Chromatin Remodeling by the Thyroid Hormone Receptor in Regulation of the Thyroid-stimulating Hormone α-Subunit Promoter*

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The chromatin architecture of a promoter is an important determinant of its transcriptional response. For most target genes, the thyroid hormone receptor (TR) activates gene expression in response to thyroid hormone (T₃). In contrast, the thyroid-stimulating hormone α-subunit (TSHα) gene promoter is down-regulated by TR in the presence of T₃. Here we utilize the capacity for the Xenopus oocyte to chromatinize exogenous nuclear-injected DNA to analyze the chromatin architecture of the TSHα promoter and how this changes upon TR-mediated regulation. Interestingly, in the oocyte, the TSHα promoter was positively regulated by T₃. In the inactive state, the promoter contained six loosely positioned nucleosomes. The addition of TR/retinoid X receptor together had no effect on the chromatin structure, but the inclusion of T₃ induced strong positioning of a dinucleosome in the TSHα proximal promoter that was bordered by regions that were hypersensitive to cleavage by methidiumpropyl EDTA. We identified a novel thyroid response element that coincided with the proximal hypersensitive region. Furthermore, we examined the consequences of mutations in TR that impaired coactivator recruitment. In a comparison with the Xenopus TRβA promoter, we found that the effects of these mutations on transactivation and chromatin remodeling were significantly more severe on the TSHα promoter.

The molecular mechanism of nuclear hormone receptor-mediated gene regulation and the importance of chromatin structure to this process have been intensively investigated over the past decade. Although the packaging of DNA into dense chromatin is a barrier to transcription factor access, multiple mechanisms exist to overcome this obstacle and thereby facilitate regulation of gene expression (1–3). Regulation of the acetylation state of core nucleosomal histone proteins influences their interaction with DNA and, subsequently, the nucleosomal packing density and transcription factor accessibility to chromatin. Transcriptional repression by DNA-bound thyroid hormone receptor (TR) in the absence of hormone (T₃) involves the recruitment of histone deacetylase-containing complexes that facilitate the formation of repressive chromatin structure. The addition of T₃ causes the release of the deacetylase complexes and stimulates transcriptional activation by the recruitment of coactivators that include acetyltransferase components (1–3). The acetyltransferases and deacetylases are numerous, but occur in discrete subcomplexes that may exhibit cell type and promoter context dependence (4).

The role of ATP-dependent mechanisms such as SWI/SNF, Mi2/NURD, and ISWI (5) in nuclear receptor-mediated regulation has also been demonstrated. Studies using the glucocorticoid receptor on the mouse mammary tumor virus promoter, which has been shown to have positioned nucleosomes (6), have demonstrated a requirement for SWI-SNF complexes and their ligand-induced targeting to the promoter to activate gene expression (7–11). More recently, it has been demonstrated that glucocorticoid receptor activation induces nucleosome translational positioning on the mouse mammary tumor virus promoter (12). From these studies and the additional observations that additional cofactors such as the DRIP/ARC complex require a chromatin environment in which to exert their effect on gene expression (13, 14), it is clear that chromatin architecture plays a key role in nuclear receptor-mediator gene regulation.

In contrast to the majority of TR-regulated genes, for which T₃ induces up-regulation of promoter activity, the thyroid-stimulating hormone α-subunit (TSHα) promoter is regulated by a negative feedback loop in which unliganded TR activates TSHα expression and the addition of T₃ results in repression (15). This negative regulation in response to T₃ presents a conundrum when considering TR action in the context of the mechanisms described above. This raises the question as to what are the mechanistic determinants of positive versus negative transcriptional responses to T₃.

A recent report detailed a novel mechanism whereby recruitment of deacetylases by unliganded TR is associated, paradoxically, with histone acetylation and activation of transcription, but whereby subsequent overexpression of deacetylase reverses this effect, as does the addition of T₃ (16). It was proposed that the mechanism involves active exchange of repressors and activators between TR and intrinsic promoter regulatory factors. However, the role of chromatin structure and how it is altered in TR-mediated regulation of the TSHα promoter have not been investigated.

