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The Runx2 transcription factor plays a key role in the 1α,25-dihydroxy Vitamin D3-dependent upregulation of the rat osteocalcin (OC) gene expression in osteoblastic cells☆

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Abstract

Bone-specific transcription of the osteocalcin (OC) gene is principally regulated by the Runx2 transcription factor and further stimulated in response to 1α,25-dihydroxy Vitamin D3 via its specific receptor (VDR). The rat OC gene promoter contains three recognition sites for Runx2 (sites A–C). Mutation of sites A and B, which flank the 1α,25-dihydroxy Vitamin D3-responsive element (VDRE), abolishes 1α,25-dihydroxy Vitamin D3-dependent enhancement of OC transcription, indicating a tight functional relationship between VDR and Runx2 factors. Additionally, the transcriptional co-activator p300 is recruited to the OC promoter by Runx2 where it up-regulates both basal and 1α,25-dihydroxy Vitamin D3-enhanced OC expression. Here, we present an overview of how in osteoblastic cells expressing OC, Runx2 modulates the 1α,25-dihydroxy Vitamin D3-dependent stimulation of the OC promoter by first recruiting transcriptional co-activators and then by further stabilizing the interaction of the VDR with the VDRE.

Keywords: Vitamin D; Runx2; Transcription; Osteocalcin

1. Introduction

The rat osteocalcin (OC) gene encodes for a bone-specific protein that is induced in osteoblasts with the onset of mineralization of extracellular matrix, at late stages of differentiation [1]. Bone-specific expression of the OC gene is principally regulated by the Runx2 transcription factor [2,3]. The rat OC gene promoter contains three recognition sites for Runx2 interactions, site A (positions −605 to −595), site B (positions −438 to −430), and site C (positions −138 to −130) (Fig. 1). Mutation of all three Runx2 sites results in significantly reduced OC expression in bone-derived cells [4]. Genetic ablation of the Runx2 gene causes developmental defects in osteogenesis [5] and hereditary mutations in this gene are linked to specific ossification defects as observed in cleidocranial dysplacia [6]. Another key regulatory element that controls OC gene expression is recognized by the 1α,25-dihydroxy Vitamin D3 receptor (VDR) complex upon ligand activation. This 1α,25-dihydroxy Vitamin D3-responsive element (VDRE) is located in the distal region of the OC promoter (positions −465 to −437) (Fig. 1) and functions as an enhancer to increase OC transcription [7]. Here, we present an overview of how in osteoblastic cells expressing OC, Runx2 plays a key role in the 1α,25-dihydroxy Vitamin D3-dependent stimulation of the OC gene promoter by first recruiting the transcriptional co-activator p300 and then by stabilizing, through a direct protein–protein interaction, the binding of the VDR to the VDRE.

2. Runx2 recruits the transcriptional co-activator p300 to the OC promoter

Several lines of evidence suggest a tight functional relationship between Runx2 and the VDR in the regulation of the OC gene transcription. Thus, it has been reported that mutation of Runx2 sites A and B (which flank the VDRE) abolishes 1α,25-dihydroxy Vitamin D3-enhancement of OC promoter activity [4]. It was also shown both in vitro and in intact osteoblastic cells, that VDR and Runx2 factors

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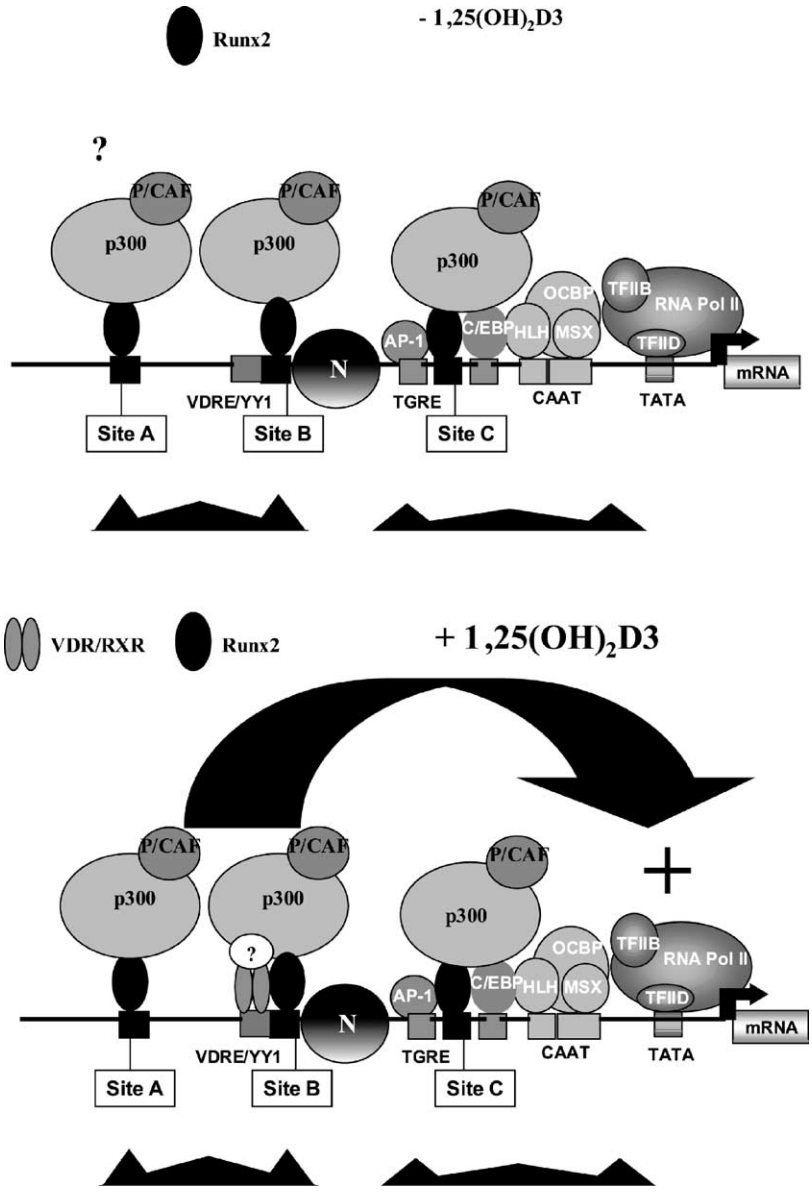

