Interactions of SKIP/NCoA-62, TFIIB, and Retinoid X Receptor with Vitamin D Receptor Helix H10 Residues*

Received for publication, December 31, 2002, and in revised form, January 15, 2003
Published, JBC Papers in Press, January 15, 2003, DOI 10.1074/jbc.C200712200

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The vitamin D receptor (VDR) is a ligand-dependent transcription factor that heterodimerizes with retinoid X receptor (RXR) and interacts with the basal transcription machinery and transcriptional cofactors to regulate target gene activity. The p160 coactivator GRIP1 and the distinct coregulator Ski-interacting protein (SKIP)/NCoA-62 synergistically enhance ligand-dependent VDR transcriptional activity. Both coregulators bind directly to and form a ternary complex with VDR, with GRIP1 contacting the activation function-2 (AF-2) domain and SKIP/NCoA-62 interacting through an AF-2 independent interface. It was previously reported that SKIP/NCoA-62 interaction with VDR was independent of the heterodimerization interface (specifically, helices H10/H11). In contrast, the present study defines specific residues within a conserved and surface-exposed region of VDR helix H10 that are required for interaction with SKIP/NCoA-62 and for full ligand-dependent transcription activity. SKIP/NCoA-62, the basal transcription factor TFIIB, and RXR all interacted with VDR helix H10 mutants at reduced levels compared with wild type in the absence of ligand and exhibited different degrees of increased interaction upon ligand addition. Thus, SKIP/NCoA-62 interacts with VDR at a highly conserved region not previously associated with coregulator binding to regulate transactivation by a molecular mechanism distinct from that of p160 coactivators.

The vitamin D receptor (VDR)† is a ligand-dependent transcription factor important in the regulation of calcium homeostasis, development, cell growth, and differentiation. VDR belongs to the nuclear receptor superfamily, members of which share a common modular structure including a highly conserved DNA binding domain (DBD) and a conserved ligand binding domain (LBD). The LBD has a predominantly a-helical structure with an activation function-2 (AF-2) domain in the COOH-terminal helix (H12). The LBD is the main site of VDR interaction with its heterodimer partner retinoid X receptor (RXR) and basal transcription factors, and the AF-2 domain in combination with the hydrophobic cleft forms an interaction surface for transcriptional corepressors and coactivators (1, 2). In the absence of its ligand 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), VDR transcriptional activity is low due to interaction with corepressors such as N-CoR and SMRT and potentially through sequestration of the basal transcription factor TFIIB. Addition of 1,25(OH)₂D₃ causes dissociation of VDR-corepressor and VDR-TFIIB complexes, thereby relieving repression (3–5). VDR ligand-dependent activation is facilitated by recruitment of at least one coactivator protein with intrinsic histone acetyl transferase activity, such as p160 coactivators. Recruitment of multiprotein coactivator complexes including CBP/p300, p/CAF, and DRIP provide further regulation, coordinating interactions of the receptor with other proteins including basal transcription factors, RNA polymerase II, and the p160 coactivators (6).

The novel coregulator Ski-interacting protein (SKIP)/NCoA-62, which is structurally distinct from the p160 coactivators, was isolated through its interactions with both the Ski oncoprotein and VDR (7, 8). It also interacts with other nuclear proteins including the repressor CBF-1 and the HPV-16 E7 oncoprotein, can bind coregulators such as SMRT, Sin3A, HDAC2, and SRC-1, and interacts with Smad proteins to augment transforming growth factor-β-dependent transactivation (7–12). SKIP/NCoA-62 functions in cell fate determination, alternatively facilitating gene repression by tethering CBF-1 to the corepressor SMRT and gene activation by tethering CBF-1 to the activator NotchIC (10). Such mutually exclusive interactions with coactivators and corepressors suggest that SKIP/NCoA-62 may facilitate coregulator switching.

SKIP/NCoA-62 augments transactivation by the vitamin D, retinoic acid, estrogen, and glucocorticoid receptors (7), selectively binding the VDR-RXR heterodimer in a ligand-enhanced manner (13). SKIP/NCoA-62 forms a ternary complex and acts synergistically with the p160 coactivator GRIP1 to augment VDR transactivation (13). Consistent with such complex formation, p160 coactivators contact VDR through the AF-2 do...
main, whereas SKIP/NCoA-62 interactions with VDR are AF-2-independent. SKIP/NCoA-62 reportedly contacts helix H1 of the VDR LBD (residues 116–164 of human VDR) and the helix H10/H11 region (residues 373–403) but not helix H12 (7). It was subsequently shown, however, that SKIP/NCoA-62 could bind to the VDR/RXR heterodimer, leading to the conclusion that the heterodimerization interface (including helix H10/H11) could be excluded as a SKIP/NCoA-62 interaction surface (13).

