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Biochemistry

SMRT isoforms mediate repression and anti-repression of nuclear receptor heterodimers

(transcriptional corepressor/dominant negative mutant)

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ABSTRACT **Transcriptional repression represents an important component in the regulation of cell differentiation and oncogenesis mediated by nuclear hormone receptors. Hormones act to relieve repression, thus allowing receptors to function as transcriptional activators. The transcriptional corepressor SMRT was identified as a silencing mediator for retinoid and thyroid hormone receptors. SMRT is highly related to another corepressor, N-CoR, suggesting the existence of a new family of receptor-interacting proteins. We demonstrate that SMRT is a ubiquitous nuclear protein that interacts with unliganded receptor heterodimers in mammalian cells. Furthermore, expression of the receptor-interacting domain of SMRT acts as an antirepressor, suggesting the potential importance of splicing variants as modulators of thyroid hormone and retinoic acid signaling.**

The steroid, retinoid, and thyroid hormones are pleiotropic mediators of diverse aspects of animal development, reproduction, and adult organ physiology. The action of these hormones is mediated by nuclear receptors, which compose a large family of ligand-dependent transcription factors that display considerable specificity and selectivity in regulating the genetic programs they ultimately influence (1). Over the past several years, it has been established that retinoic acid receptors (RARs) and thyroid hormone receptors (TRs) function via the formation of heterodimeric complexes with retinoid-X receptors (RXRs) (2). In these heterodimeric complexes, RXR appears in some cases as a "silent partner" to help produce the appropriate DNA-binding and ligand-binding properties of the individual complexes (3). The ligand-binding domain (LBD) is highly complex, mediating not only ligand binding but also receptor homo- and heterodimerization as well as transcriptional activation and repression.

Previous studies have demonstrated that the TR can be a transcriptional repressor in the absence of ligand and a potent activator in the presence of T3 (4, 5). Using combinations of DNA-binding and functional assays, the repressor activity of the nonliganded TR was demonstrated to depend on an appropriate thyroid hormone response element as well as the intact LBDs of both TR and RXR (4–7). v-erbA represents an oncogenic form of TR that is one of the two oncogenes of the avian erythroblastosis virus (8, 9). Previous studies have demonstrated that v-erbA acts as a constitutive repressor of the basal promoter activity and that a transformation defective form of v-erbA (Td359) fails to suppress basal transcription (10). Thus, the oncogenic activity of v-erbA is directly linked to its constitutive repressor activity. Work by Damm and Evans (11) demonstrated that a transformation defective form of v-erbA arises as a result of a single Pro → Arg substitution in the LBD at a position equivalent to residue 160 in the rat TRα. Introduction of this single change into TRα abolishes its ability to suppress basal transcription but does not interfere with either hormone-binding, DNA-binding, or transcriptional activation. These studies suggest that the repressor activity resides in the LBD and is functionally separable from the C-terminal activation function and ligand-binding properties. We have demonstrated that deletion of the activation domain of RAR converts it into a potent transcriptional repressor (12). This repression is as potent as the v-erbA oncogene, and when overexpressed *in vivo*, it was shown to have dramatic functional consequences leading to defects in cellular differentiation *in vitro* and lethal developmental effects *in vivo* (12–14).

By using the yeast two-hybrid screening system, we have identified a nuclear-receptor-interacting protein that functions as a transcriptional corepressor for both TR and RAR (15). This corepressor, named SMRT, is a novel protein and is distinct from other described corepressors found in yeast (16–18), *Drosophila* (19), or higher organisms (20, 21). Interestingly, the carboxyl terminus of SMRT is related to RIP13 (22), which was later identified as a portion of the nuclear receptor corepressor N-CoR (23). Here, we compare the sequences between SMRT and N-CoR, and their similarity suggests that they belong to the same family of receptor-interacting proteins. We also demonstrate the interaction between SMRT and receptor heterodimers in mammalian cells. Furthermore, our results identify a dominant negative form of SMRT that functions as an antirepressor and suggest an alternative mechanism of releasing the transcriptional repression activity.

MATERIALS AND METHODS

Plasmids. The Gal-receptor and Gal-SMRT constructs contain the yeast GAL4 DNA-binding domain (amino acids 1–147) fused N-terminal to receptors or C-SMRT (amino acids 981-1495Δ; Δ indicates the absence of the alternative spliced insert between amino acids 1330–1375). The virus protein (VP) fusions contain the last 78 amino acids comprising the transactivation domain of Herpes VP16 fused to either C-SMRT or various receptors.

