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FUNDAMENTALS OF VITAMIN D HORMONE-REGULATED GENE EXPRESSION

J. Wesley Pike and **Mark B. Meyer**
Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706

Abstract

Initial research focused upon several known genetic targets provided early insight into the mechanism of action of the vitamin D hormone (1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$)). Recently, however, a series of technical advances involving the coupling of chromatin immunoprecipitation (ChIP) to unbiased methodologies that initially involved tiled DNA microarrays (ChIP-chip analysis) and now Next Generation DNA Sequencing techniques (ChIP-Seq analysis) has opened new avenues of research into the mechanisms through which 1,25$(OH)_2D_3$ regulates gene expression. In this review, we summarize briefly the results of this early work and then focus on more recent studies in which ChIP-chip and ChIP-seq analyses have been used to explore the mechanisms of 1,25$(OH)_2D_3$ action on a genome-wide scale providing specific target genes as examples. The results of this work have advanced our understanding of the mechanisms involved at both genetic and epigenetic levels and have revealed a series of new principles through which the vitamin D hormone functions to control the expression of genes.

Keywords

Transcription; 1,25$(OH)_2D_3$; VDR; unbiased genome-wide analysis; ChIP-seq methods; epigenetics

1. INTRODUCTION

The vitamin D hormone 1,25$(OH)_2D_3$ exerts its diverse biological effects in target tissues by regulating gene expression (1). Research over the past several decades focused upon several specific genes has shown that this activity is mediated the vitamin D receptor (VDR) which, following activation by 1,25$(OH)_2D_3$, binds as a retinoid X receptor (RXR) heterodimer directly to specific nucleotide sequences (vitamin D response elements or VDREs) located in regulatory regions of DNA (2-6). This binding triggers the recruitment of diverse sets of

Please send correspondence to: Professor J. Wesley Pike, Department of Biochemistry, University of Wisconsin-Madison, Biochem Addition, Room 543D, 433 Babcock Drive, Madison, WI 53706. Tele: (608) 262-8229; Fax: (608) 263-9609; pike@biochem.wisc.edu..

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enzymatic coregulatory complexes that remodel chromatin, facilitate the epigenetic modification of histones and influence the local concentration of RNA polymerase II (RNA pol II) at target gene promoters (7). While this mechanism represents a general characterization drawn from the existing collection of gene targets, numerous gene-specific variations on this theme exist, perhaps the most important being the means through which the vitamin D hormone suppresses gene expression. Here, few clear examples exist and much less information is currently available. In the case of the cytochrome P450, family 27, subfamily B, polypeptide 1 (*CYP27B1)* and parathyroid hormone (*PTH)* genes, their downregulation appears to involve the ability of the VDR to interact directly with and to nullify the activity of a prebound transcription factor that is essential for the expression of these genes (8-10). Other negative regulatory mechanisms are likely to exist, however, as VDREs that permit negative regulation have been suggested and other modes of suppression that directly influence DNA structure are also possible (11). However, the role of the ligand in VDR activation under many of these circumstances remains ill-defined. Finally, the DNA-independent interaction of the VDR with numerous transcription factors that are the regulatory end points of various signaling 74 pathways defines additional sets of general mechanisms for both positive and negative signal integration through which the VDR modulates gene expression (reviewed in 12). As is clear from this brief summary, while the central role of the VDR is quite clear, the underlying actions of the vitamin D hormone to modulate gene expression are indeed complex and much remains to be learned.

Delineation of the mechanism of action of $1,25(OH)_2D_3$ defined over the past several decades has relied upon a cohort of standard molecular biological and biochemical methodologies *in vitro* (2). These include mapping the activities of wildtype and mutant gene promoter/reporter plasmids in response to $1,25(OH)_2D_3$ following transient transfection into cultured cells, determining the influence of various transcription factors including the VDR on reporter plasmids following co-transfection of plasmids overexpressing these factors, and examining the ability of these factors to interact with each other as well as with DNA sequences using biochemical interaction assays. While these and other assays have provided considerable insight into how genes are regulated, the methods display considerable limitations. First, the cloning and analysis of segments of genetic material is highly biased towards short regions of DNA (1-3 kb) located near gene promoters despite considerable genetic and clinical evidence that regulatory regions can occur distal to these transcriptional start sites. Second, segments of cloned DNA are restricted in size, rarely contain the entire span of DNA that constitutes an entire boundary-limited gene locus, and, following transient transfection, are unlikely to be properly chromatinized or to contain the appropriate epigenetic marks that characterize regulatory and other key landmark features of endogenous gene loci. Biochemical interaction assays are also flawed for a multitude of reasons, the least of which is the consequence of gene overexpression and the flagrant use of exceptionally high concentrations of reactant proteins. Many of these analytical difficulties have been overcome recently through the development of chromatin immunoprecipitation (ChIP) assays which now permit the detection and localization of transcription factors and both epigenetic DNA and histone modifications 99 at specific sites on genomes without significant cellular modification both *in vitro* and *in vivo* (13,14). Coupled to tiled microarrays (ChIP-chip analysis) (15,16) and

