Alternative mRNA Splicing of SMRT Creates Functional Diversity by Generating Corepressor Isoforms with Different Affinities for Different Nuclear Receptors*

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Many eukaryotic transcription factors are bimodal in their regulatory properties and can both repress and activate expression of their target genes. These divergent transcriptional properties are conferred through recruitment of auxiliary proteins, denoted coactivators and corepressors. Repression plays a particularly critical role in the functions of the nuclear receptors, a large family of ligand-regulated transcription factors involved in metazoan development, differentiation, reproduction, and homeostasis. The SMRT corepressor interacts directly with nuclear receptors and serves, in turn, as a platform for the assembly of a larger corepressor complex. We report here that SMRT is expressed in cells by alternative mRNA splicing to yield two distinct variants or isoforms. We designate these isoforms SMRTα and SMRTγ and demonstrate that these isoforms have significantly different affinities for different nuclear receptors. These isoforms are evolutionarily conserved and are expressed in a tissue-specific manner. Our results suggest that differential mRNA splicing serves to customize corepressor function in different cells, allowing the transcriptional properties of nuclear receptors to be adapted to different contexts.

Nuclear receptors are transcription factors that play multiple roles in metazoan development and physiology (1–6). Nuclear receptors operate by binding to specific promoter elements on DNA and by modulating transcription of adjacent target genes in response to hormone ligand (3, 7–9). The nuclear receptors include, among others, the thyroid hormone receptors (TRs),1 the retinoic acid receptors (RARs), and the retinoid X receptors (RXRs) (3–5, 7, 10, 11). Each of these receptors localizes to the nucleus and binds to DNA in both the absence and presence of hormone ligand. These receptors can repress transcription of their target genes in the absence of hormone, but activate target gene transcription upon binding to hormone agonist (3, 7–9, 12, 13). This bimodal transcriptional regulation is accomplished through a hormone-regulated exchange of a corepressor complex, found on the nuclear receptor in the absence of hormone, for a coactivator complex recruited in the presence of hormone agonist (14). Corepressor and coactivator protein complexes regulate transcription through direct interaction with the basal transcription machinery and through modification of chromatin structure (15).

Both activation and repression are essential for correct receptor function. For example, RAR-mediated repression is required for appropriate anterior/posterior segregation in vertebrates, and disruption leads to aberrant head formation during murine development (16). TR-mediated repression is required for correct Xenopus larval development, and abrogation of repression leads to premature metamorphosis (17). Aberrations in the regulation of repression can result in human disease. For example, resistance to thyroid hormone syndrome, an inherited endocrine disorder, has been mapped to mutations in TRs that disrupt the hormone-driven release of corepressor (18–23); similarly, mutant RARs that fail to release corepressor correctly in response to hormone ligand play a causal role in human acute promyelocytic leukemia (24–28).

SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) and its paralog, N-CoR (nuclear receptor corepressor), are central mediators of transcriptional repression by TRs, RARs, and RXRs (29–32). SMRT and N-CoR make direct contact with their nuclear receptor partners and serve, in turn, as platforms for the recruitment of additional components of a larger corepressor complex that includes histone deacetylases, TBL1, TBLR1, and GPS2 (33–37). A series of at least four “repression domains” within the N-terminal portion of SMRT and N-CoR (denoted RD1 to RD4) serve as docking surfaces for these additional corepressor subunits, whereas a series of more C-terminal receptor interaction domains (denoted S1 and S2 in SMRT and N1 to N3 in N-CoR) mediate contacts with the nuclear receptors (see Fig. 1A) (38–42). In the absence of hormone, conserved “CoRNR box” amino acid motifs, located within each of these receptor interaction domains, tether to a hydrophobic groove on the surface of the unliganded nuclear receptors (38–42). In the presence of hormone agonist, a conformational change in the C-terminal helix 12 of the nuclear receptors occludes this corepressor docking surface, causing release of SMRT or N-CoR, release of the remainder of the corepressor complex, and derepression of target gene expression (43–47). This repositioning of helix 12 by agonist simultaneously forms a new surface that recruits coactivators, thereby conferring transcriptional activation (43, 46). Many nuclear receptors bind to DNA as protein dimers, and it is believed that two CoRNR motifs within a single SMRT or N-CoR are em-

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1 The abbreviations used are: TRs, thyroid hormone receptors; RARs, retinoic acid receptors; RXRs, retinoid X receptors; GST, glutathione S-transferase; EST, expressed sequence tag; GFP, green fluorescent protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MEKK1, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-1; EMSA, electrophoretic mobility shift assay; T3, triiodothyronine.
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employed in tethering these corepressors to the two receptors that compose the dimer (41, 42, 48, 49). Previous work has suggested that RAR preferentially interacts with the S2 domain, RXR preferentially interacts with the S1 domain, and TR can interact with both the S1 and S2 domains (31, 38, 42, 50).

Many nuclear receptors are expressed as a series of interrelated isoforms or isoforms. For example, two genetic loci in vertebrates encode TRs (denoted TRα and TRβ); three genetic loci encode RARs (denoted RARα, RARβ, and RARγ); and three genetic loci encode RXRs (denoted RXRα, RXRβ, and RXRγ) (51–55). Alternative mRNA splicing and promoter utilization result in further diversification of the receptors that are produced from a given locus (56–60). These various nuclear receptor isoforms are expressed in tissue- and development-specific patterns, display distinct interactions with corepressors and coactivators, and exhibit distinct transcriptional properties (50, 61). N-CoR and SMRT can similarly be considered isoforms of one another, thereby paralleling the multiple isoforms found in their nuclear receptor partners. We report here a further extension of this concept by demonstrating that SMRT is itself expressed by alternative mRNA splicing to generate at least two distinct isoforms (denoted SMRTα and SMRTβ) that are expressed at different levels in different tissues (see Fig. 1A). SMRTα contains 47 amino acids in its C-terminal domain that are absent from SMRTβ (30, 31). The SMRT-specific 47 amino acids map only 5 residues away from the S1 CoRNR box. As a consequence, although both SMRT isoforms interact nearly equally with RARα, they differ significantly in the ability to interact with and mediate repression by different isoforms of TR. We conclude that the receptor interaction properties of SMRT can be modified by alternative mRNA splicing and that, as a result, the repression properties of different nuclear receptors are likely to differ in different cell and tissue contexts. These observations also help reconcile apparent discrepancies in the literature as to the relative affinity of TRs for N-CoR versus SMRT (17, 35, 42, 48, 62, 63).