Cell Culture and Transfection. Monkey kidney CV-1 cells were grown in DMEM supplemented with 10% resin-charcoal stripped fetal bovine serum (FBS), 50 units/ml penicillin G, and 50 μg/ml streptomycin sulfate at 37°C in 7% CO_2. One day before transfection, cells were plated in 24-well tissue culture dish, and transfection was performed by calcium phosphate precipitation method or by lipofection using *N*-(2-(2,3)-dioleoyloxy)propyl-*N*,*N*,*N*-trimethyl ammonium methyl sulfate according to the manufacturer's instructions (Boehringer Mannheim). The amount of plasmids used in each transfection are as follows: GAL fusion plasmid (20 ng), VP fusion plasmid (50 ng), GAL4 reporter (150 ng), pCMX-βgal (350 ng), and

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Abbreviations: RAR, retinoic acid receptor; TR, thyroid hormone receptor; RXR, retinoid-X receptor; LBD, ligand-binding domain.

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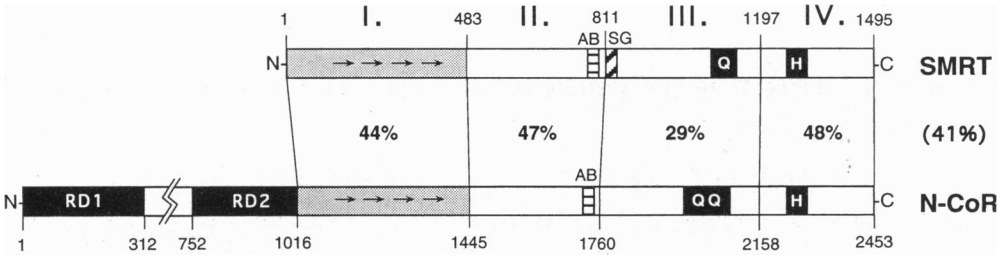

FIG. 1. Comparison between SMRT and N-CoR. The overall amino acid sequence identity between SMRT and N-CoR is 41% through the entire SMRT sequence. The identities within individual domains are as indicated. In N-CoR, two repression domains (RD1 and RD2) are found at the further N-terminal region. The acidic-basic motif (AB) and the serine-glycine-rich segment (SG) at the central region are as indicated. The glutamine-rich region (Q) and a predicted amphipathic α-helix (H) are as indicated.

carrier DNA pGEM4 (200 ng). All the points were at least duplicated. Six hours after transfection, cells were washed and fed with fresh media containing indicated amount of ligand. After 30 hr, cells were harvested for β-galactosidase and luciferase assay. The relative luciferase activity was calculated by normalizing to the β-galactosidase activity.

Northern Blot Analysis. The premade multi-tissue Northern blots (CLONTECH) were hybridized with ^{32}P-labeled SMRT cDNA probe according to the manufacturer's instruction. The filter was prehybridized in 50% formamide, 5× saline phosphate/EDTA, 5× Denhardt's solution, 0.1% SDS, and 100 μg/ml single-stranded DNA at 42°C for 4 hr, and then the labeled probe was added and hybridization was allowed to proceed at 42°C for 24 hr. The hybridized filter was washed under high stringency condition (0.1× standard saline citrate, 0.1% SDS) and exposed to x-ray film and developed. The filter was striped and rehybridized for actin.

Immunofluorescence Analysis. A rabbit anti-SMRT polyclonal antibody was raised against the purified GST-C-SMRT protein. For immunofluorescence assay, cells were fixed in 1:1 methanol/acetone 24 hr after plating and immunolabeled with a rabbit anti-SMRT polyclonal antibody as described (24).