The Xenopus oocyte has been shown to provide a useful paradigm in which to study the determinants of chromatin assembly and TR-mediated alteration of the chromatin structure, particularly of the Xenopus TRβA promoter (17–20).
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high capacity for chromatinization of DNA templates injected into oocytes makes this an ideal system in which to study transcription factor-mediated effects on promoter structure and activity. In this study, we sought to compare the effects of TR on the chromatin architecture of the TSHα promoter with those of the TRβA promoter, which is ordinarily up-regulated by T3. In addition, mutations in TR identified in individuals with the clinical disorder of Resistance to Thyroid Hormone and shown to be deficient in their capacity to interact with coactivators (21) were used to investigate the role of coactivator recruitment in the modification of chromatin structure.

EXPERIMENTAL PROCEDURES

Reporter Plasmid Constructs and mRNA Synthesis—The reporter plasmid TRβA-CAT, containing the Xenopus laevis TRβA promoter linked to the chloramphenical acetyltransferase reporter gene, has recently been described in detail (22). The reporter construct TSHα-Luc contains the human TSHα proximal promoter (−846 to +44) linked to the luciferase reporter gene (23), whereas the TRH-Luc reporter construct contains the human thyrotropin-releasing hormone promoter (−900 to +55) also linked to luciferase (24). For mRNA synthesis, cDNA encoding human TRβ1 was cloned into pT7TS (25) between the X. laevis β-globin 5′- and 3′-untranslated regions. This template was linearized in vitro and then transcribed in vitro using T7 polymerase (Ambion Inc.). Mutations were introduced into TRβ1 by site-directed mutagenesis. cDNA encoding the human TRβ2 isofrom was cloned into pSP64(A) (Promega), and the linearized template was transcribed in vitro using SP6 polymerase (Ambion Inc.). The X. laevis retinoid X receptor (RXR)-α construct has been described previously (17) and was transcribed in vitro using SP6 polymerase.

Xenopus Oocytes and Microinjection—Stage V–VI Xenopus oocytes were prepared as previously described (26) and stored in MBSH buffer (27) at 18 °C for the duration of each experiment (up to 18 h). 0.5–5 ng of each mRNA was microinjected into the cytoplasm. 1 ng of reporter DNA template was injected into the nucleus. Oocytes were then incubated in the presence or absence of 3,3′,5-triiodo-l-thyronine (Sigma). Where specified, 33 mM trichostatin A or 50 μg/ml cycloheximide was added to the medium. Typically, 20 oocytes were injected for each test sample.

Analysis of Transcription by Primer Extension—RNA was extracted essentially as previously described (17). For transcript quantitation, 3–5 oocyte eq of RNA was annealed with 0.2 pmol of 32P-end-labeled primer in 30 mM Tris-Cl (pH 8.3), 45 mM KCl, 1.8 mM MgCl2, and 3 mM dithiothreitol. The primer used for TRβ1 was Primer I, described previously (17). For both TSHα-Luc and TRH-Luc, primer Luc64 was used, which corresponds to a region in the luciferase proximal gene (5′-TGGCGTCTTCCATTGCCAACAG-3′). Endogenous histone H4 was also measured as an internal loading control using primer H4 (5′-GAGGCGGAGATGCGTTCGAC-3′). Primer extension analysis of mRNA levels was performed as previously described (17). The specific signal for each reporter transcript was normalized against the histone H4 level.

