitamin D responsive proteins. And here, rather than being inclusive, we will focus on several concepts and experimental approaches associated with mechanisms by which the structural organization of vitamin D-regulated genes supports responsiveness to physiological signals that determine the level of transcription. The bone-specific osteocalcin gene will be used to illustrate the biological and molecular parameters of vitamin D-mediated transcriptional control and where information is available, we will present the extent to which analogous mechanisms support transcription of other vitamin D-regulated genes.

BIOLOGICAL PARAMETERS OF VITAMIN D RESPONSIVENESS

Despite the extensive efforts that have been directed towards experimentally addressing the influence of vitamin D on biological processes, we are only beginning to appreciate the complexity of the regulatory mechanisms impinged upon by the hormone. We are becoming acutely aware of the diversity of cellular information exchange pathways that are involved in transducing vitamin D-mediated signals to specific genes [reviewed in Ozono et al., 1991]. The extensive integration of cellular signals are functionally related to both the timing and extent to which vitamin D-regulated genes are transcribed [Owen

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et al., 1991; Studzinski et al., 1985]. Borrowing from the vocabulary of our clinical colleagues, we can now responsibly make a preliminary diagnosis of the biological problems associated with vitamin D-regulated gene expression and write a prescription for experimental approaches that will further contribute to resolution of a series of important molecular mechanisms operative in physiological control.

From a historical perspective, it has been well known for a number of years that vitamin D anabolically and catabolically modulates bone cell metabolic activities, and more recently it has become apparent that this occurs through selective expression of a series of vitamin D-responsive genes. Therefore, several fundamental questions involve 1) the identification and characterization of the regulatory elements in skeletal associated gene promoters that respond to the hormone; 2) the mechanisms by which vitamin D-responsive sequences of specific skeletal genes are selectively rendered competent to bind the vitamin D receptor complex in a cell and tissue specific manner and/or at particular stages of differentiation; and 3) the combined influence of multiple physiological mediators on vitamin D responsiveness at the level of transcriptional control.

The vitamin D responsiveness of osteocalcin gene expression during progressive development of the bone cell phenotype in primary cultures of normal diploid osteoblasts provides a striking example of the complex biological parameters that contribute to the transcriptional level of a vitamin D-regulated gene [Owen et al., 1990a, 1991]. Developmentally regulated gene expression is indicated by the absence of osteocalcin gene transcription, cellular mRNA levels, or osteocalcin biosynthesis in proliferating osteoblasts. Osteocalcin gene expression is induced post-proliferatively and expressed at an elevated level in mature osteoblasts at the onset of extracellular matrix mineralization. Elevated levels of osteocalcin biosynthesis are paralleled by an upregulation of transcription and cellular accumulation of osteocalcin mRNA. Transcriptional control of osteocalcin gene expression involves an apparently non-steroid hormone-dependent activation and subsequent maintenance of basal levels of osteocalcin mRNA synthesis, rendering the osteocalcin gene competent for increased transcription in response to $1,25(OH)_2D_3$. The requirement of basal transcription for vitamin D responsiveness of the osteocalcin gene indicates that the hormone contributes to the level of transcription as an enhancer rather than as an activation macromolecule. The complexity of the relationship between basal expression and vitamin D-mediated enhancement of osteocalcin gene transcription is further reflected by a reciprocal relationship between the level of basal transcription and the extent to which transcription by hormone can be upregulated [Owen et al., 1991]. The interrelationship of basal expression to vitamin D enhancement is further suggested by a growing body of experimental results indicating that while several independent promoter domains serve as the principal regulatory elements responsible for basal and vitamin D-enhanced components of transcription, each shares overlapping activities of other known regulatory sequences. Furthermore, following transcriptional activation, evidence from both deletion mutagenesis [Demay et al., 1990; and Kerner et al., 1989] and in vivo competition experiments with specific regulatory sequences (our laboratory) indicate that the VDRE contributes to physiologic transcription controlled by proximal promoter sequences.

