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In vivo transcription factor recruitment during thyroid hormone receptor-mediated activation

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ABSTRACT **Thyroid hormone receptor (TR) can act as both a transcriptional activator and a silencer. Optimal activation by TR requires synergism with activator(s) bound to the promoter (promoter proximal activator). It is thought that liganded TR either helps to recruit preinitiation complexes (PIC) to the promoter or activates the PIC already recruited. However, the studies analyzing the TR action on the PIC formation were done *in vitro* and, therefore, it is not clear how relevant they are to the *in vivo* TR action. For example, *in vivo*, the TR can act from distances equal to or greater than a kilobase from the promoter, but such distant effect is not reproducible *in vitro*. In this study, we used the PIN*POINT (ProteIN POsition Identification with Nuclease Tail) assay to define the molecular mechanism of TR action on transcription from the thymidine kinase promoter in the cellular context. We demonstrate that the recruitment of promoter-proximal activator Sp1, and the components of the basal transcription factors such as TBP, TFIIB, and Cdk7, is enhanced with thyroid hormone activation. Our results suggest that DNA forms a loop with TR-mediated activation to accommodate interactions between the liganded TR complex and the complex formed on the promoter. We also show that Sp1 bound to the promoter is essential for the DNA looping and recruitment of basal transcription factors such as TFIIB and Cdk7 but not for recruitment of TBP. On the basis of these findings, we present a model that illustrates the molecular mechanism of TR-mediated activation *in vivo*.**

The regulation of thyroid hormone (T3)-inducible genes by T3 receptor (TR) has served as an important paradigm for understanding transcriptional silencing and ligand-inducible gene activation. TRs, which exist as α and β isoforms, bind to T3 response element (TRE) and regulate transcription in two ways (1–9). In the absence of T3, TR associates with a corepressor complex that includes proteins such as SMRT/N-CoR, mSin3A/B, and histone deacetylase (10–15), resulting in silencing of transcription. In the presence of T3, TR undergoes a conformational change and associates with a coactivator complex that includes proteins such as CBP/p300, pCAF, ACTR/TRAM1, SRC-1/TIF2, and RIP140 (7, 16–19) to activate TRE-regulated genes. The TREs in the genes activated by T3 are occupied by TR both before (when it acts as a silencer) and after T3 induction (when it acts as an activator). The TR-mediated silencing–activation switch is essential for normal physiology, as conversion of TR to the oncogenic v-ErbA is thought to result partly from mutations in TR that block T3 binding and prevent it from becoming an activator (20–22). The clinical symptoms of generalized resistance to T3 are also thought to result partly from mutations in TRβ that make TR a constitutive silencer (23, 24).

Despite the physiological importance of the TR-mediated silencing-activation switch of gene expression, the exact molecular mechanisms of TR action *in vivo* are not well understood. Although TR and its associated cofactors are thought to regulate transcription initiation by affecting the assembly of the basal transcription complex, the exact step of the assembly which is targeted by TR *in vivo* is not known. On the basis of results from *in vitro* studies, two models have been proposed. In the first model, the unliganded TR complex recruits TBP/TFIIB but blocks subsequent steps of the preinitiation complex (PIC) formation by means of inhibitory interactions with TBP or TFIIB (16, 25, 26). In this model, T3 activation reduces such inhibitory interactions and allows the recruitment of the remaining basal transcription factors to complete the PIC formation. In the second model, the unliganded TR complex inhibits transcription after the recruitment of the PIC by locking the PIC in a repressed state (27). In the presence of T3, the liganded TR converts the PIC from a repressed state to an active state. It is not known, however, whether these models, which are based on *in vitro* studies, reflect the TR-mediated activation or silencing *in vivo*.

Optimal TR-mediated activation requires synergistic interactions between TR and various activators binding to the promoter (promoter proximal activators) such as Sp1, CREB, AP1, AP2, NF1, Oct-1, NFκB, and Pit-1, depending on the promoter context (28). The exact nature of this synergism remains a mystery. For example, if the liganded TR can stimulate the formation of PIC as has been shown *in vitro*, it is not clear what role the promoter-proximal activator plays in T3-induced transcription. Such questions have been difficult to address because the capability to detect *in vivo* the recruitment of individual proteins to DNA did not exist.

