A group of transcriptional cofactors for nuclear hormone receptors, referred to as corepressors (CoRs) and coactivators (CoAs), has been shown to induce transcriptional silencing and hormone-induced activation, respectively, of genes that contain positive hormone response elements. Transcriptional silencing by CoRs involves the recruitment of histone deacetylases (HDACs), whereas ligand-dependent activation is associated with the recruitment of CoAs, which possess or recruit histone acetyltransferases (HATs). In a reciprocal manner, negatively regulated genes are stimulated by nuclear receptors in the absence of ligand and are repressed in response to ligand binding to receptors. We show here that negative regulation of the thyroid-stimulating hormone α (TSHα) promoter by the thyroid hormone receptor (TR) involves a novel mechanism in which the recruitment of CoRs by TR is associated with transcriptional stimulation and histone acetylation. Expression of excess HDAC reverses the stimulation mediated by the TR-CoR complex, consistent with a pivotal role for acetylation in this event. Addition of the ligand, 3,5,3'-triiodothyronine (T3), induces transcriptional repression of the TSHα promoter and is associated with the loss of histone acetylation. T3-dependent repression is blocked by phosphorylation of cAMP response element binding protein, or by inhibition of HDAC, indicating that receptor action is subverted by maneuvers that stimulate histone acetylation of the target gene. We propose that negative regulation of a subset of genes by TR involves the active exchange of CoRs and CoAs with intrinsic promoter regulatory elements that normally strongly induce histone acetylation and transcriptional activation.

Nuclear receptors are transcription factors that regulate the expression of a wide array of target genes. An intriguing aspect of their action is that, within the same cell, some target genes are stimulated whereas others are repressed. This phenomenon is most readily explained by differences inherent in the regulatory elements of these target genes.

In the case of positively regulated genes, the mechanism of transcriptional control is relatively well understood. These genes contain hormone response elements that bind nuclear receptors, usually as homo- or heterodimers. For receptors such as the thyroid hormone receptors (TRs) or the retinoic acid receptors, receptor binding in the absence of ligand is associated with transcriptional repression. This property of nuclear receptors involves the recruitment of corepressors (CoRs) like silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor corepressor (NCoR). These CoRs in turn assemble a repression complex that includes Sin3 and histone deacetylases (HDACs), among other proteins (6–8). Transcriptional silencing by this receptor-assembled complex is thought to involve chromatin remodeling caused by histone deacetylation.

The binding of ligand to these nuclear receptors reverses silencing and induces transcriptional activation. Ligand binding causes conformational changes in the receptor that dissociate the CoRs and allows the recruitment of an array of coactivators (CoAs). These CoAs include steroid receptor coactivator 1 (SRC1), transcriptional intermediary factor 2 (TIF2), glucocorticoid receptor interacting protein 1, amplified in breast cancer 1, receptor-associated coactivator 3, p300/CBP-cointegrator-associated protein, nuclear receptor coactivator (ACTR), thyroid receptor activator molecule 1, p300/CBP associated factor, and cAMP response element binding protein (CREB) binding protein. The CoAs possess intrinsic histone acetyltransferase (HAT) activity and recruit additional HAT enzymes that alter chromatin structure and modulate gene transcription. Thus, the control of positively regulated genes is dictated by receptor binding to DNA regulatory elements and the recruitment of CoRs in the absence of ligand; in the presence of ligand, CoRs are dissociated and CoAs bind to stimulate gene transcription.

In contrast to positively regulated genes, the mechanism by which nuclear receptors control the transcription of negatively regulated genes is less well understood. Proposed mechanisms include competition of nuclear receptors with other transcription factor-binding sites, receptor binding to so-called negative regulatory elements, direct interactions of nuclear receptors with transcription factors like AP1 or NFκB, and competition for transcriptional cofactors like CBP.

