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Recruitment and subnuclear distribution of the regulatory machinery during $1\alpha,25$ -dihydroxy vitamin D_3 -mediated transcriptional upregulation in osteoblasts

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Abstract

The architectural organization of the genome and regulatory proteins within the nucleus supports gene expression in a physiologically-regulated manner. In osteoblastic cells ligand activation induces a nuclear punctate distribution of the $1\alpha,25$ -dihydroxy vitamin D_3 ($1\alpha,25(OH)_2D_3$) receptor (VDR) and promotes its interaction with transcriptional coactivators such as SRC-1, NCoA-62/Skip, and DRIP205. Here, we discuss evidence demonstrating that in osteoblastic cells VDR binds to the nuclear matrix fraction in a $1\alpha,25(OH)_2D_3$ -dependent manner. This interaction occurs rapidly after exposure to $1\alpha,25(OH)_2D_3$ and does not require a functional VDR DNA binding domain. The nuclear matrix-bound VDR molecules colocalize with the also nuclear matrix-associated coactivator DRIP205. We propose a model where the rapid association of VDR with the nuclear matrix fraction represents an event that follows $1\alpha,25(OH)_2D_3$ -dependent nuclear localization of VDR, but that precedes $1\alpha,25(OH)_2D_3$ -dependent transcriptional upregulation at target genes.

Keywords

Vitamin D Receptor; Intranuclear Trafficking; Nuclear Matrix

1. Introduction

The role that vitamin D plays in bone metabolism provides a paradigm for understanding molecular mechanisms that operate in vitamin D action. Vitamin D, in particular its active form $1\alpha,25$ -dihydroxy vitamin D_3 [$1\alpha,25(OH)_2D_3$], promotes osteoblast differentiation by regulating the transcription of a number of genes associated with the osteoblast phenotype [1,2]. Increasing evidence support a relevant role of the nuclear architecture and genome organization within the nuclei in the physiological regulation of eukaryotic genes [3].

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Therefore, in this article we focus on recent evidence that demonstrate the association of the VDR and VDR-bound coregulators with the nuclear matrix fraction in osteoblastic cells.

1.1 Components of $1\alpha,25(\text{OH})_2\text{D}_3$ -dependent regulatory complexes

$1\alpha,25(\text{OH})_2\text{D}_3$ exerts its genomic effects through the $1\alpha,25(\text{OH})_2\text{D}_3$ receptor (VDR) which is a member of the superfamily of nuclear receptors [1,2]. As in other nuclear receptors, binding of the ligand changes the C-terminal ligand-binding domain (LBD) of the VDR, thus establishing competency for VDR interaction with coactivators of the p160/SRC family, which contain intrinsic histone acetyl transferase (HAT) activity and therefore modify chromatin structure [4]. The multisubunit DRIP (VDR-Interacting Protein) complex also binds to VDR in response to the ligand $1\alpha,25(\text{OH})_2\text{D}_3$ [2,4]. This interaction occurs through the LBD of VDR in the same manner as the p160/SRC coactivators, resulting in transcriptional enhancement. In contrast to p160/SRC coactivators, DRIP is devoid of HAT and other chromatin remodeling activities and interacts with nuclear receptors through a single subunit designated DRIP205, which anchors the other DRIP subunits to the receptor LBD. As several of the DRIP subunits are part of the Mediator complex, which interacts with the C-terminal domain (CTD) of RNA polymerase II [5], the DRIP complex appears to function by forming a molecular bridge between the VDR and the basal transcription machinery. The coactivator NcoA62/SKIP can also interact with VDR in a ligand-dependent manner. However, this protein-protein interaction occurs through a domain of VDR that is different from that recognized by p160/SRC or DRIP205 coactivators [6]. Moreover, NcoA62/SKIP can form a ternary complex with VDR and SRC-1 to cooperatively stimulate VDR-mediated transcription activation.