now almost exclusively to the use of Next Generation Sequencing (NGS) methods (ChIP-seq analysis) (17,18), chromatin immunoprecipitation is capable of providing detailed, unbiased transcription factor as well as epigenetic data on a genome-wide scale. The use of these techniques permits overarching assessment of the consequences of vitamin D hormone action at target cell genomes at the level of VDR DNA binding (termed the VDR cistrome), requirements for RXR co-localization at VDR binding sites, differences that emerge as a result of activation vs. suppression, and identification of numerous additional features of vitamin D action not previously approachable (19,20). Perhaps as important, these mechanistic data sets can be linked directly through DNA microarray or RNA sequencing methods (21) to parallel genome-wide measurements of the transcriptome under basal and induced conditions such as those which occur during treatment with $1,25(OH)_2D_3$. They also permit a reassessment of the tenets of $1,25(OH)_2D_3$ action that have emerged through single gene studies conducted over the past several decades. This review, generally of our own research, provides a summary of the results of such genome-wide studies which highlight features of vitamin D hormone action that are consistent with earlier tenets and features that are novel.

2. GENERAL METHODOLOGIES

2.1. Chromatin Immunoprecipitation

Chromatin immunoprecipitation was conducted as previously described (22,23).

2.2. Tiled Microarray (ChIP-chip) Analysis

ChIP-chip analysis was conducted as described (24,25).

2.3. DNA Sequencing and ChIP-seq Analysis

ChIP-seq analysis and NGS was carried out as documented in Meyer et al. (23)

2.4. Bioinformatic Processing and Analyses

Bioinformatic analyses were carried out as previously described (23).

3. RESULTS AND DISCUSSION

3.1 Genome Wide Analysis of Vitamin D Hormone Action

3.11 The VDR and RXR Cistromes—ChIP-chip and ChIP-seq analyses have been conducted in target cell lines to determine the number of DNA binding sites occupied by the VDR (the cistrome) in the absence and presence of $1,25(OH)_2D_3$ (Table 1) (22,23,26-30). In several of these studies, co-occupancy at these sites by several of the isoforms of RXR has also been determined (22,23). These analyses have revealed that despite the potential for 10^5 to 10^6 binding sites on either the mouse or human genomes based upon an *in silico* determination of putative VDRE-like sequence motifs (Meyer and Pike, unpublished data), less than 1000 sites or so are generally occupied by the VDR in the absence of $1,25(OH)_2D_3$ and between 2000 and 8000 sites are occupied following treatment with the hormone. An important determinant of the cistrome itself is the level of expression of the VDR itself. A large percent of these binding sites also contain RXR as well, supporting the view that RXR

on a genome-wide scale is indeed a heterodimer partner of VDR. Of course, RXR binding also occurs at sites on genomes that are never occupied by the VDR regardless of whether $1,25(OH)_2D_3$ is present. This highlights the diverse role that RXR plays as a permissive heterodimer partner for other nuclear receptors. It is important to note that the determination of transcription factor cistromes in general are heavily dependent analytically upon the technical quality of the study, the properties of the antibodies used to conduct the precipitations, and the bioinformatic 145 programs and statistical cut offs that are utilized. Regardless of this technical aspect, when the individual binding data summarized in Table 1 are examined in more detail, the data collectively support the conclusion that genomic VDR binding is strongly induced by $1,25(OH)_2D_3$ in all cells and, more importantly, that the sites of binding within each VDR cistrome differ considerably depending upon cell-type. The latter observation is consistent with early observations indicating that while overlap exists, $1,25(OH)_2D_3$ regulates the expression of different subsets of genes depending upon the type of cell/tissue that is targeted (31,32). The data also provide unequivocal support for the idea that *in silico* analysis cannot be used as the primary predictor of the presence of a functional VDRE. The reduction in functional VDR binding sites to a small percentage of those determined by genomic sequence alone, as determined by direct ChIP-chip or ChIP-seq analysis, highlights the considerable role of chromatin structure as a negative regulator of gene expression (33).

