Corepressor SMRT Functions as a Coactivator for Thyroid Hormone Receptor T3Rα from a Negative Hormone Response Element*

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Nuclear receptors are ligand-modulated transcription factors that transduce the presence of lipophilic ligands into changes in gene expression. Nuclear receptor activity is regulated by ligand-induced interactions with coactivator or corepressor molecules. From a positive hormone response element (pHRE) and in the absence of hormone, corepressors SMRT and N-CoR are bound to some nuclear receptors such as the thyroid hormone (T3Rs) and retinoic acid receptors and mediate inhibition of basal levels of transcription. Ligand binding results in dissociation of corepressors and association of coactivators, resulting in the reversal of inhibition and a net activation of transcription. However, the role of cofactors on the activity of nuclear receptors from negative HREs (nHREs) is poorly understood. Here we show that corepressor SMRT can act as a potent coactivator for T3Rα from a nHRE; N-CoR has a similar but significantly attenuated activity. Mutagenesis of residues in the hinge region of T3Rα that block binding of SMRT and N-CoR inhibits ligand-independent transcriptional activation by T3Rα from a nHRE. These mutations also abrogate SMRT-mediated increase in transcriptional activity by T3Rα at a nHRE without significantly affecting ligand-dependent activation at a pHRE. Partial protease digestion coupled to the mobility shift assay indicate differences in the conformation of T3Rα-SMRT complexes bound to a pHRE versus a nHRE. These results suggest that allosteric changes resulting from binding of T3Rα to different response elements, i.e. pHREs versus nHREs, dictate whether a cofactor will function as a coactivator or a corepressor. This, in turn, greatly expands the repertoire of mechanisms used in modulating transcription without the need for expression of new regulatory molecules.

Transcriptional regulation is fundamental to the normal functioning of the cell and is achieved through positively or negatively acting transcription factors (1, 2). Whereas in some cases the transcription factors that activate and repress gene expression are encoded by different genes, increasingly more transcription factors are now recognized to function both as an activator and a repressor depending on the nature of the response element that they interact with and the cellular context (1, 2). However, the details of how an activating transcription factor can become a repressor and vice versa is not well understood.

One of the factors that can serve as a transcriptional activator or repressor depending on the response element and cellular context is the thyroid hormone receptor (T3Rα) (3, 4), which belongs to the nuclear receptor superfamily of ligand modulated transcriptional factors (for reviews, see Refs. 5 and 6). Recent studies have begun to uncover the molecular details of this bimodal activity on a positive hormone response element (pHRE). In the absence of hormone, T3Rα associates with corepressor molecules, such as SMRT and N-CoR (7–9), which assemble a repressive complex that shuts down transcription (reviewed in Refs. 5 and 6). This is likely to be due the activity of histone deacetylases, which are recruited to this complex. In the presence of ligand, this corepressor complex dissociates and is replaced by a coactivator complex that then activates transcription at the promoter, which is probably because of the recruitment of histone acetyltransferases (5, 6).

To gain insight into the molecular details of transcriptional activation at a nHRE, we have compared the contribution of corepressors to the activities of T3Rα from pHREs and nHREs. We found that interaction of corepressor SMRT with T3Rα is essential for transcriptional activation at the RSV nHRE (10). Our results suggest that the interaction of SMRT with T3Rα on a pHRE is topologically different from SMRT-T3Rα interactions on a nHRE, which may account for these differential effects.

MATERIALS AND METHODS

Cell Culture, Transient Transfection, and Luciferase Assays—HeLa and CV-1 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum, antibiotics, and glutamine. The calcium phosphate coprecipitation method was used to transfec HeLa cells with 0.25 μg of reporter plasmid, 50–125 ng of expression vector, and pUC18 to a total of 1 μg of DNA/well in a 12-well dish. After 5–8 h of incubation with the precipitates, cells were shocked with 10% glycerol in phosphate-buffered saline, washed once with phosphate-