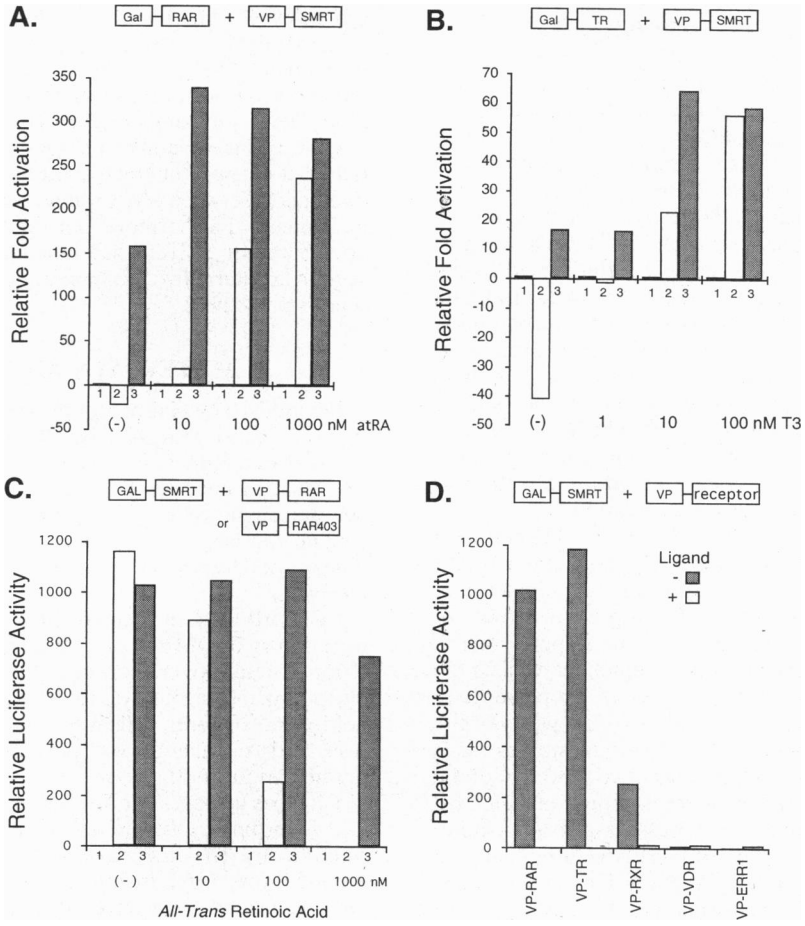

FIG. 2. Interactions between SMRT and receptors in mammalian cells. (*A*) Interactions between Gal-RAR and VP-SMRT in a mammalian two-hybrid assay. Column 1, Gal-DBD; column 2, Gal-RAR; column 3, Gal-RAR cotransfected with VP-SMRT. (−), solvent alone. The relative fold activation was determined by comparing the reporter activity to Gal-DBD alone. Note that the Gal-RAR alone shows a 25-fold repression in the absence of ligand. (*B*) Interactions between Gal-TR and VP-SMRT. The assay and each column are as described in *A*. (*C*) Interactions between Gal-SMRT and VP-F-RAR (blank columns) or VP-F-RAR403 (gray bars) in a mammalian two-hybrid assay. Column 1, Gal-SMRT; column 2, Gal-SMRT cotransfected with VP-RAR; column 3, Gal-SMRT cotransfected with VP-RAR403. (*D*) Interactions between Gal-SMRT and VP fusions of indicated receptors in a mammalian two-hybrid assay. The ligands used in these experiments are atRA for RAR and ERR1, T3 for TR, 9-cis RA for RXR, and 1,25 hydroxyvitamin D3 for VDR.

RESULTS

SMRT is Highly Related to N-CoR. A direct sequence comparison between SMRT and N-CoR reveals high degree of similarity with the exception of an N-terminal extension unique to N-CoR (Fig. 1). The homologous region is empirically divided into four domains based on the sequence similarities and functional properties. The most N-terminal region of SMRT (amino acids 1–483) contains four putative repeated motifs and it has 44% identity with N-CoR. The second domain (amino acids 484–811) is also highly conserved (47% amino acid identity) and it contains an alternative charged motif (the acidic-basic or AB motif). The third domain is less conserved (29% amino acid identity) and it contains a short serine-glycine-rich (SG) segment unique to SMRT and a glutamine-rich (Q) region. The most C-terminal domain (domain IV, amino acids 1197–1495) is involved in receptor interaction and is also highly conserved (48% amino acid identity). The overall amino acid identity between these two proteins is 41%, suggesting that SMRT and N-CoR are highly related proteins.

Interaction between SMRT and Receptors in Mammalian Cells. To test whether interactions between SMRT and receptors occur in mammalian cells, a two-hybrid protein–protein interaction assay was performed in tissue culture cells. Fig. 2*A* shows the relative reporter activities in such a test between Gal-RAR and VP-SMRT. In the absence of ligand, Gal-RAR behaves as a repressor of the basal promoter activity (lane 2; approximately 20-fold). When VP-SMRT is coexpressed with Gal-RAR, the luciferase activity increases dramatically (lane 3), whereas VP-SMRT alone does not have an effect on the reporter activity (not shown). *All-trans* RA treatment stimulates Gal-RAR activity in a dose-dependent manner (Fig. 2*A*, lanes 2), whereas ligand diminishes the fold of enhancement mediated by VP-SMRT over Gal-RAR (Fig. 2*A*, compare lanes 3 over lanes 2 at different ligand concentration).

