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Emerging insights into the coactivator role of NCoA62/SKIP in Vitamin D-mediated transcription☆

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Abstract

NCoA62/SKIP was discovered as a nuclear protein that interacts with the Vitamin D receptor (VDR) and the SKI oncoprotein. NCoA62/SKIP expresses properties consistent with other nuclear receptor transcriptional coactivator proteins. For example, NCoA62/SKIP interacts selectively with the VDR–RXR heterodimer, it forms a ternary complex with liganded VDR and steroid receptor coactivator (SRC) proteins, and it synergizes with SRCs to augment 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$]- and VDR-activated transcription. Chromatin immunoprecipitation studies show that NCoA62/SKIP is recruited in a 1,25-$(OH)_2D_3$-dependent manner to native Vitamin D responsive gene promoters and it enters these promoter complexes after VDR and SRC entry. This suggests that NCoA62/SKIP functions at a distal step in the transactivation process. Recent studies indicate that NCoA62/SKIP is a component of the spliceosome machinery and interacts with important splicing factors such as prp8 and the U5 200 kDa helicase. Functional studies also support an involvement of NCoA62/SKIP in mRNA splicing. Collectively, these data suggest a pivotal role for NCoA62/SKIP in coupling transcriptional regulation by VDR to RNA splicing. They further solidify an important role for VDR/NR-interactors downstream of the transcription process in determining the overall response of Vitamin D and steroid hormone regulated genes.

Keywords: Vitamin D receptor; Nuclear receptor; Cholecalciferol; NCoA62; Ski interacting protein; mRNA splicing; Coactivator; Transcription splicing coupling

The diverse effects of 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] are mediated through the Vitamin D receptor (VDR). The VDR is member of the superfamily of receptors for steroid hormones and, as such, it functions as a ligand-activated transcription factor [1,2]. That is, the 1,25-$(OH)_2D_3$ hormonal ligand selectively binds to and "activates" the VDR. One aspect of ligand activation is promoting the selective heterodimerization of VDR with retinoid X receptor (RXR), the obligate dimeric partner in VDR-mediated transcription (Fig. 1). The liganded VDR–RXR heterodimer interacts with specific DNA response elements located in the promoter regions of Vitamin D-responsive genes (i.e. VDREs). The interaction of the receptors with promoter DNA ultimately influences the rate of RNA polymerase II-directed transcription. Important molecular details of this transactivation process have been revealed over the past several years. An emerging concept is that the VDR transactivation process involves a complex series of protein–protein interactions with a variety of discrete comodulator proteins and large protein complexes that may occur in a temporally coordinated fashion ultimately leading to 1,25-$(OH)_2D_3$-induced alterations in chromatin structure and enhanced transcription of target genes (Fig. 1). Here, we briefly review several features of discrete coactivators and coactivator complexes and their potential roles in VDR-activated transcription.

1. Protein–protein interactions in nuclear receptor-mediated transcription

The transcriptional components that are known to interact with VDR and other nuclear receptors may be classified into several broad categories (Fig. 2) including; the general transcription factors (GTFs), the TBP-associated factors (TAFs) in the TFIID complex, and the comodulatory proteins or the nuclear receptor coactivators and corepressors. Protein–protein contacts between nuclear receptors and the basal transcriptional machinery are important for ligand-mediated transactivation or repression by nuclear receptors (NR). In this regard, TFIIB and TAFs appear to be a central targets for nuclear receptor (NR) interaction.