3.12 Intronic and Intergenic Locations of Regulatory Regions—As described above, data generated on a genome-wide basis as well as at specific loci has confirmed many single gene studies suggesting that VDR DNA binding is largely ligand dependent and involves an RXR heterodimer (see Figure 1 for examples at the vitamin D receptor (*Vdr*, A), tumor necrosis factor superfamily, member 11 (*Tnfsf11*, B) and secreted phosphoprotein 1 (*Spp1*, C) gene loci). Surprisingly, however, and with significant consequence, the widely held view that regulatory regions are located predominantly at or near transcriptional start sites of $1,25(OH)_2D_3$-regulated genes (within several kilobases) has not proven to be correct. Indeed, while VDR/RXR binding sites across the genome are found near *bone fide* target gene promoters (see Figure 1C for example, *Spp1*), the majority have been discovered located within the introns of genes (see Figure 1A for example, *Vdr*) and within intergenic regions upstream (see Figure 1B for example, *Tnfsf11*) or downstream of the transcription unit itself (23,28). Most strikingly, many sites are often located 10's if not 100's of kilobases from transcriptional start sites of potential target genes (170 see Figure 1B for example, Tnfsf11). Perhaps as important, these and additional studies also support the idea that the transcriptional targets of many of these regulatory regions are not the genes located proximally, but rather are targets that are separated by one or more interspersed genes. Regardless of this, these observations point out the flaws inherent in using transfected plasmids to identify the overall locations of regulatory regions. Unbiased ChIP-seq analysis also highlights the frequency with which genes are regulated by multiple control regions (see Figure 1 for examples). Indeed, many, if not most genes, contain more than one regulatory region and in some cases as many as 10 or more. This finding is perhaps the most important to emerge from unbiased studies of gene expression, and has considerable implications for the study of the mechanisms through which the vitamin D hormone regulates transcription. Indeed, an immediate question emerges as to how one might directly

link the activity of a distal regulatory region(s) to the gene whose expression it regulates. An additional question is whether the regulatory activity of a specific control region is restricted to a single target or whether it can contribute to the expression of more than one nearby target gene (23).

3.13 Modes of VDR/RXR DNA Binding—Interestingly, while $1,25(OH)_2D_3$-induced VDR binding sites frequently contain co-localized RXR (Figure 1), the latter is not always present (23). Whether this arises as a unknown technical issue inherent to ChIP-seq analysis or represents the presence of a distinct pathway of $1,25(OH)_2D_3$ activation that does not require RXR is unclear. One obvious possibility is that VDR-mediated gene suppression may not involve RXR in every case. Unfortunately, our understanding of the mechanism(s) that underlies $1,25(OH)_2D_3$'s ability to downregulate gene expression is currently incomplete and limited to that defined by its actions at the *CYP27B1* and the *PTH* genes. These actions involve the tethering of VDR to a pre-DNA bound basal activator termed vitamin D interacting repressor (VDIR) rather than direct binding of the VDR to DNA (8,9). Although this indirect mechanism is utilized by many transcription factors, 195 it may represent only one of several methods through which gene downregulation by $1,25(OH)_2D_3$ occurs. Indeed, binding of the VDR within the vicinity of genes whose expression is simultaneously suppressed by $1,25(OH)_2D_3$ is multifaceted. In some cases, VDR DNA binding appears in response to $1,25(OH)_2D_3$, suggesting active repression; in others the VDR is prebound in the absence of $1,25(OH)_2D_3$ and is either unaffected by ligand treatment or displaced (34). In these cases, RXR is frequently present. Although these observations clearly reveal multiple modes of VDR and RXR binding to DNA, linking the actions of the VDR at these regulatory regions specifically to the genes whose expression is affected by the hormone will require additional experimentation because, as outlined above, the gene that is located in closest proximity to a transcriptional control region may not be its direct regulatory target. In addition, while VDR DNA binding is largely ligand-induced (Figure 1), there are many examples where the VDR is prebound to known regulatory regions of previously described $1,25(OH)_2D_3$ target genes in the absence of ligand (23,35). At these sites, $1,25(OH)_2D_3$ treatment can upregulate the concentration of DNA-bound VDR or manifest no activity at all. Similar actions can be seen with RXR as well. Perhaps the action of $1,25(OH)_2D_3$ in these cases is to facilitate the recruitment of coregulatory factors by the VDR, as often occurs for other prebound transcription factors. What distinguishes ligand-independent DNA binding of the VDR at these sites remains unknown.