In this report, we use the PIN*POINT assay (ProteIN POsition Identification with Nuclease Tail; ref. 29) to study T3-dependent recruitment of transcription factors to the thymidine kinase (TK) promoter in the cellular context. We find that when the TR complex is activated with T3, the recruitment of Sp1, the promoter-proximal activator of the TK promoter, and basal transcription factors such as TBP, TFIIB, and Cdk7 (a component of TFIIH) occurs. Our results also suggest that with T3 activation, the liganded TR complex and the complex formed on the promoter DNA come into close proximity, thereby looping out the intervening DNA. It appears that Sp1 bound to the promoter region plays an essential role in stabilizing the DNA loop and recruiting basal transcription factors such as TFIIB and Cdk7 but not TBP.

MATERIALS AND METHODS

Plasmid Construction. The expression vector for Sp1 pointer has been described previously (29). The expression

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Abbreviations: T3, thyroid hormone; TR, T3 receptor; PIC, preinitiation complex; TRE, T3 response element; CAT, chloramphenicol acetyltransferase; PIN*POINT, protein position identification with nuclease tail assay; LM-PCR, ligation-mediated PCR; TK, thymidine kinase.

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vectors for TBP, TFIIB, Cdk7, Oct-1, and GAL4 DNA-binding protein (DBD) were constructed by linking the corresponding cDNA fragments to the nuclease domain of Fok1 from pCB FOK IR (30). As described previously for the construction of Sp1 pointer, the cDNA fragments were connected to the nuclease domain through a flexible linker encoding 14 amino acids (AGGGGGGGGGGARL). For construction of the target plasmid 3XF2 TRE, a 1-kb nonspecific fragment derived from pBR322 was fused to the 5′ *Kpn*I site of the pBL TK/CAT plasmid (CAT, chloramphenicol acetyltransferase) and a 90-bp fragment containing three copies of the F2 TRE derived from the chicken lysozyme gene (31) was inserted immediately upstream of the 1-kb nonspecific fragment. The mutated targets used in Fig. 3*A* were created by replacing the wild-type TK promoter (*Bam*HI/*Xho*I fragment) with PCR fragments containing the corresponding deletions in the TK promoter.

Transfection and Isolation of the Transfected DNA. One day before transfection, HeLa cells were supplemented with fetal calf serum treated with AG1-X8 resin and with activated charcoal to eliminate thyroid and steroid hormones. By using the calcium phosphate method, HeLa cells (5×10^6 cells) were cotransfected with 5 μg of the pointer expression vector, 2–5 μg of the target plasmid, and 2 μg of the expression vector for human TRβ (wild-type or KS as indicated for some experiments). For T3 induction, cells were treated with 10 μM T3. CAT activity was measured as previously described (32). For PIN*POINT analysis, cells were harvested 24–36 hr after transfection and low molecular weight DNA was isolated according to the Hirt extraction technique (29). Briefly, the cells were washed with PBS and resuspended in 500 μl of 10 mM Tris·HCl/10 mM EDTA. After addition of 50 μl of 10% SDS and incubation for 10 min at room temperature, 140 μl of 5 M NaCl was added and the mixture was gently mixed. The resulting mixture was kept at 4°C overnight. The supernatant was collected after centrifugation at 15,000 $\times$ *g* for 30 min, and 40 μg of proteinase K was added to the supernatant. After incubation at 50°C for 2 hr, the sample was extracted first with phenol/chloroform and then with chloroform. The DNA was isolated by ethanol precipitation and resuspended in 20–30 μl of 10 mM Tris·HCl (pH 8.0).