Several research groups have shown that coactivator complexes including p160/SRC and DRIP are recruited to steroid hormone-regulated genes by nuclear receptors in a sequential and mutually exclusive manner [reviewed in 2] (Figure 1A). These results provided the basis for a model in which cyclical association of different coactivator complexes reflects the dynamics of the transcription activation process of nuclear receptor-regulated genes [2]. Alternatively, other reports indicate that occupancy at the target gene regulatory regions by nuclear receptor-associated coactivator complexes may also occur gradually and at a significantly lower rate [2,7].

1.2 VDR functions in association with the nuclear architecture

Increasing evidence indicates that components of nuclear structure, including nuclear pores, the nuclear matrix and subnuclear domains, contribute to subnuclear distribution and activities of genes and regulatory factors [3]. In mammalian cells, including osteoblastic cells, the VDR exhibits a nuclear punctate signal distribution that is significantly enhanced upon ligand stimulation [8,9], suggesting that the VDR is interacting with components of the nuclear architecture.

Several reports indicate that transcriptional coactivators that normally interact with nuclear receptors are associated with the nuclear matrix fraction. GRIP/SRC-2 is associated with specific subnuclear domains [10] and colocalizes with either the glucocorticoid receptor (GR) or the estrogen receptor (ER) upon activation by dexamethasone or estradiol, respectively [Reviewed in 11]. Similarly, SRC-1 is associated with specific subnuclear domains in human breast carcinoma cells and is bound to the nuclear matrix fraction in response to estrogen [12]. In addition, NCoA-62/Skip is bound to the nuclear matrix in osteoblastic cells that respond to $1\alpha,25(\text{OH})_2\text{D}_3$ [13], further indicating that nuclear regulatory complexes including VDR are associated with subnuclear domains where $1\alpha,25(\text{OH})_2\text{D}_3$ -dependent transcriptional control occurs.

We have recently demonstrated that in osteoblastic cells, the VDR binds to the nuclear matrix in a $1\alpha,25(\text{OH})_2\text{D}_3$ -dependent manner [14] (see Figure 1B). This VDR-NMIF interaction occurs rapidly after addition of $1\alpha,25(\text{OH})_2\text{D}_3$ and does not require a functional VDR DNA binding domain (DBD). A significant fraction of the NMIF-bound VDR molecules are found to colocalize with the transcriptional coactivator DRIP205 (Figure 1B), which interacts with the VDR in a highly dynamic ligand-dependent manner to upregulate transcription of bone-related target genes [2,4]. DRIP205 [14], as well as the NcoA62/Skip coactivator [13], is bound to the nuclear matrix of osteoblastic cells in the absence of $1\alpha,25(\text{OH})_2\text{D}_3$ (Figure 1B), indicating that the association of VDR and these transcriptional coactivators with components of the nuclear architecture is controlled through different mechanisms. Interestingly, we find that the coactivator SRC-1 is not bound to the nuclear matrix fraction in osteoblastic cells (Figure 1B), irrespective of the presence of $1\alpha,25(\text{OH})_2\text{D}_3$. As SRC-1 colocalizes with the estrogen receptor (ER) at subnuclear compartments in the human breast cancer cells incubated with estradiol [12], these results indicate that the association of SRC-1 with subnuclear structural components may be a cell- and ligand-specific event.

Paschal and colleagues have reported that upon androgen stimulation the androgen receptor (AR) is transiently bound to a subnuclear compartment. This transient association appears to facilitate protein-protein interactions between the receptor and coactivators localized at these subnuclear domains before ligand-induced transcription of target genes occurs [15]. Interestingly, these authors find that specific mutations in the DBD that prevent the AR from interacting with its DNA element result in the arrest of AR at specific subnuclear foci and strong colocalization with transcriptional coactivators. This distribution pattern, however, was not found when other nuclear receptors were analyzed, including the glucocorticoid receptor, retinoic acid receptor and thyroid receptor. Hence, these results raise the possibility that different nuclear receptors may follow alternative subnuclear distribution pathways once activated by their cognate ligands.