EXPERIMENTAL PROCEDURES

Plasmids—PCR was used to introduce BamHI and XhoI restriction sites at the ends of DNA fragments representing the S1 domain (amino acids 2313–2517), the S2 domain (amino acids 2077–2312), or both the S1 and S2 domains (amino acids 2077–2517) of SMRTα (GenBankTM/EBI accession number AF113003) or the corresponding fragments of SMRTβ. These PCR products were cloned into the corresponding BamHI and XhoI sites in pGEX-KG (64). For expression of pGEX-KRα or pGEX-KRβ, the sequence encoding the S1 domain and HBD is hormone-binding domain; amino acids 177–461) of the receptor was amplified by PCR using a pair of primers (5′-acccagcaacgctggatg-3′ and 5′-ggtgaggggctggatg-3′) and a commercial Taq polymerase. The resulting protein DNA complexes were resolved by native gel electrophoresis and visualized using a Storm 840 PhosphorImager. The ability of the SMRT constructs to retard ("supershift") the mobility of the nuclear receptor-DNA complexes was quantified using the PhosphorImager. Apparent dissociation constants were calculated using Prism Version 4.0 software (GraphPad Software, San Diego, CA) to fit the equation

\[ Y = B_{0} - X/\text{K}_{d} + X; \quad B_{0} \text{ was constrained to be the same for all SMRTα and SMRTβ in all cases. Relative affinities are expressed as the ratio of SMRTα K_{d}}/\text{K}_{d}\text{.}

Subcellular Localization of SMRTα and SMRTβ—Green fluorescent protein (GFP)-fusions of full-length SMRTα and SMRTβ were generated by ligation of the appropriate corepressor coding regions into a pCMV-GFP vector.2 These constructs were introduced into CV-1 cells (1,0 × 10^{4} cells in a 6-well plate) using the Effectene transfection reagent (Qiagen). The cells were fixed 48 h after transfection in acetone/methanol (1:1) for 10 min. The cells (on glass coverslips) were then stained with 50 ng/ml 4′,6-diamidino-2-phenylindole to visualize nuclei, mounted on slides (30 μl of Vectashield (Vector Laboratories, Burlingame, CA), and sealed with fingernail polish. The slides were visualized using a Nikon Microphot epifluorescence microscope. Digital images were captured with a Nikon Cool Pix 4500 digital camera.

Dominant-negative Derepression Assay—CV-1 cells (3 × 10^{4}cells) were plated in 24-well culture plates and incubated overnight in a humidified 5% CO2 atmosphere at 37 °C in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% fetal bovine serum. The medium was then replaced with Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum (hormone-depleted); a total of 250 ng of plasmid DNA was introduced per well using the Effectene transfection reagent according to the manufacturer’s protocol. The DNA mixture included 50 ng of the appropriate luciferase reporter plasmid, 50 ng of either plasmid pSG5-TRβ1 or pSG5-Gal4DBD-TRβ1HBD, 50 ng of pCH110-β-galactosidase vector (employed as an internal transfection control), and 0–100 ng of pSG5Myc-SMRTα (S1/S2) or pSG5Myc-SMRTβ (S1/S2). Total plasmid DNA was adjusted by addition of an empty pSG5 vector so that it is equal in all samples. After an additional 24 h incubation at 37 °C, the cells were harvested, lysed, and analyzed for luciferase activity using the Promega luciferase assay system and for β-galactosidase activity as described previously (43). Duplicate transfections were immunoblotted with anti-Myc antibody (Gallma1 Laboratories, Lexington, KY). The immunoblot was visualized using horseradish peroxidase-conjugated anti-Myc antibody (Cell Signaling Technology) and chemiluminescence substrate (Alpha Innotech Corp.). Images of the immunoblot were captured using an Alpha Innotech FluorChem 8900 densitometer and quantified using AlphaEaseFC software.

Reverse Transcription—Organs were harvested from 6-week-old male C57BL/6 mice and quick-frozen on dry ice before brief storage at −80 °C. Mouse organs were homogenized in TRIzol reagent (Invitrogen) using a T 8 ULTRA-TURRAX homogenizer (IKA Works, Wilmington, NC); total RNA was prepared according to the manufacturer’s protocol. cDNA was synthesized using random hexamer primers, 4 μg of total RNA, and avian myeloblastosis virus reverse transcriptase (Promega) as described previously (74). cDNAs corresponding to SMRTα and SMRTβ were selectively amplified by PCR using a set of primers for SMRTα-S starts at 5′-gctctggagggatgtagg-3′ and a common SMRTα-S ends at 5′-ctgtggagggatgtagg-3′. PCR was carried out for the number of cycles given in each figure legend using the following cycling parameters: 94 °C for 30 s, 62 °C for 45 s, and 72 °C for 1 min. SMRTα and SMRTβ mRNAs generated products of 442 and 301 bp, respectively, which were resolved on 2% agarose gel (0.5× Tris borate/EDTA running buffer, 100 V for 75 min), visualized by ethidium bromide staining, and quantified using an Alpha Innotech FluorChem 8900 densitometer and AlphaEaseFC software. The identity of each PCR product was confirmed by DNA sequencing. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (GenBankTM/EBI 2 Jonas, B. A., and Privalsky, M. L. (2004) J. Biol. Chem. 279, 54676–54686.)

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accession number NM_008084) was amplified using the same cycle parameters described for SMRT and oligonucleotides GAPDH-Up (5'-gctgaacgggaagctcactgg-3') and GAPDH-Down (5'-gcctgcttcaccaccttcttgatg-3'), producing a 125-bp product. Both the SMRT and GAPDH primers span introns such that genomic DNA would produce significantly larger DNA products; however, no evidence of genomic DNA contamination was observed.