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1 The abbreviations used are: T3R, thyroid hormone receptor; pHRE, positive hormone response element; nHRE, negative hormone response element; SMRT, silencing mediator of retinoid and thyroid receptors; N-CoR, nuclear receptor corepressor; RSV, Rous sarcoma virus; LUC, luciferase; GST, glutathione S-transferase; MHC, myosin heavy chain; LTR, long terminal repeat; TSH, thyroid-stimulating hormone (thyrotropin).
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buffered saline, and then maintained in Dulbecco's modified Eagle's medium supplemented with 0.5% charcoal-treated fetal bovine serum in the presence or absence of T3 (10^{-7} M). For CV-1 cells, the same procedure was followed except that the glycerol shock was not performed. Luciferase (LUC) enzyme activities were determined as described previously (10).  

Plasmids—Reporter plasmids 2XT3RE-LUC (8) and RSV_{50}LUC (10) and the expression vectors for T3Ra (pSG5-c-ErbA, referred to here as pSG5-T3Ra) (15), SMRT (7), and N-CoR (9) have been described. For the generation of the mutant T3Ra constructs, single-stranded mutagenic primers were synthesized corresponding to the amino acids centered around the BstE II site in the hinge region of T3Ra. These primers and a primer corresponding to the SV40 polyadenylation sequence (residues 1109–1130 in pSG5) were used in PCR with pSG5-T3Ra as the template, and the amplified fragments were digested with BstE II and BamHI and exchanged with the corresponding fragment of pSG5-T3Ra. All mutants were confirmed by sequencing. For the generation of the GST-SMRT-(914–1495), pGEX-KG-TRAC2 (7) was cut with XhoI and NotI, filled in with Klenow polymerase, and religated. To generate GST-N-CoR-(1873–2453), pCEP-N-CoR (9) was cut with NotI and BgIII (partial), and the fragment was inserted into the BamHI and NotI sites of pGEX4TI (Promega).  

GST Pull-down Assay—The production and purification of the GST fusion proteins and the in vitro interactions between T3Rs and SMRT or N-CoR were examined by the GST pull-down assay as described previously (7).  

Mobility Shift Analysis—Preparation of recombinant T3Ra and the conditions for the mobility shift analyses were essentially as described previously with minor modifications (10). Briefly, recombinant T3Ra (25 and 105 ng for the MHC- and RSV-T3REs, respectively) was incubated with GST-SMRT-(914–1495) (100 ng) for 10 min at room temperature to allow heterodimer formation. The probes and rest of the DNA binding mix were then added (0.3 μg of poly[dI-dC] in 5 mM HEPES, pH 7.9, 25 mM KCl, 6.25 mM MgCl₂) and the reactions were continued at room temperature for 10 min. Increasing amounts of the indicated probease were then added, and the reactions were incubated at room temperature for an additional 5 or 10 min for chymotrypsin and carboxypeptidase Y, respectively, followed by immediate loading on a 5% nondenaturing polyacrylamide gel. After electrophoresis, gels were dried, and bands were visualized by PhosphorImager analysis (Amer sham Biosciences). To facilitate comparison, the GST-S3RE portion of the gels were exposed longer to give an intensity of bands approximately similar to those of the MHC-T3RE portion.  

T3 Binding Assay—{eq}_{1495}^{2453}T3 binding assays were performed on the in vitro translated wild-type and mutant receptors as described (16). The wild-type T3Ra and mutant receptors translated in vitro using the TNT expression system (Promega) were used in the T3 binding assay, and % values were calculated using the Prism computer program (GraphPad Software, Inc.).  