3.14. The Predominance of the Classic VDRE—As summarized previously, the VDR/RXR heterodimer interacts directly with VDRE sequences located in target gene loci that are comprised of two directly-repeated hexameric nucleotide halfsites separated by three base pairs (36). This motif is similar to that of other nuclear receptor/RXR heterodimers with the exception that the spacing between the half-sites differs; this spacing represents the primary determinant of nuclear receptor specificity (37). The immediate question that arises is whether the VDRE motif identified on a genome-wide scale is similar. As transcription factor binding by ChIP-seq analysis is 220 resolved to between 100 to 200 bp, a *de novo* motif finding algorithm is necessary to identify in an unbiased manner the most frequent sequences that are located in these larger collections of VDR/RXR binding regions. This

type of analysis has revealed that in the genome-wide studies conducted thus far, the most frequent sequence found is a classic consensus VDRE motif comprised of two directly repeated hexameric half-sites separated by 3 bp (Figure 2) (23). This frequency ranges from 35% to 65%, however, suggesting that motifs other than that of the classic VDRE may be involved as well. Some of these regions may mediate gene suppression, as described above, and thus may not contain sites that interact directly with the VDR. Others may contain sites that mediate activation, yet are comprised of VDREs that have been reported previously and whose configurations diverge from those of the classic VDRE (38). Many of these sequences were identified and characterized using traditional *in vitro* methods of analysis, however, and should be considered suspect. The authenticity of these sites will require re-examination using ChIP-seq approaches capable of revealing whether VDR and its partner RXR are indeed bound at these unusual sites of action. In that context, it is worth noting that while *de novo* analysis does not reveal the classic VDRE motif at all high confidence VDR binding sites across the genome identified by ChIP-seq analysis, a position weight matrix based search does in fact yield VDRE-like sequences at over 95% of these biological sites (Meyer and Pike, unpublished data). This secondary analysis suggests that the identification of VDREs following a genome-wide study is not trivial. Perhaps as important, while many of these sites contain multiple candidate VDREs, additional functional analyses of these elements must be conducted to confirm their regulatory capabilities.

3.15. VDR Binding Sites Are Located in *Cis* Regulatory Modules (CRMs)—

While the classic VDRE is often the most frequently identified sequence motif found in genome-wide collections of VDR/RXR binding sites, additional transcription factor binding motifs are also identified at a high percentage of these sites as well (23,27). In 245 bone cells, these motifs include those for RUNX2, C/EBPα and β, and several others (39,40). Importantly, the authenticity of many of these sites has been confirmed through direct ChIP-seq analysis of the factors predicted to bind at these sites (see Figure 1A-C for examples of RUNX2 and C/EBPβ). Often, these transcription factors will interact within several hundred base pairs of the identified VDRE. These findings support the idea that VDR/RXR binding occurs within distinct open regions that are structurally complex, are modular with respect to additional transcription factor binding, do not display a repressive chromatin architecture and are able to mediate and likely to integrate synergistically the activities of multiple signaling pathways. These sites are termed cis regulatory modules (CRMs) or enhancers/silencers and likely represent key structures through which gene expression is controlled (41,42). The observation that coregulatory proteins such as steroid receptor coactivator 1 (SRC1), mediator protein 1 (MED1), silencing mediator of retinoid and thyroid receptors (SMRT) and nuclear receptor co-repressor (NCoR) are also recruited to these regions during VDR/RXR binding supports this view (27). Importantly, while CRMs are often located in linear terms many kilobases distant from the genes they regulate, studies over the past few years have demonstrated that distal regulatory regions interact directly with their target gene promoters and that the large DNA segments located between them are looped away from the transcriptionally active site (43). The structural basis for these three dimensional interactions is not known, but appears to involve transcription factors such as the CCCTC binding factor CTCF, cohesin and perhaps components of mediator complex (44,45). How these interactions are regulated also remains to be defined.