Ligation-Mediated PCR (LM-PCR) and Southern Blotting. The DNA sample (5 μl) prepared by the Hirt extraction method described above was treated with the Klenow fragment of DNA polymerase in the presence of dNTP to create blunt ends. After blunting, the DNA was ligated to a double-stranded synthetic linker at 15°C overnight. The synthetic linker was made by annealing MK21 (sense, 5′-GAAACACT-TCAGATCTCCCGAGTCACCGC-3′) and MK22 (antisense, 5′-phosphorylated, 5′-GCGGTGACTCGGGAGATCT-GAAGTG-3′). One fourth of the DNA from the ligation reaction was subjected to 20–25 cycles of PCR. The PCR was performed by denaturation at 94°C for 5 min, annealing at 55°C for 5 min, and extension at 72°C for 5 min with *Taq* DNA polymerase, 5′ primer MK21, and one of the 3′ primers indicated in Fig. 1*A* (primer 1 or primer 2) in a total volume of 100 μl. After LM-PCR, 20 μl of the sample was run on a 3:1 (Nusieve/agarose) gel (FMC) and transferred to a nitrocellulose membrane by the alkaline transfer method. The blot was probed with one of the two ^{32}P-end-labeled internal primers (MK24 or MK25). Primer MK24, which was derived from the $-15/+25$ region of the TK promoter, was used for the promoter region analysis, and primer MK25, which was derived from a region 11–50 bp downstream of the 3′ end of the 3XF2 TRE, was used for the TRE region analysis. For the analysis of DNA recovery in each sample, 5% of the recovered DNA from the Hirt preparation was cleaved with *Eco*RI (position -80 in the TK promoter) and primer extension was performed with the end-labeled antisense primer MK 23 (5′-TGAGCATTCATCAGGCGGGCAAGAATGTGA-3′) derived from a sequence immediately 5′ of the *Eco*RI site in the CAT gene. The expected ≈380-bp product was examined on an agarose gel (indicated as "recovery" in Fig. 1 *B* and *C*; for Fig. 3 *B* and *C*, data not shown).

RESULTS AND DISCUSSION

To study the role of TR and T3 in the recruitment of transcription factors to the TRE-containing promoter in the cellular context, we used a recently described assay termed PIN*POINT (29). In this assay, an expression vector for a chimeric protein (referred to as the "pointer") composed of a given transcription factor fused to the nuclease domain of the restriction endonuclease *Fok*I is cotransfected with a TRE-containing "target plasmid" into HeLa cells. Restriction endonuclease *Fok*I is composed of two domains: the DNA-binding domain, which targets the endonuclease to the recognition sequence (GGATG), and the nuclease domain, which cleaves 9 and 13 bp from the recognition sequence on Watson and Crick strands, respectively (29). In designing the pointer, we fused the transcription factor to the nuclease domain of *Fok*I through a flexible linker that contains more than 10 glycine residues to confer a high degree of freedom to the nuclease for movement and DNA cleavage. When the pointer binds to the target DNA, the nuclease domain cleaves the DNA near the binding site. One useful feature of the PIN*POINT assay is that the nuclease tail that is fused to transcription factors that do not bind DNA directly but are recruited to DNA through protein–protein interaction can also cleave the nearby DNA (29). DNA cleavage is then detected by primer extension or LM-PCR. LM-PCR is more sensitive than primer extension, and therefore we used LM-PCR for all of the studies presented in this report. The resulting PCR product was detected by Southern blotting and hybridization with a radioactively labeled internal primer.

TR-mediated activation and silencing have been demonstrated in cotransfection experiments using a number of different cell lines, including HeLa cells, where TR is thought to regulate transcription either as a TR homodimer or as a TR-RXR (retinoid X receptor) heterodimer (33). Because HeLa cells do not express TR but express RXR and the cofactors for TR function (33, 34), the expression vector for human TRβ was cotransfected with the target plasmid and pointer expression vectors. The cells were treated with or without T3. For the target plasmid (Fig. 1*A*), we chose the widely used TRE-containing reporter gene, F2-TRE-TK-CAT, in which the high-affinity TRE from the chicken lysozyme silencer was inserted 1 kb upstream of the TK promoter ($-105/+55$) (31). The pointers that we tested in this report included basal transcription factors TBP, TFIIB, and Cdk7, and Sp1, the promoter-proximal activator for the TK gene that has been shown to be essential for the TK gene activation both *in vivo* and *in vitro* (35).