The sequential expression of genes during osteoblast differentiation further provides an example of a biological context within which regulatory mechanisms that render genes transcriptionally responsive to vitamin D is operative. The hormone is a physiologic mediator of collagen gene expression in proliferating osteoblasts and post-proliferative expression of the alkaline phosphatase gene [Owen et al., 1991]. Osteopontin and matrix Gla protein are vitamin D regulated throughout osteoblast development. Here, an understanding of the utilization of vitamin D-responsive promoter regulatory sequences and unquestionably a diverse series of cellular signalling pathways to modulate the activity of vitamin D gene promoter regulatory elements is required. Resolution of transcription regulatory mechanisms from a biological perspective is not restricted to identification and characterization of gene regulatory elements and their cognate binding factors. Rather, insight is required into the tissue-specific and developmental stage-specific mechanisms by which a single species of vitamin D receptor selectively binds to specific vitamin D-responsive promoter elements in a broad spectrum of vitamin D-responsive genes. Thus, an understanding of the biological parameters of vitamin D-mediated transcriptional control must be addressed from the standpoint of a highly integrated cascade of

regulatory events associated with specific cellular factors that are modulated to accommodate both homeostatic regulatory adjustments and long-term commitments to gene expression that support specialized cellular functions.

Vitamin D-mediated transcriptional control is functionally related to the representation of transcription factors in the nucleus that reflect the cellular phenotype, with both positive and negative control operative, together with coordinate control of genes expressed concomitantly. It therefore follows that there appears to be multiple rate limiting steps for determining the extent of vitamin D-responsive transcriptional activity. A reasonable expectation is that the regulation of phenotype-associated "accessory factors" [Liao et al., 1990; Ross et al., 1992] that complex with the vitamin D receptor and/or receptor-binding promoter sequences, establish permissive sequence-specific regulation at vitamin D responsive promoter elements.

MOLECULAR ANATOMY OF VITAMIN D-RESPONSIVE SEQUENCES

Sequence Organization of Vitamin D-Responsive Gene Promoters Reflects Hormone-Mediated Control

The complexity of vitamin D-mediated transcriptional control is illustrated by direct sequence-specific interactions of hormone-receptor complexes in the vitamin D-responsive gene promoters, which are accompanied by and functionally related to modifications in protein/DNA interactions at non-vitamin D receptor promoter regulatory sequences [Markose et al., 1990; Bortell et al., in press]. Sequence analysis of the osteocalcin gene and tissue-restricted expression in bone [Lian et al., 1989; Yoon et al., 1988] provides an indication of the complexity of transcriptional and to some extent the post-transcriptional regulation that mediates the level at which the osteocalcin gene is expressed (Fig. 1). The sequence organization of the mRNA coding region of the gene reflects the biochemical events associated with processing of the initially synthesized 10,000 kD precursor. The representation of consensus sequences in the promoter for regulatory elements that are responsive to vitamin D and other steroid hormones, basal regulatory factors and tissue-specific transactivation factors contributes to our understanding of regulatory mechanisms and biological activity [Lian et al., 1989]. Identification and characterization of proteins that interact in a sequence-specific manner with their cognate regulatory elements in the osteocalcin gene promoter further establishes the physiological mediators of osteocalcin gene transcription and the circumstances under which they participate in regulation during development, tissue maintenance and in skeletal disorders.

Consensus sequences occur in the osteocalcin gene promoter for responsiveness to a series of physiological mediators (e.g., cAMP and γ-interferon [Nanes et al., 1990]) of expression. In addition to a vitamin D-responsive element (VDRE), sequences bearing homology to other steroid receptor binding complexes (e.g., thyroid hormone, estrogen, and glucocorticoids) [Lian et al., 1989; Pike, 1990] are represented. As with many genes transcribed by RNA polymerase II, a TATA motif is located at −31 to −27, a CCAAT element at −91 to −87, and AP-1 sites are present at several sites in the osteocalcin gene promoter. However, while such consensus sequences provide an indication of potential regulatory mechanisms that may be operative, several lines of evidence are necessary to establish function, among which are 1) demonstration of an influence on transcriptional activity by deletion, substitution or site-specific mutagenesis; 2) identification and characterization of sequence-specific regulatory element occupancy by cognate transcription factors; and 3) modifications in protein/DNA interactions as a function of biological activity.