3.16. VDR-containing CRMs Display Distinct Epigenetic Histone Signatures—

As discussed above, VDR binding sites are located within CRMs that frequently contain sites for multiple transcription factors whose binding activities may be cooperative and whose functional activities may be synergistic. Additional studies using digital DNAse1 hypersensitivity (DHS) measurements suggest that these regions contain 270 proteins prebound to DNA (thus, hypersensitive to DNase1) and are unique in structure (46). Enhancers are also known to display a distinct epigenetic signature on the N-terminal tails of histones H3 and H4 that includes mono- and dimethylation at H3K4 (H3K4me1 and H3K4me2, respectively) (See Figure 1 for examples at *Vdr, Tnfsf11* and *Spp1*) and acetylation at H3K9 (H3K9ac), H4K5 (H4K5ac), and H3K27 (H3K27ac) (47,48). These and perhaps other epigenetic marks are frequently placed at regulatory sites during development and/or differentiation, are dynamically modulated by chromatin regulatory factors and play a role in manipulating chromatin architecture and transcription factor accessibility. Additional histone modifications are now known to highlight other structural or functional features at gene loci, and in some cases, reflect the overall transcriptional activity of the gene itself. How these histone marks participate directly in the regulation of gene expression is unclear, although many are recognized specifically by members of a diverse set of chromatin "reader" proteins that contain unique enzymatic activities and act downstream of transcription factors such as the VDR. They likely participate in coordinating the changes necessary for altered transcriptional output (49). Early studies revealed that high levels of histone acetylation are often present at known sites of VDR action (specific target genes) and that $1,25(OH)_2D_3$-induced binding of the VDR to these sites is associated with an increase in the level of this histone modification as well (14). Recent ChIP-seq studies using antibodies to a wide variety of histone modifications have shown that VDR binding sites are indeed enriched on a genome-wide scale for H3K4me1, H3K4me2, H3K27ac and others, components of the epigenetic CRM signature (see Figure 1A-C for a subset of examples). These studies also support the observation that treatment with $1,25(OH)_2D_3$ together with VDR binding correlates directly with an upregulation in H3 and H4 acetylation at enhancers that are known to be associated with the expression of specific vitamin D hormone target genes (Figure 1A-C). These results contribute significantly to our understanding of the mechanism of action of $1,25(OH)_2D_3$ and provide additional clues as to the nature of the downstream mechanisms that facilitate the actions of the hormone to 296 regulate gene expression. Perhaps of equal importance, the ability to detect regions that contain "enhancer" signatures provides a means of identifying the regulatory sites of action of hormones, growth factors and other regulatory molecules without knowing what specific transcription factor(s) may be involved (50). Regardless, the simultaneous detection of these key dynamic histone modifications together with the VDR/RXR heterodimer at genetic loci provides knowledge of the two elements that are essential for further exploration of how the vitamin D hormone regulates the expression of genes at target gene loci.

3.2 Linking CRMs to Genes They Regulate

One consequence of the discovery that regulatory regions are frequently located large distances from transcriptional start sites is that although their identification on the genome has become easier, verification of the genes they regulate has become more difficult. While several ChIP-based approaches may ultimately provide detailed genome-wide level linkage

(41), current bioinformatic-based data is of low resolution and largely correlative. Several approaches have been made to link the actions of specific regulatory regions directly to the genes they regulation. These include using bacterial artificial chromosomes (BACs) which may contain up to 250 kb of linear DNA and therefore generally span specific target gene loci that include surrounding regulatory components. Importantly, a BAC clone can be genetically modified through bacterial recombinant engineering to contain a reporter function and then stably integrated as a minigene into the genome of cells in culture (51) or as a transgene into mice (52,53). Following characterization of the activity of the recombineered wildtype BAC clone, enhancers can be removed and the consequence of their removal assessed following reintroduction of the clone into either the same cells or into the mouse genome. An additional approach has been to delete regulatory regions directly from the genome (54). While this approach is similarly useful, it is both expensive and difficult to thoroughly investigate genes at this level if they contain multiple regulatory regions. Recent 321 advances in genome-editing methods that include the use of TALENs (55) or of RNA-directed CRISPR associated protein 9 (Cas9) nucleases (56) will likely expedite this approach and facilitate simultaneous examination of more than one regulatory region. We and others have used both BAC clone analysis (40) as well as genomic deletion in mice (54) to explore the properties of several $1,25(OH)_2D_3$ target genes. Importantly, these studies confirm the functional role of many of these control regions identified initially by ChIP-chip and ChIP-seq methodologies.