When cells transfected with the expression vectors for the pointer, TR, and the target plasmid were treated with T3, Sp1 (Fig. 1*B*), and basal transcription factor pointers (TBP, TFIIB, and Cdk7) (Fig. 1*C*) cleaved the DNA near the promoter (Fig. 1*B*, lanes 5, 6, and 8 for Sp1; Fig. 1*C*, lanes 7–9 for basal transcription factors). The cleavage site for each pointer is shown schematically in Fig. 1*D*. Such DNA cleavage was not detected when the TRE was replaced with the mutated TRE that does not bind TR (31) (data not shown). Cleavage by Sp1 pointer was undetectable when the Sp1-binding sites were deleted (see Fig. 3*B*) and cleavage by TBP, TFIIB, and Cdk7 pointers was undetectable when the TATA box was deleted, indicating that the pointers were targeted properly (data not shown). Without T3, cleavage by the pointers for Sp1, TBP, TFIIB, and Cdk7 was not detectable (Fig. 1*B*, lane 4, Sp1; Fig. 1*C*, lanes 4–6, basal transcription factors). T3 did not affect the expression of the pointers as detected by immunoblotting (data

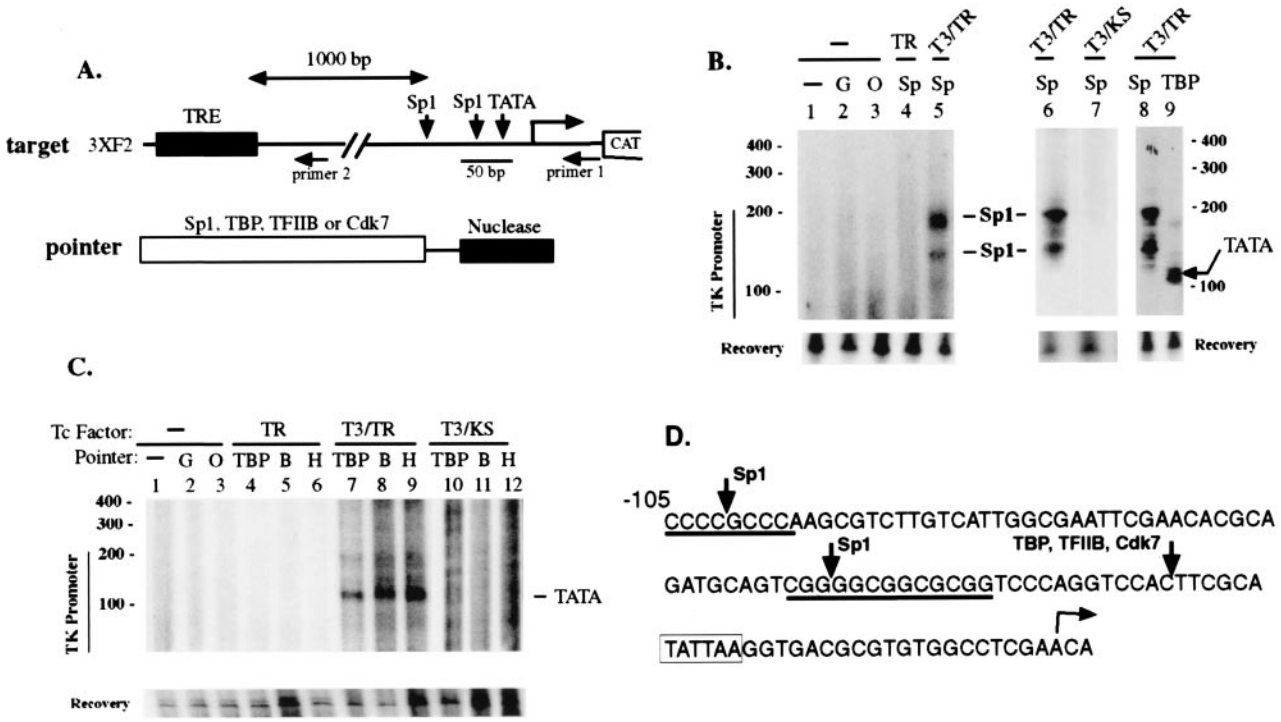

FIG. 1. Using PIN*POINT to study *in vivo* transcription factor recruitment during transcriptional silencing and activation of the TRE-regulated reporter gene. (*A*) Diagram of the target plasmid and pointers. The target DNA, 3XF2 TRE, contains three copies of the high-affinity TRE (30 bp) (ttatTGACCCCAgcTGAGGTCAagttacga, capital letters indicate inverted TREs) from the chicken lysozyme silencer F2 (31) inserted 1-kb 5′ upstream of the herpes simplex virus TK −105/+55 promoter. The TK promoter contains two Sp1 sites at −105/−95 and −56/−45 and the TATA box at −26/−21 position. Horizontal arrows mark the positions of the 3′ antisense primers (primer 1 and primer 2) used for LM-PCR in this study. Primer 1 was derived from the +26/+55 region of the TK promoter and was used to detect cleavage within the TK promoter; primer 2 was derived from a region 51–80 bp downstream from the 3′ end of the 3XF2 TRE and was used to detect cleavage within the TRE region. The pointers were composed of the 25-kDa nuclease domain of *Fok*I restriction endonuclease fused to the carboxyl terminus of Sp1, TBP, TFIIB, or Cdk7 through a flexible glycine linker and were expressed by cotransfecting with the target plasmid. (*B*) Analysis of recruitment of Sp1 pointer to the TK promoter during the TR-mediated activation and silencing. HeLa cells were cotransfected with target plasmid 3XF2, a TR expression vector (wild type, TR; mutant KS, KS) and one of the pointer expression vectors (G, GAL4 DNA-binding domain; O, Oct-1; Sp, Sp1). Treatment of transfected cells with T3 is indicated. Approximately 24 hr after transfection, the target plasmid was harvested and LM-PCR was performed with primers 1 and MK21 (from the linker). The amplified fragment was detected with Southern blotting followed by hybridization with a radioactively labeled internal probe MK24. The amount of target plasmid was similar in each