In the case of the osteocalcin gene promoter, to date only four regulatory sequences, which are schematically illustrated in Figure 1, have been established by the above criteria. The basal elements all contribute to vitamin D-mediated transcriptional control of osteocalcin gene expression. The osteocalcin box, a 24 nucleotide element with a CCAAT motif as the central core, is a highly conserved and essential basal regulatory sequence required for expression [Lian et al., 1989; Demay et al., 1990]. The TATA sequence residing at −31 to −27 is also required for rendering the osteocalcin gene transcribable. For both of these elements, a direct involvement in transcriptional control has been provided by several experimental approaches in intact cells [Owen et al., 1990b; Kerner et al., 1989; Demay et al., 1990] and sequence-specific protein/DNA interactions have been demonstrated which exhibit variations with modifications in biological activity, notably during development of the osteoblast phenotype and/or following vitamin D treatment [Markose et al., 1990; Lian and Stein,

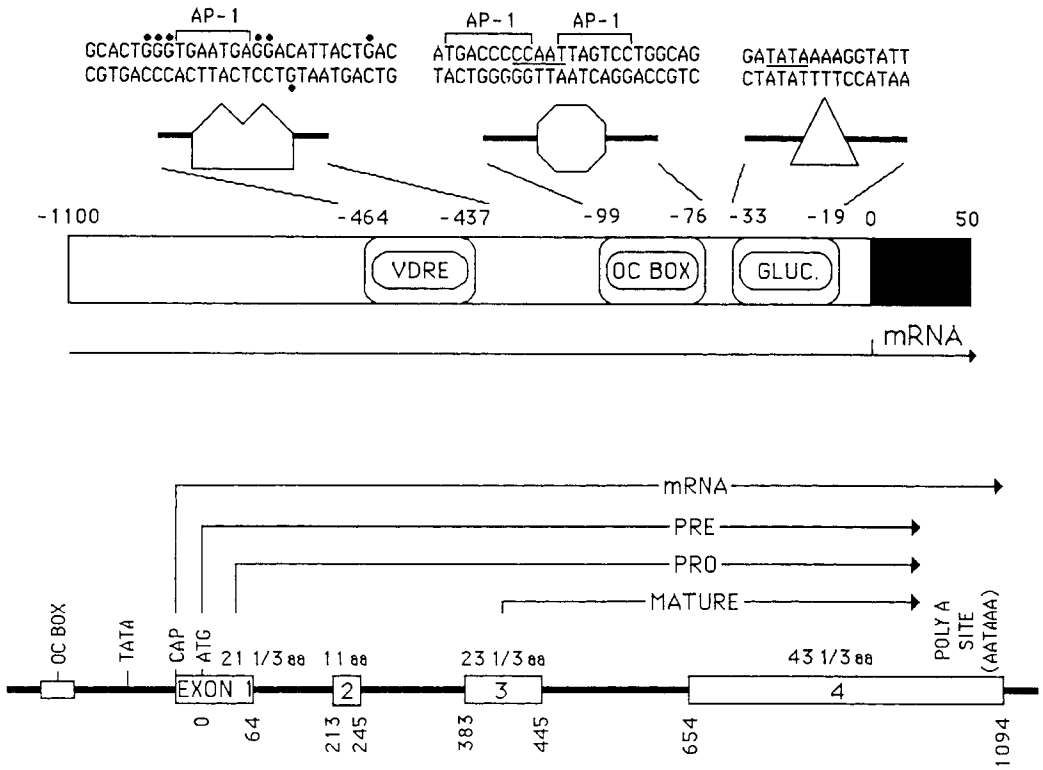

Fig. 1. Structural organization of the rat osteocalcin gene. **Top:** Sequences of those regulatory elements in the proximal promoter that have been defined and partially characterized. These include the vitamin D-responsive element (VDRE); the osteocalcin box (OC box), which is a primary proximal transcription regulatory element containing the CCAAT motif as a central core; and the glucocorticoid response element containing a TATA motif (GLUC), which is analogous to that defined in the human gene [Stromstedt et al., 1991]. Within the OC box and VDRE elements are also found active AP-1 sites which bind the oncogene-encoded Fos/Jun protein complex. The solid circles above or below G residues indicate vitamin D receptor protein/DNA interactions defined at single nucleotide resolution within the vitamin D-responsive element [Markose et al., 1990]. **Bottom:** With reference to the OC box and TATA promoter elements, intron and exon organization and location of the pre-, propeptide, and mature osteocalcin forms are illustrated.

in press]. The vitamin D-responsive element (−464 to −437) and a glucocorticoid-responsive element (GRE) associated with the TATA domain have been directly shown to modulate steroid hormone effects on osteocalcin gene transcriptional activity [Stromstedt et al., 1991]. These independent regulatory elements of the modularly organized osteocalcin gene promoter are influenced not only by basal levels of expression but by mutual interaction of the steroid responsive elements [Bortell et al., in press]. For example, a synergistic action of vitamin D with dexamethasone on osteocalcin transcription in normal diploid osteoblasts and the abrogation of the vitamin D upregulation of the osteocalcin gene by dexamethasone in osteosarcoma cells has been observed [Schepmoes et al., 1991; Stein et al., 1992].

The Vitamin D-Responsive Element (VDRE)