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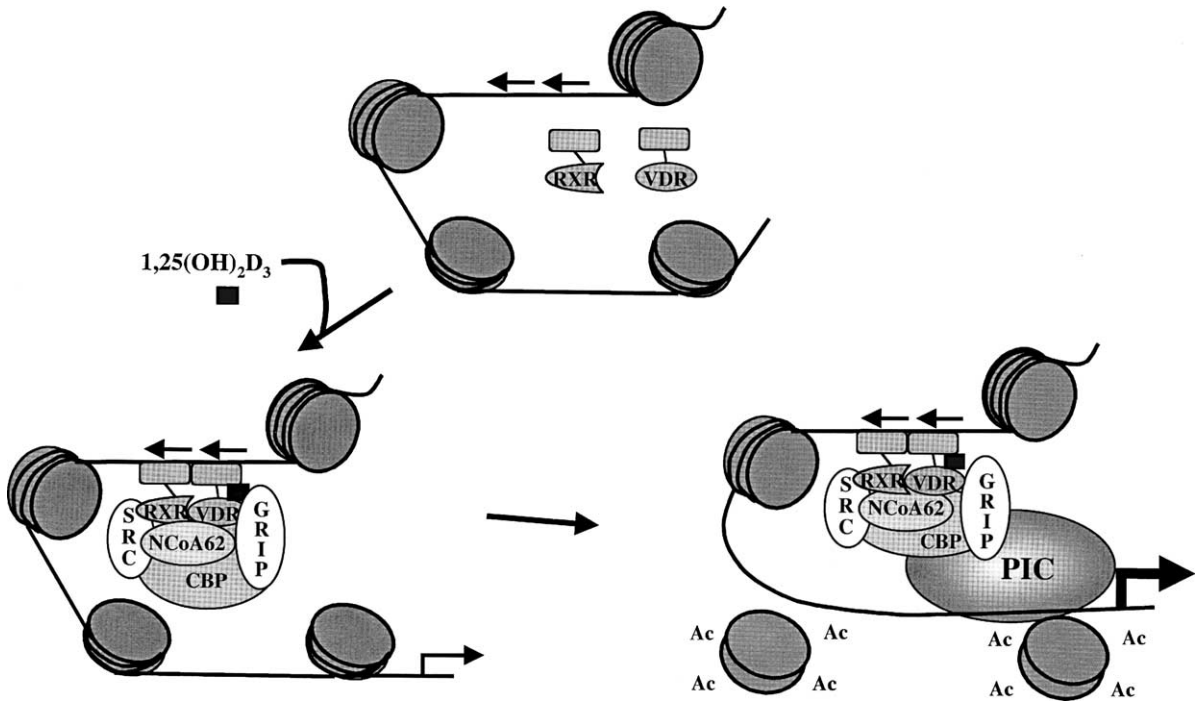

Fig. 1. Nuclear receptor coactivator proteins in Vitamin D-mediated transcription. 1,25-Dihydroxyvitamin D_3 promotes VDR–RXR heterodimerization and tight association with DR-3 type elements located in the promoter regions of Vitamin D responsive genes. Ligand binding also induces interactions between VDR and SRC coactivators. SRCs and CBP express HAT activities that acetylate histones resulting in a loosening of chromatin structure in the hormone responsive and basal promoter regions of the gene. Different classes of coactivators, such as NCoA62/SKIP, also play important roles in this transactivation process.

Direct interactions of TFIIB with the estrogen, progesterone, thyroid hormone, and Vitamin D receptors have been reported [3–7]. This interaction is functionally important since TFIIB expression augments Vitamin D-activated transcription in transient gene expression studies [7] and a dominant-negative inhibitor of TFIIB–VDR complexes selectively impairs VDR-activated transcription [8]. Indeed, differences in the activities of NH_2-terminal VDR isoforms are attributed to differential interactions with TFIIB [9] thus, supporting an important role for the VDR–TFIIB interaction in determining the overall transactivation potential of the liganded VDR.