Analysis of Chromatin Supercoiling—The method was used essentially that described previously (19). Typically, five injected oocytes (1 ng of DNA each) were homogenized in 50 μl of 0.25 M Tris-Cl (pH 7.5), followed by the addition of an equal volume of stop buffer (20 mM Tris-HCl (pH 7.5), 30 mM EDTA, 1% SDS, and 0.5 mg/ml proteinase K (Roche Molecular Biochemicals)), and incubated for at least 1 h at 37 °C, followed by two phenol/chloroform extractions and then ethanol precipitation. The centrifuged pellet was resuspended in 10 μl of Tris/EDTA buffer containing 100 μg/ml RNase A and incubated for 1 h at 37 °C. DNA topoisomers were resolved on a 1.2% agarose gel in 1× Tris phosphate/EDTA buffer in the presence of 90 μg/ml chloroquine diphosphate (Sigma) for 16 h at 45 V. The gel was then washed for 1–2 h in water to remove chloroquine before performing Southern analysis using a 32P-labeled random-primer HindIII/ EcoRI fragment (615 base pair) from TSHα-Luc spanning from position +46 of the TSHα promoter into the luciferase reporter gene. TRβ1 was probed with a 266-base pair Xbal/EcoRI fragment from within the chloramphenical acetyltransferase reporter gene. Blots were scanned using a PhosphorImager.

Western Densitometry—Whole extract samples were homogenized in 10 μl/oocyte 0.25 M Tris-Cl (pH 7.5), and the lysate was microcentrifuged for 15 min at 4 °C. 0.5 oocyte eq was run on a 10% SDS-polyacrylamide gel and transferred to a membrane. Western analysis of TRβ expression was performed using a polyclonal antibody directed against Xenopus TRα, followed by a chemiluminescent secondary antibody (Amersham Pharmacia Biotech).

RESULTS

Classical “Negative” Promoters Can Be Up-regulated by T3 in the Xenopus Oocyte—The initial analysis of the transcriptional response of the TSHα promoter to T3 in the Xenopus oocyte was performed as a comparative study alongside the well-characterized positively regulated Xenopus TRβA promoter (17–19, 28). Following the microinjection paradigm described in Fig. 1a, we confirmed earlier observations that the Xenopus TRβA promoter exhibits a high basal transcriptional activity that is repressed by unliganded TR/ RXR to ~20% of the basal level, but is de-repressed and activated to twice the basal level in the presence of T3, giving a 9-fold range in activity (Fig. 1b). The TSHα promoter exhibited a much lower basal activity than Xenopus TRβA but, in contrast to its normal in vivo response, was also derepressed by unliganded TR/RXR (of the basal level) and stimulated ~13-fold in the presence of T3, permitting a 33-fold-range in activity (Fig. 1b). To ascertain the generality of T3-induced activation of a promoter normally repressed by T3, we performed identical studies using the TRH promoter, which is also ordinarily down-regulated by T3 in vivo. Again, we observed a low basal activity with a strong T3-dependent stimulation, giving a 33-fold range of activity (Fig. 1b). The positive T3 response observed with these negative promoters is not a property of the reporter plasmid (pA2LUC) since we have shown these very same plasmid constructs to be down-regulated by T3 when transfected into mammalian cells (21, 29).
Furthermore, we tested the keratin promoter K17, on the background of a chloramphenicol acetyltransferase reporter plasmid, which has also been shown to bind TR and to be downregulated by T$_3$ in mammalian cell culture (30), but found this too to be stimulated by T$_3$ in the Xenopus oocyte (data not shown). These observations provide strong evidence that the cellular biochemical environment of a promoter is critical in determining both the magnitude and the direction of response to regulatory factors.

