INTRODUCTION

In eukaryotes, genomic DNA is packaged around nucleosomes, i.e., simplistic schemes of “naked” DNA do not correctly describe its status within the nucleus of a cell. Nucleosomes are complexes of two copies each of the histone proteins H2A, H2B, H3, and H4 and represent a basic, every 200bp repeating unit of chromatin. The complex of nucleosomes and genomic DNA, referred to as chromatin, largely differs in its degree of packaging (Fig. 13.1) [1]. Histones are rather small proteins (molecular mass below 15kD) that are...
FIGURE 13.1 Chromatin stages. Chromatin is distinguished into open chromatin (euchromatin, left) with loose nucleosome arrangement and closed chromatin represented by dense nucleosome packing (heterochromatin, right). There are several stages between these extremes that are summarized as facultative heterochromatin (center). Each stage is characterized by a set of chromatin-modifying enzymes, such as HATs and HDACs, HMTs and HDMs, and DNA methyltransferases (DNMTs) and the demethylating ten–eleven translocation (TET) proteins, and CoAs and CoRs of transcription factors, such as VDR, which lead to the schematically indicated scenarios of acetylation (ac) and methylation (me) of histone tails and genomic DNA. CoAs, coactivators; CoRs, corepressors; HATs, histone acetyltransferases; HDACs, histone deacetylases; HDMs, histone demethylases; HMTs, histone methyltransferases.

In this chapter, I will discuss—from a genome-wide perspective—the interaction of vitamin D and its receptor with chromatin. This will demonstrate the significant impact of vitamin D on the epigenome both in cell culture models in vitro as well as within the human body in vivo.

II. MECHANISM OF ACTION

The human genome comprises approximately 1600 genes for transcription factors, i.e., proteins that (1) recognize sequence-specifically genomic DNA and (2) modulate the activity of RNA polymerase II in gene transcription [9,10]. VDR belongs to a unique family of structurally related transcription factors, referred to as the nuclear receptor superfamily, most members of which are modulated in their activity by small lipophilic molecules in the size of cholesterol [11,12] (for details on VDR please refer to Chapter 9 and other chapters in this section). The endocrine subgroup of the nuclear receptor superfamily, to which VDR is a member, contains the intensively investigated receptors for estrogen, testosterone, and cortisol, i.e., ER, AR and GR, respectively, all of which share similar mechanisms of action. These are (1) the sequence-specific recognition of genomic targets via protein-DNA interactions of their DNA-binding domains and (2) the highly specific protein-ligand interactions via the ligand-binding pocket located within their ligand-binding domains (LBD). The latter results in minor but significant conformational changes on the surface of the LBD that alter the protein-protein interaction profile of the receptor with nuclear adaptor proteins such as corepressors (CoRs), coactivators (CoAs) and the mediator complex [13,14]. In total, more than 50 nuclear proteins interact with the VDR’s LBD [15]. In this way, activated VDR acts as “crystallization core” for the assembly of nuclear proteins such as (1) chromatin modifiers that read, write, or erase of posttranslational marks on histones or (2) chromatin remodelers that rearrange nucleosome positions. This means that the location of VDR at specific genomic regions makes these chromatin regions sensitive to stimulation by vitamin D. The resulting epigenomic changes in turn facilitate looping of the VDR-marked genomic regions, i.e., 1,25(OH)2D3-inducible enhancers, toward accessible

Chromatin has an intrinsic repressive potential [3]. In an average terminally differentiated cell only less than 10% of the chromatin is in the euchromatin stage. This means that the so-called “epigenetic landscape” of a differentiated cell is restricted to some 50–100,000 accessible chromatin loci that primarily contain TSS and enhancer regions [4]. The remaining genomic DNA is located within far more densely packed, largely inaccessible heterochromatin that protects the genome from unintentional activation of genes located within these regions (Fig. 13.1). A subset of the latter regions is facultative heterochromatin that can be opened (and again closed) via the action of chromatin-modifying enzymes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs) or histone methyltransferases (HMTs) and histone demethylases (HDMs) [5]. At a given chromatin locus, the activities of chromatin-modifying enzymes are controlled by transcription factor binding across these regions and via signal transduction cascades originating from intra- and extracellular signaling molecules that modulate the activity of the enzymes [6]. The vitamin D receptor (VDR) is one of many transcription factors that communicate with chromatin-modifying enzymes via direct and indirect interaction, such as transcriptional up- and down-regulation. Therefore, VDR’s unique natural high affinity ligand 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3) can act as a chromatin-modifying signal [7,8].
TSS regions in their relative vicinity [16]. This assembly of enhancer and TSS regions enables the formation of a large protein complex containing VDR, nuclear adaptor proteins, and RNA polymerase II that modulate the transcription of primary vitamin D target genes. Therefore, vitamin D acts as a far more specific chromatin modulating compound than HDAC inhibitors such as trichostatin A that induce more global activation of chromatin [17,18]. Nevertheless, 1,25(OH)2D3 and trichostatin A act synergistically in their global gene-regulatory effect, for example, in THP-1 human monocytic leukemia cells [19].