RESULTS  

To assess the potential contribution of SMRT and N-CoR to activation of transcription from a nHRE, we studied the activities of T3Ra from a well characterized nHRE found in the Rous sarcoma virus long terminal repeat (LTR), termed RSV-T3RE (10). We reasoned that if SMRT and/or N-CoR had a role in ligand-independent activation from the RSV-T3RE, mutations that block T3Ra-SMRT or T3Ra-N-CoR interactions should abrogate this activity. To that end, we generated point mutations within the hinge region of T3Ra based on previous mutational studies with T3Rβ and N-CoR that have established the residues important for this interaction (9). We targeted two residues that are conserved between T3Ra and T3Rβ within
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To determine whether the mutant receptors are altered in their ability to interact with SMRT and N-CoR, we performed glutathione S-transferase (GST) pull-down experiments. The receptor interaction domain (RID) of SMRT (8) was expressed as a GST fusion protein in Escherichia coli (GST-SMRT(914–1495)), purified, and used in the GST pull-down assay with cell-free translated, 35S-labeled wild-type T3Rα or its mutants in the presence or absence of T3. As shown in Fig. 2A, although there was no significant interaction with GST alone, wild-type T3Rα displayed significant binding to GST-SMRT(914–1495) in the absence of T3, which was lost in the presence of T3. In contrast, all of the mutants exhibited a significantly compromised ability to bind SMRT either in the presence or absence of T3. An essentially identical pattern of interaction of the T3Rα mutants with N-CoR was observed in a similar GST pull-down assay (Fig. 2B).

To assess whether the mutations that disrupt the interaction of SMRT with T3Rα have a role in ligand-independent activation from an nHRE, we performed transient transfection experiments. HeLa cells were first cotransfected with RSV180LUC and the expression vector encoding wild-type T3Rα or one of the mutants. As shown in Fig. 3A, whereas wild-type T3Rα efficiently activated RSV180LUC in the absence of T3 that was relieved in the presence of T3, none of the mutants significantly activated RSV180LUC in the presence or absence of T3.

The mutants having lost their ability to bind SMRT in vitro suggested that corepressor interactions may be involved in ligand-independent activation by T3Rα from an nHRE and prompted us to determine whether SMRT could serve as a coactivator for T3Rα in this context. To that end, the same transient transfection assay as described above was performed in the presence of an expression vector encoding SMRT. Coexpression of T3Rα and SMRT gave rise to ~5-fold greater activation of RSV180LUC expression when compared with expression of T3Rα alone. SMRT coexpression did not significantly affect ligand-induced relief of RSV180LUC activation as it was relieved back to basal levels or lower in the presence of T3. On the other hand, the mutant receptors did not show any significant activity in response to SMRT coexpression, even at higher levels of SMRT expression (Fig. 3A, and data not shown). This finding is consistent with the significantly diminished in vitro...
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Fig. 4. T3Ra-SMRT complexes assume different conformations on a pHRE compared with an nHRE. A, 32P-labeled positive or negative response elements, either from the myosin heavy chain (MHC-T3RE) or the RSV LTR (RSV-T3RE) promoter, respectively, were used in the mobility shift assay with partially purified recombinant T3Ra and purified GST-SMRT. The reactions were carried out as described under “Materials and Methods.” After complex formation and binding, increasing amounts of carboxypeptidase Y (CarbY) were added to the reactions, which were incubated for 10 min at room temperature and immediately loaded on the gel. The migration positions of the free probe (F), homodimeric T3Ra (T-T), and T3Ra-SMRT (T-T-S) complexes are indicated on the left. The partially digested bands of interest are indicated by arrowheads on the right. The RSV-T3RE panel was exposed longer to facilitate comparison. The weak band that appears above the homodimeric complex is due to an E. coli contaminant (data not shown). Data shown are from a representative experiment that was repeated three times. B, same as in A, but increasing amounts of chymotrypsin (Chy) were used as the protease, and the digestion was for 5 min. Data shown are from a representative experiment that was repeated four times.

Fig. 5. A model illustrating the differential role of SMRT on the activity of T3Ra from two different response elements: pHRE versus nHRE. Unliganded T3Ra dimers (shown as homodimers here, but they can also be heterodimers with retinoid X receptor) bind to the MHC-T3RE (an example of a pHRE). Subsequent SMRT binding then recruits other polypeptides, which then inhibit the transcriptional initiation complex (TIC). On the other hand, when the T3Ra dimers are bound at the RSV-T3RE (an example of an nHRE) they also bind SMRT, but the T3Ra-SMRT complex assumes a different conformation then that formed on the MHC-T3RE. Consequently, a different set of proteins are recruited to the complex, which then productively contact the transcriptional initiation complex and activate transcription.