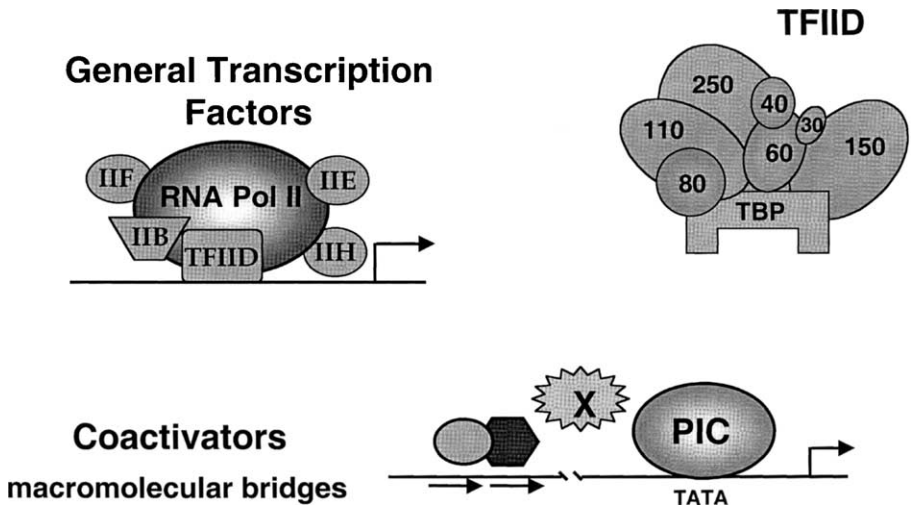

Fig. 2. General protein targets for VDR and other nuclear receptors in the transactivation mechanism. Nuclear receptor contacts with the preinitiation complex (PIC) include central factors such as transcription factor IIB and the TBP-associated factors (TAFs) in the TFIID complex. Critical associations with nuclear receptor comodulators also occur including ligand-dependent interactions with the coactivator proteins which may serve as a bridge between the nuclear receptor and the PIC.

NR comodulatory proteins (i.e. coactivators and corepressors) have emerged as central players in the communication process connecting ligand-activated receptors to the preinitiation complex and to chromatin structure (Fig. 1) [10,11]. The general functional properties of the NR comodulators are their ability to interact with nuclear receptors and control their transcriptional responsiveness to ligand. NR coactivators may function as macromolecular bridges between the receptor and the transcriptional machinery that aid in the assembly or promote the stability of the preinitiation complex. Moreover, several coactivator proteins either possess intrinsic histone acetyltransferase (HAT) activities [12,13], or recruit other proteins that possess HAT activity (e.g. CBP/P300). Acetylation of histones near the promoter presumably results in a loosening of chromatin structure and greater accessibility of the promoter for the transcriptional machinery (Fig. 1). The best-characterized coactivators are the steroid receptor coactivator (SRC) family of nuclear receptor coactivators [14] that includes three members at present: SRC-1 (NCoA-1), SRC-2 (GRIP-1, TIF2, NCoA-2), and SRC-3 (pCIP, RAC3, ACTR, AIB-1, TRAM-1).

2. The SRC family of coactivators

The SRC family members interact in a ligand-dependent manner with the nuclear receptors [15–17]. This interaction is mediated through three leucine-rich motifs located in the central region of SRC which have the consensus sequence LXXLL and are termed nuclear receptor boxes or NR-boxes [18]. Mutation of the NR-boxes abolishes the coactivator activity by disrupting their interaction with nuclear receptors [18]. With regard to the nuclear receptors, the activation function-2 (AF-2) domain is critical for interaction with LXXLL-containing coactivators [15]. Deletion of or mutations within the AF-2 domain selectively abolish ligand-activated transcription by disrupting receptor interaction with the SRC or other NR-box coactivators. The VDR AF-2 domain is a COOH-terminal amphipathic α-helix with a centrally conserved glutamic acid residue (E420 in the hVDR sequence) flanked on either side by hydrophobic residues. Mutation of residues in the hydrophobic or hydrophilic face abolishes 1,25-$(OH)_2D_3$-activated transcription as well as SRC interaction with the VDR [19,20], illustrating the mechanistic importance of coactivator contacts with the AF-2 domain.

Structural studies of related receptors provide insight into the mechanism of ligand-induced interaction of VDR with SRC coactivators [21,22]. It is hypothesized that the 1,25-$(OH)_2D_3$ ligand promotes coactivator interaction by inducing a repositioning of the AF-2 activation helix, or helix H12 (Fig. 3). In the unliganded state, the AF-2 domain (helix H12) projects out away from the globular core of the LBD, while in the liganded state the AF-2 domain is folded over onto the LBD globular core domain. One outcome of

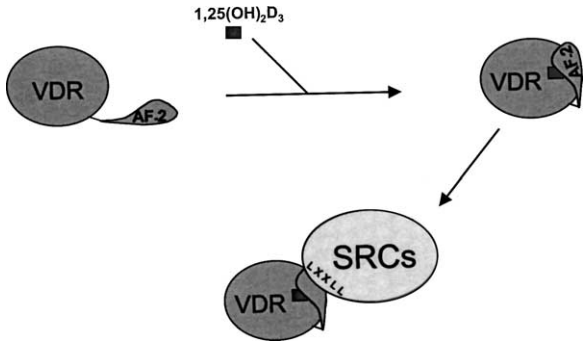

Fig. 3. Ligand-induced conformational changes in the activation function-2 (AF-2) helix of the Vitamin D receptor and interaction with LXXLL-containing coactivators. This model is based on the crystal structures of the VDR ligand binding domain as well as the structures of numerous related receptors. 1,25-$(OH)_2D_3$ binding induces a repositioning of helix H12 so that it folds over and contacts the globular LBD core. This AF-2 repositioning creates a platform or docking surface that mediates the interaction with the LXXLL NR-box in the SRC and DRIP coactivators.

