Signal-dependent N-CoR Requirement for Repression by the Ski Oncoprotein*

Received for publication, April 3, 2003, and in revised form, April 23, 2003
Published, JBC Papers in Press, April 25, 2003, DOI 10.1074/jbc.M303447200

Nobuhide Ueki‡ and Michael J. Hayman
From the Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, New York 11794-5222

The oncoprotein Ski represses transforming growth factor-β (TGF-β) and nuclear receptor signaling. To achieve this, Ski has been proposed to recruit the corepressor N-CoR to either the TGF-β-regulated Smad transcription factors or nuclear receptors. Here we define the role of the Ski/N-CoR interaction in Ski-mediated repression of TGF-β and vitamin D signaling. We show that Ski can negatively regulate vitamin D-mediated transcription by directly interacting with the vitamin D receptor. More importantly, a Ski single point mutant lacking N-CoR binding revealed that the Ski/N-CoR interaction is essential for repression of vitamin D signaling, but, surprisingly, not TGF-β signaling. Thus, Ski modulates transcription in either an N-CoR-dependent or -independent manner depending on the signaling pathways targeted.

Cell proliferation and differentiation are controlled by growth regulatory factors such as the cytokine transforming growth factor-β (TGF-β) and the biologically active metabolites of retinoic acid and vitamin D (1, 2). These extracellular stimuli are conveyed through specific signaling pathways to cause diverse physiological responses that are essential for the normal regulation of development and homeostasis.

TGF-β ligands signal through cell-surface serine/threonine kinase receptors (types I and II) and intracellular signal transducer Smad proteins (1). Upon TGF-β induction, Smad2 and Smad3 are phosphorylated by the type I receptor and released from cytoplasmic retention. Phosphorylated Smad2 and Smad3 form a heteromeric complex with Smad4, translocate into the nucleus, and bind to specific promoter elements through interaction with specific DNA-binding and non-DNA-binding partners (3). The assembled core Smad complexes then regulate transcription either positively or negatively, depending on the recruitment of coactivator or corepressor complexes, including CBP/p300, TGFIP, and Ski/Sno (3). In addition, protein turnover by degradation such as the ubiquitin-proteasome pathway also participates in the regulation of TGF-β signaling (4–8).

In nuclear receptor signaling, the actions of retinoic acid and vitamin D are mediated by the specific intracellular retinoic acid, retinoid X, and vitamin D (VDR) receptors (2). These receptors belong to the nuclear receptor superfamily composed of many structurally related transcription factors that regulate gene expression in a ligand-dependent fashion. Upon ligand binding, both the retinoic acid receptor and VDR can form a heterodimeric complex with the retinoid X receptor and selectively bind to their cognate promoter elements, causing transcriptional activation (9). This activation is believed to occur through a ligand-dependent conformational change in the receptors that leads to the dissociation of corepressors such as N-CoR/SMRT and the subsequent association of coactivators, e.g. SRC-1 and TRAP/DRIP/ARC (10).

The related corepressors N-CoR and SMRT were found to actively repress the basal level of transcription through interaction with unliganded nuclear receptors such as the retinoic acid and thyroid hormone receptors and other classes of transcription factors (11). N-CoR/SMRT-mediated repression involves chromatin modifier histone deacetylases (12). Another corepressor, Sin3, has also been implicated in the histone deacetylase-mediated transcriptional repression by the Myc/Mad/Max family proteins and several other transcription factors (13, 14).

Recently, the nuclear oncprotein Ski was shown to act as a negative regulator in several distinct signal transduction pathways, including TGF-β (15–19) and nuclear receptor (20) signaling, through its direct interaction with Smad2/3/4 proteins and the retinoic acid receptor, respectively. Ski can act as a transcriptional corepressor primarily due to multiple direct and indirect interactions with histone deacetylase complexes containing N-CoR/SMRT and Sin3 (21, 22).