RESULTS

Two Distinct Forms of SMRT That Differ in Their C-terminal Receptor Interaction Domains Are Expressed—Inspection of the known SMRT cDNA sequences revealed an interesting heterogeneity in the C-terminal corepressor domain, defined by the inclusion or exclusion of a 47-codon sequence immediately flanking the S1 receptor interaction domain (Fig. 1, A and B) (30, 31, 75, 76). To examine this phenomenon in more detail, we searched the expressed sequence tag data base (dbEST) for the 141-nucleotide sequence that encodes these additional 47 amino acids and, separately, for the 100-nucleotide sequence that represents the junction sequence created in the absence of this insert. In both cases, we found multiple entries containing sequence identical to either SMRTα or SMRTγ and the library tissue sources for each EST. D, schematic representation of the alternative mRNA splicing events that give rise to SMRTα and SMRTγ. The nucleotide positions within the open reading frames of SMRTα and SMRTγ are indicated. Nucleotide numbers within the human genome chromosome 12 HS12_9912 segment sequence are also indicated (GenBankTM/EBI accession number NT_009755).

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FIG. 1. Schematic representation of SMRT isoforms. A, the amino acid sequence of the SMRTα exon and surrounding sequences. The CoRNR box sequence of the SMRT S1 domain is indicated in boldface and boxed. The numbers indicate the amino acid positions in SMRTα. B, schematic alignment of the domains of SMRTα and SMRTγ as well as N-CoR. Black boxes indicate the repression domains, and vertical bars indicate the positions of the CoRNR box sequences within each receptor interaction domain. The amino acid positions for each isoform are indicated. C, EST clones that contain sequences identical to either SMRTα or SMRTγ and the library tissue sources for each EST. D, schematic representation of the alternative mRNA splicing events that give rise to SMRTα and SMRTγ. The nucleotide positions within the open reading frames of SMRTα and SMRTγ are indicated. Nucleotide numbers within the human genome chromosome 12 HS12_9912 segment sequence are also indicated (GenBankTM/EBI accession number NT_009755).
and accumulated at steady state to similar levels (Fig. 2A). We also created GFP fusions of both SMRT variants and introduced these into CV-1 cells to visualize the subcellular localizations of these proteins. As anticipated, unmodified GFP exhibited a diffuse, primarily cytoplasmic subcellular distribution (Fig. 2B). In contrast, both GFP-SMRTα and GFP-SMRTβ were primarily nuclear in distribution, displaying a diffuse localization over the nucleoplasm that was excluded from the nucleoli (Fig. 2B). Visible within this diffuse nucleoplasmic signal, both SMRTα and SMRTβ also displayed a brighter punctate pattern (Fig. 2B) that has been noted before for SMRTα and that has been proposed to represent clusters of corepressor complexes that also contain HDAC3, TBL1, TBLR1, and GPS2 (15). We have reported previously that SMRTβ responds to the epidermal growth factor receptor/Ras/MEKK1 cascade signaling by a change from a nuclear to a cytoplasmic distribution in many of the cells (77); SMRTα displayed a similar relocational response in co-introduction of an activated MEKK1 construct (Fig. 2C). We conclude that both the SMRTα and SMRTβ variants are synthesized, accumulate, distribute, and respond to growth factor signaling in similar fashions under the conditions tested.

SMRTα and SMRTβ Diverge in Their Ability to Interact with TR—The 47-amino-acid insert found in the SMRTα splice variant occurs only 5 amino acids C-terminal to the CoRNR box in the S1 receptor interaction domain (Fig. 1A) (41, 42). We tested the ability of each isoform of SMRT to interact with different nuclear receptors. Previous studies have demonstrated that SMRT and N-CoR preferentially interact with nuclear receptors when the latter are bound as dimers to their respective DNA response elements and that DNA binding can influence the relative affinities of these corepressor paralogs for different nuclear receptors (41, 62, 78). We therefore used an electrophoretic mobility shift assay (EMSA) that permitted us to investigate the interactions of the SMRT corepressors with nuclear receptors in the context of their DNA response elements. A fixed amount of receptor was added to a radiolabeled DNA probe in the presence of increasing amounts of a construct containing the receptor interaction domains of either the SMRTα or SMRTβ isoform (purified as a bacterially produced GST fusion protein). The ability of the SMRT construct to bind to and supershift the receptor-DNA complex was analyzed by native gel electrophoresis and quantified by PhosphorImager analysis.

In the absence of the SMRT corepressor, TRβ1 bound to the DR4 probe as a receptor homodimer (Fig. 3A, third lane). This is consistent with prior studies (13, 62, 79, 80) and was confirmed by the following criteria. (a) Little or no protein-DNA complex was observed when using non-recombinant baculovirus/Sf9 extracts with the DR4 probe or when using the TRβ1 preparation with an irrelevant DNA probe or with a DNA probe containing only a single half-site (Fig. 3A, first and second lanes) (data not shown). (b) The TRβ1-DNA complexes were supershifted to a slower mobility by anti-TR antibody or by addition of RXR (which forms a heterodimer with TRs) (Fig. 3A, compare third and fourth lanes with seventh lane and compare sixteenth and eighteenth lanes). (c) The homodimeric TRβ1-DNA complex was destabilized by addition of triiodothyronine (T3) (Fig. 3A, compare third and fourth lanes with tenth lane). Addition of the SMRTα isoform to the homodimeric TRβ1-DNA complex resulted in formation of a new tertiary complex indicative of an interaction between SMRTα and the TRβ1 homodimer (Fig. 3A, compare third and fifth lanes). The amount of tertiary complex formed was proportional to the amount of GST-SMRTα added (Fig. 3B, tenth through eighteenth lanes), and no tertiary complex formation was observed when using non-recombinant GST constructs (Fig. 3A, fourth lane). SMRTβ did not form a complex with the DNA probe in the absence of the nuclear receptor (Fig. 3A, fourteenth lane), and the SMRTβ-TRβ1-DNA complex was further supershifted by anti-TR antibody (Fig. 3A, compare fifth and seventh lanes). Finally, the SMRTβ-TRβ1-DNA complex was disrupted, as expected, by addition of T3 (Fig. 3A, compare fifth and eleventh lanes).