We have found that single aminoacid mutations in the DBD of VDR that prevent direct DNA sequence-specific recognition and $1\alpha,25(\text{OH})_2\text{D}_3$ -mediated transcriptional enhancement in osteoblastic cells, do not affect the ability of this receptor to interact with the nuclear matrix fraction in a ligand-dependent manner [14]. Moreover, these mutations do not affect the ability of VDR to colocalize with the transcriptional coactivator DRIP205 at the nuclear matrix. These results support a model (Figure 1B) where two $1\alpha,25(\text{OH})_2\text{D}_3$ -dependent properties of VDR, nuclear matrix association and DNA binding-transcriptional activation, represent two independent and perhaps sequential, events occurring in osteoblastic cells exposed to the ligand.

A mechanism for targeting the VDR to subnuclear compartments remains undefined. In addition, it is necessary to establish whether specific signaling pathways, including those activated by $1\alpha,25(\text{OH})_2\text{D}_3$ through non-genomic actions at the cell membrane [16], are also contributing to VDR subnuclear localization. It is interesting that a recent report indicates that treatment of osteoblastic cells with $1\alpha,25(\text{OH})_2\text{D}_3$ results in VDR accumulation in the nucleus through a process that involves a protein-protein interaction with the tumor suppressor BRCA1 [17]. Moreover, in osteoblastic cells in which BRCA1 expression is depleted, $1\alpha,25(\text{OH})_2\text{D}_3$ -dependent accumulation of VDR in the nucleus is significantly reduced. Interestingly, BRCA1 has been shown to be localized in subnuclear domains [18] and bound to the nuclear matrix [19], raising the possibility that it may be contributing to the targeting of the VDR to subnuclear structural components in $1\alpha,25(\text{OH})_2\text{D}_3$ -treated cells. This, as well as other alternative nuclear matrix-targeting mechanisms for VDR, are currently under evaluation.