The prototypic model for the mechanism of repression by Ski adopts a simple ternary Ski:N-CoR.Sin3 complex (19) based on early observations of the direct Ski/N-CoR, Ski/Sin3, and N-CoR/Sin3 interactions (21–26). However, the functional relevance of these interactions has not been addressed. Furthermore, accumulating biochemical data suggest that N-CoR and Sin3 may actually be present in distinct histone deacetylase-containing complexes (27–31), and thus, cooperative repression through the direct N-CoR/Sin3 interaction has recently come into question. These issues prompted us to reconsider the role of the direct Ski/N-CoR interaction in Ski-mediated repression of TGF-β and nuclear receptor signaling.

In this report, we define the role of Ski/N-CoR interaction in the Ski-mediated repression of the TGF-β and vitamin D signaling pathways. A Ski mutant lacking N-CoR binding revealed that Ski/N-CoR interaction is essential for repression of vitamin D (but not TGF-β) signaling. Thus, we propose that Ski executes at least two distinct modes of repression through N-CoR-dependent and -independent mechanisms depending on the signaling pathways involved.

* This work was supported by Grant CA42573 from the National Institutes of Health (to M. J. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 631-632-4314; Fax: 631-632-8891; E-mail: nueki@ms.cc.sunysb.edu.

1 The abbreviations used are: TGF-β, transforming growth factor-β; CBP, cAMP-responsive element-binding protein-binding protein; VDR, vitamin D receptor; AD, activation domain; wt, wild-type; mt, mutant; 5-POA, 5-fluoroorotic acid; TRITC, tetramethylrhodamine isothiocyanate; 1,25-(OH)2D3, 1,25-dihydroxyvitamin D3; NBT, nitro blue tetrazolium; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

24858 This paper is available on line at http://www.jbc.org
**EXPERIMENTAL PROCEDURES**

**Chemicals and Oligonucleotides**—All chemicals were purchased from Sigma or Fisher unless indicated. Oligonucleotides were purchased from Integrated DNA Technologies.

**Plasmid Constructs**—The LexA expression plasmid pPCA was created by replacing the SphiI fragment of the activation domain (AD) expression cassette in pPC86 (Invitrogen) with the LexA expression cassette in pLexA (Clontech). LexA-N-CoR* (N-CoR* represents the Ski-binding domain of N-CoR, amino acids 1571–1725) was generated by replacing the PCR-generated mouse N-CoR cDNA (amino acids 1571–1725) into pPCA. Gal4-Smad4 was generated by incorporating Smad4 cDNA into pDBLue (Invitrogen). The C-terminal AD fusion plasmid was created by inserting the blunt-ended SphiI fragment encoding the LexA-AD expression cassette into the EcoRV site of pYCM6/CT (Invitrogen), yielding pTY-LexA-AD. Wild-type v-Ski-AD was constructed by replacing the LexA-HpaI/SstI fragment in pYC-LexA-AD with the PCR-generated chicken v-Ski HpaI/SstI fragment through blunt-ended HpaI/SstI sites in the vector. The three-hybrid constructs (LexA-N-CoR*; Gal4-Smad4, and v-Ski-AD) have individual selection markers in yeast (TRP1, LEU2, and blastidicin) as well as Escherichia coli (ampicillin, kanamycin, and ampicillin/blastidicin), respectively. Random mutagenesis of v-Ski was carried out by PCR using Taq DNA polymerase (Invitrogen) under standard conditions, except that the dNTP concentration was 600 μM. PCR products were then processed into pYC-LexA-AD as described above. A mutant v-Ski-AD library was prepared by pooling ~2 × 10^6 independent E. coli transformants. pCMV-T7 was created by replacing the Nhel/BglII fragment of enhanced green fluorescent protein in pEGFP-C1 (Clontech) with double-stranded DNA encoding the T7 epitope tag (MAGTGGQMQMG). pCMV-T7-c-Ski was generated by incorporating c-Ski cDNA into pCMV-T7. FLAG-N-CoR and FLAG-N-CoR-C were created by cloning the corresponding cDNAs from pCMX-N-CoR and VP16-N-CoR into the pCMV-Tag2 vector (Stratagene). pM-N-CoR-CBD was generated by cloning N-CoR* into pM (Clontech). Site-directed mutagenesis of c-Ski (L110P) cDNA into the appropriate cloning sites in RIGB, respectively. All PCRs were performed using Turbo DNA polymerase (Stratagene) unless indicated, and all cloning procedures were verified by DNA sequencing.