In the case of the thyroid hormone receptor, negative regulation of gene expression in response to its ligand, 3,5,3'-triiodothyronine (T3), is an essential part of its physiological action. For example, the hypothalamic thyrotropin-releasing hormone (TSHα) gene contains a thyroid-stimulating hormone α response element (TSHα-αRE), which is essential for the negative regulation of this gene in response to T3. This mechanism is mediated by the thyroid hormone receptor (TRα), which undergoes a conformational change upon T3 binding, resulting in its dimerization with TRβ and subsequent recruitment of corepressors like SMRT and NCoR. This corepressor complex then recruits histone deacetylases (HDACs) to the TSHα-αRE, leading to histone deacetylation and repression of gene expression.

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Transcriptional Repression by Thyroid Hormone Receptor

hormone (TRH) and the pituitary thyroid-stimulating hormone (TSH) α- and β-subunit genes are inhibited by T3 as a physiologic feedback mechanism for modulating circulating thyroid hormone levels. These genes are stimulated in the absence of T3, and the addition of hormone induces rapid and strong transcriptional repression (25). Previous studies have localized hormone-responsive regions to the proximal promoter regions of these negatively regulated genes (26–29). However, the nature of the response elements and the mechanism of ligand-dependent repression remain poorly defined. Recently, we reported (30) the unexpected finding that CoRs are involved in the basal activation of the TSH and TRH genes by unliganded TRs. TR mutations that prevent its ability to interact with CoRss eliminated stimulation of these promoters in the absence of ligand, and overexpression of NCoR or SMRT enhanced rather than suppressed the basal activity of these genes.

In this report, we describe a potential mechanism for transcriptional control of genes that are negatively regulated by TR. In this model, TR retains the fundamental features of interactions with CoRs and CoAs, but the functional consequences of these interactions are reversed in comparison to positively regulated genes. We demonstrate that the negatively regulated TSHα gene is unusually sensitive to the state of histone acetylation and appears to be controlled by partitioning of HDACs and HATs between TR and other transcription factors that bind to the TSHα promoter. We propose that a subset of negatively regulated genes are controlled by two-step mechanism as follows: 1) the unliganded TR recruits CoRs and withdraws HDAC from the basal promoter to cause activation; 2) T3 binding to the TR dissociates the CoRs/HDACs and recruits CoAs to restrict access of HATs to CREB and other components of the basal promoter, thereby causing ligand-dependent repression. The specificity of this effect is encoded by the promoter elements and transcription factors that bind to negatively regulated genes, as opposite effects are seen with positively regulated promoters studied under identical conditions.

MATERIALS AND METHODS

Plasmid Constructions—The mutant kTRβ1 cDNAs were prepared by oligonucleotide-directed mutagenesis, subcloned into pCMX, and verified by DNA sequencing as described previously (27, 31). The numbering of the amino acid residues of TRβ is based on a consensus nomenclature (32). The pCMX-NCoR expression vector was provided by M. G. Rosenfeld (University of California, San Diego, CA) (9), and pCMX-ACTR expression vector was provided by R. M. Evans (Salk Institute, San Diego, CA) (15). The HD1 (HDAC-1) cDNA was provided by C. A. Hassig (Harvard University, Cambridge, MA) (33); SRC-1 cDNA was provided by B. W. O’Malley (Baylor College of Medicine, Houston, TX) (9); F-SRC1 and TRAM-1 cDNAs were provided by A. Takeshita and W. W. Chin (Brigham and Womens Hospital, Boston) (16, 34); and CBP was provided by R. H. Goodman (Vollum Institute, Portland, OR) (35). The CREB mutant (S119A) was provided by J. M. Greenberg (National Institutes of Health, Bethesda) (41). TSHα reporter plasmid, Gal4-tk-Luc, was provided by P. M. Yen (National Institute of Health, Bethesda) (41). TSHα-Luc and the 5′ deletion constructs created from it have been described (27). The Gal4 reporter plasmid, UAS-tk-Luc, contains two copies of the Gal4 recognition sequence (UAS) upstream of tk109, and UAS-E1BATA-Luc (42) contains five copies of UAS upstream of E1BATA in pA3-Luc.