sample (recovery). The positions of the Sp1 binding sites and the TATA box are indicated. (*C*) Recruitment of basal transcription factors to the TK promoter during the TR-mediated activation and silencing. Experiments were performed as described for *B* except that pointers for TBP, TFIIB (B), or TFIIH (H) were used. (*D*) The cleavage sites for the Sp1 and basal transcription factor pointers as determined against a sequence ladder. Primer extension was performed on the LM-PCR products with radioactively labeled primer 1 and electrophoresed next to a DNA sequence ladder as described previously (29). The cleavage site by each pointer was deduced by subtracting the length of the ligated 29-bp linker (MK21/MK22). By comparing the intensities of the LM-PCR band generated from pointer cleavage with the LM-PCR band generated from the recovered DNA that has been digested with *Eco*RI, we estimate that approximately 3–4% of the target plasmid was cleaved by the pointers (data not shown).

not shown). The DNA cleavage was not observed with control pointers such as GAL4 (G) and Oct-1 (O) pointers (Fig. 1*B*, lanes 2 and 3; Fig. 1*C*, lanes 2 and 3). These experiments were repeated with a TR mutant TRβ (KS), which was isolated from a patient with a syndrome called the general resistance to thyroid hormone (24). TRβ (KS) binds to TRE but cannot bind T3 and, therefore, functions as a constitutive silencer. With TRβ (KS), we detected no cleavage for the four pointers that we tested (TBP, TFIIB, Cdk7, and Sp1) even in the presence of T3 (Fig. 1*B*, lane 7 for Sp1; Fig. 1*C*, lanes 10–12 for basal factors). These findings suggest that the activation of TR by T3 causes the recruitment of Sp1 and basal transcription factors TBP, TFIIB, and Cdk7 to the promoter. This is consistent with the previous *in vivo* genomic footprinting study of T3-responsive genes, which shows an increased protection of the transcription factor binding sites such as Pit-1 or Sp1 upon T3 induction (36). These results argue that liganded TR activates transcription by recruiting TBP and the remaining basal transcription factors of PIC rather than by activating the PIC already formed on the promoter.

We did not detect any cleavage by Sp1 pointer in the basal state (i.e., in the absence of TR), but this may be a reflection of what the PIN*POINT assay actually detects (data not shown). For a pointer to cleave DNA, the pointer must be first targeted by specific protein–DNA interaction and/or protein–protein interaction. After being targeted to a particular site, the pointer may be incorporated into a more stable complex through protein–protein interaction with other transcription factors. Because the probability of cleavage after recruitment depends on the length of time the pointer stays on the DNA, it is likely that the PIN*POINT assay detects stable complexes only. In light of this, the results shown in Fig. 1 suggest that the liganded TR stabilizes the Sp1 complex on the promoter.