GENOME-WIDE LOCATION OF VITAMIN D RECEPTOR-BINDING SITES

During the last 25 years numerous in vitro studies demonstrated that VDR binds most efficiently to DNA when it (1) is complexed with the nuclear receptor retinoid X receptor (RXR) [20–22] and (2) uses binding sites formed by a direct repeat of the sequence RGKTSA (R=A or G, K=G or T, S=C or G) spaced by three nucleotides (DR3) [23–25]. In fact, a largely unbiased genome-wide method, chromatin immunoprecipitation (ChIP) combined with tiled microarrays (ChIP-Chip, reviewed in Ref. [26]) or massive parallel sequencing (ChIP-seq, reviewed in Ref. [27]), confirmed that DR3-type-binding sites are found with highest preference below the summits (±100 bp) of VDR peaks produced by this technique (Fig. 13.2).

In human cellular systems VDR ChIP-seq was performed in B cells (GM10855 and GM10861) [28], monocytes (THP-1) [29], macrophages (lipopolysaccharide-differentiated THP-1) [30], colon cancer cells (LS180) [31], and hepatic stellate cells (LX2) [32]. In parallel, in mouse systems VDR ChIP-seq was performed in preadipocytes (3T3-L1) [33], osteocytic cells (IDG-SW3) [34], preosteoblastic and differentiated osteoblastic cells (MC3T3-E1) [35], mouse intestine [36] and bone marrow-derived mesenchymal stem cells differentiating into bone and fat cells [37] (for details on these mouse differentiation models please refer to Chapter 14). A harmonized reanalysis of all the human VDR ChIP-seq datasets indicated more than 23,000 VDR-binding sites [30], 70% of which are unique for only one cellular model. DNA motif screening algorithms, such as HOMER [38] at a score of 9.1, identified only at 11.5% of these VDR loci DR3-type-binding site [30]. However, both the total number of identified VDR sites both the percentage of DR3-binding sites carrying loci critically depends on the threshold settings of the respective bioinformatic methods used for the analysis. For example, our latest version of the VDR-binding site compilation lists more than 66,000 VDR-binding sites with a DR3 rate of 20.6% (HOMER score 7, unpublished results). This indicates that by far not all VDR-binding sites contain a

![Figure 13.2](image)

**FIGURE 13.2** Vitamin D response of the *LFNG* locus in cell culture. THP-1 cells were treated for 24h with 1,25(OH)2D3 (1,25D) or vehicle (EtOH) and ChIP-seq, FAIRE-seq, and RNA-seq were performed at least in three biological repeats. The Integrative Genomics Viewer browser [72] was used to visualize the TAD around the vitamin D target gene *LFNG*. The peak tracks display data from ChIP-seq for VDR (red), PU.1 (purple), CTCF (light green), H3K27ac (orange), and H3K4me3 (dark green) as well as FAIRE-seq (turquoise). Gene structures are shown in blue. The change in expression of the four genes within the TAD was measured by RNA-seq. Only the *LFNG* gene was significantly (*P* < .05) up-regulated (red). The presence or absence of a DR3 motif below the VDR peaks is indicated. ChIP-seq, chromatin immunoprecipitation coupled with massive parallel sequencing; CTCF, CCCTC-binding factor; FAIRE-seq, formaldehyde-assisted isolation of regulatory elements sequencing; *LFNG*, LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase; PU.1, spleen focus forming virus proviral integration oncogene; RNA-seq, RNA sequencing; TAD, topologically associating domain; VDR, vitamin D receptor.
Dr3 motif. However, the Dr3 percentage largely depends on the level of conservation of VDR-binding loci between different tissues. Thus, the 282 most conserved VDR sites have a Dr3 rate of 73.0% (HOMER score 7, based on seven different cell types, unpublished results). Fig. 13.2 exemplifies the locus of the vitamin D target gene Lfng O-fucosylpeptide 3-beta-N-acetylglosaminyltransferase (Lfng) that in THP-1 cells has four VDR-binding sites, two of which carry a Dr3 motif.