RESULTS

Corepressors Mediate Basal Activation and Coactivators Mediate T3-induced Suppression of the TSHα Gene—Previous studies have implicated CoRs in the basal activation of negatively regulated genes, such as the TSHα, TSHβ, and TRH genes (30). Specifically, TR mutations (e.g. P214R) that impair interactions with CoRs reduce basal stimulation by unliganded TR but do not affect T3-dependent repression. In view of the apparently paradoxical effect of the CoRs on basal stimulation by the unliganded TR, we considered the possibility that CoAs might be involved in the T3-dependent repression of negatively regulated genes. For this purpose, the E457A mutant of TRβ is informative. It binds T3 with near normal affinity, but it selectively lacks binding to CoAs, and it retains interactions with CoRs (47). In parallel, we examined the effects of another CoR mutant (TRβ AHT), which contains three amino acid substitutions in the CoR interaction domain of TR but retains the ability to bind CoAs (3). The AHT mutation exhibits more complete abrogation of CoR binding than the P214R mutation in TRβ (data not shown).

By using the positively regulated promoter, TREp-tk-Luc, wild-type TR and the E457A mutant exhibited strong transcriptional silencing in the absence of T3, whereas the silencing expressed as of AHT CoR mutant was abolished (Fig. 1A). Wild-type TR and the AHT mutant induced strong transcriptional suppression in the presence of T3 but the T3-induced stimulation by the E457A mutant was reduced. These controls confirm the expected properties of these TR mutants in the context of positively regulated genes. By using the negatively regulated promoter, TSHα-Luc, wild-type TR and the E457A mutant...
exhibited a comparable degree of basal activation in the absence of T3. However, basal activation was abolished using the AHT CoR mutant (Fig. 1B), confirming our previous observation using the P214R CoR mutant (30). Of note, T3-induced repression (below the basal level) was impaired using the E457A TR mutant but not with the AHT mutant. These results are consistent with a role for CoRs in basal activation, and CoAs in T3-induced repression, of negatively regulated genes.

Histone Deacetylase Plays a Pivotal Role in TR Regulation of the TSHα Gene—One potential mechanism for basal activation of the TSHα promoter by unliganded TR could involve partitioning of NCoR, SMRT, or other components of the repressor complex. Previously, we attempted to reverse TR-dependent activation of the TSHα promoter by overexpression of NCoR or SMRT (30). Unexpectedly, CoRs enhanced, rather than attenuated, basal activation by unliganded TR. This finding indicates that basal activation of these negatively regulated promoters is not caused by titration of limited quantities of CoRs. Recently, it was shown that the CoR/Sin3 complex also recruits histone deacetylases (HDACs) (6–8), which are important for inducing alterations in histone structure and perhaps other transcription factors. We therefore hypothesized that the stimulatory effect of excess CoRs or Sin3 (data not shown) might involve the recruitment of HDAC by the TR-CoR complex.

To test this idea, the TSHα promoter was transfected with TR along with various combinations of CoRs and HDAC1. As found previously, cotransfection of NCoR enhanced basal activation by unliganded TR in comparison to the expression vector alone (Fig. 2A). In contrast to NCoR, expression of HDAC1 reduced TR-mediated basal stimulation. Since HDAC1 did not alter basal expression in the absence of TR, this effect is TR-specific. Strikingly, coexpression of HDAC1 eliminated the NCoR-mediated enhancement of basal activation by unliganded TR. A similar effect of HDAC1 was seen in the presence of SMRT and Sin3 (data not shown). Of note, T3-dependent repression was maintained in the presence of NCoR or HDAC1. These results suggest that the TR-CoR complex may deleteriously affect HDAC1 in the absence of T3, leading to stimulation of the TSHα promoter. In the presence of T3, when CoR complex dissociates from TR, overexpression of these factors does not appear to affect T3-mediated suppression of the TSHα promoter.

Coactivators Enhance the T3-induced Suppression of the TSHα Gene—In view of the finding that the E457A mutation in TRβ (which selectively alters TR binding to CoAs) appears to abrogate T3-dependent repression, we tested the effect of coexpression of CoAs on negative regulation by TR. Expression of these CoAs (SRC1, FSRC1, glucocorticoid receptor interacting protein 1, TRAM1, and ACTR) enhanced T3-induced stimulation of positively regulated genes (Fig. 2B). Each of the CoAs tested enhanced the degree of T3-induced gene suppression by 2–3-fold. However, they had little or no effect on basal activation by unliganded TR. Thus, a role for CoAs in T3-mediated repression of the TSHα promoter is supported by mutations in TR that eliminate CoA binding and by overexpression of CoAs.