In the present study, we further investigated structural aspects of VDR-SKIP/NCoA-62 interaction to determine how structure may mediate SKIP/NCoA-62 function. An interaction site was mapped to helix H10 of mouse VDR by mutational analysis. Alteration of structurally exposed amino acids of this helix markedly reduced 1,25(OH)2D3-dependent interactions with SKIP/NCoA-62 but not ligand-dependent interactions with the p160 coactivators GRIP1 and RAC3. These same VDR residues were required for interaction with RXR regardless of ligand status and for ligand-independent interaction with TFIIB. The results indicate that a transcriptional coactivator, a basal transcription factor, and the VDR heterodimerization partner each interact with VDR at the same helix H10 interface, with varying ligand sensitivities. As SKIP/NCoA-62 interacts with both unliganded and liganded VDR, the data also suggest that it may modulate the ligand-regulated occupancy of this VDR surface by TFIIB and RXR.

EXPERIMENTAL PROCEDURES

Plasmids and Mutagenesis—Mouse VDR (mVDR) was amplified from full-length cDNA (T. Kawada, Kyoto University) using wild type or mutant primers containing stop codons for truncation mutants or point mutations and cloned into pSG5 (Stratagene) and pAS2–1 (Clontech). Mutant Δ389 was further mutated to create Δ389Q380E, Δ389R384K, and Δ389A3X, the last being a triple point mutant with coordinate Q380A, D384A, and R386A changes. Full-length wild type cDNA was similarly mutated to produce mutant AX3. Constructs pACTII/SKIP (amino acids 145–530) (12), pACTII/TFIIB (14), pACTII-RXRα, pGAD424-GRIP1 (nucleotides 204–4878), pGAD10-RAC3 (nucleotides 1289–3698), pSG5GRIP1, and pSG5RAC3 (15) have been described.

Molecular Modeling—A model of the mouse VDR LBD was built using the 1.5 Å resolution crystal structure of the ligand bound human VDR LBD as a template (Protein Data Bank accession code 1DB1; Ref. 2). Initial OLSUSTALW (16) alignments were performed using the subsequent sequences of hRXRα, hRXRβ, and hRXRγ. hTRα, and hTRβ; hPPARα, hPPARγ, and hPPARG; hVDR, and mVDR, with subsequent analyses performed using hRARα, hRARγ, hVDR, mVDR, and mVDR sequences. Modeling was performed using InsightII and Homology version 98 software (Molecular Simulations Inc., San Diego, CA). The model of the LBD was built from residue 124 and required no insertions or deletions. Side chain rotamer positions were searched by retaining identical residue. Asp-337, stacked on Arg-338, making the aliphatic side chains of the conserved residues Gln-380, Asp-384, and Arg-389 context (Fig. 1, A and B). Each point mutation diminished VDR interaction with SKIP/NCoA-62 by 60–80% compared with wild type and Δ389 controls (Fig. 2B). Similarly, coordinate mutation of these residues to alanine in the Δ389 context (Δ389A3X mutant) decreased this interaction by 80%. Interaction of the full-length VDR AX3 mutant was limited to ~25% of wild type in the absence of ligand, but approached wild type levels with 1,25(OH)2D3 (Fig. 2C). Wild type VDR interaction with GST-SKIP in vitro was also comparable both with and without ligand (Fig. 2D). Mutant AX3 bound less strongly, although without the ligand-induced increase in SKIP/NCoA-62 interaction seen in yeast.

VDR Helix H10 Not Required for p160 Coactivator Interaction—In contrast to SKIP/NCoA-62, interactions of the p160 coactivators GRIP1 and RAC3 with wild type and AX3 mutant VDR were entirely ligand-dependent (Fig. 3A). Interestingly,
p160 coactivator interactions with AX3 were elevated by \( \frac{1}{2} \), suggesting that residues Gln-380, Asp-384, and Arg-386 may negatively affect access of p160 coactivators to the hydrophobic cleft on the LBD. Neither AX3 nor AX3 interacted with GRIP1 or RAC3, consistent with the AF-2 deletion. In vitro interactions between the p160 coactivators and VDR mutants were consistent with the yeast data (Fig. 3B). Thus, VDR helix H10 is important for interaction with SKIP/NCoA-62 but not p160 coactivators.