helix H12 folding is the creation of a platform or protein interaction surface through which LXXLL-containing NR coactivator proteins such as SRCs effectively dock with the VDR. Scanning mutagenesis of the thyroid hormone receptor (TR) defined a coactivator interaction surface composed of helices H12, H3, H4, and H5 [23] and structural analysis of the estrogen, thyroid hormone, and PPAR receptors complexed to NR-box peptides supported this model [22,24,25]. Consequently, docking of SRC coactivators to the NR is a ligand-dependent-, AF2 (helix H12)-dependent process that is mediated through direct, selective LXXLL-binding of the coactivator to the H3–H5, H12 docking surface of the NR.

3. Vitamin D receptor interacting proteins (DRIPs)

In addition to SRCs, a large multiprotein complex called DRIP (Vitamin D receptor interacting protein) is a coactivator for VDR and other nuclear receptors [26]. DRIP is nearly identical to the transcriptional coactivators of the thyroid receptor activating protein (TRAP) complex and the mammalian Mediator complex [27,28]. The DRIP complex is composed of at least ten different proteins anchored by DRIP205/TRAP220, which interacts directly with ligand-activated VDR/RXR heterodimers through one of two LXXLL motifs [29]. DRIP is essential for in vitro VDR-mediated transcription from chromatinized templates [30]. However, as the DRIP complex does not contain any SRCs and does not possess HAT activity, it appears to potentiate NR-mediated transcription through distinct mechanisms [29]. In particular, DRIP directly recruits the RNA polymerase II holoenzyme to 1,25-$(OH)_2D_3$-activated VDR, indicating that DRIP may serve as a bridge between VDR and the core transcriptional machinery [31]. Indeed, chromatin immunoprecipitation studies show that DRIPs enter estrogen receptor-containing transcriptional

complexes at a later time than SRC coactivators, supporting the non-redundant roles of these coactivator classes in NR-mediated transactivation [32].

4. NCoA62/SKIP, discovery of a unique, multifunctional coactivator protein

In 1996, we isolated a putative coactivator protein that interacted with the VDR using yeast 2-hybrid screening strategies. The expressed human protein was 62,000 Da, it interacted with VDR and other nuclear receptors and it augmented VDR- and other NR-activated transcriptional processes in transient reporter gene assays. Thus, this protein expressed the relevant properties to be broadly classified as a NR coactivator protein. Consequently, the protein was termed NCoA62, for nuclear coactivator of molecular mass 62,000 Da. During the time we were performing our initial studies, another group entered a GenBank sequence that matched our NCoA62 clone. The GenBank clone was termed SKIP for Ski-interacting protein. Ski is an important oncoprotein involved in muscle cell differentiation [33], and it is a potent transcriptional repressor protein, particularly in TGFβ signaling [34,35]. Thus, from the very early stages, it was evident that NCoA62/SKIP may function in a variety of transcriptional regulatory pathways. Both initial reports on NCoA62/SKIP were published several months apart in 1998 [36,37].

Sequences related to NCoA62/SKIP are found in a wide range of organisms including *C. elegans*, *S. pombe*, and *D. melanogaster*. These orthologs are highly conserved and indicate a crucial role of NCoA62/SKIP in fundamental biological processes. Indeed, genetic studies point to the essentiality of these NCoA62/SKIP orthologs in maintaining viability of these lower eukaryotic organisms [38–40]. Genetic ablation of murine NCoA62/SKIP has not been reported, though it is reasonable to predict a similar essential role in mammals as well. For example, BLAST searches of the public human genome database do not reveal other human sequences that are related to hNCoA62/SKIP. Thus, it is unlikely that related family members for NCoA62/SKIP exist in humans. It is also important to note that NCoA62/SKIP is unrelated to other NR coactivators, such as the SRCs, that have been described thus far.