Evidence That TR Is Not Tightly Bound to the Promoters of Negatively Regulated Genes—The TSHα promoter contains weak binding sites for TR, when assessed by gel mobility shift assays (27, 48). However, site-directed mutagenesis of these putative binding sites fails to alter T3-dependent repression, casting doubt on their functional importance (48). To explore this issue further, we used two novel experimental approaches
to detect whether TR is bound to the promoter of the TSHα gene. In the first approach, full-length wild-type TRβ was fused to the transcriptional activation domain of VP16 to generate a constitutively active receptor, even in the absence of T3 (Fig. 4A). As controls, several positively regulated genes containing known thyroid hormone response elements (TREs), such as DR4, F2, and TREp, were tested for responsiveness to this constitutively active version of TRβ. The VP16-TR construct strongly stimulated each of these reporter genes. VP16 alone had no effect, indicating a specific requirement for the TR portion of the fusion gene. Addition of T3 stimulated transcription of these promoters further, probably by recruiting additional CoAs to the TR-LBD portion of this chimeric protein (data not shown). In contrast, UAS-tk-Luc, which contains Gal4-binding sites (UAS) and lacks a TRE, was not activated by VP16-TR. In the case of TSHα promoter, no activation by the constitutively active VP16-TR construct was observed, consistent with the idea that it does not contain high affinity TR-binding sites.

In an independent approach, a modified mammalian two-hybrid assay was used to detect whether TR is bound to DNA (Fig. 4B). For example, the TR interaction domains of either NCoR (residues 1552–2453) or retinoid X receptor (residues 199–462) were used to seek TR bound to various promoters. When the native TR was cotransfected with the positively regulated reporters, VP16-NCoR induced transcriptional activation in the absence of T3, reflecting the TR-NCoR interaction (Fig. 4B). Similarly, VP16-NCoR stimulated the activity of Gal4-TR, using the UAS-tk-Luc reporter gene. In contrast, VP16-NCoR did not stimulate the activity of the TSHα promoter in the presence of native TR. Similar results were seen when VP16-retinoid X receptor was used as the interacting protein (data not shown). These results indicate that TR is not bound to the TSHα gene in a manner that is detected either by direct receptor binding to the promoter or by TR interactions with other binding proteins.

**CREB Is Involved in Negative Regulation of the TSHα Promoter by T3**—There are two consensus CREs between −156 and −100 bp of TSHα promoter (49). Since there is a marked difference in the degree of negative regulation by TR after deletion of the CREs, we tested the possibility that the CRE-binding protein, CREB, might be a target of TR action. A dominant negative mutant of CREB (S119A), which is not capable of phosphorylation (36), was used to assess further the functional significance of CREB for TR regulation of the TSHα gene. Expression of the CREB S119A mutant completely blocked basal activation by TR and NCoR. T3-dependent repression was reduced from 12- to 1.8-fold in the presence of the CREB S119A mutant (Fig. 5A). This finding suggests that occupancy of the CREs by the inactive form of CREB precludes effective negative regulation of the promoter by TR.

CBP is a target protein of both phosphorylated CREB (35)
and nuclear hormone receptors (50, 51). We therefore examined the effects of CBP and catalytic subunit of protein kinase A (PKA) on negative regulation by T3. Coexpression of CBP attenuated the negative regulation of $2^{156}$-Luc by T3, decreasing repression from 12- to 3-fold. In contrast, CBP enhances T3-stimulated activity of positively regulated genes (data not shown) (50). Interestingly, activation of the TSH$\alpha$ promoter by PKA completely eliminated the T3-dependent suppression of the promoter by T3. This effect of PKA is selective for the negatively regulated gene as T3-induced stimulation of positively regulated genes was preserved, or enhanced, in the presence of PKA (data not shown). In contrast, using the $2^{100}$-Luc construct, which does not contain CREs, there was little or no effect of the CREB S119A mutant, CBP, or PKA (data not shown). These results support a role for activated CREB in TR regulation of the TSH$\alpha$ gene.