Having established the overall validity of the supershift EMSA, we next used it to compare the relative binding of SMRTα and SMRTβ to the TRβ1-DNA complex. Notably, the S1 domain of SMRTα displayed a much higher affinity for the TRβ1-DNA complex than did the S1 domain of SMRTβ, manifested as a requirement for significantly less SMRTα S1 construct compared with SMRTβ S1 construct to generate a TRβ1-DNA supershift (Fig. 3, A, compare fifth and sixth lanes; and B, compare first through ninth lanes with tenth through eighteenth lanes). It should be noted that the amounts of SMRTα and SMRTβ constructs were carefully quantified, that the constructs were used as purified preparations at equal concentrations, and that comparable results were obtained using independently isolated preparations (data not shown). As observed with SMRTβ, the SMRTα′-TRβ complex could be supershifted by anti-TR antibody (Fig. 3A, compare sixth and ninth lanes) and was dissociated by saturating T3 (compare sixth and twelfth lanes).

Quantification of repeated experiments confirmed the reproducibility of these results (Fig. 3C) and verified that the data fitted a hyperbolic binding equation. Using this model, apparent dissociation constants (Kd,app) for the interaction between TRβ1 and SMRTα and SMRTβ were determined as described under “Experimental Procedures”; analogous supershift EMSAs have been used to determine the apparent affinity constants for several other transcription factors, including Sp1 and the lac and trp repressors (81–85). Based on this form of analysis TRβ1 interacted 10-fold more strongly with SMRTβ than with SMRTα (Fig. 3C and Table I). It should be noted that this mathematical representation provided a close fit to the actual data; however, the EMSA method is not a true equilibrium assay, and these numbers represent apparent rather than absolute dissociation constants. Nonetheless, these apparent dissociation constants are a useful means to describe the rela-

**Fig. 2.** Protein accumulation and nuclear localization of SMRTα and SMRTβ. A, Western blot of CV-1 cells either untransfected or transfected with plasmids expressing Myc-tagged full-length SMRTα or Myc-tagged SMRTβ. B, fluorescent micrograph of CV-1 cells transfected with plasmids expressing GFP, GFP-SMRTα, or GFP-SMRTβ. C, change in the subcellular localization of SMRTα or SMRTβ in response to MEKK1.
The relative affinity of SMRT/H9251 and SMRT/H9270 for different receptors and will be cited, acknowledging their limitations, in the remainder of this work.

TRs can interact with either the S1 or S2 domain of SMRT, and TR homodimers are believed to be able to contact both the S1 and S2 domains simultaneously. The S2 domain is identical in the SMRT/H9251 and SMRT/H9270 isoforms. We therefore also examined the effect of the isoform-specific differences in the S1 domain when tested in combination with the S2 domain. Whereas the difference in the relative affinities of the SMRT/H9251 and SMRT/H9270 isoforms for TR/H9252 was most readily observed using GST-SMRT constructs limited to the S1 domain (Fig. 4A), it was still easily discernable with GST-SMRT constructs containing the S2 domain.

Table I

| Affinities of SMRT isoforms for receptors |  
| S1 |  
|  
| τ |  
| α |  
| S1/S2 |  
| τ |  
| α |  
| TRα1 homodimers |  
| S1 |  
| τ |  
| α |  
| S1/S2 |  
| τ |  
| α |  
| Chicken lysozyme F2 element |  
| TRβ2 homodimers |  
| S1 |  
| τ |  
| α |  
| S1/S2 |  
| τ |  
| α |  
| RXRα/TRβ1 heterodimers |  
| S1 |  
| τ |  
| α |  
| S1/S2 |  
| τ |  
| α |  
| DR5 element |  
| RXRα/RXRA heterodimers |  
| S1 |  
| τ |  
| α |  
| S1/S2 |  
| τ |  
| α |  

Fig. 3. EMSA interaction between TRβ1 and the SMRT S1 domains. A, human TRβ1 derived from a recombinant baculovirus/Sf9 cell system (T) or equivalent non-recombinant preparations (N) were incubated with radiolabeled DR4 oligonucleotide DNA and GST (G), GST-SMRTα(S1) (ω), or GST-SMRTα(S1) (τ). Anti-TRβ1 antibody (Ab; catalog no. MA1–215, Affinity BioReagents, Golden, CO), 1 μM T3, and/or human RXRα (from baculovirus/Sf9 preparations) was also added to certain samples as indicated.

B, varying amounts of purified GST-SMRTα(S1) or GST-SMRTα(S1) protein were added to a constant amount of TRβ1 protein and radiolabeled DR4 probe. The TRβ1-DNA complexes supershifted to a slower mobility by the SMRTα or SMRTα(S1) were quantified relative to the amount of SMRT protein added to each binding reaction. From these data, the apparent dissociation constants for both the SMRTα and SMRTα(S1) domains were determined. The graph represents the mean of n > 3 replicates. Error bars indicate S.E. RID, receptor interaction domain.

The affinity of SMRTα and SMRTα(S1) for different receptors and will be cited, acknowledging their limitations, in the remainder of this work.

TRs can interact with either the S1 or S2 domain of SMRT, and TR homodimers are believed to be able to contact both the S1 and S2 domains simultaneously. The S2 domain is identical in the SMRTα and SMRTα(S1) isoforms. We therefore also examined the effect of the isoform-specific differences in the S1 domain when tested in combination with the S2 domain. Whereas the difference in the relative affinities of the SMRTα and SMRTα(S1) isoforms for TRβ1 was most readily observed using GST-SMRT constructs limited to the S1 domain (Fig. 4A), it was still easily discernable with GST-SMRT constructs con-
Fig. 4. Interaction between SMRT receptor interaction domains and TRα1 or TRβ1 on a DR4 DNA element. Varying amounts of purified GST-SMRT(α1/S1) or GST-SMRT(γ1) (S1) were added to binding reactions containing TRβ1 (A), or TRα1 (C) together with a radiolabeled DR4 oligonucleotide probe. Alternatively, varying amounts of purified GST-SMRTα(S1/S2) or GST-SMRTγ(S1/S2) were added to binding reactions containing TRβ1 (B) or TRα1 (D) together with the radiolabeled DR4 oligonucleotide probe. The receptor-DNA complexes supershifted by addition of the SMRT constructs were quantified relative to the amount of SMRT protein added to each binding reaction. Error bars indicate S.E. of three replicate experiments. RID, receptor interaction domain.

taining both the S1 and S2 domains (Fig. 4B). In the latter assays, TRβ1 had a 2.7-fold greater affinity for the S1 and S2 domains of SMRTα compared with those of SMRTγ.