It is curious that TBP, TFIIB, and Cdk7 pointers cleave the same site. But, like other non-sequence-specific nucleases such as DNase I and micrococcal nuclease, the flexible *Fok*I nuclease tail of transcription factor pointers may preferentially cleave certain sites due to the local DNA–protein architecture near the binding site of the transcription factors. Cleavage of the same site by different pointers in the multiprotein complex is not unique to the pointers that we have used here. For example, the pointers for BRG1 and BAF170, two subunits of the human SWI/SNF complex, also cleave the same site (20 bp 3′ of the transcription initiation site) when recruited to the β-globin promoter (data not shown).

How does the liganded TR enhance the recruitment of these transcription factors to the promoter? The most likely possibility is that the liganded TR/coactivator complex interacts with these factors and stabilize them on the promoter. Because the TRE is 1 kb upstream of the promoter region (see Fig. 1*A*), such interaction would require looping of the intervening DNA. If such looping does occur, the TRE and the proximal promoter region will be brought into close proximity and the flexible nuclease tail of the pointers recruited to the proximal promoter region will cleave the TRE region as well (Fig. 2*A*). Because neither Sp1 nor the basal transcription factors used in this study can bind TRE, such cleavage would likely indicate protein–protein interactions between the TRE and the promoter. When we analyzed the cleavage pattern within the TRE by using primer 2, we detected cleavage for all of the pointers recruited to the proximal promoter when the TR was activated with T3 (Fig. 2*B*, lanes 1–4) but not without T3 (lanes 5–8) or when TR was replaced by the TRβ (KS) mutant (data not shown). We have also attempted to study whether TR pointer that is bound to the distal TRE cleaved the promoter region, a result one would expect if DNA looping occurred. However, this study was not possible because the TR pointer did not even cleave the TRE region, either in the presence or in the absence of T3 (data not shown). As occasionally happens when two proteins are fused together, the nuclease domain might not fold properly when fused to TR.

Throughout this study, the DNA cleavage by the transcription factor pointers was detected only in the TK promoter (Fig. 1) and the TRE region (Fig. 2). Such cleavage was not detected either in the 1-kb region between the TRE and the promoter or in the CAT gene, when LM-PCR using the same DNA samples in Fig. 1 *B* and *C* was performed with various primers designed to detect DNA cleavage in these regions (data not shown). These findings (Fig. 2*B*) suggest that, *in vivo*, transcriptional activation by T3 may occur with looping of the DNA between the TRE and the promoter.

The results from Fig. 1 suggest that the liganded TR stabilizes the Sp1 complex and the PIC on the promoter. To determine the specific role of Sp1 in T3 activation, we deleted either the proximal or the distal Sp1-binding site and examined the effect on transcription and the recruitment of the pointers (Fig. 3*A*). When one of the Sp1 binding sites (target plasmids 1 or 2) was deleted, transcriptional induction by T3 as measured by CAT activity was significantly reduced. The

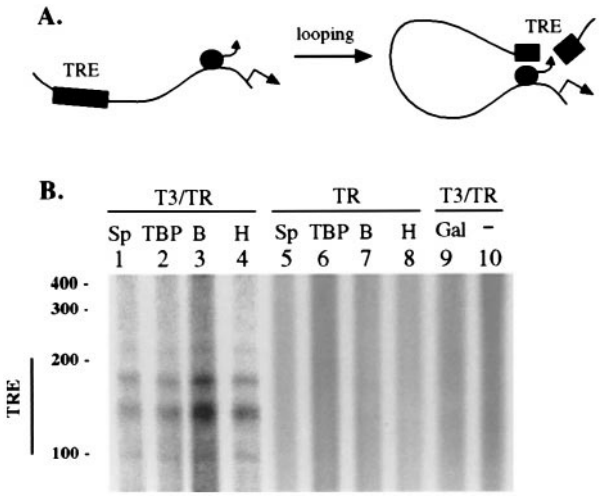

FIG. 2. Detection of DNA looping. (*A*) Diagram shows how DNA looping will cause the pointers bound to the promoter to cleave within the TRE (see text). (*B*) To detect cleavage within the TRE region, the DNA samples used in Fig. 1 *B* and *C* were analyzed by LM-PCR using primer 2. If cleavage occurs within the 90-bp 3XF2 TRE region, the size of the LM-PCR product with primer 2 is expected to be 110–200 bp.