It is the VDRE of the osteocalcin gene that was the first vitamin D receptor promoter binding sequence to be identified [Kerner et al., 1989; Demay et al., 1990; Markose et al., 1990; Terpening et al., 1991; Morrison et al., 1989]; and, as we gain further insight into the structural and functional properties of the osteocalcin gene VDRE, the complexity of regulatory events at this transcription control element is becoming increasingly apparent. Perhaps this is an indication of the basis for involvement of vitamin D as a mediator of osteocalcin gene expression within the context of a broad spectrum of physiological responses of the osteoblast.

The VDRE of the rat and human osteocalcin genes have been identified and characterized by two approaches—determining the ability of systematically introduced deletion or nucleotide substitutions to influence vitamin D upregulation of osteocalcin gene transcription [Terpening et al., 1991; Morrison et al., 1989; Demay et al., 1990; Kerner et al., 1989] and defining at single nucleotide resolution, the binding of vita-

min D receptor complexes [Markose et al., 1990]. As shown in Figure 2, the osteocalcin gene VDRE of mammals with and without tails contain steroid half-element motifs with a 3 nucleotide spacer. Of interest, the recent identification of osteopontin [Noda et al., 1990] VDRE shows a similar motif that can confer vitamin D responsiveness. Figure 2 also indicates consensus sequence for vitamin D responsive element in the calbindin promoters (S. Christakos, New Jersey Medical School, Newark, NJ, personal communication). Similar VDRE consensus sequences have been located within vitamin D-responsive regions of the collagen (D. Rowe laboratory, University of Connecticut, Farmington, CT), calcitonin (R. Gagel laboratory, Baylor University, Houston, TX, personal communication) and parathyroid hormone (H. Kronenberg Laboratory, Harvard University, Boston, MA, personal communications). These, however, have not been definitively defined by mutagenesis. Additionally, consensus sequences for another steroid hormone, retinoic acid, and for the nuclear proto-oncogene-encoded Fos and Jun proteins [Lian et al., 1989] overlap the vitamin D receptor binding domain of the osteocalcin gene VDRE. Results support the ability of retinoic acid [Schüle et al., 1990a] and the Fos-Jun complex [Owen et al., 1990b] to modulate osteocalcin gene transcription, providing a functional basis for synergistic and/or antagonistic control of multiple regulatory activities within the VDRE. Such activities within the VDRE in a broader context reflect the contribution of multiple promoter binding factors competing for or complexing with and thereby promoting and/or inhibiting interactions at a single regulatory element. Further, an explanation is in part provided for positive or negative activity of a single regulatory sequence under different biological conditions on the basis of variations in the representation of factors that have the potential for binding.