The possibility that CREB is involved in negative regulation by TR was also examined using Gal4-CREB and the UAS-E1BTATA-Luc reporter, which contains five Gal4-binding sites upstream of the minimal adenovirus E1B promoter (Fig. 5B). TR and T3 exhibit little effect on this promoter in the presence of the control, Gal4-DBD. The addition of Gal4-CREB induces basal activity and confers modest repression (3.9-fold) in the presence of TR and T3. In contrast, there was no effect of T3 using the Gal4-CREB S119A mutant. Similar to the native TSH$\alpha$ promoter, cotransfection of CBP or PKA with Gal4-CREB markedly stimulated its activity. In the context of Gal4-CREB, PKA or coexpression of CBP eliminated T3-induced repression by native TR. These results indicate that CREB is a functional target of TR and that it is sufficient to confer T3-dependent repression. In addition, stimulation of CREB activity by PKA, which is expected to generate a CREB-CBP complex, inhibits T3-mediated repression.

Negative Regulation of the TSH$\alpha$ Gene and CREB Are Sensitive to a Histone Deacetylase Inhibitor, Trichostatin A—In light of the data that coexpression of HDAC1 reverses TR-NCoR-mediated basal activation of the TSH$\alpha$ promoter and that CREB-CBP might be target for T3-dependent repression, we used a histone deacetylase inhibitor, trichostatin A (TSA) (52), to assess further the role of histone deacetylase in TSH$\alpha$ gene regulation. The effects of TSA were initially tested using the Gal4-CREB constructs (Fig. 6A). TSA markedly stimulated the activity of Gal4-CREB in comparison to its effects on transcription mediated by Gal4-DBD or the Gal4-CREB S119A mutant, suggesting that inhibition of HDAC enhances the ability of CREB-CBP to activate transcription. The sensitivity to TSA was also examined using various deletion mutants of the TSH$\alpha$ promoter (Fig. 6B). The $-156\alpha$-Luc construct, which...
contains the CREs, was stimulated about 200-fold by treatment with TSA. The shorter TSHα promoter constructs (−100α–Luc, −30α–Luc), which lack the CREs, were also stimulated 20–30-fold by TSA, but the degree enhancement was greatly reduced. Cotransfection of the CREB S119A mutant with −156α–Luc markedly decreased its induction by TSA, likely because of a dominant negative effect to block the binding of CREB. These results indicate that inhibition of HDAC activity enhances the activity of CREB-mediated transcription, suggesting a dynamic equilibrium between the processes of histone acetylation and deacetylation.

We next examined whether inhibition of HDAC by TSA would alter the ability of T3 to repress transcription of the TSHα gene. As shown in Fig. 6C, treatment with TSA markedly increased the basal activity of the TSHα promoter in the presence of unliganded TR (note the different scale on the y axis). TSA also abolished the negative regulation by T3, suggesting that deacetylation is an essential feature of negative regulation of the TSHα gene by T3.

Effects of Unliganded TR and T3 on Histone AcetylationAssociated with the TSHα Gene—The transfection assays shown above suggest that unliganded TR can compete for HDACs that otherwise repress TSHα promoter activity. Chromatin immunoprecipitation (CHIP) assays (45, 46) were performed to examine the direct effects of TR on histone acetylation associated with TSHα promoter. In this assay, it is possible to assess the acetylation status of specific genes under different treatment conditions. The TSHα (−846/+44) plasmid was transfected into TSA201 cells along with TR in the absence or presence of T3. Extracts from the transfections were immuno-precipitated with anti-acetylated histone H4 antibody, and TSHα promoter sequences that communoprecipitate were detected by PCR amplification. The total amount of transfected plasmid in the cell was also determined by PCR using the aliquots of extracts before immunoprecipitation. The immunoprecipitated results were corrected for recovery using the total PCR products for each treatment (Fig. 7). As a control, transfection of PKA and CBP increased acetylated histone H4 antibody, and TSHα promoter sequences that communoprecipitate were detected by PCR amplification of promoter sequences. The 32P-labeled PCR product was separated by polyacrylamide gel electrophoresis and quantitated using an image analyzer. The results shown in the top panel are expressed as the relative amount of PCR products communoprecipitated with acetylated H4 corrected by the amount of total PCR products.