**Yeast Manipulation and Differential Interaction Assay**—Yeast manipulation was performed using the Proquest two-hybrid system (Invitrogen) following the manufacturer’s instructions. Yeast one-hybrid yeast transformation kit (Genentech). MaVL61 was created by replacing Gal4-binding sites of SPAL10::URA3 in the parental strain MaV203 (Invitrogen) with 12 copies of LexA-binding sites through a PCR-based homologous recombination method using parent strain MaV203 (Invitrogen) with 12 copies of LexA-binding sites in RIGB, respectively. All PCRs were performed using Pfu Turbo DNA polymerase (Stratagene) unless indicated, and all cloning procedures were verified by DNA sequencing.

**Cell Culture and Transfection**—COS-1, CV-1 cells were seeded in eight-well chamber slides (Lab-Tech II, Nalge Nunc). Twenty-four hours after transfection, cells were fixed, permeabilized, and processed for indirect immunofluorescence staining according to a standard protocol. The primary antibodies used were anti-T7, anti-FLAG, and anti-Myc. Proteins of interest were detected using chemiluminescence (ECL, Amersham Biosciences).

**Direct Immunofluorescence**—CV-1 cells were seeded in eight-well chamber slides (Lab-Tech II, Nalge Nunc). Twenty-four hours after transfection, cells were fixed, permeabilized, and processed for indirect immunofluorescence staining according to a standard protocol. The primary antibodies used were mouse anti-T7 and rabbit anti-FLAG (Sigma), and the secondary antibodies used were fluorescein isothiocyanate-conjugated goat anti-mouse IgG and TRITC-conjugated sheep anti-rabbit IgG (Sigma). Fluorescence microscopy was performed on an Axioskop 2 microscope (Carl Zeiss, Inc.) through a ×100 objective equipped with a digital imaging processor.

**Growth Inhibition Assay**—Each pool of CCL-64 cells was seeded at a density of 4 × 10^4 cells/60-mm dish and transfected with each plasmid DNA. Virus-producing cells transfected with RIGB were grown for 48 h to confluence prior to harvesting the viral supernatant. CCL-64 and U937 cells were infected with the supernatant supplemented with Polybrene (10 μg/ml) and incubated for 6 h. The cells infected with the viruses were cultured for 48 h in fresh medium and then selected for 1 week in the presence of blastidicin (10 μg/ml).

**Growth Inhibition Assay**—Each pool of CCL-64 cells was seeded at a density of 4 × 10^4 cells/well on 12-well plates. Cells were treated with (4 ml HCl and 1 mg/ml bovine serum albumin) or TGF-β1 (1 ng/ml) for 2–3 days. The growth of cells was determined by cell counting (Coulter Z particle counter) and compared with that of unstimulated cells.