TRs are expressed as two different isotypes from two distinct genetic loci: TRβ and TRα. To determine whether the preference displayed by SMRTα for TRβ1 extends to the other TR isotype, we repeated our EMSA experiments using TRα1 in place of TRβ1. TRα1 had a 5.1-fold greater affinity for GST-SMRTα(S1) and a 2.1-fold greater affinity for GST-SMRTγ(S1/S2) than for equivalent constructs of SMRTγ (Fig. 4, C and D; and Table I). Controls confirmed that non-recombinant GST failed to supershift the TRα1-DNA complex and that the SMRT-TRα-DNA complex was dissociated by addition of TRβ (data not shown). We conclude that SMRTα exhibits a significantly stronger interaction with TRs than does SMRTγ and that this preference for SMRTα over SMRTγ can be observed for both TRα1 and TRβ1.

The Relative Affinities of SMRTα and SMRTγ for TRs Are Further Influenced by the Nature of the DNA Response Element and by Heterodimer Formation—In addition to the prototypic DR4 element studied above, TRs also interact with and regulate transcription of target genes through divergent repeats separated by a 6-base spacer (referred to as DIV6), such as that found in the lysozyme F2 silencer element. Given that receptor binding to DNA can alter the relative affinity of different nuclear receptors for SMRT versus N-CoR, we determined whether the nature of the DNA response element could also affect the preference of TRs for SMRTα versus SMRTγ. We examined the affinity of TRβ1 and TRα1 for SMRTγ using a probe containing the DIV6 element. On this element, TRβ1 displayed a 48-fold greater affinity for the SMRTα S1 domain (a 4.4-fold greater affinity for the S1/S2 domains of SMRTα) than for the equivalent SMRTγ construct (Fig. 5, A and B; and Table I). TRα1 also exhibited an increased ability to discriminate between SMRTα and SMRTγ on the DIV6 element. TRα1 had a 2.1-fold greater affinity for the SMRTα S1 domain and an ~2.4-fold greater affinity for the S1/S2 domains of SMRTα than for SMRTγ (Fig. 5, C and D; and Table I). The ability of TRs arrayed on the DIV6 lysozyme F2 element to bind strongly to SMRT is consistent with studies implicating this element in TR-mediated repression in the absence of hormone (e.g. Ref. 62).

The above experiments were all performed using TR homodimers. We next examined the ability of heterodimers of TR and RXR to recruit SMRTα versus SMRTγ. Notably, heterodimers of TR and RXR have been implicated in transcriptional activation rather than repression (62). Consistent with these previous results, we observed that the SMRTγ(S1/S2) construct interacted with RXRα/β heterodimers with significantly less avidity than with TRα homodimers (Fig. 6A). In contrast, the SMRTα(S1/S2) construct not only interacted with TRα homodimers more strongly than did the SMRTγ form, but also interacted strongly with RXRα/β heterodimers (Fig. 6A). The ability of the SMRTα to construct to interact with heterodimers was somewhat more pronounced for RXRα/β/TRβ1 than for RXRα/β/TRα (Fig. 6B). These results suggest that the nature of the SMRT isoform has significant influence on the ability of the corepressor to be recruited by heterodimeric versus homodimeric versions of TRs.

Previous reports have suggested that N-CoR has a much greater affinity for TRs than does SMRT, leading to the suggestion that N-CoR serves as the preferred corepressor partner for this receptor in vivo (15). We re-examined this question in light of the enhanced interaction properties of the SMRTα isoform. We compared the avidities of the SMRTα(S1/S2) and N-CoR(N1/N2/N3) constructs for TRα1 and TRβ1. Under these conditions, N-CoR displayed only a 1.8–2.7-fold higher apparent affinity for TRs than did SMRTα, a much smaller difference than that seen upon comparison of N-CoR and SMRTγ (data not shown). We suggest that at least some of the prior reports demonstrating much greater discrepancies between N-CoR and SMRT may have employed SMRTγ and that SMRTα has the potential to function as an authentic corepressor partner for TRs, if at a slightly lower efficiency than N-CoR.

Unlike TRα1 and TRβ1, RARα Interacts Nearly Equally with Both the SMRTα and SMRTγ Constructs—TRs can interact with either the S1 or S2 domain of SMRT, in contrast, RARα preferentially interacts with the SMRTγ S2 domain and displays comparatively less binding to the S1 domain of this isoform (31, 41). Consistent with these reports, we observed a significantly weaker interaction of RARα homodimers with the S1 domain of SMRTγ compared with the S2 domain of SMRTγ (which is invariant in both SMRTα and SMRTγ) (Fig. 6, compare C and D; note the change in scale of the ordinate). Although the interaction of RARα homodimers with the isolated S1 domain of SMRTα was somewhat stronger than that with
The isolated S1 domain of SMRTα (Fig. 6C), this modest SMRT isoform specificity was abolished when the ability of RARα homodimers to interact with the combined S1 and S2 domains of SMRT was assayed (Fig. 6E and Table I). RARα heterodimers formed with RXRα also bound efficiently to the S1 and S2 domains of both SMRTα and SMRTγ (although at somewhat reduced levels compared with the corresponding RARα homodimers) (Table I). We conclude that, unlike TRs, there is relatively little effect of this SMRT mRNA splicing event on the ability of the corepressor to bind to RARα.