NCoR enhanced H4 acetylation by unliganded TR. These effects were reversed by the addition of T3. Similar results were obtained using anti-histone H3 antibody (data not shown). Thus, the state of acetylated histone H3 and H4 associated with the TSHα promoter reflects the transcriptional effects seen in transient expression assays.
In this report, we make the following observations about T3-dependent regulation of the TSHα gene by TR. 1) The property of negative regulation is promoter-dependent as other positively regulated promoters are stimulated by T3 under the same experimental conditions. 2) Direct interaction of TR with the TSHα promoter is not necessary for its control, implicating protein-protein interactions as a primary mechanism of its regulation. 3) Ligand-independent stimulation of the promoter involves CoRs and is associated with increased histone acetylation. 4) Ligand-dependent inhibition of the gene involves CoAs and is associated with decreased histone acetylation. 5) The CRE is a critical regulatory element that contributes to the reversed pattern of regulation by CoRs and CoAs, and its properties can be replicated using Gal4-CREB and a heterologous promoter. 6) In addition to the CRE, the basal TSHα promoter is intrinsically subject to negative regulation. 7) Shifting the balance of histone acetylation, by inhibition of histone deacetylation with TSA, or by treatment with PKA, prevents T3-dependent repression. Taken together, these data support a mechanism in which the TSHα gene is controlled by TR-mediated partitioning of HATs and HDACs.

In previous studies, we demonstrated that proximal TSHα promoter is sufficient for negative regulation by T3 (27, 48). Although in vitro translated or recombinant TR binds to several sites within this region, these binding sites are not high affinity, at least in comparison to positive TREs (48). In addition, mutagenesis of the low affinity TR-binding sites did not alter negative regulation by T3. These experiments raised the possibility that negative regulation of this promoter by TR might be mediated by TR interactions with promoter-bound proteins, as opposed to TR binding directly to DNA sequences. This concept is supported by a recent finding that the DNA binding domain of the TR is not required for negative regulation of this promoter (30). When the ligand binding domain of TR was linked to Gal4, the Gal4-TR LBD fusion protein was sufficient to mimic negative regulation by the native TR. Specifically, Gal4-TR induced activation in the absence of ligand, and the addition of T3 resulted in ligand-dependent repression. In this report, we provide additional evidence for the absence of direct binding of the TR to the TSHα promoter by using one- and two-hybrid assays to identify potential TR-binding sites. No TR-binding sites were detected using either VP16-TR in the one-hybrid assay or using VP16-NCOR and TR in the two-hybrid assay. Taken together, these observations provide several independent lines of evidence to support the idea that negative regulation of this promoter does not involve direct interactions with DNA. These findings therefore share certain mechanistic elements in common with glucocorticoid repression of genes that are regulated by AP-1 or NFκB (24).

The absence of direct DNA binding of the TR raises interesting concerns regarding how the receptor acts to inhibit transcription and how specificity for certain promoters is achieved. Clearly, the effects of unliganded and T3-bound TR must be restricted to certain genes, even if the receptor is not tightly bound to DNA. Parallel experiments with positively regulated promoters demonstrate that TR affects them in a manner that is diametrically opposed to the effects on the TSHα promoter. Thus, the nature of the promoter and the transcription factors that bind to it are sufficient to encode positive or negative regulation by TR. What are the key regulatory elements for negative regulation of the TSHα promoter? Unfortunately, the apparent importance of protein-protein interactions in this process makes it difficult to localize these sequences by traditional mutagenesis (48). However, we were able to take advantage of the newly discovered feature that unliganded TR and NCoR enhance basal activation of the TSHα promoter to investigate sequences involved in this process, as well as T3-mediated repression. These experiments confirm previous findings that both the CREs (−156 to −100) and the proximal promoter elements (−30 to +44) play a role in the action of TR (27, 48). For example, deletion of the CREs in the TSHα promoter greatly reduces stimulation by unliganded TR and T3-induced repression. However, it is notable that features of negative regulation remain, even after deletion of the CREs, confirming previous studies in other cell lines (27). This feature of negative regulation by TR appears to be intrinsic to the proximal promoter, since T3-dependent repression is retained when it is linked to other enhancers (data not shown) (48). It seems likely that this region contains transcription factors that recruit HDAC. This idea is supported by the activation of this region of the promoter by the HDAC1 inhibitor, TSA. Several repressors have been reported to recruit HDAC to the promoter, including YY1 (53), Ume6 (54), retinoblastoma protein (45, 55, 56), and PLZF (57), among others. At present, it is not known whether these factors interact with the TSHα promoter. Recently, MeCP2, which binds to methylated CpG in genomic chromatin (58), was reported to bind to HDAC (59). The minimal TSHα gene contains several CpG sequences, and it is possible that MeCP2, or general transcription factors that bind to the TSHα promoter, are involved in the recruitment of HDAC. Thus, the configuration of regulatory elements, perhaps relative to positions of nucleosomes, and the nature of the transcription factors that are recruited to the promoter may have an important influence on whether TR acts in a positive or negative manner.