VDR Helix H10 Mutations Inhibit Transactivation Function—Effects of these helix H10 mutations on VDR function were assessed by transient transfection. Transactivation by either wild type or AX3 mutant VDR was \( \frac{1}{2} \) dose-responsive, but AX3 activity was limited to about 40% of wild type at the highest dose tested, despite comparable VDR protein levels (Fig. 3C). Thus, the integrity of helix H10 residues Gln-380, Asp-384, and Arg-386 is necessary for efficient VDR transactivation.

Other Interactions at Helix H10 Interface—In addition to SKIP/NCoA-62, TFIIB and RXR also interact with this region of VDR (5, 22-24), so their interactions with the helix H10 mutants were investigated. Two-hybrid interaction of wild type
liganded AX3 did not interact with RXR, and 1,25(OH)2D3 or 10−8 M 1,25(OH)2D3 (solid bars) treatment. B, in vitro binding of mVDR wild type or AX3 with GST-TFIIB, GST-RXR, or GST-0, with or without 10−7 M 1,25(OH)2D3. Input was 10% of VDR used in binding reactions. Electrophoresed products were probed for VDR protein levels.

VDR with TFIIB was inhibited by 30% upon addition of 1,25(OH)2D3 (Fig. 4A), consistent with a previous report that VDR-TFIIB contact is inhibited by ligand (5). Interaction of AX3 with TFIIB in the absence of ligand was −25% of wild type, but reinstated to wild type level with 1,25(OH)2D3. Interaction of Δ389 with TFIIB was independent of ligand and at least as strong as wild type. In contrast, interaction of Δ389AX3 with TFIIB was notably diminished, with and without ligand. Binding of wild type VDR with GST-TFIIB in vitro was also inhibited by ligand (Fig. 4B). AX3-TFIIB interaction was weaker than wild type, but with no ligand-induced increase as in yeast.

Unliganded wild type VDR interacted with RXR and was augmented −3-fold by 1,25(OH)2D3 (Fig. 4A). In contrast, unliganded AX3 did not interact with RXR, and 1,25(OH)2D3 induced interaction to only 30% of wild type level. Mutant Δ389-RXR interaction was comparable with wild type VDR without ligand and was unaffected by ligand treatment, as Δ389 does not bind 1,25(OH)2D3 (data not shown). Coordinate alanine mutation completely abrogated Δ389AX3 interactions with RXR. In vitro binding data of VDR with RXR correlated with yeast results, as binding of wild type and AX3 to GST-RXR increased with ligand, with AX3 binding lower than wild type (Fig. 4B). Together these data indicate that mVDR helix H10 residues 380, 384, and 386 are important for interactions with TFIIB and RXR as well as SKIP/NCoA-62. Ligand effects on these interactions of wild type VDR differed, as VDR-SKIP/NCoA-62 interactions were ligand-independent, whereas VDR-TFIIB interactions were inhibited by 1,25(OH)2D3 and those for VDR-RXR were augmented.

**DISCUSSION**

SKIP/NCoA-62 is unique among nuclear receptor coregulators in that its interactions with VDR appear to be independent of the hydrophobic cleft/AF-2 domain interface, relying instead on an undefined LBD site. In this study we defined the structural nature of this interaction interface and highlighted its importance in VDR function and protein interactions. COOH-terminal mutagenesis of mVDR identified three helix H10 residues, Gln-380, Asp-384, and Arg-386, required for full interaction with SKIP/NCoA-62, but not with p160 coactivators. Distinct differences in ligand-responsive interactions between helix H10 mutants and SKIP/NCoA-62, TFIIB, or RXR were observed. Helix H10 mutation also attenuated VDR transactivation function, presumably as a result of disrupted interactions not only with TFIIB and RXR, but also with SKIP/NCoA-62. Wild type VDR interacted with SKIP/NCoA-62 in both the absence and presence of 1,25(OH)2D3, whereas its interaction with TFIIB was partially inhibited and with RXR was augmented by 1,25(OH)2D3, consistent with earlier reports (5, 7, 13).