The interaction between Gal-TR and VP-SMRT was analyzed in a similar assay (Fig. 2*B*). The Gal-TR strongly represses the basal promoter activity in the absence of ligand (approximately 40-fold), whereas coexpression of VP-SMRT relieves the suppressive effect and results in a further stimulation of the reporter activity. Addition of T3 stimulates Gal-TR activity while it diminishes the fold of enhancement mediated by VP-SMRT. These results indicate that SMRT interacts with both TR and RAR in mammalian cells in a ligand-independent fashion. The interactions between SMRT and receptors were also analyzed in a reciprocal combination; SMRT fused to a GAL4-DBD (Gal-SMRT) and receptors fused to the VP16 activation domain. In the absence of hormone, Gal-SMRT interacts strongly with VP-RAR fusion and VP-RAR403 mutant (Fig. 2*C*, lanes 2 and 3, respectively). Addition of ligand releases the interaction between Gal-SMRT and VP-RAR (Fig. 2*C*, lanes 2). In contrast, addition of ligand to VP-RAR403 mutant does not result in a significant decrease in the reporter activities (Fig. 2*C*, lanes 3). These results indicate that, in addition to its ligand-inducible transactivation activity, the C-terminal transactivation domain of RAR is also required for dissociation of SMRT *in vivo*. The interaction between Gal-SMRT and VP-RXR is much weaker than that with VP-RAR or VP-TR, and we observed no luciferase reporter activity in the combination between Gal-SMRT and VP-VDR or VP-ERR1 (Fig. 2*D*).

SMRT Interacts with Receptor Heterodimers in Mammalian Cells. We tested whether SMRT can interact with RXR-RAR heterodimers in mammalian cells by coexpressing RAR-LBD with Gal-RXR and VP-SMRT. Fig. 3*A* shows that Gal-RXR has little effect on the basal promoter activity (lane 2), whereas coexpression with VP-SMRT enhances the luciferase reporter activity slightly (lane 3). When the RAR-LBD is coexpressed with Gal-RXR, the basal promoter activity is reduced more than 10-fold (lane 4), suggesting the interaction

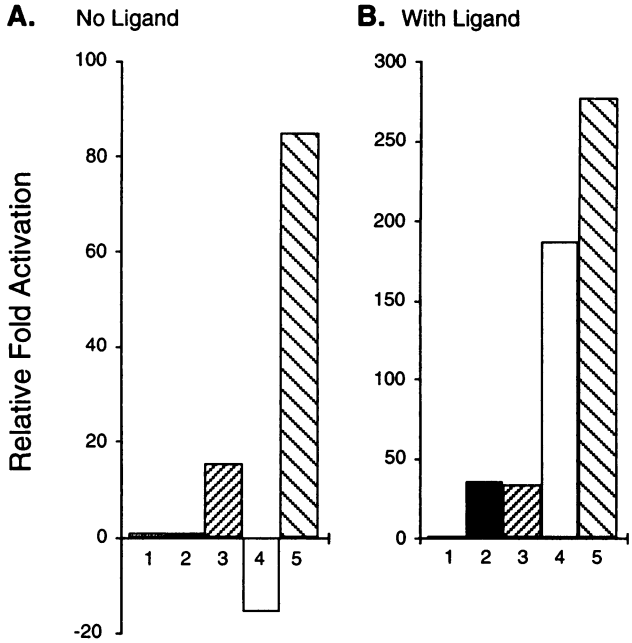

FIG. 3. Evidence that SMRT interacts with receptor heterodimers *in vivo*. Effect of RAR-LBD on the interaction between Gal-RXR and VP-SMRT in the absence (*A*) or presence (*B*) of ligand (atRA, 1 μM). Column 1, control plasmid; column 2, Gal-RXR; column 3, Gal-RXR cotransfected with VP-SMRT; column 4, Gal-RXR cotransfected with RAR-LBD; column 5, Gal-RXR cotransfected with RAR-LBD and VP-SMRT.