4. CONCLUSIONS

Early studies of a few selected target genes provided significant insight into how $1,25(OH)_2D_3$ functions to regulate gene expression. The advent of ChIP, linked ultimately to unbiased methodologies that include the use of tiled microarrays and deep sequencing analysis, has revolutionized our approach to the study of gene regulation and provided new insight into how $1,25(OH)_2D_3$ as well as other systemic and local factors operate to control the expression of genes. These unbiased methods have revealed not only novel features of VDR and RXR binding, but the identity of the regions to which they bind. They have also revealed novel genetic and epigenetic features of these regions and their potential impact on regulatory function. These findings indicate that much remains to be discovered in our ongoing effort to understand how the vitamin D hormone functions to modulate the expression of genes.

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Abbreviations

VDR	vitamin D receptor
ChIP	chromatin immunoprecipitation
ChIP-chip	ChIP coupled to tiled microarrays

ChIP-seq	ChIP coupled to deep sequencing methods
VDRE	vitamin D response element
NGS	Next Generation Sequencing
RXR	retinoid X receptor
VDIR	vitamin D receptor interacting repressor
CTCF	CCCTC binding factor
MED	Mediator complex
SRC1	steroid receptor coactivator 1
SMRT	silencing mediator retinoid and thyroid hormones
NCoR	Nuclear receptor corepressors
DHS	digital hypersensitive sites
CRM	*Cis* Regulatory Module
H3K4me1	monomethylated histone H3K4
H3K4me2	dimethylated histone H3K4
H3K9ac	acetylated histone H3K9
H4K5ac	acetylated histone H4K5
H3K27ac	acetylated histone H3K27
BAC	bacterial artificial 49 chromosome
TALEN	transcription activator-like effector nucleases
CRISPR	clustered regularly interspersed short palindromic repeats
Cas9	CRISPR associated protein 9

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Highlights

- Novel genome-wide approaches provide new insight into the actions of $1,25(OH)_2D_3$
- VDR DNA binding is dependent upon $1,25(OH)_2D_3$ and generally involves RXR
- VDR binding sites are located predominantly within introns and intergenic regions
- The DNA sequence to which the VDR binds is comprised of a classic VDRE
- VDR binding regions are modular and marked by specific histone modifications