Mutagenesis of mVDR H10 residues Gln-380, Asp-384, and Arg-386 markedly reduced ligand-independent interactions with SKIP/NCoA-62 and TFIIB and completely abrogated RXR interaction. Ligand treatment restored interactions with TFIIB fully, and with SKIP/NCoA-62 and RXR partially. Thus, in the absence of ligand the bulky side chains of these helix H10 residues appear to present a prominent interaction site, as their removal in AX3 attenuated protein-protein interactions. The reinstatement of interactions upon ligand treatment may result from a conformational change that brings additional interacting regions into play. This proposal is consistent with a requirement for hVDR helix H11 residues 116–165 for SKIP/NCoA-62 interaction (7), and for sites in the DNA binding and ligand binding domains of VDR for TFIIB and RXR interactions (22–26).

Interaction of SKIP/NCoA-62 with the VDR helix H10 interface would be compatible with concurrent binding of GRIP-1 to the AF-2-dependent hydrophobic cleft and thus consistent with ternary complex formation in the presence of ligand (13). SKIP/NCoA-62 interaction with helix H10 of unliganded VDR would also be compatible with formation of a repressive ternary complex in the absence of ligand, as SKIP/NCoA-62 and unliganded VDR both interact with NCoR and SMRT (3, 4, 10). Furthermore, by analogy with its tethering function in the alternate recruitment of corepressors and coactivators in Notch pathway regulation (10), SKIP/NCoA-62 may perform a similar function in VDR signaling, i.e. coordinating interactions with unliganded VDR to mediate transcriptional repression and with liganded receptor to mediate a switch to transcriptional activation. This proposal is supported by the finding that SKIP/NCoA-62 can augment or repress VDR transactivation in a cell-specific manner (27).

Modulation of VDR protein interactions by SKIP/NCoA-62 may also help regulate its interactions with TFIIB and RXR. It has been proposed that ligand-induced disruption of VDR-TFIIB interactions releases TFIIB for incorporation into the transcription complex (5). SKIP/NCoA-62 may regulate this transition through dynamic helix H10 interactions, as it can interact directly with TFIIB (data not shown) in addition to both unliganded and liganded VDR. Similarly, SKIP/NCoA-62 may facilitate RXR interaction with VDR when ligand is present, consistent with formation of a ternary complex between SKIP/NCoA-62 and the liganded VDR:RXR heterodimer (13).

Such a modulator role for SKIP/NCoA-62 may also be important for nuclear receptors other than VDR. Residues Gln-380, Asp-384, and Arg-386 are in the ninth heptad repeat region, which is highly conserved among the nuclear receptors. Consistent with this conservation, TFIIB interacts with RXRβ and TRβ (14, 28), and RXRα contacts PPARγ and RARα (29, 30) through heptad 9 residues analogous to mVDR Gln-380, Asp-384, and Arg-386. By extension, therefore, SKIP/NCoA-62 may also interact with nuclear receptors other than VDR via the helix H10 interface.
In summary we have found that the coregulator SKIP/NCoA-62 interacts with VDR via a helix H10 interface that is functionally and physically distinct from the region bound by the AF-2-dependent p160 coactivators. This previously unidentified coactivator interaction surface is required for full VDR transactivation activity and comprises conserved residues from helix H10 that are also involved in TFIIB interaction and heterodimerization with RXR. Moreover, the interactions of SKIP/NCoA-62, TFIIB, and RXR with the VDR helix H10 interface are dynamic, with varying responses to ligand. This study elucidates the structural basis of VDR-SKIP/NCoA-62 interaction and suggests that SKIP/NCoA-62 may regulate the exchange between TFIIB and RXR at the VDR helix H10 interface. Furthermore, it highlights the novel role of SKIP/NCoA-62 in VDR transcriptional regulation, a role that may also extend to other nuclear receptors.