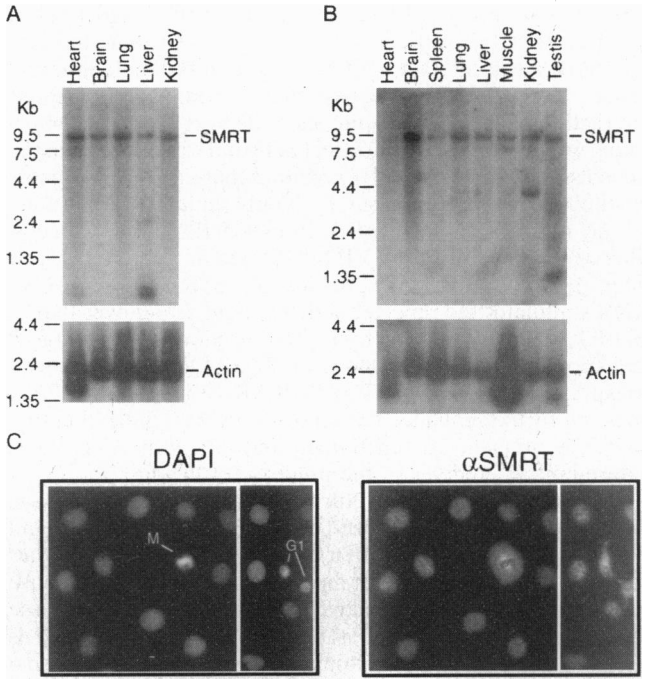

FIG. 4. SMRT is a ubiquitous nuclear protein. The expression pattern of SMRT in a human fetal Northern blot (*A*) and a mouse multiple tissue Northern blot (*B*) were analyzed as described in the text. The filters were stripped and rehybridized with a human actin probe (*Lower*). Note the existence of several smaller messages in addition to the full-length SMRT. (*C*) CV-1 cells grown on coverslips were processed for immunofluorescence analysis using a rabbit anti-SMRT polyclonal antibody (αSMRT). The same cells were counterstained by DNA binding dye DAPI for the location of the nucleus. One mitotic cell (M) and two cells in early G1 phase (G1) are indicated.