Fig. 1. Schematic representation of the rat OC promoter, including key regulatory elements and the cognate transcription factors that modulate basal tissue-specific (upper panel) and 1α,25-dihydroxy Vitamin D3-enhanced (lower panel) OC transcription in osteoblastic cells. The question mark indicates that recruitment of p300 through the Runx2 site A has not been formally shown. N marks the presence of a positioned nucleosome and the black bars below indicate the presence of the two DNase I hypersensitive sites (proximal and distal). VDRE, 1α,25-dihydroxy Vitamin D3-responsive element; TGRE, TGFβ-responsive element.

interact simultaneously with their cognate sequences located in close proximity within the distal region of the OC promoter (VDRE and Runx2 site B, respectively) [8,9]. In addition, we have reported that in osteoblastic cells the transcriptional co-activator p300 is recruited to the OC promoter by Runx2, where it up-regulates both basal and 1α,25-dihydroxy Vitamin D3-enhanced OC expression [9]. p300 over expression studies have also shown that the Runx2 recognition site B, is required for the p300-dependent up-regulation of the 1α,25-dihydroxy Vitamin D3-enhanced OC promoter activity. p300 has been demonstrated to interact directly, or through other molecules such as the members of the p160 family of co-activators, with nuclear receptors in a ligand-dependent manner [10]. Therefore, we propose that Runx2-mediated recruitment of p300 to the OC promoter may be facilitating the subsequent interaction of p300 with VDR upon ligand stimulation [9] (Fig. 1).

3. Runx2 interacts with VDR

As described above there is a tight functional relationship between Runx2 and VDR factors in the 1α,25-dihydroxy Vitamin D3-dependent transcriptional enhancement of the OC gene expression in osteoblastic cells [4,8,9]. We have found that Runx2 and VDR are components of the same nuclear

protein complexes in bone-derived cells cultured in the presence of 1α,25-dihydroxy Vitamin D3. We immunoprecipitated VDR from nuclear extracts of ROS 17/2.8 osteoblastic cells treated with 10^{-8} M 1α,25-dihydroxy Vitamin D3 for 4 h, but not from cells cultured in the absence of the hormone. The bone-specific transcription factor Runx2 was found present in the immunoprecipitated material strongly indicating that the VDR and Runx2 proteins interact. This direct protein–protein interaction was further confirmed by GST pull down analyses using recombinant Runx2 and VDR proteins expressed in bacteria. Using this same experimental approach, we determined that the N-terminus of VDR is necessary for its interaction with Runx2. We also mapped the region of Runx2 responsible for the interaction with VDR and found it to be located C-terminal of the *runt* homology DNA binding domain of Runx2.

4. Conclusion

In conclusion, our results indicate that in osteoblastic ROS 17/2.8 cells cultured in the presence of 1α,25-dihydroxy Vitamin D3, the bone-specific transcription factor Runx2 interacts directly with VDR. This protein–protein interaction may be highly relevant for the stabilization of the VDR complex as it binds to the OC promoter VDRE upon ligand stimulation [8,11]. Moreover, we have found that p300 is recruited to the OC promoter by Runx2 [9]. p300 is capable of interacting simultaneously with several transcription factors, including nuclear steroid receptors, thus forming large multi-molecular protein complexes that regulate transcription [10]. Together, these results provide a basis for postulating that following 1α,25-dihydroxy Vitamin D3 treatment of bone-derived cells expressing OC, the p300 molecules recruited to the OC promoter by Runx2, can functionally interact with the VDR complex bound to the VDRE, and together enhance basal OC transcription (Fig. 1).

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