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References

1. Van Driel DM, Pols HA, van Leeuwen JP. Osteoblast differentiation and control by vitamin D and vitamin D metabolites. *Curr Pharm Des* 2004;10:2535–2555. [PubMed: 15320744]
2. Montecino M, Stein G, Cruzat F, Marcellini S, Stein J, Lian J, van Wijnen A, Arriagada G. An architectural perspective of vitamin D responsiveness. *Arch Biochem Biophys* 2007;41:272–278.
3. Zaidi SK, Young DW, Choi JY, Pratap J, Javed A, Montecino M, Stein JL, van Wijnen AJ, Lian JB, Stein GS. The dynamic organization of gene-regulatory machinery in nuclear microenvironments. *EMBO Rep* 2005;6:128–133. [PubMed: 15689940]
4. Rachez C, Freedman LP. Mechanisms of gene regulation by vitamin D(3) receptor: a network of coactivator interactions. *Gene* 2000;246:9–21. [PubMed: 10767523]
5. Kornberg RD. Mediator and the mechanism of transcriptional activation. *Trends Biochem Sci* 2005;30:235–239. [PubMed: 15896740]
6. Barry J, Leong G, Church B, Issa L, Eisman J, Gardiner E. Interactions of SKIP/NCoA-62, TFIIB, and retinoid X receptor with vitamin D receptor helix 10 residues. *J Biol Chem* 2003;278:8224–8228. [PubMed: 12529369]
7. Carvallo L, Henriquez B, Paredes R, Olate J, Onate S, van Wijnen AJ, Lian JB, Stein GS, Stein JL, Montecino M. 1 α ,25-dihydroxyvitamin D₃-enhanced transcription of the bone-specific osteocalcin gene involves preferential recruitment of the coactivator SRC-1. *J Cell Physiol* 2008;214:740–749. [PubMed: 17786964]
8. Barsony J, Renyi I, McKoy W. Subcellular distribution of normal and mutant vitamin D receptor in living cells. *J Biol Chem* 1997;272:5774–5782. [PubMed: 9038191]
9. Paredes R, Arriagada G, Cruzat F, Villagra A, Olate J, Zaidi SK, van Wijnen AJ, Lian JB, Stein GS, Stein JL, Montecino M. The bone-specific transcription factor RUNX2 interacts with the 1 α ,25-dihydroxyvitamin D₃ receptor to up-regulate rat osteocalcin gene expression in osteoblastic cells. *Mol Cell Biol* 2004;24:8847–8861. [PubMed: 15456860]
10. Baumann CY, Ma H, Wolford R, Reyes JC, Maruvada P, Lim C, Yen PM, Stallcup MR, Hager GL. The glucocorticoid receptor interacting protein 1 (GRIP1) localizes in discrete nuclear foci that associate with ND10 bodies and are enriched in components of the 26S proteasome. *Mol Endocrinol* 2001;15:485–500. [PubMed: 11266502]
11. Day RN, Nordeen SK, Wan Y. Visualizing protein-protein interactions in the nucleus of the living cells. *Mol Endocrinol* 1999;13:517–526. [PubMed: 10194758]
12. Stenoien D, Mancini M, Patel K, Allegreto E, Smith C, Mancini M. Subnuclear trafficking of estrogen receptor- α and steroid receptor co-activator-1. *Mol Endocrinol* 2000;14:518–534. [PubMed: 10770489]
13. Zhang C, Dowd DR, Staal A, Gu C, Lian JB, van Wijnen AJ, Stein GS, MacDonald PN. Nuclear coactivator-62 kDa/Ski-interacting protein is a nuclear matrix-associated coactivator that may couple vitamin D receptor-mediated transcription and RNA splicing. *J Biol Chem* 2003;278:35325–35336. [PubMed: 12840015]
14. Arriagada G, Paredes R, van Wijnen AJ, Lian JB, van Zundert B, Stein GS, Stein JL, Montecino M. 1 α ,25-dihydroxy vitamin D₃ induces nuclear matrix association of the 1 α ,25-dihydroxy vitamin D₃ receptor in osteoblasts independently of its ability to bind DNA. *J Cell Physiol*. 2009 In press.
15. Black BE, Paschal BM. Intranuclear organization and function of the androgen receptor. *Trends Endocrinol Metab* 2004;15:411–417. [PubMed: 15519887]
16. Norman AW, Mizwicki MT, Norman DP. Steroid-hormone rapid actions, membrane receptors and conformational ensemble model. *Nat Rev Drug Discov* 2004;3:27–41. [PubMed: 14708019]

17. Deng C, Ueda E, Chen KE, Bula C, Norman AW, Luben RA, Walker AM. Prolactin blocks nuclear translocation of VDR by regulating its interaction with BRCA1 in osteosarcoma cells. *Mol Endocrinol* 2009;23:226–236. [PubMed: 19074549]
18. Scully R, Livingston DM. In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature* 2000;408:429–432. [PubMed: 11100717]
19. Huber LJ, Chodosh LA. Dynamics of DNA repair suggested by the subcellular localization of BRCA1 and BRCA2 proteins. *J Cell Biochem* 2005;96:47–55. [PubMed: 16052489]

Figure 1.

VDR interacts with co-activators and the nuclear matrix in a ligand-dependent manner. A) Schematic representation showing the mutually-exclusive association of VDR with co-regulators of the p160/SRC and DRIP/Mediator families at target genes. VDRE: Vitamin D-Responsive Element; HAT: Histone Acetyl Transferase activity. B) Proposed model for intranuclear trafficking of the VDR. Upon $1\alpha,25(\text{OH})_2\text{D}_3$ stimulation VDR rapidly heterodimerizes with RXR, preferentially localizes in the nucleus and bind to the nuclear matrix and/or target genes. The arrow represents the transcription initiation site and indicates the level and direction of transcription. NM: Nuclear Matrix; N: Nucleus; C: Cytoplasm.