On the basis of its primary amino acid sequence, NCoA62/SKIP is a unique protein. Functionally, it is also distinct from other NR comodulators described thus far. One key difference is the lack of LXXLL motifs which are characteristic of a large variety of coactivators including the SRCs, CBP/p300 and the DRIP/TRAP coactivator complex. In contrast to most other LXXLL-containing coactivators, NCoA62 interacts with VDR and other NRs in a ligand-independent and in an AF2-independent manner (Fig. 4) [36,41]. Accumulating evidence strongly supports an important and unique role for NCoA62/SKIP in NR-activated transcription. NCoA62/SKIP interacts with and augments transactivation mediated by a variety of NRs [36]. NCoA62/SKIP interacts selectively with the VDR–RXR heterodimer as compared to its weaker interaction with VDR homodimers or monomers [42]. Thus, NCoA62/SKIP shows a marked preference for the functionally relevant heterodimeric complex. Although NCoA62/SKIP does not interact directly with SRC coactivators, NCoA62/SKIP, VDR, and SRCs form a ligand-dependent ternary complex (Fig. 5) [42]. Finally, NCoA62/SKIP also functions cooperatively with SRC coactivators to augment VDR-activated transcription and dominant negative inhibitor studies point to the essentiality of both classes of coactivator proteins (i.e. AF2-dependent and independent proteins such as SRCs and NCoA62, re-

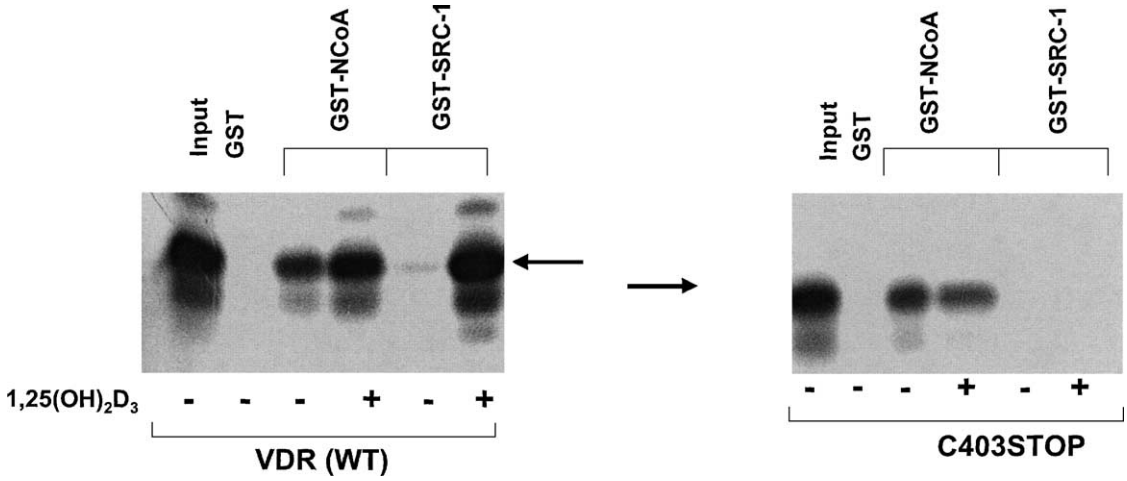

Fig. 4. NCoA62/SKIP interacts in an AF-2 and ligand-independent manner with the VDR. Radiolabeled wild-type VDR (left panel) or the VDR AF-2 deletion mutant (C403STOP, in the right panel) were incubated with GST, GST-NCoA62, or with GST-SRC1 in the absence or presence of 1,25-$(OH)_2D_3$. Protein complexes were resolved on SDS-PAGE gels and exposed to film. In contrast to SRC1, NCoA62/SKIP interaction with VDR is not dependent on the ligand or on the AF-2 domain of the VDR.