To demonstrate that the observed effect of T$_3$ on the TSH$_{a}$ promoter in the oocyte was directly mediated by TR rather than a secondary effect, TR/RXR mRNA and the TSH$_{a}$ reporter plasmid were injected into oocytes and incubated for 12 h in the absence of T$_3$ to permit full translation of the mRNA and the formation of a receptor-bound chromatinized TSH$_{a}$ promoter. Prior to the subsequent addition of T$_3$, the oocytes were incubated for 4 h to permit cycloheximide-mediated inhibition of translation and the message levels of each reporter were assayed by primer extension. The level of each reporter message (Tsx) was normalized against that of histone H4 (H4). In each case, the transcriptional activity is reported relative (rel.) to the basal activity for each promoter, i.e. in the absence of both T$_3$ and injected mRNA. c, effect of cycloheximide on TR-mediated activation of TSHA. Oocytes were injected with mRNA and TSH$_{a}$-Luc DNA as described for b, but without the addition of T$_3$. 12 h after the DNA injection, 50 µg/ml cycloheximide (CHX) was added where appropriate, and the oocytes were incubated a further 4 h to permit cycloheximide-mediated inhibition of translation. T$_3$ was then added to activate TR-mediated transcription, followed by a further 4-h incubation, after which RNA was extracted and assayed for TSH$_{a}$-Luc activity. d, analysis of the level of TR/RXR protein present after the treatment with cycloheximide in the experiment described for c. The lysate from the oocytes used for transcription analysis in c was resolved on an SDS-polyacrylamide gel and subjected to Western blot analysis using an antibody to TR. ns, nonspecific band. e, effect of TSA on promoter activity. Oocytes were treated as described for b, except that TSA (33 µM) was added, as appropriate, immediately after DNA injection.

In Fig. 1e, cycloheximide had no effect on T$_3$ stimulation of TSH$_{a}$, and it did not affect the level of TR protein, as shown by Western analysis in Fig. 1d. This indicates that regulation of the TSH$_{a}$ promoter by liganded TR in the Xenopus oocyte is direct. This would be anticipated because the oocyte genome is tetraploid, and the capacity to generate adequate transcripts to cause secondary effects is very limited.

The Xenopus TR$\beta$A, TSH$_{a}$, and TRH Promoters Exhibit a Differential Response to the Histone Deacetylase Inhibitor Trichostatin A—From the data in Fig. 1b and earlier work (17), it is apparent that in the case of the TR$\beta$A promoter, repression of basal transcription by unliganded TR accounts for a large proportion (~50%) of the observed transcriptional control in oocytes. As discussed earlier, many studies have linked repression with the recruitment of histone deacetylase activity (31). In Fig. 1e, we examined the relative contributions of acetylation on each of the TR$\beta$A, TSH$_{a}$ and TRH promoters by incubating the oocytes in the presence or absence of the deacetylase inhibitor trichostatin A (TSA). We found that for the TR$\beta$A promoter, maximal activation could be achieved by TSA alone, irrespective of the presence of TR, as seen previously (28), and that this activity was not further enhanced by the addition of T$_3$. This indicates that the acetylation state of the TR$\beta$A promoter is a key factor in its regulation. However, for both the TSH$_{a}$ and TRH promoters, TSA gave only partial activation, as did ligand-bound TR. Maximal activity was seen only with the combination of both T$_3$ and TSA. This indicates that mechanisms other than those involving histone acetylation, e.g. ATP-dependent regulators, may play a relatively greater role on
TSHα and TRβ than they do on TRβ6A.

Identification of a Novel T3 Response Element in the TSHα Promoter—We have confirmed a direct effect of TR on regulation of the TSHα promoter (Fig. 1c). However, to date, no definitive thyroid response elements (TREs) have been reported in this promoter, either in a chromatin context or on naked DNA. To investigate this issue in an in vivo configuration, we injected the TSHα promoter into oocytes in the presence or absence of TR/RXR, with or without T3, and performed in vivo DNase I footprinting on the chromatinized DNA. As illustrated in Fig. 2a, the presence of TR/RXR protected a region of chromatinized TSHα promoter between positions −200 and −240. Furthermore, this footprint was retained upon the addition of T3, as might be expected for ligand-bound receptor to activate transcription in a direct manner and in accordance with earlier observations on the TRβ6A promoter (32). We analyzed the sequence of the TSHα promoter that was protected by TR/RXR and found that, within a 23-base pair stretch, it contained one perfect consensus half-site, AGGTCA (site A), and two degenerate half-sites (B and C). All three half-sites are arranged in a direct repeat orientation. Interestingly, the half-site spacing is unusual in that sites A and B are spaced by 5 base pairs, an arrangement more typical of a retinoic acid response element, whereas there is no spacing between sites B and C (33).