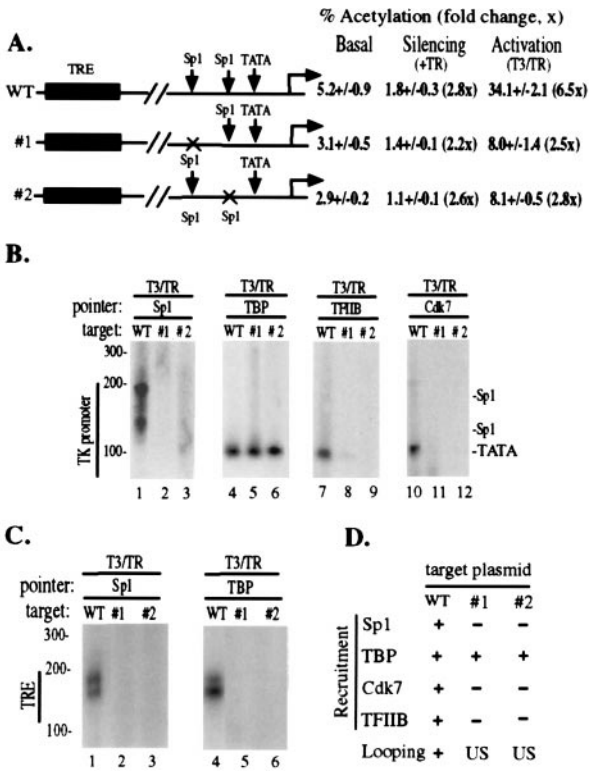

FIG. 3. Role of the Sp1 binding sites in the recruitment of various pointers to the promoter and on DNA looping. (*A*) The expression levels of the target/reporter plasmids containing a deletion ($\times$) of the distal (#1) or the proximal (#2) Sp1 site were determined by measuring CAT activities (percent acetylation) after transient transfection (32). For these transfections, 5 μg of the reporter gene and 2 μg of the expression vector for TR were used, and cells were treated with or without T3. The extent (fold, in parenthesis) of silencing with TR and activation with T3/TR is in comparison with the basal level of expression (without TR). The data for CAT activities were obtained from three to six independent transfections. (*B*) Recruitment of Sp1, TBP, TFIIB, and Cdk7 pointers to the target plasmids [wild type (WT), #1, and #2] were analyzed as described in the legend for Fig. 1*B*. (*C*) Analysis of DNA looping in target plasmids #1 and #2. Cleavage in the TRE region was analyzed by performing LM-PCR on the samples from *B* with primer 2. (*D*) Summary of pointer recruitment and detectable DNA looping for target plasmids WT, #1, and #2. "US" indicates unstable or transient DNA looping.

PIN*POINT assays shown in Fig. 3*B* indicate that stable recruitment of Sp1 was not observed at either Sp1 site in target #1 or #2 (Fig. 3*B*, lanes 2 and 3), suggesting that two separate sites with Sp1 bound stabilize each other for T3 activation. Interestingly, the stable recruitment of TBP to targets 1 and 2 was detectable but not that of TFIIB or Cdk7 (Fig. 3*B*, lanes 5 and 6 for TBP; lanes 8 and 9 for TFIIB; lanes 11 and 12 for Cdk7) which may explain, at least in part, their reduced transcriptional induction with T3 (Fig. 3*A*). Moreover, this result suggests that although TBP, TFIIB, and Cdk7 are components of the basal transcriptional machinery, they may be recruited differently. The liganded TR appears to be sufficient for the recruitment of TBP, whereas the liganded TR and Sp1 are both required for the stable recruitment of TFIIB and Cdk7.

These findings are consistent with what is known about Sp1 and the general transcription factors. Although it has been known for some time that Sp1 binding to two sites acts synergistically in activating transcription by direct interaction between tetramers of Sp1, the effect of this interaction is unknown (37). The result shown in Fig. 3*B* (lanes 2 and 3) suggests that the interaction between the two tetramers of Sp1 help stabilize Sp1 on the DNA.