On Dr3-type motifs VDR most likely forms heterodimers with Rxr, but on none-Dr3 sites the receptor may be using other binding modes, such as indirect binding “backpack” to other transcription factors, and alternative partner proteins [39]. HOMER motif screening below VDR peaks showed a significant enrichment for binding sites for the transcription factor spleen focus forming virus proviral integration oncogene (Sp1, also called Pu.1) [30]. Pu.1 is well-known for its role as pioneer transcription factor in the hematopoietic system and works together with Vdr in the differentiation into monocytes and granulocytes [41]. ChIP-seq for Pu.1 in THP-1 cells demonstrates the colocation of 6488 Vdr and Pu.1-binding sites [42]. This means that at 63.7% of its genomic-binding sites Vdr overlapped with Pu.1 (Fig. 13.2). Similar overlap rates have been reported for Rxr and Pu.1 [43], Er and Foxa1 [44], Gr and Foxa1 [45], Vdr and Runx2 as well as for Vdr and Cebpβ [37]. This suggests that, at least in monocytes, Vdr seems to need Pu.1 at the majority of its binding sites to locally keep the chromatin transcriptionally permissive. However, there is no evidence that Pu.1 acts, such as Rxr, as a heterodimerizing partner protein that increases Vdr’s DNA-binding affinity.

Interestingly, at 6498 genomic loci (representing 5.3% of all Pu.1 sites in THP-1 cells) a stimulation with 1,25(OH)2D3 significantly (P < .05) increases Pu.1 binding [42]. This suggests that there is also an inverse relationship between Vdr and Pu.1 where Vdr supports Pu.1 binding.

CTCF AS ORGANIZER OF VITAMIN D-DEPENDENT CHROMATIN DOMAINS

The multizinc finger transcription factor Ccctc-binding factor (Ctcf) [46,47] is the major protein binding to genomic insulator regions [48] and therefore involved in organizing chromatin into active and inactive regions. Ctcf binding stimulates the formation of a three-dimensional chromosomal network of topologically associating domains (TADs), i.e., of genomic DNA loops in the size of hundreds of kilobases to a few megabases [49]. The whole human genome is subdivided into at least 2000 TADs [50]. Ctcf binding is highly conserved between tissues and cell types and genome-wide every cell contains at least some 20,000 Ctcf loci, only some 15% of which are involved in forming TAD anchor regions. Interestingly, at 1321 Ctcf loci the association strength of the protein with genomic DNA was significantly (P < .05) modulated by stimulation of THP-1 cells with 1,25(OH)2D3 [51]. More than 60% of these ligand-sensitive Ctcf sites contribute to DNA looping and mark the anchors of 425 putative TADs containing at least one VDR-binding site and one 1,25(OH)2D3 target gene. Fig. 13.2 shows the example of two vitamin D-sensitive Ctcf sites in a distance of some 200 kb that form a TAD containing four VDR-binding sites and one vitamin D target gene (Lfng). The assumption that these Ctcf sites form TAD borders, i.e., that they are involved in DNA looping, is derived from chromatin interaction analysis by paired-end tag sequencing (ChIA-Pet) data of the Encode tier 1 cell line K562 (human monocytes [4]). Since genome-wide Ctcf binding is to 95% identical between K562 and THP-1 cells [51], it is possible to extrapolate the ChIA-Pet data from one cellular model to the other, i.e., the three-dimensional loops mediated by Ctcf are most likely largely identical both in K562 and THP-1 cells [52].

The observation that ligand-dependent Ctcf sites support the action of Vdr in more than 400 vitamin D-sensitive TADs suggest that Ctcf contributes to the epigenome-wide process of Vdr signaling [53]. Interestingly, Ctcf functionally interacts also with other members of the nuclear receptor superfamily. For example, thyroid hormone receptors collocate with Ctcf [54] and on a few example sites this association has been shown to be thyroid hormone dependent [55].