Several lines of evidence point to a critical role for transcription factor CREB in negative regulation of the TSHα promoter by TR. In addition to mutations of the CRE, which reveal a partial loss of TR regulation, maneuvers that alter the function of CREB have profound effects on negative regulation. The dominant negative CREB mutant, S119A, which cannot be activated by PKA, blocked stimulation by TR and repression by T3. Cotransfection of CBP, a coactivator of CREB, attenuated negative regulation, and maximal activation of CREB by coexpression of PKA abolished T3-mediated repression. These findings suggest that part of the mechanism of T3-mediated repression involves reversal of the activation state of CREB. Several aspects of negative regulation can be recapitulated using Gal4-CREB, in the context of a heterologous promoter. The activity of Gal4-CREB is stimulated by unliganded TR and NCoR, and these effects are reversed by T3. It is clear that, in the context of a native gene, CREB is not sufficient to dictate negative regulation by TR, as it binds to numerous other cellular genes that are not negatively regulated. However, these experiments underscore its potential importance as a target of TR action, perhaps because both TR and CREB interact with additional proteins involved in the process of protein acetylation. These results echo the findings of Kamei et al. (50) who reported that CBP is a limiting factor for nuclear receptor inhibition of AP-1. In this respect, it is notable that many genes that are negatively regulated by TR contain AP-1-binding sites. These include the collagenase (60–62), prolactin (63), c-fos (61), and TSHβ (64) promoters. The TRH promoter appears to contain a composite site for both CREB and AP-1 (65). In the GHP-1 pit-1 gene, the CRE has been reported to be a target for negative regulation by TR through protein-protein interactions (66). Thus, genes that contain AP-1/CRE sites may be poised for negative regulation by nuclear receptors, perhaps reflecting competition for CoAs like CBP.

Although additional studies will be necessary to define exactly how TR interacts with transcription factors that bind to CREB and to the basal region of the TSHα gene, it is apparent...
that TR interactions with CoRs and CoAs are involved in this process. Previously, we reported (30) the paradoxical observation that CoRs are involved in basal stimulation of negatively regulated genes by unliganded TR. These findings were supported by the fact that a TR mutation (P214R) that selectively disrupts interactions with CoRs prevented basal stimulation by TR. This result was confirmed in this study using a different mutation in TR (AHT) that more completely disrupts TR interactions with CoRs. Initially, we postulated that stimulation of the TSHα promoter by unliganded TR might be accounted for by competition for a limiting amount of CoRs that were bound to other factors on the promoter. However, when excess CoRs were cotransfected with TR, we found that they enhanced, rather than reversed basal activation (30). In view of recent findings that CoRs, like NCoR and SMRT, form a complex with Sin3 and HDAC to mediate transcriptional repression via histone deacetylation (6–8), we hypothesized that the ability of CoRs to enhance further basal activity might reflect the recruitment of a limiting amount of HDAC by the TR-CoR complex. Consistent with this idea, cotransfection of excess HDAC abolished the basal activation by unliganded TR and CoRs. Supporting this result, inhibition of HDAC activity by TSA abolished the T3-induced repression by TR. These findings strongly implicate HDAC as a pivotal factor that controls TR regulation of the TSHα gene. It is notable that this promoter is highly sensitive to HDAC inhibition. ATR-mediated alterations in histone acetylation, likely applies to CREB-CBP versus HDAC can induce marked changes in the activity of this gene.