Interactions between the T3Ra mutants and SMRT in the GST pull-down assay (Fig. 2A). These data suggest that SMRT serves as a coactivator for T3Ra from a nHRE.

In a similar series of experiments, we tested the possible effect of N-CoR coexpression on ligand-independent activity of T3Ra from the RSV180LUC reporter. N-CoR coexpression with T3Ra resulted in a modest increase of RSV180LUC expression that was less than 2-fold compared with the levels achieved in the presence of T3Ra alone (Fig. 3B). We did not find any more significant increase in stimulation of T3Ra activity from RSV180LUC at a wide range of lower or higher levels of N-CoR coexpression (data not shown). These results suggest that N-CoR has only a modest effect on the activity of T3Ra on a nHRE in vivo.

One possible explanation for how SMRT can act as a corepressor for T3Ra from a pHRE and a coactivator from a nHRE is that the complexes formed on these two response elements have different conformations, resulting in different contributions to the transcriptional initiation complex. To assess this possibility, we used a combination of the mobility shift and partial protease digestion assays with a pHRE (MHC-T3RE) compared with a nHRE (RSV-T3RE). When recombinant T3Ra expressed in E. coli was used in the mobility shift assay, a major shifted band corresponding to the T3Ra homodimers was formed with both probes (Fig. 4; also see Refs. 10, 15, and 18). When GST-SMRT-(914–1495) was included in the binding reaction, an additional band migrating more slowly was formed corresponding to T3Ra-SMRT complexes. GST-SMRT-(914–1495) alone did not bind either probe (data now shown). Both T3Ra homodimers and T3Ra-SMRT complexes were specific as shown by competition studies, and they contained T3Ra as indicated by supershift analysis (Refs. 10, 15, and 18 and data not shown).

To assess whether there are conformational differences between the heterodimers formed on the two T3REs, the mobility shift binding reactions were carried out first to allow heterodimeric complex formation on DNA; these were then subjected to partial proteolysis with increasing amounts of either carboxypeptidase Y (CarbY, Fig. 4A) or chymotrypsin (Chy, 4B). As shown in Fig. 4A, increasing amounts of carboxypeptidase Y quickly converted the T3Ra-SMRT complex into a form that migrated faster, running just above the migration point of the T3Ra-T3Ra homodimers on both response elements (compare lane 2 with lanes 5–7). However, the faster
migrating band formed on the MHC-T3RE at higher concentrations of carboxypeptidase Y was resistant to further digestion; this band is also wide, indicating the presence of more than one polypeptide complex bound to DNA. In contrast, the band formed upon carboxypeptidase Y digestion on the RSV-T3RE is a sharp, single-species band in which mobility is clearly increased in a stepwise fashion as the amount of enzyme is increased (Fig. 4A; compare lanes 5–7 for the two T3REs).

Similar results were obtained when the experiment was repeated with chymotrypsin. The addition of increasing amounts of chymotrypsin to the binding reaction resulted in an increase in the mobility of the T3Ra-SMRT complex for both probes (Fig. 4B). However there were differences in the pattern of bands generated and their rate of appearance. With increasing amounts of chymotrypsin, a band that migrated faster than the T3Ra homodimers was formed that was more resistant to digestion on the RSV-T3RE compared with the MHC-T3RE (Fig. 4B; compare lanes 5–7 between the two panels, lower arrow). In addition, this band was wide for the MHC-T3RE, as opposed to a tight, single-species band on the RSV-T3RE, indicating the presence of multiple polypeptides in the complex formed with the MHC-T3RE. Limited protease digestion of the homodimers alone under the same conditions gave rise to different, faster migrating bands compared with the experiments described above, indicating that the bands appearing upon proteolysis in the experiments presented in Fig. 4 are primarily the products of the T3Ra-SMRT complex (data not shown).

**DISCUSSION**

We have studied the possible role of the corepressors SMRT and N-CoR on transcriptional activation by T3Ra from a well characterized nT3RE in the RSV promoter (10). Our results indicate a direct role of the corepressor SMRT in transcriptional activation by T3Ra from a nHRE, whereas N-CoR had a significantly attenuated effect. At present, the basis for the differences between SMRT and N-CoR on regulating T3Ra action from a nHRE are not clear.