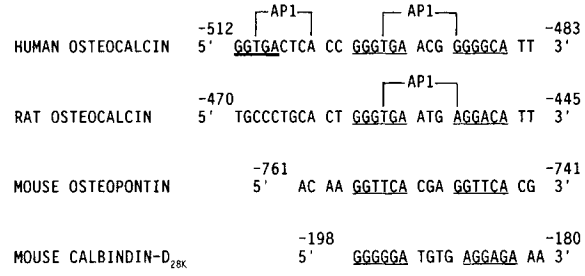

Fig. 2. Sequence comparison of VDREs in 4 promoters of vitamin D-responsive genes that have been identified and partially characterized. The homologous half steroid elements are indicated by the underlined sequences. AP-1 motifs, within the rat osteocalcin gene VDRE and both within and upstream from the human osteocalcin gene VDRE, that have been established as binding sites for the Fos/Jun complex are bracketed. The AP-1 containing sequences within the additional steroid half element (designated by double underline) upstream of human osteocalcin gene VDRE is not required for vitamin D receptor binding [Ozono et al., 1990]. The mouse osteopontin VDRE contains two identical half elements [Noda et al., 1990]. In contrast to a 3 nucleotide spacer between the steroid half elements in the VDRE elements of the osteocalcin, osteopontin and calbindin genes, the calbindin-D_{28K} promoter VDRE contains a 4 nucleotide spacer between the steroid half elements (S. Christakos, New Jersey Medical School, Newark, NJ, unpublished observations).

Competency for occupancy of the VDRE by the vitamin D–vitamin D receptor complex is requisite for enhancement of osteocalcin gene transcription by the hormone. Equally important is the mechanism by which the VDRE is rendered refractory to binding of the regulatory complex in immature osteoblasts not expressing osteocalcin and in non-osteoblastic cells. The answers to these longstanding biological questions are undoubtedly to a large extent the complement of proteins that complex with the VDRE. Protein/DNA interactions and protein/protein interactions must both be considered. Results of Markose et al. [1990], from studies where the protein/DNA interactions at the VDRE were mapped at specific guanine residues using nuclear extracts from osteosarcoma cells expressing osteocalcin under the influence of vitamin D, provided the first indication of the complexity of transcription factor binding at the VDRE. Subsequent results from the Pike [Liao et al., 1990] and H. DeLuca [Ross et al., 1992] laboratories, demonstrating the requirement of an accessory factor for binding of the vitamin D receptor complex to the osteocalcin gene VDRE, and the findings of DeLuca and co-workers [Brown and DeLuca, 1990] and the Haussler research group [Haussler et al., 1988] establishing vitamin D receptor phosphorylation as essential for hormone-receptor complex formation and transactivation (H. DeLuca, personal communication), reflects the complexity of events associated with activity of the VDRE. Modifications mediating and/or as a reflection of activity at the osteocalcin gene VDRE under various biological circumstances is suggested by striking differences in VDRE protein/DNA interactions in normal diploid osteoblasts compared with osteosarcoma cells and during progression of osteoblast differentiation [Stein et al., 1992].

Phenotype Suppression: A Postulated Mechanism for the Coordinate Regulation of Vitamin D-Mediated Enhancement and Basal Transcription of the Osteocalcin Gene by Fos-Jun Interactions at AP-1 Sites Within the VDRE and Other Promoter Elements

A mechanism by which vitamin D modulates the extent to which the osteocalcin is transcribed post-proliferatively in osteoblasts may involve regulation of Fos-Jun binding at the VDRE and at the OC box where we identified a series of AP-1 consensus sequences [Lian et al., 1989; Markose et al., 1990; Owen et al., 1990b] and demonstrated sequence-specific interactions of these promoter binding sequences with a stable heterodimeric Fos-Jun complex [Owen et al., 1990b]. The possibility therefore arises that Fos and Jun proteins expressed in proliferating osteoblasts could suppress osteocalcin gene transcription until late in the development of the bone cell phenotype.