**Fig. 5. Interaction between SMRT receptor interaction domains and TRα1 or TRβ1 on a DIV6 DNA element.** Varying amounts of purified GST-SMRTα(S1) or GST-SMRTγ(S1) were added to binding reactions containing TRα1 (A) or TRβ1 (C) together with a radiolabeled DIV6 oligonucleotide probe. Alternatively, varying amounts of purified GST-SMRTα(S1/S2) or GST-SMRTγ(S1/S2) were added to binding reactions containing TRα1 (B) or TRβ1 (D) together with the radiolabeled DIV6 oligonucleotide probe. The receptor-DNA complexes supershifted by addition of the SMRT constructs were quantified relative to the amount of SMRT protein added to each binding reaction. Error bars indicate S.E. of two or more replicate experiments. **RID**, receptor interaction domain.
 Isoforms That Differ in Their Receptor Interaction Domains—Notably, the alternative spliced sequences in SMRTα and SMRTτ map within the S1 receptor interaction domain of the corepressor, only 5 amino acids downstream from a CoRNR box that serves as a key binding surface between corepressor and its nuclear receptor partners. The CoRNR box itself appears to form an α-helical domain that fits into a docking surface composed of helices 3, 5, and 6 of the receptor hormone-binding domain. Corepressor sequences immediately flanking the CoRNR box are known to play a role in defining the specificity of the corepressor for different nuclear receptors; and indeed, SMRTα and SMRTτ differ substantially in their affinity for TRs bound to DNA response elements. The most dramatic differences were seen in the relative affinity of the two SMRT isoforms for TRβ1 homodimers bound to the lysosome F2 element, with SMRTα displaying a 48-fold greater interaction compared with SMRTτ. SMRTα also exhibited a strong preference for TRα. The preferential ability of the SMRTα isoform to interact with TRs in vitro was also mani-

![Diagram](https://example.com/diagram.png)

**Fig. 6. Interaction between SMRT receptor interaction domains and RXRα/Tr heterodimers or RARα homodimers.** Varying amounts of purified GST-SMRTα(S1/S2) or GST-SMRTτ(S1/S2) were added to binding reactions containing TRα1 and RXRα (A) or TRβ1 and RXRα (B) and a DR4 oligonucleotide probe. Alternatively, varying amounts of purified GST-SMRTα(S1) or GST-SMRTτ(S1) (C), GST-SMRTτ(S2) (D), or GST-SMRTα(S1/S2) or GST-SMRTτ(S1/S2) (E) were added to binding reactions containing RARα and a radiolabeled DR5 oligonucleotide probe. The receptor-DNA complexes supershifted by addition of the SMRT constructs were quantified relative to the amount of SMRT protein added to each binding reaction. Error bars indicate S.E. of three replicate experiments. RID, receptor interaction domain.

could be visualized simultaneously in a single analysis. The identity of both PCR products as SMRTα and SMRTτ was confirmed by isolating and sequencing the amplified DNA band from the electrophoretogram. Levels of GAPDH mRNA were also determined for each tissue to normalize for mRNA recovery and cDNA synthesis efficiency. Notably, mRNAs corresponding to SMRTα and SMRTτ were detectably expressed in all tissues tested; however, the relative amounts of each isoform varied considerably among tissues (Fig. 9B). Heart, lung, and skeletal muscle all expressed higher levels of SMRTτ, whereas brain, kidney, and testis expressed higher levels of SMRTα. Comparable results were obtained using an RNase protection assay (data not shown). We conclude that the ratio of SMRTα to SMRTτ differs significantly in different cell types and, based on these and our other results, suggest that the ability of a given nuclear receptor to recruit SMRT and to repress transcription is likely to differ correspondingly in different tissues.

**DISCUSSION**

**The SMRT Corepressor Is Expressed as at Least Two Distinct Isoforms That Differ in Their Receptor Interaction Domains**—In this study, we have provided evidence for the existence of two distinct isoforms of the corepressor SMRT that differ by the presence or absence of an in-frame 47-amino acid insert in the most C-terminal receptor interaction domain (S1 domain). This conclusion (based on alignment of published SMRT sequences) was further confirmed by analysis of data from the EST data base. Additional characterization of genomic sequence data revealed the presence of consensus splice donor sequences flanking this 141-base insert, indicating that the two SMRT isoforms arise from alternative mRNA splicing through the use of alternative 5′-splice donor sites. We refer to the longer form as SMRTα and to the shorter form as SMRTτ. When introduced ectopically, these two different SMRT isoforms accumulated in cells to comparable extents and exhibited similar stabilities. Both isoforms displayed very similar nuclear localization when expressed as GFP fusions, observable as a punctate pattern within a more diffuse nucleoplasmic distribution; both forms of SMRT were excluded from nucleoli. This pattern is consistent with prior descriptions of the subcellular localization of both SMRT and N-CoR using GFP fusions, such as those employed here, or by immunofluorescent visualization of the endogenous corepressors (15).

**SMRTα and SMRTτ Differ in Their Interactions with Different Nuclear Receptors**—Notably, the alternative spliced sequences in SMRTα and SMRTτ map within the S1 receptor interaction domain of the corepressor, only 5 amino acids downstream from a CoRNR box that serves as a key binding surface between corepressor and its nuclear receptor partners. The CoRNR box itself appears to form an α-helical domain that fits into a docking surface composed of helices 3, 5, and 6 of the receptor hormone-binding domain. Corepressor sequences immediately flanking the CoRNR box are known to play a role in defining the specificity of the corepressor for different nuclear receptors; and indeed, SMRTα and SMRTτ differ substantially in their affinity for TRα bound to DNA response elements. The most dramatic differences were seen in the relative affinity of the two SMRT isoforms for TRβ1 homodimers bound to the lysosome F2 element, with SMRTα displaying a 48-fold greater interaction compared with SMRTτ. SMRTα also exhibited a strong preference for TRα. The preferential ability of the SMRTα isoform to interact with TRs in vitro was also mani-
fixed amount of purified GST-SMRT together with a radiolabeled DR4 oligonucleotide probe. Alternatively, a were added to binding reactions containing TR mone.

The S2 domain of SMRT is unaltered by the alternative splicing event that generates the SMRT isoforms; these receptors display preferential interaction with SMRT or N-CoR when assayed as receptor dimers bound to DNA in a supershift EMSA protocol (42). A similar phenomenon was observed here in our studies of the two different SMRT isoforms; this is not unexpected that both SMRT isoforms exhibit comparable interactions with RAR homodimers. The RXR molecule in an RAR/RXR heterodimer is thought to interact with the corepressor S1 domain, leaving the S2 domain available to interact with the RAR moiety; nonetheless RARα/ RXRα heterodimers also displayed nearly equal apparent affinities for SMRTα and SMRTγ in our assays.