This paradoxical finding can be explained by the different conformations that SMRT assumes on a pHRE (exemplified by the MHC-T3RE in this study) compared with a nHRE (the RSV-T3RE). This is indicated by the fact that the sensitivity of the T3Ra-SMRT complex to proteases is different depending on the response element on which it is formed. This is expected to differentially affect the proteins recruited to the promoters, thus giving rise to diametrically opposite transcriptional outcomes.

This hypothesis is depicted schematically in Fig. 5. In the absence of hormone, when the T3Ra-SMRT complex is bound to the MHC-T3RE, it assumes a different conformation than when bound to the RSV-T3RE. This, in turn, results in the recruitment of a different collection of polypeptides to the complex, one that activates the transcriptional initiation complex, and the other that represses it.

Recent reports suggested that a putative splicing variant of N-CoR, N-CoR-I (19), which lacks the N-terminal repressor domain, can act as an activator for the mouse preprothyrotropin-releasing hormone gene (TRH) (13), which is known to be negatively regulated by thyroid hormone. N-CoR-I also inhibits the ligand-independent repression by T3Ra at pHREs, presumably through competition with the endogenous N-CoR and SMRT that are involved in this process (27). In addition, N-CoR and SMRT are involved in basal activation of the promoters that are negatively regulated by thyroid hormone (11, 12). This finding was suggested to be due to protein-protein interactions involving a non-DNA bound T3Ra, allowing the association of coactivators with the DNA-bound transcription factor cAMP-response element-binding protein (CREB) at the TSH promoter in the absence of hormone; the coactivator is removed from the DNA by a hormone-activated T3Ra, resulting in repression (12).

The findings we have presented here suggest that the effect of SMRT on T3Ra action on a nHRE is direct and that DNA binding by T3Ra is required for these activities. Indeed, whereas the nHREs on the TSH promoter are poorly defined and therefore the negative effects of T3Ra action in the presence of hormone are likely to be mediated by protein-protein interactions, as suggested previously (12), the RSV-T3RE that is used in our studies is well characterized and is essential for this activity (7). Furthermore, by using an artificial construct in which the RSV-T3RE is fused to a minimal promoter driving expression of the LUC gene, similar affects of SMRT on potentiation of T3Ra action is observed.3 Taken together, these findings suggest that there may be two distinct mechanisms by which a corepressor may mediate transcriptional activation by T3Ra at a negatively regulated promoter, dictated by the nature of the cis elements present. One mechanism involves the removal of the corepressors from DNA by the receptor, and the other involves recruitment of the corepressors to the DNA and assembly of a coactivating complex. Future work will be needed to define the components of these complexes and how they activate transcription.

It has recently been shown that coactivators of the nuclear receptor superfamily to which T3Ra belongs either recruit or are themselves histone acetyltransferases; conversely, the corepressors recruit histone deacetylases (for reviews, see Refs. 5 and 6). It has therefore been suggested that changes in histone acetylation and subsequent effects on chromatin structure may be the mechanism through which cofactors mediate regulation of transcription (for reviews, see Refs. 20–23). It will now be important to determine what the differences are in the complexes that are formed by T3Ra and SMRT on the pHRE versus the nHRE in the absence of hormone and whether they contain histone acetyltransferases or histone deacetylases.

To our knowledge, this is the first report in which a corepressor is shown to function as a coactivator that is dependent on DNA binding by its transcription factor. The role of the allosteric effects of DNA on the transcription factors that bind to it has been recognized for many years (for a review, see Ref. 24). The findings we report here extend the importance of these allosteric effects by demonstrating that even the cofactors that bind to the transcription factor will be affected, such that they can function in a diametrically opposite fashion when tethered to transcription factors bound to different response elements. The fact that a cofactor previously identified as a corepressor can also function as a coactivator in another context through these allosteric effects greatly increases the repertoire of responses that the cell can mount to various signals without additional gene activation or protein synthesis.

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