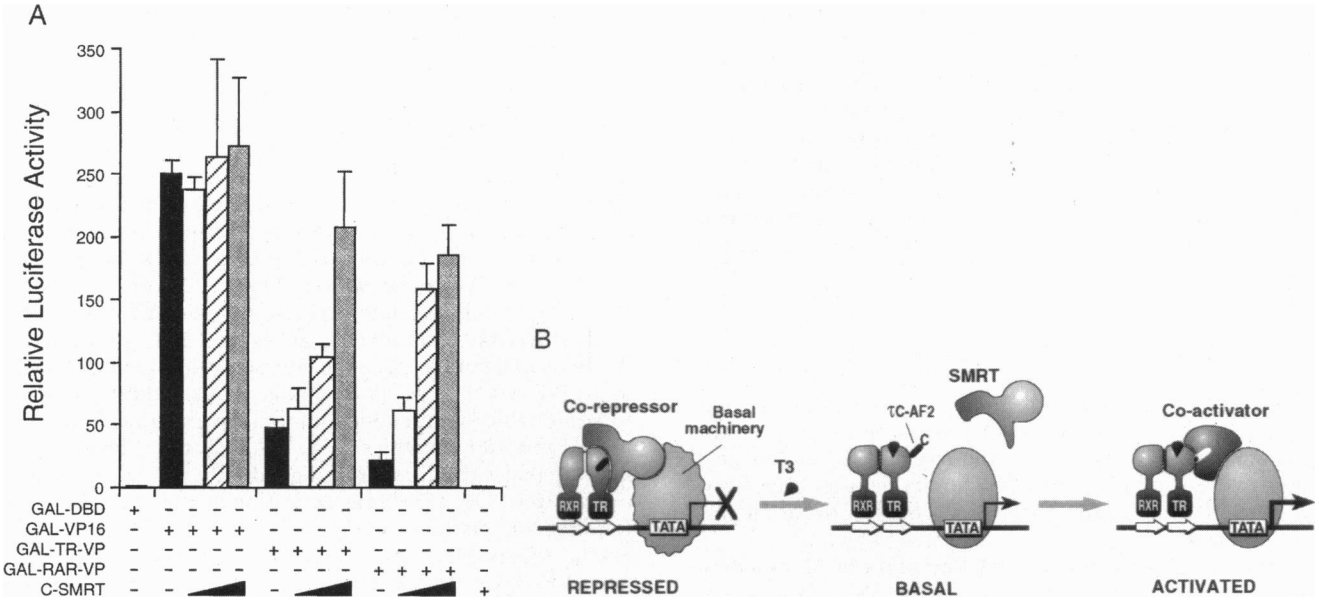

FIG. 5. (*A*) The receptor-interacting domain of SMRT acts as an anti-repressor. Effects of overexpression of the receptor-interacting domain of SMRT (C-SMRT) on the transactivation activities of GAL-VP16, GAL-TR-VP, and GAL-RAR-VP were analyzed by transient transfection assay. One nanogram of Gal-DBD fusion construct was transfected where indicated (+) together with increasing amount of C-SMRT expression plasmid (1, 5, and 15 ng). The relative luciferase activities were determined as described in the text. (*B*) Model of SMRT in hormone signaling. In the absence of hormone, SMRT associates with unliganded DNA-binding receptor heterodimer and represses basal promoter activity of target genes by interfering with the basal transcription machinery. Addition of hormone results in a conformational change in the receptor-LBD, which exposes the C-terminal ligand-inducible transactivation domain (AF2/τc) of the receptor to release SMRT from the receptor-DNA complex and to recruit coactivator(s) for transactivation.

between Gal-RXR and RAR-LBD and the ability of this heterodimeric complex to suppress basal promoter activity. When VP-SMRT is coexpressed with Gal-RXR and RAR-LBD, this suppressive effect is released, and a high level of reporter activity is observed (lane 5). These results indicate that SMRT can interact with receptor heterodimers in mammalian cells. This also suggest that the interface of RAR interacting with SMRT is physically distinct from that interacting with RXR. When the effect of ligand was analyzed in the same assay system (Fig. 3*B*), we found that atRA was capable of stimulating Gal-RXR activity slightly (lane 2), perhaps due to the isomerization of atRA into 9-cisRA in CV-1 cells, whereas coexpression with VP-SMRT had no additional effect (lane 3). In contrast, coexpression of RAR-LBD with Gal-RXR stimulates the reporter activity about 5-fold over that of Gal-RXR alone (lane 4). This represents more than a 500-fold induction relative to that shown in Fig. 3*A*, lane 4. However, coexpression of VP-SMRT with RAR-LBD and Gal-RXR does not further enhance the reporter activity, indicating that SMRT is not able to functionally interact with RXR-RAR heterodimeric complex in the presence of ligand.

SMRT Is a Ubiquitous Nuclear Protein. The expression pattern of SMRT was analyzed by Northern blot analysis and a message of about 9 kb is detected in all human fetal tissues as shown in Fig. 4*A*. A similar message is detected in the mouse adult tissues by using a human SMRT probe (Fig. 4*B*). These results suggest that SMRT is ubiquitously expressed and is highly conserved between human and mouse. A polyclonal antibody against the C-SMRT polypeptide was generated and used to analyze the subcellular localization of the endogenous SMRT in an immunofluorescence assay. Fig. 4*C* shows that SMRT is located in the nuclei of interphase cells. In the mitotic cell, SMRT is dispersed in the cytoplasm and is excluded from the metaphase chromosomes, whereas in the early G1 phase, SMRT is present in both the cytoplasm and the nucleus. These subcellular localizations of SMRT are consistent with a putative role of SMRT as a transcriptional corepressor for unliganded nuclear receptors.

The Receptor-Interacting Domain of SMRT Functions as an Anti-Repressor. The functional interaction between the receptor repression domain and VP16 transactivation domain was tested by using a trimeric fusion of TR or RAR inserted between the GAL4 DBD and the VP16 activation domain. The transactivation activities of the resulting constructs, Gal-TR-VP and Gal-RAR-VP, were tested in a transient transfection assay using a Gal4-dependent luciferase reporter (Fig. 5*A*). We found that the constitutive activity of VP16 can be dramatically reduced when linked to the receptor LBDs, presumably due to the recruitment of endogenous corepressor by the LBD. We then tested the effect of over expression of the receptor-interacting domain of SMRT (C-SMRT) on the receptor-mediated repression, and the results show that C-SMRT can reverse the repression effect efficiently in a dose-dependent manner. Similarly, C-SMRT also reverse the repression effect mediated by Gal-RAR and Gal-TR fusions but has no effect on the activity of Gal-VP16 or Gal-DBD (not shown). The dominant negative activity of C-SMRT suggests the potential importance of a recently isolated splicing variant which encodes only the C-terminal receptor-interacting domain of SMRT (25).

DISCUSSION

It was not until 1989 that transcriptional silencing was recognized as a critical component of the TR action and as an essential activity of the v-erbA oncoprotein (5, 26). Subsequently, transcriptional silencing by v-erbA has been well-characterized and shown to play an important role in development, cell differentiation and cellular transformation (27, 28). We propose that the effect of hormone in nuclear receptor signaling is to relieve silencing by inducing a dissociation of corepressor(s) and to activate transcription by recruiting transcriptional coactivator(s) such as Trip1 (29), RIP140 and RIP160 (30, 31), TIF1 (32), or SRC-1 (33).