**Nitro Blue Tetrazolium (NBT) Reduction Assay**—Each pool of U937 cells was treated with solvent (ethanol) or 1.25-(OH)_2D_3 (100 nm) for 5 days. The cells (1 × 10^6 cells) were incubated in RPMI 1640 medium containing NBT (1 mg/ml) and 12-O-tetradecanoylphorbol-13-acetate (100 nm) at 37 °C for 1–2 h. The percentage of NBT-positive cells

---

**FIG. 1. Generation of a Ski point mutant deficient in the N-CoR interaction.** A, MaVL61 was transformed with the three-hybrid plasmids as indicated. Phenotypes were assessed by His+ prototrophy (left panel), X-gal assay (middle panel), and 5-FOA-resistance/His+ combination (right panel). Serial dilutions of exponentially growing cell cultures were spotted on the plates (top row and right panels). A representative of the wt-v-Ski-AD clone was isolated (row 5). B, a standard two-hybrid assay was carried out in L40 to confirm the interaction of wt- or m-v-Ski with N-CoR*. L40 was transformed with pairs of plasmids as indicated. Phenotypes were monitored by X-gal assay. A representative of the wt-Ski-AD clone was isolated (row 4). 3-AT, 3-amino-1,2,4-triazole; vector, pYCM6/CT.
distinct modes of repression by ski

Results

generation of a ski point mutant deficient in the n-coR interaction—to create a ski mutant deficient in N-CoR binding, we modified a three-hybrid method developed by grossel et al. (34). it is composed of dual independent DNA-binding domains (LexA and Gal4) and one common AD. the yeast strain MaVL61 harbors one LexA and two Gal4 DNA-binding domain-responsive reporters (ura3 and his3/lacZ, respectively) to allow differential selection. the target proteins are randomly mutagenized by PCR and expressed with an AD tag at the C terminus to ensure authentic protein expression. interaction between LexA and AD fusion moieties confers URA3 expression, leading to the 5-FOA-sensitive phenotype (no growth in the presence of 5-FOA). interaction between Gal4 and AD fusion moieties confers HIS3/lacZ expression, leading to His+ growth (on −His plate) and β-galactosidase-positive phenotypes. theoretically, mutant proteins that lose the interaction with the LexA fusion moiety but retain the interaction with the Gal4 fusion moiety can be monitored by 5-FOA resistance (growth in the presence of 5-FOA) and His+/β-galactosidase-positive phenotypes.

we created Ski mutants that were deficient in N-CoR binding but that retained the interaction with Smad4 using LexA-N-CoR*, Gal4-Smad4, and an AD fusion library to express the mutagenized v-Ski region (v-Ski, amino acids 22–458 in c-Ski).

A series of control experiments verified the system (Fig. 1). Reporter assays in MaVL61 were performed using the −His plate, X-gal assay, or the dual selection plate (−His/5-FOA). In the presence of wt-v-Ski-AD, Gal4-Smad4, and LexA-N-CoR*, the reporter strain conferred His+/β-galactosidase-positive and 5-FOA-sensitive phenotypes, resulting in no growth on the dual selection plate (Fig. 1A, row 1). In the absence of Smad4, which is equivalent in context to v-Ski-Smad4 dissociation, it lost the His+/β-galactosidase-positive phenotypes, leading to no growth on either plate (Fig. 1A, row 2), whereas in the absence of N-CoR*, which is equivalent in context to v-Ski-N-CoR* dissociation, the reporter strain showed His+/β-galactosidase-positive and 5-FOA resistance phenotypes, leading to growth on the dual selection plate (row 3). As a negative control, v-Ski-AD alone did not grow on either selection plate (Fig. 1A, row 4). In addition, specific interaction between v-Ski-AD and LexA-N-CoR* was confirmed by the X-gal assay using another yeast strain, L40 (35), which harbors two LexA-inducible reporters (HIS3/lacZ) (Fig. 1B, rows 1–3). These control experiments demonstrated the validity of the screening system.