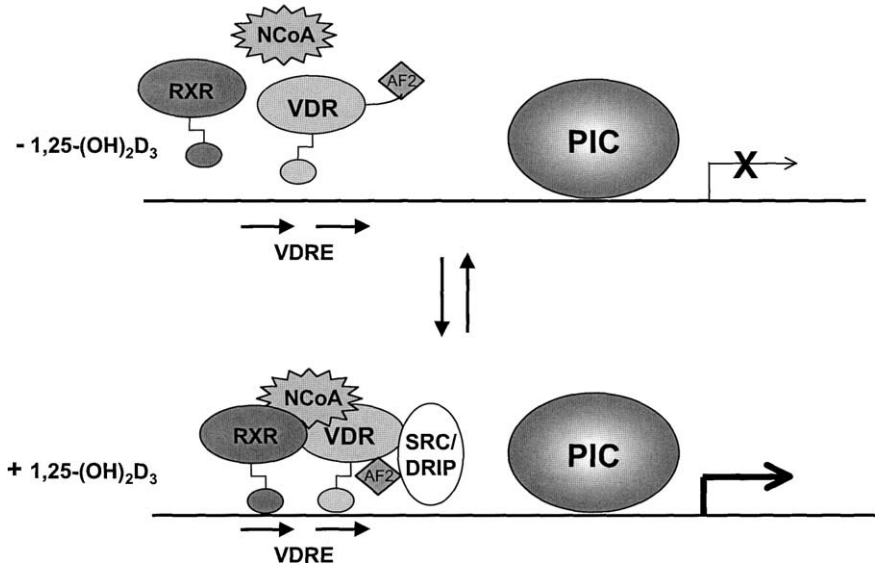

Fig. 5. Model for the interaction of NCoA62/SKIP and SRCs in VDR-activated transcription. This model depicts the preference of NCoA62/SKIP for interaction with the VDR–RXR heterodimer as well as its interaction with the VDR LBD through a region that is distinct from the H3–5/H12 interaction surface for LXXLL-containing coactivators such as SRCs and DRIPs. It also represents the requirement and interplays of both coactivator classes (i.e. AF-2 dependent and AF-2 independent) in VDR-mediated transactivation.

spectively) in VDR-activated transcription [42]. Presently, the mechanisms involved in this synergistic action of these two coactivator classes are unknown.

The studies described above strongly support the concept of NCoA62/SKIP as a NR coactivator. However, the major caveat has been that this concept is based on overexpression studies in mammalian cells and on in vitro protein–protein interaction studies, conditions that are far removed from a native cellular context. Importantly, our recent use of the chromatin immunoprecipitation approach provides strong support for the involvement of NCoA62/SKIP in VDR-activated transcription of relevant target genes in osteoblasts [43]. These studies show a 1,25-$(OH)_2D_3$-mediated recruitment of endogenous NCoA62/SKIP to the Vitamin D responsive promoter regions of the native 24-hydroxylase and osteocalcin target genes in osteoblast cells. Thus, NCoA62/SKIP is physically recruited and present during the 1,25-$(OH)_2D_3$/VDR-activated transcription in target cells. Importantly, NCoA62/SKIP recruitment to these promoters is markedly delayed relative to SRC entry. This further supports the notion that NCoA62/SKIP and the SRCs are functionally distinct coactivators that may play different roles in VDR-activated transcription.

5. NCoA62/SKIP, a putative transcription/splicing coupling factor

To gain insight into potential mechanistic differences between NCoA62/SKIP and SRCs, we purified and identified HeLa cell nuclear proteins that interacted with GST-NCoA62/SKIP [43]. The majority of NCoA62/SKIP-interacting proteins represented components of the spliceosome, a huge multi-component cellular machine involved in processing pre-mRNA into mature mRNA. Prominent NCoA62/SKIP-interacting proteins were identified as prp8, U5snRNP 200 kDa helicase, prp28, and 116 kDa U5 snRNP component. These studies are in agreement with previous studies showing that NCoA62/SKIP is present in global spliceosome complexes [44–46]. Moreover, a dominant-negative inhibitor of NCoA62/SKIP provided the initial evidence that NCoA62/SKIP has a functional role in mRNA splicing. Expression of a dominant negative NCoA62/SKIP resulted in a 1,25-$(OH)_2D_3$-dependent transient accumulation of unspliced transcripts generated from a Vitamin D responsive mini-gene cassette [43]. These studies indicate that disrupting native NCoA62/SKIP through the expression of the dominant-negative NCoA62/SKIP derivative interferes with the proper splicing of transcripts from a 1,25-$(OH)_2D_3$-inducible reporter gene in mammalian cells. Collectively, these most recent data indicate a putative role for NCoA62/SKIP as a factor that may couple transcription regulation to RNA splicing events in Vitamin D-mediated transcription.

Considerable evidence supports the concept of a functional coupling between RNA polymerase II-directed transcription and RNA processing events, including RNA capping, polyadenylation, and splicing [47–49]. Putative candidates that may aid in the coupling process might be predicted to associate with and alter the functional properties of proteins involved in both transcription and RNA processing. NCoA62/SKIP expresses properties that are consistent with this putative role and other NR coactivator proteins were recently proposed to play a similar role. Auboeuf et al.