Several experimental results support the concept of coordinate occupancy of the Fos-Jun protein complex at the VDRE and OC Box regulatory elements providing a potential molecular mechanism to account for the absence of osteocalcin gene expression in proliferating osteoblasts. Expression of c-fos and c-jun have been shown to occur primarily during the proliferative period of the osteoblast developmental sequence [Owen et al., 1990a; Shalhoub et al., 1989]. Secondly, AP-1 binding activity is observed primarily in proliferating osteoblasts and dramatically decreases after the down-regulation of proliferation and the initiation of extracellular matrix maturation and mineralization at which time osteocalcin gene transcription is initiated [Owen et al., 1990b]. Clearly distinct patterns of protein-DNA interactions occur at the VDRE and OC box with nuclear protein extracts from proliferating (day 5) osteoblasts which do not express osteocalcin compared to differentiated cells. Moreover, mutations introduced into the VDRE of the osteocalcin gene that block vitamin D receptor binding result in Fos-Jun interactions at the AP-1 site within the VDRE. This Fos-Jun interaction is not observed when the vitamin D-receptor complexes are bound to the VDRE sequence (this laboratory, unpublished abstract). Even in tumor cells (ROS 17/2.8) where osteocalcin expression is deregulated, in that transcription is ongoing in proliferating cells, a reciprocal relationship between AP-1 activity and OC gene expression is maintained. In confluent culture AP-1 activity is markedly diminished and osteocalcin mRNA and transcription is increased tenfold. The loss of AP-1 activity during the developmental sequence of osteoblast maturation coincides with marked changes in protein-DNA interactions in osteocalcin gene promoter elements having AP-1 sites. Further, we observed that protein-DNA contacts do not occur at G residues within the AP-1 consensus sequences of the VDRE and OC box when the gene is actively transcribed [Markose et al., 1990]. Additionally, experiments in which transfection of c-fos and c-jun into cells expressing osteocalcin resulted in the downregulation of osteocalcin gene transcription further supports a Fos-Jun-mediated suppression of the osteocalcin gene [Schüle et al., 1990a]. These results are consistent with a model in which coordinate occupancy of the AP-1 sites in the VDRE and osteocalcin box in proliferating osteoblasts may suppress both basal level and vitamin D-enhanced osteocalcin gene transcription, a phenomenon described as phenotype suppression [Owen et al., 1990b; Lian et al., 1991]. A remaining question is the mechanism by which the osteocalcin gene is rendered transcribable and vitamin D responsive following the downregulation of proliferation. Possibilities include release or modification of the Fos-Jun complex or association with other DNA binding proteins or protein-protein interactions.

We have demonstrated Fos-Jun binding to the internal AP-1 site in the VDRE of the human gene [Lian and Stein, in press]. However, the human VDRE in contrast to the rat VDRE has an additional upstream AP-1 site (Fig. 2). While the internal AP-1 site may function in suppression of transcription, the upstream AP-1 site in the human VDRE has been shown to enhance vitamin D-stimulated gene transcription, but is not necessary for vitamin D regulation [Ozono et al., 1990]. It is therefore not surprising that responsiveness of the human and rat osteocalcin genes, to factors that influence AP-1 activity, may in fact differ. Thus, the presence of 2 AP-1 sites may contribute to different functional responses of the human gene compared to the rat. Yet, the similar organization of the internal AP-1 sites of the VDRE and the identical AP-1 organization in the osteocalcin box for the human and rat osteocalcin genes suggests that there are some similar functional properties of

the elements, perhaps in regulating expression in relation to the proliferative state of the cells.