Subsequently, we screened a mt-v-Ski-AD library generated by PCR. A total number of ~1 × 10⁶ yeast transformants were directly applied to the dual selection plate, yielding five positive clones. Three of five isolated plasmids reproducibly conferred His+/β-galactosidase-positive and 5-FOA resistance phenotypes in MaVL61 (Fig. 1A, row 5) and the β-galactosidase-negative phenotype in L40 (Fig. 1B, row 4).

characterization of the isolated ski mutant in mammalian cells—DNA sequencing determined that the three clones con-
tained an identical mutation from leucine to proline at residue 110 (L110P) in c-Ski. This residue is located within the N-terminal segment of c-Ski that has been identified as the N-CoR-binding domain (Fig. 2A) (21). To further characterize the interaction capacity of the mutant obtained, we performed a co-immunoprecipitation assay using N-CoR, Smad4, and Sin3. wt-c-Ski was efficiently precipitated with both N-CoR and a truncated form of N-CoR lacking the N-terminal repression domains (N-CoR-C). In contrast, only minor amounts of mt-c-Ski were recovered (Fig. 2B). This indicates that the L110P mutation significantly affects the interaction with N-CoR. Similar results were obtained using the N-CoR-related co-repressor SMRT (data not shown). In contrast, the mutation minimally affected the interactions of Ski with Smad4 and Sin3 (Fig. 2, C and D, respectively). These results demonstrate that the L110P mutation in c-Ski specifically affects the N-CoR interaction while retaining the capacity to bind to Smad4 and Sin3.

To further confirm the effect of the L110P mutation on the N-CoR interaction, indirect immunofluorescence analysis in CV-1 cells was performed (Fig. 3). Whereas N-CoR alone localized to the small nuclear granular structures (Fig. 3A), coexpression with wt-c-Ski influenced the distribution pattern of N-CoR, resulting in numerous dot structures distinct from those of N-CoR alone and showing a clear colocalization pattern with wt-c-Ski (Fig. 3, B–D). In contrast, mt-c-Ski had no detectable effect on N-CoR localization (Fig. 3, E–G). Furthermore, in the case of N-CoR-C, wt-c-Ski had a more prominent effect on N-CoR-C localization (Fig. 3, H–N). Whereas N-CoR-C alone localized mainly in the cytoplasm (Fig. 3H), coexpression with wt-c-Ski dramatically altered the distribution pattern of N-CoR-C, relocating it into punctate structures in the nucleus that showed a clear colocalization pattern with wt-c-Ski (Fig. 3, I–K). Again, mt-c-Ski had little effect on N-CoR-C distribution, leaving it in the cytoplasm (Fig. 3, L–N). These results demonstrate that the L110P mutation substantially impairs the capacity of c-Ski to interact with N-CoR in vivo. Taken together, our results clearly indicate that Leu110 in c-Ski is crucial for the interaction with N-CoR.

N-CoR-independent Repression by Ski in TGF-β Signaling—To determine whether the Ski/N-CoR interaction is necessary for Ski-mediated repression of TGF-β signaling, we compared the repressive activity of mt-c-Ski with that of wt-c-Ski. Because Ski has been reported to repress TGF-β-induced and Smad-dependent transcriptional activation (15–18), we assessed the effect of the L110P mutation by an established reporter gene assay using TGF-β-responsive 3TP-Luc and Smad3-dependent 4xSEB-Luc reporter constructs (17) in HEK293 and CCL-64 cells. Activation of TGF-β signaling was done either by transfection of a constitutively active type I receptor, TβRI(TD)/ALK5, or by addition of TGF-β ligand, which markedly increased luciferase activity (Fig. 4, A–D, vector). This TGF-β-dependent activation was further enhanced in the presence of Smad3 and Smad4 (Fig. 4, A and B, vector). This TGF-β-dependent activation was further enhanced in the presence of Smad3 and Smad4 (Fig. 4, A and B, vector). As previously reported (15–18), wt-c-Ski strongly repressed this activation (Fig. 4, A–D, wtSki). However, regardless of the demonstrated effect in the N-CoR interaction, mt-c-Ski repressed the reporter gene expression to a comparable extent compared with wt-c-Ski, showing no notable difference (Fig. 4, A–D, mtSki). Similar results were obtained using HepG2 and COS-1 cells (data not shown). Therefore, these data strongly indicate that the Ski/N-CoR interaction is not required for repression of TGF-β signaling.