Given the involvement of CoRs/HDAC and CREB/CoR in negative regulation, we propose a two-step model for TR control of the TSHα promoter (Fig. 8). In the absence of ligand, TR recruits CoRs, which in turn form a complex that includes HDAC. The withdrawal of HDAC from other target sites, such as the basal promoter, results in a net increase in histone acetylation, and consequently, increased basal transcription. Consistent with this step, CoR mutants such as P214R and AHT, only impair TR-mediated stimulation, without altering the degree of T3 suppression below the basal activity. The second step of negative regulation, involving T3-induced repression, is proposed to involve both CoRs and CoAs. The ligand-dependent release of CoRs and HDAC may facilitate deacetylation of the promoter. The ability of the HDAC inhibitor to block T3-mediated repression is consistent with this idea. In addition, T3-induced recruitment of CoAs may restrict their access to other transcription factors like CREB. Supporting this possibility, a CoA mutant, E457A, was able to reverse CoR-mediated stimulation, but it did not exhibit T3-dependent suppression below basal activity. CoAs like SRC1 appear to be involved in this step, since their addition selectively enhances the degree of T3-dependent repression. In addition, CBP appears to be a critical factor in this process as treatment with PKA, or overexpression of CBP, abrogates T3-dependent repression. In the case of TSHα gene, it is likely that phosphoCREB is a critical factor that participates in the binding of CBP. This novel mechanism for negative regulation, involving TR-mediated alterations in histone acetylation, likely applies to other nuclear receptors and genes that are repressed, rather than stimulated by nuclear receptor ligands.

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REFERENCES
1. Chen, J. D., and Evans, R. M. (1995) Nature 377, 454–457
2. Sande, S., and Privalsky, M. L. (1996) Mol. Endocrinol. 10, 813–825
3. Hore nip, A. J., Naar, A. M., Heintzel, T., Torchia, J., Gloss, B., Kurukawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K., and Rosenfeld, M. G. (1995) Nature 377, 397–404
4. Kurukawa, R., Soderstrom, M., Heintzel, A., Halachmi, S., Brown, M., Rosenfeld, M. G., and Glass, C. K. (1995) Nature 377, 451–454
5. Lee, J. W., Ryan, F., Swaffield, J. C., Johnston, S. A., and Moore, D. D. (1995) Nature 374, 91–94
6. Alland, L., Muhe, R., Hou, H., Jr., Petes, J., Chin, L., Schreiber-Agus, N., and DePinho, R. A. (1997) Nature 387, 49–55
7. Heintzel, T., Laviy, R., Mullen, T.-M., Soderstrom, M., Laherty, C. D., Torchia, J., Yang, W.-M., Brand, G., Ngo, S. D., Davie, J. R., Sets, E., Eisenman, R. N., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1997) Nature 387, 43–48
8. Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans, R. M. (1997) Cell 89, 373–380
9. Onate, S. A., Tsai, S. Y., Tsai, M. J., and O’Malley, B. W. (1995) Science 267, 1354–1357
10. Voge el, J. J., Heine, M. J. S., Zechel, C., Chambon, P., and Grommeh Meyer, H. (1996) EMBO J. 15, 3667–3675
11. Hong, H., Kohli, K., Trivedi, A., Johnson, D. L., and Stallcup, M. R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4948–4952
12. Anzick, S. L., Komminen, J., Walker, R. L., Aznara, D. O., Tanner, M. M., Guan, X.-Y., Sauter, G., Kulliomeni, O.-P., Trent, J. M., and Meltzer, P. S. (1997) Science 277, 965–968
13. Li, H., Gomes, P. J., and Chen, J.-D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8479–8484
14. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K., and Rosenfeld, M. G. (1997) Nature 387, 677–684
15. Chen, H., Lin, R. J., Schulitz, R. L., Chakravarti, D., Nash, A., Nagy, L.,