To demonstrate the functional relevance of this putative TRE, we mutated simultaneously the first of the two guanine residues (shown in boldface in Fig. 2a) in each half-site to adenine. Previous studies have shown that this residue is highly conserved in nuclear receptor-binding sites (33). We then examined the transcriptional activity of this promoter compared with that of the wild-type promoter (Fig. 2b) and found that the triple mutation lowered T3-induced activation to ~50% of the wild-type level, supporting the notion that this region represents a functional TRE. To further demonstrate the role of this putative TRE in receptor binding to the TSHα promoter, we performed competitive band shift analysis. Highly purified recombinant TR and RXR were bound to duplex oligonucleotides containing an authentic high affinity direct repeat TRE from the malic enzyme gene promoter (34). DNA comprising a 260-base pair fragment from the wild-type or mutant promoter including the putative novel TRE was used as an unlabeled competitor. Fig. 2c shows that the mutant promoter, TSH(ΔTRE), was notably impaired in its ability to disrupt the receptor-probe complex, even at a 450-fold molar excess (lanes 2 and 6–8), whereas the wild-type fragment was an effective competitor at the high concentration (lanes 2 and 3–5). Quantitation of the receptor-probe complexes (Fig. 2d) reveal that, at the high concentration of competitor, wild-type TSH displaced 51% of the bound probe, whereas TSH(ΔTRE) displaced only 23%, supporting this region as one playing a role in TR binding.

T3 Induces Alteration in the Chromatin Architecture of the TSHα Promoter—We examined the receptor-mediated effects of T3 on the chromatin architecture of the TSHα promoter under both repressed and active transcriptional states. In Fig. 3a, we utilized the susceptibility of chromatinized DNA to chemical cleavage by MPE to map nucleosome positions and to examine the T3-induced changes. In the absence of receptor (lanes 1 and 2), a diffuse banding pattern was observed with no apparent nucleosome positioning. However, this was not due to a simple lack of chromatization, as Fig. 2 shows that topoisomers were still generated, either in the absence or presence of TR, indicating effective chromatization of exogenous TSHα promoter in the Xenopus oocyte. The presence of additional diffuse bands in the middle of nucleosomes A, C, and D in lanes 4–8 suggests that the positioning preference for those nucleosomes in the basal state is relatively weak and that the nucleosomes exist in more than one position. In the presence of unliganded TR (Fig. 3a, lanes 3 and 4), no significant change in
chromatin structure was detected when compared with lanes 1 and 2, indicating that the relatively small repression of transcriptional activity imparted by unliganded receptor occurs without major changes in chromatin structure. This lack of chromatin structural change in the presence of unliganded TR/RXR is supported by the lack of change to promoter supercoiling shown in Fig. 3c, indicating that the overall nucleosome density is not altered. However, upon the addition of ligand, a dramatic remodeling of the chromatin architecture was observed (Fig. 3a, lanes 5 and 6). First, the generation of a strongly positioned dinucleosome (C + D) occurred between positions −220 and −570. With the exception of the band at position −390, which presumably represents the linker region between the two nucleosomes, all other bands seen in lanes 1–4 in this region disappeared, indicating that this dinucleosome is acutely positioned only in the transcriptionally active state. Second, nucleosome A was also repositioned upon activation, as demonstrated by the disappearance of the mid-nucleosomal band at position +40 in the extreme 3' end of this TSHα promoter fragment that encompasses the transcription start site. Third, there was a dramatic manifestation of MPE hypersensitivity at positions −220 and −570 (either side of the positioned C + D dinucleosome) and, to a lesser degree, at position −60 in the vicinity of the TATA element located from positions −23 to −29. These ligand-induced changes in the chromatin architecture of the TSHα promoter as revealed by MPE accessibility are in keeping with the T3-induced change in supercoiling shown in Fig. 3c (lane 4), which reflects a decrease in nucleosome density. It should be noted that since these analyses were performed in the presence of a-amanitin, which inhibits RNA polymerase II action, the structural changes are occurring independent of transcription.