II. MECHANISM OF ACTION

The human genome expresses tissue-specifically hundreds of chromatin modifiers that add (“write”), interpret (“read”), or remove (“erase”) posttranslational histone modifications [5] (Fig. 13.3). Chromatin acetylation is generally associated with transcriptional activation, while the exact amino acid residue of the histone tails that is acetylated seems not to be very critical. The acetylation state of histones within a given chromatin locus is controlled by two classes of antagonizing histone modifying enzymes, HmtS and HdmS. Although histone methylation mainly mediates chromatin repression, at certain residues, such as H3K4, it results in activation [57]. Therefore, for histone methylation the exact residue in the histone tail and its degree of methylation (mono-, di- or tri-methylation) is of critical importance. Genome-wide maps of histone modification, as obtained by ChIP-seq, from cells undergoing differentiation or comparisons different human cell types, as obtained by the Encode project [4], provide...
major insights into the principles of tissue- and gene-specific chromatin organization. These data suggest that most histone marks are assigned to functional regions of the chromatin, such as TSS regions, enhancers, or heterochromatin [58]. For example, H3K27ac indicates active chromatin in general and H3K4me3 active TSS regions.

The functional readout of the histone code are different levels of accessibility of a given chromatin region, so that transcription factors and their associated cofactors bind more (or less) efficiently to the respective genomic region [59]. Chromatin accessibility is experimentally monitored by the method DNase I hypersensitivity sequencing (DNase-seq) [60], its successor formaldehyde-assisted isolation of regulatory elements sequencing (FAIRE-seq) [61] and the latest version assay for transposase-accessible chromatin using sequencing [62]. Thus, alterations in the profile of a given genomic region, such as measured by any of these methods, indicate epigenomic changes.

VITAMIN D-TRIGGERED EPIGENOME CHANGES

FAIRE-seq of 1,25(OH)\(_2\)D\(_3\)-stimulated THP-1 cells demonstrated more than 62,000 accessible chromatin loci in the basal state of this cellular model [53]. The vast majority of VDR-binding sites colocalize with these loci of open chromatin [63] (Fig. 13.2). Genomic loci at which a stimulation with 1,25(OH)\(_2\)D\(_3\) leads to a significant (\(P < .05\)) increase in chromatin accessibility provide indications for the location of vitamin D target genes within the same genomic region. Interestingly, nearly 9000 FAIRE sites are significantly (\(P < .05\)) modulated by 1,25(OH)\(_2\)D\(_3\) [53]. The exact molecular mechanisms of these 1,25(OH)\(_2\)D\(_3\)-triggered epigenome changes are not yet understood, but it is obvious that they are secondary consequences of VDR’s binding to genomic sites. For example, CTCF- and PU.1-binding sites are the top-ranking transcription factor-binding motifs below the summits of these 1,25(OH)\(_2\)D\(_3\)_modulated FAIRE peaks, i.e., vitamin D-mediated chromatin opening seems to be associated with the presence of CTCF and/or PU.1. However, probably many other chromatin-associated proteins are involved in these epigenomic events. For example, in osteoblasts the transcription factors RUNX2 and CEBP\(\beta\) are acting as pioneer factors for VDR [37].

A subset of 1,25(OH)\(_2\)D\(_3\)_modulated chromatin sites, in particular those at TSS regions, are fully open already 2h after onset of stimulation, but majority of the sites reach maximal accessibility only after 24h [53]. This indicates that the process of chromatin opening via 1,25(OH)\(_2\)D\(_3\) includes multiple steps, i.e., chromatin responses observed after 24h are most likely secondary effects of genes and proteins that are primary vitamin D targets [64]. Interestingly, after 48h most 1,25(OH)\(_2\)D\(_3\)-sensitive chromatin sites return back to basal levels, i.e., many epigenome-wide effects of vitamin D are transient. A multistep process also applies in the mouse IDG-SW3 osteocyte cell line model, in which the effect of 1,25(OH)\(_2\)D\(_3\) on osteocyte differentiation was demonstrated [34]. Changes in gene expression over the 35 days of the differentiation process were accompanied by posttranslational modifications to histones H3 and H4, i.e., in alterations of the epigenetic landscape, that in turn alter the transcriptomic response to 1,25(OH)\(_2\)D\(_3\). Similarly, in the mouse bone marrow-derived mesenchymal stem cell model the differentiation of 7 and 15 days into adipocytes and osteocytes, respectively, is a multistep process that is epigenetically modulated by 1,25(OH)\(_2\)D\(_3\) [37].