To further characterize the role of the Ski/N-CoR interaction in a more physiological fashion, we performed TGF-β-induced growth inhibition assays using CCL-64 cells. Ski has been shown to antagonize TGF-β-mediated growth inhibition in certain types of cells such as CCL-64 cells, which are highly responsive to the growth inhibitory effect of TGF-β (17). We generated CCL-64 cells stably expressing wt- or mt-c-Ski by retrovirus-mediated gene transfer. All retroviral constructs contained a blasticidin resistance marker fused to an enhanced green fluorescent protein cDNA driven by an internal ribosome entry site for the purpose of selecting infected cells (Fig. 4E). Several pools of CCL-64 cells infected with empty vector or wt- or mt-c-Ski were analyzed. The levels of protein expression after blasticidin selection were assessed by Western blotting and showed comparable expression and stability of the Ski proteins (Fig. 4F, wt-cSki and mt-cSki). Then, each pool was treated with either solvent or TGF-β to test the growth inhibitory effect of TGF-β (Fig. 4G). Upon TGF-β treatment, the vector control cells showed ~70% growth inhibition (Fig. 4G, vector). Expression of wt-c-Ski conferred a partial resistance to the growth inhibitory effect of TGF-β in that inhibition was only ~40% (Fig. 4G, wt-cSki), an inhibition level similar to that previously reported (17). Importantly, the mt-c-Ski-expressing cells and the wt-c-Ski-expressing cells showed comparable resistance (Fig. 4G, mt-cSki). Taken together, our data strongly indicate that Ski-mediated repression of TGF-β signaling is N-CoR-independent.

N-CoR-dependent Repression by Ski in Vitamin D Signaling—We previously reported that Ski transforms hematopoietic cells by interfering with nuclear receptor signaling (20). To determine whether the Ski/N-CoR interaction is necessary for Ski-mediated repression of nuclear receptor signaling, we in-
investigated the effect of the mutation in Ski. Because we have found that Ski can negatively regulate vitamin D-induced responses in certain cells, the transcriptional properties of wt- and mt-c-Ski on VDR-responsive promoters were assessed. We used the human osteocalcin promoter construct, which confers responsiveness to 1,25-(OH)2D3 through the canonical response element for VDR. Transient transfection of wt-c-Ski and a human osteocalcin promoter-luciferase reporter construct (OS2-Luc) (36) revealed a clear repressive effect of wt-c-Ski on 1,25-(OH)2D3-dependent transcription (Fig. 5A). In contrast, transfection of mt-c-Ski resulted in only partial repression. In addition, albeit to a lesser extent, similar results regarding 1,25-(OH)2D3-induced transcription were obtained using a VDR-responsive rat 1,25-(OH)2D3 24-hydroxylase promoter construct (24HO-Luc) (37) (data not shown), suggesting that the repressive action of wt-c-Ski and its compromise by the mutation are not specific to the osteocalcin promoter.

To demonstrate the direct involvement of N-CoR in Ski-mediated repression of VDR signaling, we assessed the dominant-negative effect of overexpressed N-CoR* (amino acids 1571–1725), which could specifically abrogate the interaction between wt-c-Ski and N-CoR (21). As expected, overexpression of N-CoR* in 1,25-(OH)2D3-induced cells compromised the repressive effect of wt-c-Ski to the equivalent level of mt-c-Ski, which was unaffected by N-CoR* (Fig. 5B). N-CoR* also enhanced the transcription of the vector control, presumably through affecting endogenous Ski protein. In addition, a comparable effect was observed using the equivalent region of SMRT (data not shown). Taken together, the data provide evidence showing that the repression by wt-c-Ski in VDR signaling is at least in part mediated by the Ski/N-CoR interaction.