Comparison of the TSHα promoter with the TRβ1 promoter revealed a notable difference in the effects conferred on chromatin structure by liganded TR (Fig. 3b). In contrast to the TSHα promoter, TRβ1 exhibited the clearly defined periodicity indicative of organized nucleosomal packaging seen previously (19), both in the absence and presence of unliganded receptor (lanes 1–4). Both promoters exhibited induced hypersensitivity to MPE upon the addition of T3 (see arrow in Fig. 3c). For TRβ1, this induced hypersensitive region disrupted an existing nucleosome between two TREs (compare lanes 3 and 4 with lanes 5 and 6). This is in agreement with the ligand-induced change in supercoiling observed in Fig. 3c (lane 8). However, unlike with the TSHα promoter, the presence of T3 did not appear to stabilize the position of other nucleosomes, suggesting a differing requirement for structural reorganization of chromatin upon activation of these two promoters in the oocyte.

Natural TR Mutants Are Differentially Impaired in Their Capacity to Regulate the TSHα and TRβ1 Promoters—The wild-type receptor and the mutants used in this study are all the human TRβ1 isoform. Another isoform, TRβ2, differs from

Fig. 3. TR-mediated effects on the chromatin architecture of the TSHα and TRβ1 promoters. Oocytes were left uninjected or were injected with 5 ng each of TRβ and RXRα mRNAs, followed 4 h later by 1 ng of pTRβA or TSHα-Luc DNAs, and incubated in the presence or absence of 100 nM T3 for 12 h. Oocytes were then harvested and subjected to MPE treatment (a and b) or supercoiling assay (c) as described under “Experimental Procedures.” a, human TSHα (hTSHα) promoter. Solid arrows denote MPE-hypersensitive regions in the linker DNA between nucleosomes. Dashed arrows denote the T3-induced loss of MPE sensitivity. The schematic shows the positions of nucleosomes A–F on the TSHα promoter (−846 to +44) in the T3-activated state. Note that nucleosomes A and F continue into the vector backbone. b, Xenopus TRβ1 (xTRβ1) promoter. The arrow denotes the T3-induced MPE-hypersensitive site. Black dots indicate the positions of thyroid hormone response elements. c, effect of TR and T3 on supercoiling of TSHα and TRβ1 promoters.
TRβ1 at the amino terminus and is expressed primarily in the pituitary, where it is believed to be a major regulator of TSHα (35, 36). Given the unexpected up-regulation of TSHα by TRβ1 in the oocyte, we sought to ascertain whether this result was isoform-dependent. We expressed TRβ2 in the oocyte (Fig. 4a) and compared its transactivation capacity with that of TRβ1 (Fig. 4b). We found no significant difference between the activities of these two isoforms in this system.

We have previously characterized the naturally occurring TRβ mutations L454V and L454W identified in individuals with resistance to T₃ (21), a dominantly inherited clinical disorder characterized by elevated levels of circulating thyroid hormone, but inappropriately normal TSH levels, as well as variations in goiter, attention-deficit hyperactivity disorder, reduced IQ, and growth retardation. These mutant receptors are impaired both in their T₃-dependent transactivation function and their ability to recruit coactivators such as steroid receptor coactivator-1, yet bind T₃ with near wild-type affinity (21). Since recruitment of coactivators is believed to play an integral role in the regulation of chromatin structure, we utilized the Xenopus oocyte system to examine the influence of these mutations on transcriptional activation and chromatin remodeling of both the TSHα and TRβA promoters.