The Nature of the DNA-binding Site Can Influence the Apparent Affinity of SMRTα and SMRTγ for the Nuclear Receptor Partner—Previous work has demonstrated that binding of a nuclear receptor to DNA can influence its ability to interact with corepressor (48). Although RARs and TRs exhibit nearly equal affinities for SMRT and N-CoR in the absence of a DNA response element (i.e., in a GST pull-down or two-hybrid assay), these receptors display preferential interaction with SMRT or N-CoR when assayed as receptor dimers bound to DNA in a supershift EMSA protocol (42). A similar phenomenon was observed here in our studies of the two different SMRT isoforms. When assayed in a GST pull-down assay in the absence of DNA, SMRTα displayed a somewhat lower ability compared with SMRTγ to bind to TRs (data not shown), yet SMRTα

**Fig. 7.** Disruption of SMRT/DR complexes with thyroid hormone. A fixed amount of purified GST-SMRTα(S1) or GST-SMRTγ(S1) were added to binding reactions containing TRβ1 (A) or TRα1 (C) together with a radiolabeled DR4 oligonucleotide probe. Alternatively, a fixed amount of purified GST-SMRTα(S1/S2) or GST-SMRTγ(S1/S2) were added to binding reactions containing TRβ1 (B) or TRα1 (D) together with the radiolabeled DR4 probe. Varying amounts of T3 were added to each reaction. The SMRT-TR-DNA complexes formed in the presence of the differing T3 concentrations were quantified. (SMRT-DR complexes observed in the absence of hormone were defined as 100%). Error bars indicate S.E. (n = 3).

**Fig. 8.** Dominant-negative inhibition of TRβ1-mediated repression. CV-1 cells were transfected with a TRβ1 expression vector, the lysozyme F2 element-luciferase reporter, and varying amounts of either a Myc-SMRTα(S1/S2) or Myc-SMRTγ(S1/S2) expression vector. Transfected cells were analyzed for luciferase activity. Luciferase activity for each sample is plotted versus the amount of SMRT expression vector. The unpressed level of luciferase activity (no TRβ1) is indicated. Error bars indicate S.E. of three replicate experiments. RID, receptor interaction domain.

**Fig. 9.** Analysis of expression of SMRTα and SMRTγ in various mouse tissues. A, cDNAs from heart (H), brain (B), kidney (K), spleen (S), liver (L), lung (Lu), testis (T), or skeletal muscle (M) were amplified with primers that span the SMRT exon; these are expected to produce a 442-bp product for SMRTα and a 301-bp product for SMRTγ. SMRT samples were amplified for 30 cycles. The cDNA from the same mouse tissues was also amplified for 20 cycles using a primer for GAPDH, which produces a 125-bp product. B, samples from duplicate reactions from two mice were analyzed using an Alpha Innotech FluorChem 8900 densitometer. Averages of the ratio of SMRTα to SMRTγ and S.E. for each are plotted (n > 3).
displayed a much higher apparent affinity for TRs when bound to DNA in the EMSA protocol. Consistent with the importance of the DNA-binding site in determining corepressor specificity, we also determined that the preference of both the TRα1 and TRβ1 homodimers for SMRTα over SMRTβ was highest for a DIV6 DNA element (i.e. as found in the lysozyme P2 promoter) and reproducibly less for the same receptors bound to the DR4 DNA element. This suggests that the nature of the DNA response element has the potential to influence the identity of the corepressor recruited to that element. This DNA modulation of corepressor recruitment may operate through an allosteric mechanism by which DNA binding alters the conformation or accessibility of the corepressor docking surface on the receptor, or it may arise from the alterations in the topology of the receptor dimer when arrayed on response elements bearing different half-site orientations and/or spacings.

The Identification of Two Isoforms of SMRT Supports a Role for SMRT, as Well as N-CoR, in TR-mediated Repression—The role of SMRT in TR-mediated repression has been of some debate. SMRT was first isolated by a yeast two-hybrid screen using a TR allele as “bait,” and both SMRT and N-CoR interact strongly with TRs in two-hybrid and GST pull-down procedures (29–31). It was also noted, however, that TRs preferentially interact with N-CoR over SMRT when bound to DNA response elements, leading to the suggestion that N-CoR, and not SMRT, is the primary effector of TR-mediated repression in cells (42). Paradoxically, both SMRT and N-CoR were found associated with TRs at physiologically relevant, Trp-modulated promoters during Xenopus laevis metamorphosis using chromosome immunoprecipitation (35). Differences in the isoform of SMRT employed in these analyses could account for at least some of these differences in the reported ability of SMRT to interact with TR. In our own experiments, SMRT interacted with TRs quite weakly compared with N-CoR, whereas SMRTα and N-CoR both interacted with TRs strongly. Notably, a recent publication confirmed that the alternative mRNA splicing event that gives rise to the SMRTα and SMRTβ isoforms is conserved in Xenopus as well as in mammals and that SMRTα predominates at least at some stages of Xenopus development (86). We suggest that SMRTα is the isoform most likely associated with TR in the previously published studies of Xenopus metamorphosis (35, 86).

Alternative mRNA Splicing Is a General Means of Diversifying Corepressor Expression—In common with SMRT, there are also alternatively spliced isoforms of N-CoR that differ in their interaction with nuclear receptors (87–89). Intriguingly, this alternative mRNA splicing of N-CoR operates through a very distinct mode from that described here for SMRT. There is no evidence in the EST data base for a form of N-CoR bearing a splice junction equivalent to that of SMRTβ (i.e. virtually all identified N-CoR clones contain the SMRTα-like 47-amino acid region within the N1 interaction domain). Instead, N-CoR is expressed as two isoforms (denoted N-CoRα and RIP13Δ1) that differ by the inclusion or exclusion of a third receptor interaction domain (N3) that is absent from all known SMRT isolates (32). The longer N-CoR isoform interacts strongly with TRs, primarily through N3 and N2 contacts, whereas the alternatively spliced RIP13Δ1 isoform lacks the N3 receptor interaction domain and interacts only weakly with TRs (32, 89). Conversely, RIP13Δ1 interacts more strongly than does N-CoR with the orphan nuclear receptors COUP-TF (chicken gyaelluminin upstream promoter transcription factor) and Rev-ErbA (90, 91). Thus, SMRT receptor specificity is regulated by altering the amino acid sequences flanking the S1 CoRNR box; N-CoR receptor specificity is regulated by insertion or removal of a supernumery third CoRNR box.