Both mouse differentiation models indicate that an alternative way to follow vitamin D-triggered effects on the epigenome is to monitor by H3K27ac ChIP-seq the general activation status of chromatin or more specifically by H3K4me3 ChIP-seq that of TSS regions, i.e., these markers map enhancer and promoter regions, respectively. In THP-1 cells basically all VDR-binding sites show also H3K27ac
marker activity and at a few thousand loci the marker is significantly (P<.05) increased after ligand treatment (unpublished results, see Fig. 13.2 for an example). Similar observations were made for the promoter marker H3K4me3. This suggests that both in mouse and in human models vitamin D modulate the epigenetic landscape.

A CHROMATIN MODEL OF VITAMIN D SIGNALING EMERGES

Based on the harmonized analysis of VDR ChIP-seq datasets from six human cellular models [30] in combination with ChIP-seq datasets on 1,25(OH)₂D₃-dependent PU.1 [42] and CTCF [51] binding and ligand-triggered chromatin opening as measured by FAIRE-seq in THP-1 cells [53] observations can be summarized into the following:

1. In the absence of ligand VDR already binds to a limited number of loci within accessible chromatin.
2. A stimulation with 1,25(OH)₂D₃ increases the number of DNA-bound VDR molecules as well as the percentage of binding sites carrying a DR3-type motif.
3. VDR's access to genomic DNA is supported by pioneer factors, such as PU.1 in monocytes.
4. VDR binding leads to changes in chromatin accessibility, the vast majority of which are opening of chromatin.
5. In parallel with VDR binding and chromatin opening the binding strength of TAD anchor forming CTCF sites upstream and downstream of prominent VDR-binding sites is changing in response to ligand stimulation.
6. In monocytes the borders of more than 400 TADs, which contain at least one VDR sites and one vitamin D target gene, are formed by ligand-sensitive CTCF loci.

This emerging chromatin model of vitamin D signaling (Fig. 13.4) needs to be refined by the integration of vitamin D-sensitive chromatin markers (coming soon for H3K27ac and H3K4me3, see also Fig. 13.2) and other genome-wide data, such as the vitamin D-modulated binding of CoAs, CoRs, chromatin modifiers, and remodelers. Moreover, the chromatin model needs to be integrated with the findings of mouse bone and adipocyte differentiation model systems published by the teams of Pike [34–37] and Mandrup [33]. In this context the time frame of vitamin D- and VDR-triggered epigenomics changes have to be taken into account, to differentiate primary from secondary events.

FIGURE 13.4 Chromatin model of vitamin D signaling. VDR (green) binds accessible genomic DNA in complex with a partner protein (RXR or others, blue). The DNA binding is supported by the pioneer factor PU.1 (purple) and the genomic region that can be influenced by 1,25(OH)₂D₃ (via binding to VDR) is restricted by CTCF proteins (light green) defining left and right TAD borders, i.e., only vitamin D target genes within the TAD will be stimulated to produce more mRNA copies. Further experimental results on other nuclear proteins, such as CoAs, CoRs, and chromatin modifiers, will refine the model. CoAs, coactivators; CoRs, corepressors; CTCF, CCCTC-binding factor; mRNA, messenger RNA; RXR, retinoid X receptor; TAD, topologically associating domain; VDR, vitamin D receptor.

VITAMIN D SIGNALING IN VIVO

The present models of vitamin D signaling are primarily based on experiments, in which (1) a high dose of 1,25(OH)₂D₃ was applied once to a cell culture model and (2) the reference sample was fully depleted from VDR ligands. Significant effects of this ligand stimulation are observed on the level of both the epigenome and the transcriptome in a time frame of 2–24 h [53]. For example, within 24 h the expression of the genes cytochrome P450, family 24, subfamily A, polypeptide 1 (CYP24A1), cathelicidin antimicrobial peptide (CAMP), and CD14 is increased more than 100-fold in THP-1 cells [65]. However, one should always be mindful of the fact that these experimental setups are designed for maximal effects in vitro and may not reflect the reality of the endocrinology of vitamin D in vivo where rapid responses to very high levels of hormone are not the norm [7,66].

When an individual lives in a geographic region where he/she has the chance to expose his/her skin long enough to sunlight containing a reasonable UV-B component, sufficient amounts of vitamin D₃ are produced. Alternatively, vitamin D₃ can be taken up by diet or direct supplementation. In both cases the vitamin D status of the respective human, as measured by the serum levels of 25-hydroxyvitamin D₃ (25(OH)D₃) [53], should be optimal. Seasonal variations in sun exposure may cause 2- to 3-fold changes in 25(OH)D₃ levels, but this happens over weeks and months and not within 24 h [68]. This comparison raises the question of how far results
CONCLUSION

In the past, vitamin D and its metabolites were studied preferentially in the context of calcium homeostasis and bone formation, but nowadays the genome-wide actions of the nuclear hormone are also often monitored in cells of the hematopoietic system. This emphasizes the impact of vitamin D has pleiotropic effects. Moreover, the investigation of hematopoietic in vitro cell culture models, such as THP-1, allows a direct comparison to in vivo experimental setups, where PBMCs represent a tissue that can be isolated fast and with minimal harm for the human donor.