To determine whether wt- and mt-c-Ski are involved in the VDR complex, co-immunoprecipitation assays were performed (Fig. 5C). wt-c-Ski associated with VDR in a ligand-independent fashion (Fig. 5C, wt-cSki), suggesting that wt-c-Ski can link VDR to N-CoR even in the presence of ligand. Similarly, mt-c-Ski also associated with VDR (Fig. 5C, mt-cSki), demonstrating that the capacity of Ski to bind to VDR is not affected by the mutation. These results indicate that the substantial loss of the repression capacity of mt-c-Ski is not due to a defect in VDR interaction, but to a defect in N-CoR binding.

Next, we examined the effect of the mutation in the U937 hematopoietic cell line. U937 cells are myeloid precursors that are blocked at the promonocytic stage and that proceed toward terminal differentiation upon treatment with agents such as 1,25-(OH)2D3 (38). When induced to differentiate with 1,25-(OH)2D3, U937 cells acquire NBT reduction activity, a property associated with the expression of the phagocytic NADPH oxidase of mature monocytes. Using the retrovirus-mediated gene transfer method described above, several pools of U937 cells infected with empty vector or wt- or mt-c-Ski were produced. The level of protein expression in these cells was analyzed by Western blotting, which showed equal expression and stability of N-CoR (Fig. 5D, wt-cSki and mt-cSki). Then, each pool was treated with either solvent (none) or 1,25-(OH)2D3 (VD3) for 5 days and harvested for analysis by the NBT reduction assay (Fig. 5E). Upon stimulation with 1,25-(OH)2D3, the vector control cells differentiated as assessed by the increased number of NBT-positive cells (78%) (Fig. 5E, vector). In contrast wt-c-Ski ex-
expression in the U937 cells substantially reduced the number of NBT-positive cells induced by 1,25-(OH)2D3 (to a level of 13%) (Fig. 5E, wt-cSki). Interestingly the mt-c-Ski-expressing cells showed similar numbers of NBT-positive cells compared with the vector control (79%) (Fig. 5E, mt-cSki), indicating that, in nuclear receptor signaling, the L110P mutation has practically eliminated the repressive activity of Ski. Taken together, these results indicate that the Ski/N-CoR interaction is essential for the ability of Ski to block VDR signaling in U937 cells.

DISCUSSION

We have provided compelling functional evidence that the repressive activity of Ski in VDR signaling is N-CoR-dependent. Even though the mutant Ski protein retains the capacity to bind to Sin3 (Fig. 2D), the mutation substantially impairs the inhibitory activity of Ski in VDR signaling (Fig. 5). Intriguingly, we have observed similar effects of the mutation on retinoic acid signaling (data not shown). It is tempting to speculate that N-CoR-dependent repression by Ski plays a general role in the negative regulation of nuclear receptor signaling pathways.

Contrary to the early model of Ski-mediated repression of TGF-β signaling, our results clearly indicate that the repression by Ski is N-CoR-independent. Ski was shown to link Smad3 and Sin3, forming a complex including HDAC1 (16). Thus, it is possible that Sin3 plays an important role in the Ski-mediated repression of TGF-β signaling. Furthermore, Ski was suggested to interfere with Smad/CBP/p300 coactivator complex formation (16) and functional Smad complex formation (39). Hence, these lines of repression by Ski cannot be ruled out as well. The N-CoR-independent mechanism of repression by Ski in TGF-β signaling still awaits further investigation.

Our observations have resulted in the conclusion that Ski achieves its repressive activity through signal-dependent utilization of N-CoR. However, the molecular mechanisms by which Ski specifically interacts with distinct corepressors and specific transcription factors depending on the signaling pathways involved remain unknown. Dissecting the signal-dependent regulatory mechanisms underlying Ski-mediated repression will be of considerable interest in the future.