To confirm the expression of the TR mutants in the oocyte, we performed Western blot analysis of oocyte extracts using an antibody to Xenopus TRβ. Fig. 4a shows that all three mutants were expressed to a level similar to that of the wild-type receptor. We next examined the ability of each mutant to activate the TRβA and TSHα promoters. Fig. 4c shows that on the TRβA promoter, each mutant TR was able to repress basal transcription in the absence of ligand at least as well as wild-type TR (10–20% of the basal level). In the presence of saturating levels of T₃, the more mildly affected natural mutant, L454V, was able to achieve 75% of the wild-type activity, whereas the severely affected mutant, L454W, did not activate above basal levels (<38% of the activated wild-type level). Furthermore, the artificial mutant, L454A, which has previously been shown to be inactive in mammalian cells and devoid of coactivator binding (21, 37), was even incapable of fully releasing basal repression, reaching only ~31% of the wild-type maximum. However, when tested on the TSHα promoter, the phenotype of these mutants was notably more severe, with L454V achieving only 12% of the wild-type activity, whereas both L454W and L454A showed <4% of the wild-type level. This marked difference between mutant TR responses on the two promoters suggests key promoter-dependent differences in the nature of TR-mediated regulation.

Changes in Chromatin Supercoiling Are Not Sufficient for TR-mediated Activation—To examine the capacity for the TR mutants to remodel chromatin on the TSHα and TRβA promoters, we used a DNA supercoiling assay to assess ligand-induced changes in DNA supercoiling. In this assay, a loss of supercoiling upon the addition of T₃ is represented by a general downshift in the distribution of topoisomer bands and, in the strong cases, the appearance of a cluster of diffuse bands toward the bottom of the gel. Note that the minor variation in distribution of topoisomers between each mutant in the absence of T₃ is not considered significant. The data in Fig. 5a shows that, in the presence of wild-type TR, T₃ induced a loss of supercoiling in the TRβA promoter, confirming an earlier report from this laboratory (19). The L454V mutant also exhibited a wild-type level of change in supercoiling, in keeping with its capacity for transactivation on this promoter. Furthermore, the supercoiling changes observed with L454W and L454A were moderate and poor, respectively, again correlating with the extent of T₃-induced activation of transcription (Fig. 4c). However, for the TSHα promoter, the correlation between supercoiling and transcription did not hold. Although the transcriptionally inactive L454W and L454A mutants were completely incapable of inducing significant changes in DNA supercoiling (Fig. 5b), the L454V mutant, which retains a low level of activity on this promoter, elicited a change in supercoiling that was similar to that of the wild-type reporter. This suggests that although a change in chromatin supercoiling may be a prerequisite for transactivation, it is not, by itself, sufficient and is in accord with a different regulatory mechanism.

\[\text{V. K. K. Chatterjee, unpublished data.}\]
given our observation that T3 induced activity in the presence of the protein synthesis inhibitor cycloheximide. Historically, the precise nature of the TRE has been difficult to define, with one report suggesting a region near the transcription start site that resembles a degenerate palindromic TRE (23). Although the element identified in the present study appears to confer T3 responsiveness in the oocyte, it does not account for the entire T3 response. Two possibilities could explain this. The first is the existence of other TRES. In addition to that described above (23), the observed proximity of the TRES to the MPE-hypersensitive sites in both the TSHα and TRβ promoters (Fig. 3, a and b) suggests that the other strongly T3-induced MPE-hypersensitive site in TSHα, between nucleosomes D and E, points to another region of TR binding. The second possibility is that TR may regulate the TSHα promoter through mechanisms other than direct DNA binding, as recently suggested (48).

The differing importance of activation versus repression on these promoters shown in Fig. 1 (b and c) is likely due to differences in promoter structure and utilization of regulatory factors and mechanisms. The data in Fig. 1e suggest that acetylation is the major effector of activation of the TRβ promoter and that, in the absence of deacetylase activity, non-targeted acetyltransferases may acetylate this promoter sufficiently to facilitate maximal activation. In contrast, on the TSHα and TRH promoters, full activation required the concerted action of both TSA and T3-activated TR. This suggests either that additional acetylation of these promoters, beyond that achieved by TSA alone, requires targeted acetylase recruitment or that other mechanisms in addition to acetylation are important. The latter scenario has precedent in other nuclear receptor studies. Glucocorticoid receptor-mediated remodeling of a reconstituted mouse mammary tumor virus nucleosomal array has been shown to require ATP and remodeling factors, as well as interaction with acetyltransferase coactivators, supporting the idea of distinct requirements for each of these two remodeling mechanisms (10, 11). The estrogen and retinoic acid receptors also have been shown to require ATP-dependent remodeling complexes in the chromatin context (49, 50).