Several pieces of evidence establish SMRT as a transcriptional corepressor for both TR and RAR; first, SMRT inter-

acts efficiently with unliganded TR and RAR and dissociates in a fashion that closely parallels binding of hormones. Second, the extent of interaction between SMRT and receptor mutants correlates with the repression activity of the receptors; third, overexpression of the receptor-interacting domain of SMRT antagonizes the repressive effect of both TR and RAR; and fourth, tethering of SMRT to the DNA template results in a direct transcriptional repression even in the absence of associated receptors. Together, these results strongly suggest SMRT as a transcriptional corepressor molecule that plays an important role in mediating the repressor activities of nuclear receptors.

Recently, a polarity-specific corepressor for nuclear receptors N-CoR has been identified (23, 34). The overall sequence similarity between SMRT and N-CoR suggests that they belong to a new protein family. Previous studies have indicated that the activator and repressor functions of TR and RAR are functionally distinct (13, 27, 35, 36). In fact, the major activation function of these two receptors resides in the C-terminal domain whose activity is entirely dependent on the addition of ligand. If this domain is deleted, the receptor becomes a potent transcriptional repressor as a presumed consequence of continual association with SMRT. Because ligand is capable of binding to the C terminus activation domain-deleted mutants (12, 36, 37), this domain is thus important for promoting SMRT dissociation, which results in the release of repression.

We propose a two-step mechanism for the action of thyroid hormone and retinoic acid on the modulation of nuclear receptor activity (Fig. 5*B*). In the absence of hormone, we assumed that the C-terminal domain is in a configuration that allows the receptor to interact with corepressors. Ligand binding to the receptor results in a conformational change of the LBD which in turn alters the position of the C-terminal domain. This altered domain then promotes SMRT dissociation and allows receptor to recruit transcriptional coactivator(s). Recent crystal structures on the LBD of unliganded RXR and liganded RAR and TR support the hypothesis that the C-terminal helix undergoes a relative position shift upon hormone binding (38–40). Our current results suggest a mechanistic link between transcriptional repression and activation, perhaps via competition of a common interacting surface.

The unliganded TR has been shown to interact also with general transcription factors TFIIB and TBP (41, 42). The identification of corepressor molecules for nuclear receptors suggest the involvement of other intermediate proteins between the receptor and the basal machinery. Accordingly, SMRT may interact with a distinct component of the transcription machinery or possibly stabilize the association of TR with general transcription factors in a nonfunctional configuration.