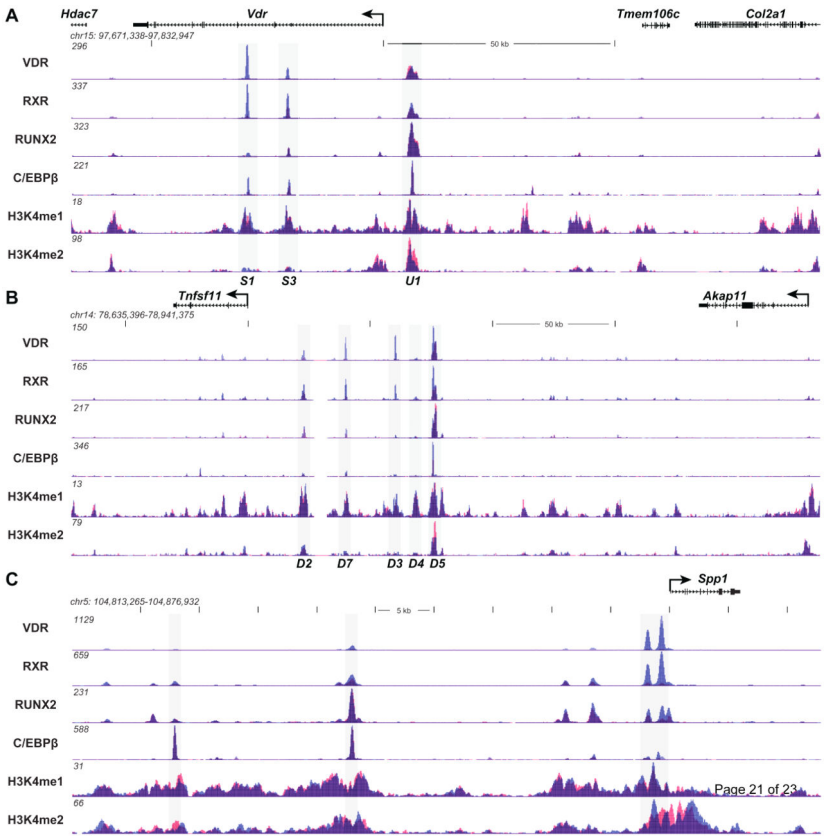

Figure 1.

ChIP-seq profiles at specific gene loci. Mouse MC3T3-E1 cells were treated for 3 hrs with either vehicle or 1,25$(OH)_2D_3$ (10^{-7}M) and then subjected to ChIP-seq analysis using antibodies to VDR, RXR, RUNX2, C/EBPβ and histone H3K4me1 or H3K4me2. ChIP-seq tag densities (normalized to 10^7 reads) were quantified and mapped to the mouse MM9 genome using MACS (Model-based Analysis for ChIP-seq), HOMER (Hypergeometric Optimization of Motif Enrichment) and Cistrome Data Analyses. The genomic loci (chromosome number and nucleotide interval are indicated) contain the *Vdr* (A), *Tnfsf11* (B), and *Spp1* (C) genes and their respective neighbors. Genomic tracks (read scales are indicated on the Y-axis) for VDR, RXR, C/EBPβ, RUNX2, H3K4me1 and H3K4me2 each contain two mapped data sets derived from vehicle- and 1,25$(OH)_2D_3$-treated cells (red and blue, respectively with overlap in purple). The transcriptional start sites and direction of transcription for each gene is indicated by an arrow; exons are indicated by vertical boxes within the gene. Shaded vertical columns highlight the locations of several known regulatory regions for each target gene, as explored in specific detail in references 25 and 40 (*Vdr*), 24 and 54 (*Tnfsf11*), and 3 and 57 (*Spp1*); numbers/letters below the data sets in (A) and (B) represent our CRM designation.

Figure 2.

The classic VDRE motif defined via genome-wide ChIP-seq analysis in human colorectal LS180 cells (23). This motif logo describes the relative nucleotide sequence of the most enriched 15 bp DNA segment located within the collection of VDR/RXR binding sites found across the LS180 genome (compared to 50,000 GC-matched random 15 bp DNA sequences).

Table 1

Genome-wide analysis of VDR binding site in diverse cell-types.

Published			
Reference	**Cell type**	**VDR sites/Genome $1,25(OH)_2D_3$ − +**	**RXR overlap**
Meyer *et al.* (22)	Osteoblast (MC3T3E1)	1325 / 8241	Yes
Ramagopalan *et al.* (26)	B Cell (Transformed)	623 / 2776	ND
Heikkinen *et al.* (28)	Monocyte (THP-1)	520/1171	ND
Meyer *et al.* (23)	CRC (LS180)	262 / 2209	Yes
Satoh *et al.* (29)*	B Cell/Monocyte	1820/2297	ND
Ding *et al.* (30)	Liver Stellate (HSC)	ND / 6281	ND
Unpublished			
studies**	**Cell type**	**VDR sites/Genome $1,25(OH)_2D_3$ − +**	**RXR overlap**
Meyer *et al.* 1	Osteoblast (MC3T3E1)	947 / 7007	Yes
Meyer *et al.* 2	MSC (BM-derived)	1752 / 10557	Yes
Meyer *et al.* 2	Osteoblast (MSC-derived)	245 / 823	Yes
Meyer *et al.* 2	Adipocyte (MSC-derived)	245 / 616	Yes
St. John *et al.* 3	Osteocyte (SW3)	409 / 2969	Yes
Goetsch *et al.* 4	Osteoblast (MG63)	261 / 7076	ND

ND = Not done

* Reanalysis of ChlP-seq data sets derived from studies in references 26 and 28.

** Binding site summary of unpublished data sets in which the indicated cell types were subjected to ChlP-seq analysis following treatment for 3 hours with either Vehicle or $1,25(OH)_2D_3$. Data are processed bioinformatically as described in reference 23.