**FIGURE 13.5** Vitamin D response of the KDM1B locus in vivo. An individual was challenged once with a vitamin D$_3$ bolus (2000μg) and PBMCs were isolated before (day 0) and at days 1 and 2. VDR ChIP-seq, FAIRE-seq, and RNA-seq were performed. The IGV browser was used to visualize the KDM1B locus. The peak tracks display data from ChIP-seq for VDR in PBMCs (orange) and in THP-1 cells (red, see Fig. 13.2) and FAIRE-seq in PBMCs (turquoise) and in THP-1 cells (dark blue, see Fig. 13.2). Gene structures are shown in blue. The change in expression of the four genes was measured by RNA-seq (in triplicate experiments with the same individual). Only the genes KDM1B and DEK were significantly ($P<.05$) upregulated (unpublished results). The presence a DR3 motif below the VDR peak is indicated. ChIP-seq, chromatin immunoprecipitation coupled with massive parallel sequencing; FAIRE-seq, formaldehyde-assisted isolation of regulatory elements sequencing; IGV, Integrative Genomics Viewer; PBMC, peripheral blood mononuclear cells; RNA-seq, RNA sequencing; VDR, vitamin D receptor.

II. MECHANISM OF ACTION

from in vitro experiments, such as most of those described above, represents the physiological reality of the actions of vitamin D in vivo.

The VitDbol (NCT02063334) intervention study presents an approach, where peripheral blood mononuclear cells (PBMCs) were isolated before (day 0) and 1 and 2 days (days 1 and 2) after healthy individuals were treated once with a high vitamin D$_3$ dose (2000μg) [69,70]. The serum 25(OH)D$_3$ levels of all individuals increased in average by some 20 nM within 2 days. This represents an elevation of the basal vitamin D level by some 20%–40%. Epigenome-wide changes were investigated by VDR ChIP-seq and FAIRE-seq, and the transcriptome was studied by RNA sequencing (RNA-seq). Fig. 13.5 displays the example of the genomic region of the lysine-specific demethylase 1B (KDM1B) gene.

As in THP-1 cells, VDR binds also in this in vivo setting to the TSS region of the KDM1B gene. Moreover, FAIRE-seq assays demonstrated that after ligand stimulation at the same genomic region chromatin opens not only in THP-1 cells but also in PBMCs. Importantly, the KDM1B gene and its neighboring gene DEK proto-oncogene (DEK) are statistically significantly ($P<.05$) up-regulated (unpublished results). The gene KDM1B encodes for an HDM-type chromatin modifier that may explain some of the epigenome-wide effects of vitamin D. In PBMCs many similar gene-regulatory scenarios can be observed (unpublished results). These first results suggest that also in an in vivo setting ligand-dependent VDR binding and chromatin opening can be observed. However, due to far less drastic changes in VDR ligand concentrations the strength of the observed effects in PBMCs is less prominent than in THP-1 cells. Moreover, PBMCs represent a mixture of monocytes, B cells, and different types of T cells, not all of which are responsive to vitamin D, i.e., effects of vitamin D may be “diluted” by nonresponse cell types. Therefore, the next step of investigations will be to use purified cell types, such as monocytes and CD4$^+$ T cells.
Epigenome-wide effects of vitamin D need to be analyzed in the context of “big biology” projects, such as the Roadmap Epigenomics Consortium, that presented the integration of 111 human epigenomes [71]. In this context, an impressive set of epigenome-wide data, such as different histone modifications and DNA methylation, have been collected from a variety of primary human tissues. As in ENCODE [4], these consortia primarily describe the basal, unstimulated state of the human epigenome in tissues and cell types. This suggests that also the vitamin D field has to adapt principles of the ENCODE and Roadmap Epigenomics projects, to reach a genome-wide understanding of in vivo vitamin D endocrinology.

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References

[27] Carlberg C. Genome-wide (over)view on the actions of vitamin D. Front Physiol 2014;5:167.