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1. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. & Evans, R. M. (1995) *Cell* **83,** 835–839.
2. Mangelsdorf, D. J. & Evans, R. M. (1995) *Cell* **83,** 841–850.
3. Kurokawa, R., DiRenzo, J., Boehm, M., Sugarman, J., Gloss, B., Rosenfeld, M. G., Heyman, R. A. & Glass, C. K. (1994) *Nature (London)* **371,** 528–531.
4. Baniahmad, A., Kohne, A. C. & Renkawitz, R. (1992) *EMBO J.* **11,** 1015–1023.
5. Damm, K., Thompson, C. C. & Evans, R. M. (1989) *Nature (London)* **339,** 593–597.
6. Holloway, J. M., Glass, C. K., Adler, S., Nelson, C. A. & Rosenfeld, M. G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8160–8164.
7. Renkawitz, R. (1993) *Ann. N.Y. Acad. Sci.* **684,** 1–10.
8. Sap, J., Munoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., Beug, H. & Vennstrom, B. (1986) *Nature (London)* **324,** 635–640.
9. Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J. & Evans, R. M. (1986) *Nature (London)* **324,** 641–646.
10. Damm, K., Beug, H., Graf, T. & Vennstrom, B. (1987) *EMBO J.* **6,** 375–382.
11. Damm, K. & Evans, R. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10668–10672.
12. Damm, K., Heyman, R., Umesono, K. & Evans, R. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2989–2993.
13. Imakado, S., Bickenbach, J. R., Bundman, D. S., Rothnagel, J. A., Attar, P. S., Wang, X. J., Salczak, V. R., Wisniewski, S., Pote, J., Gordon, J. S., Heyman, R. A., Evans, R. M. & Roop, D. R. (1995) *Genes Dev.* **9,** 317–329.
14. Tsai, S., Bartelmez, S., Heyman, R., Damm, K., Evans, R. & Collins, S. J. (1992) *Genes Dev.* **6,** 2258–2269.
15. Chen, J. D. & Evans, R. M. (1995) *Nature (London)* **377,** 454–457.
16. Keleher, C. A., Redd, M. J., Schultz, J., Carlson, M. & Johnson, A. D. (1992) *Cell* **68,** 709–719.
17. Komachi, K., Redd, M. J. & Johnson, A. D. (1994) *Genes Dev.* **8,** 2857–2867.
18. Tzamarias, D. & Struhl, K. (1994) *Nature (London)* **369,** 758–761.
19. Paroush, Z., Finley, R. L., Jr., Kidd, T., Wainwright, S. M., Ingham, P. W., Brent, R. & Ish-Horowicz, D. (1994) *Cell* **79,** 805–815.
20. Ayer, D. E., Lawrence, Q. A. & Eisenman, R. N. (1995) *Cell* **80,** 767–776.
21. Schreiber-Agus, N., Chin, L., Chen, K., Torres, R., Rao, G., Guida, P., Skoultchi, A. I. & DePinho, R. A. (1995) *Cell* **80,** 777–786.
22. Seol, W., Choi, H.-S. & Moore, D. D. (1995) *Mol. Endocrinol.* **9,** 72–85.
23. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamai, Y., Soderstrom, M., Glass, C. K. & Rosenfeld, M. G. (1995) *Nature (London)* **377,** 397–403.
24. Dyck, J. A., Maul, G. G., Miller, W., Jr., Chen, J. D., Kakizuka, A. & Evans, R. M. (1994) *Cell* **76,** 333–343.
25. Sande, S. & Privalsky, M. (1996) *Mol. Endocrinol.*, in press.
26. Sap, J., Munoz, A., Schmitt, J., Stunnenberg, H. & Vennstrom, B. (1989) *Nature (London)* **340,** 242–244.
27. Baniahmad, A., Steiner, C., Kohne, A. C. & Renkawitz, R. (1990) *Cell* **61,** 505–514.
28. Saitou, M., Sugai, S., Tanaka, T., Shimouchi, K., Fuchs, E., Narumiya, S. & Kakizuka, A. (1995) *Nature (London)* **374,** 159–162.
29. Lee, J. W., Ryan, F., Swaffield, J. C., Johnston, S. A. & Moore, D. D. (1995) *Nature (London)* **374,** 91–94.
30. Halachmi, S., Marden, E., Glover, M., Mackaay, H., Abbondanza, C. & Brown, M. (1994) *Science* **264,** 1455–1458.
31. Cavailles, V., Dauvois, S., L'Horset, F., Lopez, G., Hoare, S., Kushner, P. J. & Parker, M. G. (1995) *EMBO J.* **14,** 3741–3751.
32. LeDouarin, B., Zechel, C., Garnier, J. M., Lutz, Y., Tora, L., Pierrat, P., Heery, D., Gronemeyer, H., Chambon, P. & Losson, R. (1995) *EMBO J.* **14,** 2020–2033.
33. Ozáte, S. A., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. (1995) *Science* **270,** 1354–1357.
34. Kurokawa, R., Soderstrom, M., Horlein, A., Halachmi, S., Brown, M., Rosenfeld, M. G. & Glass, C. K. (1995) *Nature (London)* **377,** 451–454.
35. Danielian, P. S., White, R., Lees, J. A. & Parker, M. G. (1992) *EMBO J.* **11,** 1025–1033.
36. Schulman, I. G., Chakravarti, D., Jugilon, H., Romo, A. & Evans, R. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8288–8292.
37. Leng, X., Blanco, J., Tsai, S. Y., Ozato, K., O'Malley, B. W. & Tsai, M. J. (1995) *Mol. Cell. Biol.* **15,** 255–263.
38. Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H. & Moras, D. (1995) *Nature (London)* **375,** 377–382.
39. Renaud, J.-P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H. & Moras, D. (1995) *Nature (London)* **378,** 681–689.
40. Wagner, R. L., Apriletti, J. W., McGrath, M. E., West, B. L., Baxter, J. D. & Fletterick, R. J. (1995) *Nature (London)* **378,** 690–697.
41. Baniahmad, A., Ha, I., Reinberg, D., Tsai, S., Tsai, M. J. & O'Malley, B. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8832–8836.
42. Fondell, J. D., Brunel, F., Hisatake, K. & Roeder, R. G. (1996) *Mol. Cell. Biol.* **16,** 281–287.