Both the TSHα and TRβ promoters exhibited T3-induced MPE hypersensitivity around the TR-binding sites, but TSHα exhibited greater T3-dependent changes in nucleosome translational positioning (Fig. 3). Chromatin remodeling is likely to facilitate transcription factor access and formation of a transcriptionally permissive state. These additional structural changes in the TSHα promoter that are not seen with TRβ may account for the greater potential for activation of transcription. Such highly organized chromatin structures have been previously identified in the regulatory regions of many inducible genes (51–56), with both rotational positioning of DNA on the nucleosomes as well as translational positioning of the nucleosomes along the DNA being important (12, 57–59). Changes in chromatin structure appear to be generally confined to regulatory regions (60), and in vivo footprinting studies on the mouse mammary tumor virus promoter have shown that transcription factors such as nuclear factor-1 and the transcription factor IID complex do not bind unless hormone-induced chromatin remodeling mediated by the glucocorticoid receptor has occurred (61, 62). It is likely that the observed remodeling of the TSHα promoter also facilitates its binding to transcription factors.

Work from our laboratory has previously shown that the TRβ promoter in Xenopus oocytes can be fully activated by acetylation alone and that TR-dependent chromatin disruption is not required for transcriptional activation by the deacetylase inhibitor TSA (28). It was suggested that this observation would be anticipated if histone acetylation was the only alter-

**DISCUSSION**

In this study, we have shown that (i) the TSHα promoter is transcriptionally activated in Xenopus oocytes; (ii) T3 induces major changes in the translational positioning of nucleosomes in the TSHα promoter; (iii) changes in chromatin architecture are not sufficient for T3-mediated gene activation; and (iv) unlike the TRβ promoter, activation of the TSHα promoter cannot be fully accounted for by relief of deacetylase-mediated repression.

In a surprising observation, we found that in the Xenopus oocyte, the TSHα promoter was repressed by unliganded TR, but activated upon the addition of T3 (Fig. 1b), contrary to its perceived mode of negative regulation in vivo in the thyrotroph cells of the anterior pituitary (15). Furthermore, this effect was not specific to TSHα or genes active in the pituitary since the promoters for the hypothalamic TRH (Fig. 1b) and keratinocyte-specific keratin 17 (data not shown) (30) genes that are ordinarily down-regulated by T3 also demonstrated this reversal of T3 response. The most likely explanation for this would be the coexistence of specific regulatory factors that are not conserved between mammalian cells and Xenopus oocytes. The promoter region of the TSHα gene contains many regulatory elements central to its expression both in the pituitary and in the placenta that have demonstrated cell-type specificity for their usage (38–47). The concerted action of these regulatory factors in the correct combination may be the key determinant of the nature of the transcriptional response on the TSHα promoter, with TR itself functioning largely as the switch. This suggests that the mechanism of nuclear receptor-mediated control of mammalian gene expression involves receptors functioning as integrators of a regulatory pathway that is predetermined by the concerted action of specific promoter-bound transcription factors. To that end, the receptor-mediated changes in chromatin architecture seen here on the TSHα promoter may be instrumental in facilitating the coordinate DNA binding and activity of such factors.

That TR regulates the TSHα promoter directly seems clear with earlier observations on the TRβ promoter (19). Furthermore, for both activation of transcription (Fig. 4e) and topological change, the extent to which each mutation affects receptor function is promoter type-dependent.
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Chromatin Remodeling by the Thyroid Hormone Receptor in Regulation of the Thyroid-stimulating Hormone α-Subunit Promoter
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