Although the focus of this study has been on splicing events that alter the receptor specificity of SMRT (and N-CoR), additional alternative splicing events that map outside the receptor interaction domains are also known for these two corepressors. TRAC-1 and SMRTβ isoforms that lack various portions of the N-terminal repression domains found in SMRTs and N-CoR have been reported; the physiological significance of these putative isoforms has not been fully established (31, 75, 76). Similarly, a RIP13α variant of N-CoR that has an altered RD3 has been reported (32). Inspection of the EST data base provides evidence for still additional variants of both N-CoR and SMRT, and a recent report using both bioinformatic and reverse transcription-PCR approaches has confirmed that a multiplicity of alternatively spliced SMRT mRNAs are expressed in vertebrates (86).

In conclusion, two distinct genetic loci in vertebrates (SMRT and N-CoR) give rise to two distinct corepressor paralogs. Alternative splicing of each of these loci gives rise to a still broader repertoire of corepressor proteins that differ in their receptor specificities and (potentially) transcriptional regulatory properties. These diverse corepressor variants are expressed at different levels in different cell types. We suggest that, by regulating the expression of corepressor isoforms in a tissue-dependent manner, the cellular response to different hormones can be modulated. This may contribute to the known tissue selectivity of hormone response and the tissue-selective effects of nuclear receptor-mediated repression during development.

REFERENCES
1. Sucov, H. M., and Evans, R. M. (1995) Mol. Neurobiol. 10, 169–184
2. Yen, P. M. (2001) Physiol. Rev. 81, 1097–1142
3. Tsai, M. J., and O’Malley, B. W. (1994) Annu. Rev. Biochem. 63, 451–486
4. Zhang, J., and Lazar, M. A. (2000) Annu. Rev. Physiol. 62, 439–466
5. Kastner, P., Mark, M., and Chambon, P. (1995) Cell 83, 859–869
6. Gronemeyer, H., and Miturski, R. (2001) Cell. Mol. Biol. Lett. 6, 3–52
7. Chambon, P. (1996) FASEB J. 10, 940–954
8. Mangedalff, D. J., Thummler, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1995) Cell 83, 835–839
9. Glass, C. K. (1996) J. Endocrinol. 150, 349–357
10. Mangedalff, D. J., and Evans, R. M. (1995) Cell 83, 841–850
11. Whitfield, G. K., Jurutka, P. W., Haussler, C. A., and Haussler, M. R. (1999) J. Cell. Biochem. 352, 110–122
12. Aranda, A., and Pascual, A. (2001) Biochem. Physiol. 18, 1269–1304
13. Zhang, X. K., Wills, K. N., Graupner, G., Tzukerman, M., Hermann, T., and Pfahl, M. (1991) New Biol. 3, 169–181
14. Glass, C. K., and Rosenfeld, M. G. (2000) Trends Endocrinol. Metab. 11, 470–480
15. Huber, B. K., Deslozenax, M., West, B., Cuna-Lima, S. T., Nguyen, H. T., Baxter, J. D., Ingraham, H. A., and Fletterick, R. J. (2003) Mol. Endocrinol. 17, 107–116
16. Toide, T., Downes, M., Chandratrata, R. A., Blumberg, B., and Umesono, K. (2001) Genes Dev. 15, 2111–2121
17. Sachs, I. M., Jones, P. L., Havis, E., Rouse, N., Demeneix, B. A., and Shi, Y. B. (2002) Mol. Cell. Biol. 22, 8527–8538
18. Matsushita, A., Misawa, H., Andoh, S., Natsume, H., Nishiyama, K., Sasaki, S., and Nakamura, H. (2000) J. Endocrinol. 167, 483–503
19. Yoh, S. M., and Privalsky, M. L. (2000) Mol. Cell. Biol. 20, 109–124
20. Yen, P. M. (2003) Trends Endocrinol. Metab. 14, 327–333
21. Yoh, S. M., Chatterjee, V. K., and Privalsky, M. L. (1997) Mol. Cell. Biol. 11, 470–480
22. Chatterjee, V. K. (2001) Biochem. Soc. Trans. 29, 227–231
23. Huber, B. R., Deslozenaux, M., West, B., Cuna-Lima, S. T., Nguyen, H. T., Baxter, J. D., Ingraham, H. A., and Fletterick, R. J. (2003) Mol. Endocrinol. 17, 107–116
24. Tomita, A., Buchholz, D. R., Obata, K., and Shi, Y. B. (2003) J. Biol. Chem. 278, 23786–23795
25. Segalla, S., Rinaldi, L., Klüstrup-Nielsen, C., Badaracco, G., Minucci, S., Pelicci, P. G., and Landsberger, N. (2003) Mol. Cell. Biol. 23, 8795–8808
26. Lin, R. J., Egan, D. A., and Evans, R. M. (1999) Trends Genet. 15, 179–184
27. Lin, R. J., Sterndorf, T., Tani, M., and Evans, R. M. (2001) Oncogene 20, 7204–7215
28. Hong, S. H., David, G., Wong, C. W., Dejean, A., and Privalsky, M. L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9028–9033
29. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K., and Rosenfeld, M. G. (1995) Nature 377, 397–404
30. Chen, J. D., and Evans, R. M. (1995) Nature 377, 454–457
31. Chen, S., and Privalsky, M. L. (1996) Mol. Endocrinol. 10, 813–825
32. Seol, W., Malon, M. J., Lee, Y. K., and Moore, D. D. (1996) Mol. Endocrinol. 10, 3–52