INTEGRATION OF ACTIVITIES OF MULTIPLE PROMOTER ELEMENTS CONTRIBUTE TO VITAMIN D-MEDIATED TRANSCRIPTIONAL CONTROL

Transcriptional control of vitamin D-responsive genes is mediated by regulation of the factors that determine the extent to which these genes are expressed. Regulatory mechanisms include but are no means restricted to biosynthesis, post-translational modifications, composition, and recruitment. Using the vitamin D receptor as an example of a sequence-specific DNA binding protein that interacts with a key promoter regulatory element of vitamin D-responsive genes, the biological significance of biosynthesis [Chen and Feldman, 1981], phosphorylation [Haussler et al., 1988; Brown and DeLuca, 1990], and stage of the cell cycle [Kurihara, et al., 1986; and Chen and Feldman, 1981] are among the regulatory parameters that have been identified. The overlap of promoter binding proteins for multiple regulatory factors such as the glucocorticoid receptor binding sequences and TATA motif of the osteocalcin gene [Stromstedt et al., 1991] or the association of AP-1 sites within vitamin D- and glucocorticoid-responsive elements in other genes [Diamond et al., 1990; Schüle et al., 1990b] are indicative of a mechanism whereby integration of regulatory signals associated with hormonal and basal regulatory control may be operative. Similarly, overlap of binding domains for cell growth regulated oncogene-encoded proteins with those of the vitamin D receptor and basal regulatory factors (OC box) further illustrates shared promoter recognition sequences as a mechanism for facilitation and/or mutual exclusion of transcription factor interactions [Owen et al., 1991].

Mechanisms by which cross-talk between independent promoter elements participate in transactivation of vitamin D-responsive genes must be defined. From systematic analysis of responsive promoter regulatory elements by several laboratories directed towards defining the minimal sequences and functional limits of sequences contributing hormonal influences on transcription, it has become apparent that sequences within and contiguous to the VDRE as well as independent regulatory elements residing considerable distances both upstream and downstream from the VDRE are functionally related to vitamin D-mediated transcriptional control. The questions that are thereby raised within the context of vitamin D-regulated transcription include the extent to which independent transcription factor-regulatory element complexes are autonomously organized and operative as well as the mechanisms by which transcription is initiated within the nucleus of an intact cell where both the gene regulatory elements and sequence-specific transcription factors are represented in low concentration. In a restricted sense, these questions relate to understanding mechanisms associated with responsiveness of promoter activity to vitamin D and other steroid hormones, but the general biological relevance to transcriptional regulation must not be overlooked.

The three-dimensional organization of the nucleus may provide a basis for directly addressing in vivo transcriptional regulation and several features of nuclear architecture are consistent with control of hormone-dependent transcription. Chromatin structure and nucleosome organization provide a basis for reducing the distance between independent promoter regulatory elements, potentially facilitating cooperative interactions that mediate both sequence-specific protein/DNA and protein/protein interactions as well as the combined contribution of multiple elements to transcriptional activity. Findings that are consistent with an involvement of chromatin structure in vitamin D-mediated transcriptional control of the osteocalcin and calbindin gene promoters include 1) the representation of nucleosomes in the osteocalcin [Bortell et al., in press] and calbindin (A.W. Norman, unpublished observation) gene 5′ regulatory sequences; and 2) hormone-related modifications observed in nuclease accessibility of promoter sequences. Association of the progesterone-responsive ovalbumin gene [Ciejek et al., 1983] with the nuclear matrix and association of nuclear matrix proteins with sequences in the region of the VDRE in the osteocalcin gene (this laboratory, unpublished findings) and modifications in the composition of nuclear matrix proteins during bone cell phenotype development [Dworetzky et al., 1990, in press] additionally supports involvement of nuclear architecture in the imposition of structural constraints on promoter conformation and in the concentration and localization of transcription regulatory factors.

Taken together, our present understanding of the composition and organization of the cell

nucleus are beginning to provide insight into mechanisms by which vitamin D may contribute to the transcriptional activity of genes associated with structural and functional properties that support diversity of cellular phenotypes. Further insight into regulatory mechanisms related to vitamin D affects at the cellular and molecular levels can contribute to defining activities of related hormonal mediation of physiological signalling pathways that regulate and are in turn regulated by differentiation and maintenance of function in specialized cells.

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NOTE ADDED IN PROOF

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