Acknowledgments—We are grateful to R. Sternglanz and D. C. Zappulla for helpful advice on the generation of the yeast reporter...
Distinct Modes of Repression by Ski

strains. We thank T. Tagami (VP16-N-CoR), S. Kern and B. Vogelstein (4xSEB-Luc), M. Kawabata (Myc-Smad3 and Myc-Smad4), M. G. Rosenfeld (pCMX-N-CoR), J. D. Chen (pCMX-SMRTe), J. Massagué (3TP-Luc and TβR-II(TD)), B. Derynck and R. N. Eisenman (pCS2+MT-MSm3A), G. M. Leong (FLAG-VDR, OS2-Luc, and 24HO-Luc), R. M. Evans (pCMX-IXReo), O. Petrenko (REBNA), and H. Beug for kindly providing reagents; K. Donnelly for supporting our research project; and the members of the Hayman laboratory for helpful discussions and criticisms.

REFERENCES

1. Massagué, J. (2000) Nat. Rev. Mol. Cell Biol. 1, 169–178
2. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1995) Cell 83, 835–839
3. Massagué, J., and Wotton, D. (2000) EMBO J. 19, 1745–1754
4. Stroschein, S. L., Bonni, S., Wrana, J. L., and Luo, K. (2001) Genes Dev. 15, 2822–2836
5. Sun, Y., Liu, X., Ng-Eaton, E., Lodish, H. F., and Weinberg, R. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12442–12447
6. Bonni, S., Wang, H. R., Causing, C. G., Kawabata, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12442–12447
7. Wang, Y., Liu, X., and Kirschner, M. W. (2001) Mol. Cell 8, 1027–1039
8. Kawabata, M. (1999) J. Biol. Chem. 274, 4485–4488
9. Li, J., Wang, Z., Liu, J. M., Qin, J., and Wong, J. (2000) Mol. Cell Biol. 20, 4342–4350
10. Wen, Y. D., Perioli, V., Stasiewski, L. M., Yang, W. M., Krones, A., Glass, C. K., Rosenfeld, M. G., and Seto, E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7202–7207
11. Zhang, J., Kalkum, M., Chait, B. T., and Roeder, R. G. (2002) Mol. Cell 9, 611–625
12. Petrenko, O., Beavis, A., Kline, M., Kuttappa, R., Gorden, I., and Lemischka, I. R. (1999) Immunity 10, 691–700
13. Leontine, M. S., McKenzie, A., Demarini, D. J., Shah, N. G., Wach, A., Brachate, A., Philippens, P., and Pringle, J. R. (1998) Yeast 14, 953–961
14. Gressel, M. J., Wang, H., Gadea, B., Yeung, W., and Hinds, P. W. (1999) Nat. Biotechnol. 17, 1222–1233
15. Vojtek, A. B., Hollemberg, S. M., and Cooper, J. A. (1993) Cell 74, 205–214
16. Issa, L. L., Leong, G. M., Barry, J. R., Sutherland, R. L., and Eisen, J. A. (2001) Endocrinology 142, 1606–1615
17. Devi, V. S., Luck, C., O’Rourke, P. J., O’Dowd, K. L., and May, B. K. (1998) J. Mol. Endocrinol. 20, 327–335
18. Olesen, I., Gullberg, U., Wied, H., and Nilsson, K. (1983) Cancer Res. 43, 5862–5867
19. Wu, J. W., Krawitz, A. R., Chai, J., Li, W., Zhang, F., Luo, K., and Shi, Y. (2002) Cell 111, 357–367

by guest on July 25, 2018http://www.jbc.org/Downloaded from
Signal-dependent N-CoR Requirement for Repression by the Ski Oncoprotein

Nobuhide Ueki and Michael J. Hayman

J. Biol. Chem. 2003, 278:24858-24864.
doi: 10.1074/jbc.M303447200 originally published online April 25, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303447200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 39 references, 17 of which can be accessed free at
http://www.jbc.org/content/278/27/24858.full.html#ref-list-1