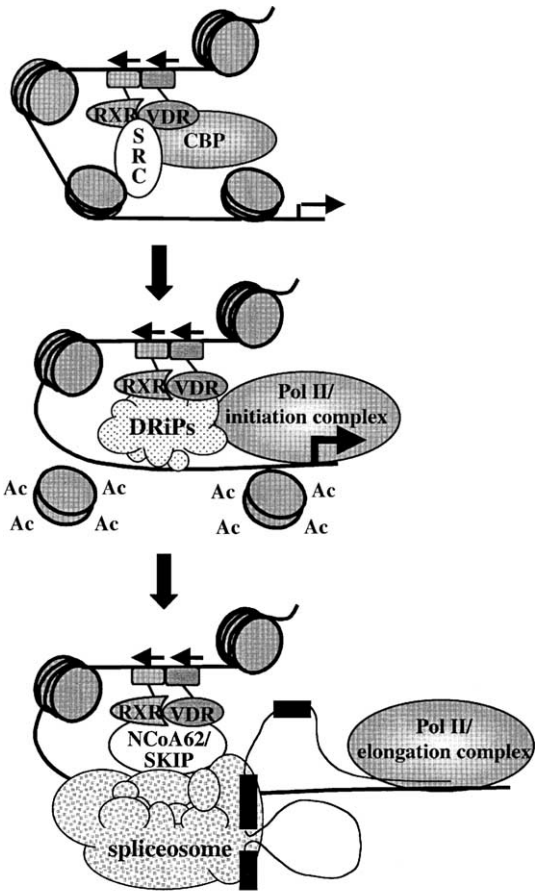

Fig. 6. Temporal entry and distinct functional involvement of various VDR coactivators in the mechanism of VDR-activated transcription. This model is based on chromatin immunoprecipitation studies and functional studies in various NR-mediated transcription systems. The general concept is that distinct coactivator classes function in different aspects of hormone-activated transcription. SRCs and CBP/P300 may be initially recruited to open the chromatin template through their HAT and HAT-associated activities. The DRIP complex, which lacks HAT activity, enters later and may recruit the RNA polymerase II holoenzyme complex. Finally, NCoA62/SKIP enters later and perhaps mediates the recruitment of the spliceosome, thus facilitating splicing of the nascent pre-mRNA. These combined actions provide for efficient 1,25-$(OH)_2D_3$-activated gene expression.

recently demonstrated that PR-activated transcription influenced splicing decisions of alternatively spliced transcripts in a PR and progesterone response element-dependent manner [50]. They and others provided evidence implicating NR coactivators in this transcription-splicing coupling role [50,51].

Although direct evidence proving a role for NCoA62/SKIP as a transcription/splicing coupling factor is lacking, several key observations support this hypothesis. First, endogenous NCoA62/SKIP is recruited to native VDR-responsive promoters in a 1,25-$(OH)_2D_3$-dependent manner in osteoblast-like target cells proving its physical association with the transcriptional regulatory machinery at the responsive promoter regions. Second, the predominant nuclear proteins interacting with NCoA62/SKIP are components of the U5 snRNP, a central snRNP involved in splicing of mRNA transcripts. Furthermore, NCoA62/SKIP is predominantly associated with the nuclear matrix, a property that is shared with numerous other splicing factors and transcriptional regulators. Finally, our data suggest a functional role for NCoA62/SKIP in mRNA splicing. These recent studies provide the basic framework for our working hypothesis that NCoA62/SKIP may couple VDR-mediated transcription to RNA splicing.

Fig. 6 presents an integrated model that incorporates these recent observations on NCoA62/SKIP with other NR coactivators, including SRCs and DRIPs. This model is based on the temporal entry of distinct coactivator classes onto target promoters following steroid hormone activation [32]. Coactivators with HAT activity (CBP and SRCs) are thought to enter first in order to open chromatin structure through histone acetylation. The DRIP complex enters later and one potential role is the recruitment of RNA polymerase holoenzyme into the complex. Our chromatin immunoprecipitation studies reveal that NCoA62/SKIP entrance is markedly delayed relative to other LXXLL containing coactivators. Thus, we propose that this delayed entry is reflective of its potential role in splicing of the nascent RNA transcript as it emerges from the elongation complex. Thus, distinct coactivator classes enter the promoter complexes at distinct times to fulfill their individual roles in the hormone-dependent transactivation process.

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