We then examined whether the DNA looping occurs in target plasmids 1 and 2 by examining whether cleavage in the TRE region by the Sp1, TBP, TFIIB, and Cdk7 pointers occurs. No cleavage was detected in the TRE region by Sp1 (Fig. 3*C*, lanes 2 and 3), TFIIB, and Cdk7 pointers (data not shown) when one of the Sp1 binding sites was deleted. This finding was expected because these pointers were not recruited to the promoter in target plasmids 1 and 2 (see Fig. 3*B*). However, the TBP pointer, which was recruited to the promoter in target plasmids 1 and 2 (Fig. 3*B*, lanes 5 and 6) also did not cleave the TRE region (see Fig. 3*C*, lanes 5 and 6). Because recruitment of the TBP pointer requires the liganded TR (see Fig. 1*C*), it is likely that DNA looping occurs without Sp1 in the target plasmids 1 and 2, but the absence of cleavage in the TRE region in these plasmids suggests that the DNA looping in these plasmids is either unstable or transient. We summarize these results in Fig. 3*D*.

On the basis of the results presented above, we propose a model for the transcription factor recruitment that occurs with TR-mediated silencing and activation of the TRE-TK gene (Fig. 4). In the absence of T3, TR appears to communicate with the promoter and actively repress recruitment of transcription factors such as Sp1 and the components of the basal transcriptional machinery. In the presence of T3, the liganded TR stabilizes the Sp1 complex and the components of the basal transcriptional machinery on the promoter. Sp1 bound to the two separate binding sites allows them to stabilize each other and promotes the recruitment of TFIIB and Cdk7. TBP recruitment appears to depend predominantly on the liganded TR and less on Sp1. Stable interactions among the liganded TR, Sp1 complex, and basal transcription machinery are likely to cause the looping out of the intervening DNA between the distal TRE and the promoter.

At this point, we do not know which proteins participate directly in the protein–protein interactions described here. Coactivators that are recruited to TR upon T3 induction have been proposed to mediate the interactions between TR and various activators or the basal transcription factors. For example, a coactivator such as CBP/p300 can directly interact with a number of sequence-specific activators, including CREB, bHLH factors, AP1, NF-κB, and Stat proteins, as well as basal transcription factors TFIIB and TBP (38–41). Sp1 directly interacts with several TBP-associated factors (TAFs) *in vitro* (42–44) but not with TR, TBP, or TFIIB (42, 45). The interactions between Sp1 and TAFs have been proposed to stabilize the transcription machinery and to activate transcription *in vitro* (44).

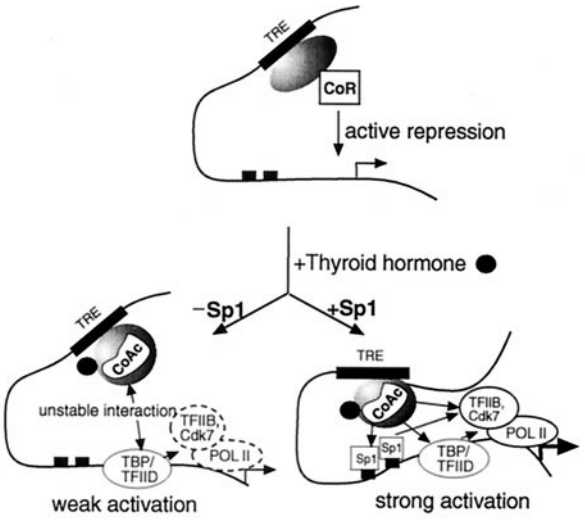

FIG. 4. Model for transcription factor recruitment and DNA looping during the TR-mediated silencing and activation (see text). TR-associated corepressors and coactivators are indicated as CoR and CoAc, respectively. Pol II, RNA polymerase II. To indicate that one protein was essential for the recruitment of another, straight arrows are used. The arrows point to the recruited protein. The transcription initiation site is indicated with a bent arrow.

Recruitment of the basal transcription factors is mediated by a complex network of protein–protein interactions with other transcription factors and serves as a central regulatory step in transcription (46). Despite this, very little is known about how individual transcription factors are recruited to the promoter *in vivo* and particularly how the enhancers control such recruitment from a distance. T3 induction, which converts TR from a silencer to an activator, is an ideal system to address these questions.

In this report, we have described the mechanism by which the TR activates transcription by examining its role in the recruitment of transcription factors to the promoter and the network of protein–protein interactions that it triggers. Moreover, we have presented evidence that distant regulatory elements such as an enhancer and a promoter come into close proximity, a phenomenon that is assumed to occur but has not been demonstrated experimentally.

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