REFERENCES
1. Haussler, M. R., Whitfield, G. K., Haussler, C. A., Hsieh, J. C., Thompson, P. D., Selanick, S. H., Dominguez, C. E., and Jurutka, P. W. (1998) J. Bone Miner. Res. 13, 325–349
2. Rochel, N., Wurtz, J. M., Mitschler, A., Klaholz, B., and Moras, D. (2000) Mol. Cell 5, 173–179
3. Dwivedi, P. P., Muscat, G. E., Bailey, P. J., Omdahl, J. L., and May, B. K. (1998) J. Mol. Endocrinol. 20, 327–335
4. Tagami, T., Lutz, W. H., Kumar, R., and Jameson, J. L. (1998) Biochem. Biophys. Res. Commun. 253, 358–363
5. Masuyama, H., Jefcoat, S. C., Jr., and MacDonald, P. N. (1997) Mol. Endocrinol. 11, 218–228
6. Glass, C. K., and Rosenfeld, M. G. (2000) Genes Dev. 14, 121–141
7. Baudino, T. A., Kraichely, D. M., Jefcoat, S. C., Winchester, S. K., Partridge, N. C., and MacDonald, P. N. (1998) J. Biol. Chem. 273, 16434–16441
8. Dahl, R., Wani, B., and Hayman, M. J. (1998) Oncogene 16, 1579–1586
9. Prathapam, T., Kuhne, C., and Banks, L. (2001) Oncogene 20, 7677–7685
10. Zhou, S. F., Fujimuro, M., Hsieh, J. J. D., Chen, L., Miyamoto, A., Weinmaster, G., and Hayward, S. D. (2000) Mol. Cell. Biol. 20, 2400–2410
11. Zhou, S. F., Fujimuro, M., Hsieh, J. J. D., Chen, L., and Hayward, S. D. (2000) J. Virol. 74, 1939–1947
12. Leong, G. M., Subramaniam, N., Figueroa, J., Flanagan, J. L., Hayman, M. J., Eisman, J. A., and Kouzmenko, A. P. (2001) J. Biol. Chem. 276, 18243–18248
13. Zhang, C., Baudino, T. A., Dowd, D. W., Tokumaru, H., Wang, W., and MacDonald, P. N. (2001) J. Biol. Chem. 276, 40614–40620
14. Leong, G. M., Wang, K. S., Marton, M. J., Blanco, J. C., Wang, I. M., Rolffes, R. J., Ozato, K., and Segars, J. H. (1998) J. Biol. Chem. 273, 2296–2305
15. Issa, L. L., Leong, G. M., Barry, J. B., Sutherland, R. L., and Eisman, J. A. (2001) Endocrinology 142, 1606–1615
16. Thompson, J. D., Higgs, D. G., and Gibson, T. J. (1994) Nucleic Acids Res. 22, 4673–4680
17. Lu, Z., Hanson, K., and DeLuca, H. F. (1997) Biochim. Biophys. Acta 1339, 99–106
18. Ha, I., Roberts, S., Maldonado, E., Sun, X., Kim, L. U., Green, M., and Reinberg, D. (1993) Genes Dev. 7, 1021–1032
19. Nunez, S. B., Medin, J. A., Braissant, O., Kemp, L., Wahli, W., Ozato, K., and Segars, J. H. (1997) Mol. Cell. Endocrinol. 127, 27–40
20. Andrews, N. C., and Faller, D. V. (1991) Nucleic Acids Res. 19, 2499
21. Mangelsdorff, D. J., Pike, J. W., and Hausessler, M. R. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 354–358
22. Masuyama, H., Brownfield, C. M., St-Arnaud, R., and MacDonald, P. N. (1997) Mol. Endocrinol. 11, 1507–1517
23. Nakajima, S., Hsieh, J. C., MacDonald, P. N., Galligan, M. A., Haussler, C. A., Whitfield, G. K., and Haussler, M. R. (1994) Mol. Endocrinol. 8, 159–172
24. Nishikawa, J., Kitaura, M., Imagawa, M., and Nishihara, T. (1995) Nucleic Acids Res. 23, 606–611
25. Whitfield, G. K., Hsieh, J. C., Nakajima, S., MacDonald, P. N., Thompson, P. D., Jurutka, P. W., Haussler, C. A., and Haussler, M. R. (1995) Mol. Endocrinol. 9, 1166–1179
26. Jurutka, P. W., Remus, L. S., Whitfield, G. K., Thompson, P. D., Hsieh, J. C., Zetler, H., Tavakkoli, P., Galligan, M. A., Dang, H. T., Haussler, C. A., and Hausessler, M. R. (2000) Mol. Endocrinol. 14, 401–420
27. Leong, G. M., Eisman, J. A., and Gardiner, E. M. (1998) Bone 22, 325–336
28. Baniamad, A., Ha, I., Reinberg, D., Tsai, S. T., Tsai, M. J., and O’Malley, B. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8832–8836
29. Bourguet, W., Vivat, V., Wurtz, J. M., Champon, P., Gronemeyer, H., and Moras, D. (2000) Mol. Cell 5, 289–298
30. Gampe, R. T., Jr., Montana, V. G., Lambert, M. H., Miller, A. B., Bledsoe, R. K., Milburn, P. A., Kluiver, S. A., Wilson, T. M., and Xu, H. E. (2000) Mol. Cell 5, 545–556
31. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
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J. Biol. Chem. 2003, 278:8224-8228.
doi: 10.1074/jbc.C200712200 originally published online January 15, 2003

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