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Review

Role of chromatin disruption and histone acetylation in thyroid hormone receptor action: implications in the regulation of HIV-1 LTR

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Summary. Thyroid hormone (TH) affects a wide variety of biological processes, from development to physiological function of different cells and organs. Alterations in plasma TH concentrations lead to developmental abnormalities and pathological consequences. Earlier studies have observed that plasma TH levels vary in AIDS patients such that low levels of TH correlate with survival rate. Furthermore, studies on the regulation of the human immunodeficiency virus type 1 (HIV-1) have shown that TH receptor (TR) is capable of binding to two regions within the long terminal repeat (LTR), which controls the transcription of HIV-1 genome. The frog oocyte is an in vivo system that allows microinjected DNA to be chromatinized in a process mimicking the process that occurs in somatic cells. Studies in the frog oocyte have provided in vivo evidence on the role of chromatin remodeling in transcriptional regulation by TR and have shown that TR utilizes similar mechanisms in the regulation of the HIV-1 LTR. That is, TR binds to LTR in chromatin in vivo and represses the LTR in the absence of TH by recruiting corepressor complexes containing histone deacetylases, and upon TH binding, TR causes chromatin remodeling and LTR activation.

Key words: Thyroid hormone receptor, HIV, AIDS, Chromatin, Histone acetylation

Introduction

Thyroid hormone (TH) plays an important role in vertebrate development and human pathology (Oppenheimer, 1979; Shi et al., 2001; Yen, 2001). The critical effects of TH on human development have been well documented. The most obvious and earliest known abnormalities of human body and behaviour associated with TH deficiency are the goiter (a lump in the neck due to thyroid gland enlargement) and cretinism (a form of severe mental deficiency together with retarded skeletal growth) (Hetzel, 1989). TH deficiency can arise from the lack of iodine (an essential element of the TH), removal of thyroid gland or absence of the gland due to diseases or congenital defects, etc. In humans, much of the developmental defects caused by TH deficiency can be reversed if TH replacement is initiated shortly after birth (Larsen, 1989), indicating that TH normally influences neonatal development mainly by acting directly on the foetus, not through the mother.

In addition to its developmental roles, TH has been found to be important for the metabolism and function of diverse organs. TH deficiency leads to reduced metabolic rate and both hyper- and hypo-thyroidism may cause abnormal function of different organs such as the heart (Guernsey and Edelman, 1983; Freake and Oppenheimer, 1995; Silva, 1995; Yen, 2001). The effects of TH on diverse tissues/organs may be expected to enable TH to influence pathological processes instigated by various viruses, which are dependent upon cellular function to propagate. Studies on TH levels in AIDS (Acquired Immuno-Deficiency Symdrome) and ARC (AIDS–Related Complex) patients suggest that TH may affect disease development (Lopresti et al., 1989; Tang and Kaptein, 1989). The long terminal repeat (LTR) of the human immunodeficiency virus type 1 (HIV-1), which causes AIDS and ARC, plays an essential role in the development of the diseases by directing the transcription of the viral genome. In vitro and tissue culture transfection studies have identified many DNA elements within the LTR as well as many host and viral

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Abbreviations: TH, Thyroid Hormone; TR, Thyroid Hormone Receptor; HIV, Human Immunodeficiency Virus; LTR, Long Terminal Repeat; ChIP, Chromatin immunoprecipitation; HAT, Histone acetyl transferase; HDAC, Histone deacetylase

proteins required for the transcriptional activation of the LTR (Vaishnav and Wong-Staal, 1991; Pereira et al., 2000). Among the DNA elements within the LTR are two regions that can be recognized by the nuclear receptor for TH (TR). TRs are TH-dependent transcription factors that can repress target gene expression in the absence of TH but activate it when TH is present. In this article, we will review some of the studies on the mechanisms of transcriptional regulation by TR and how TR regulates the LTR in the context of chromatin.

Transcriptional regulation by TR

There are two TR genes in vertebrates, TRα and TRß, both of which are capable of binding to TH with high affinities (Sap et al., 1986; Weinberger et al., 1986; Davey et al., 1994; Puzianowsak-Kuznicka et al., 1996). TRs belong to the superfamily of nuclear hormone receptors (Evans, 1988; Tsai and O'Malley, 1994; Yen and Chin, 1994; Mangelsdorf et al., 1995). Transcriptional activation by TH requires the binding of TRs, most likely as heterodimers with RXRs (9-cis-retinoic acid receptors), to the thyroid hormone response elements (TREs) in TH-response genes. TR/RXR heterodimers bind to TREs constitutively, even in the context chromatin (Perlman et al., 1982; Tsai and O'Malley, 1994; Wong et al., 1995). They repress the transcription of TH-inducible genes in the absence of TH and activate them when TH is present (Fondell et al., 1993; Tsai and O'Malley, 1994; Wong et al., 1995; Wolffe, 1997).

TR regulates gene expression by recruiting TR-interacting cofactors. Many such cofactors have been isolated, based on their ability to interact with TRs in the presence or absence of TH or under both conditions (Chen and Li, 1998; McKenna et al., 1999; Xu et al., 1999; Burke and Baniahmad, 2000; Rachez and Freedman, 2000; Zhang and Lazar, 2000; Ito and Roeder, 2001; Yen, 2001). The corepressors bind preferentially or exclusively to unliganded TR while the coactivators generally require TH for binding to TR. The characterization of these cofactors has shown that corepressors (e.g. SMRT, N-CoR) form multimeric complexes containing histone deacetylases (HDACs) while many coactivators (e.g. SRC-1, CBP/p300) are histone acetyltransferases or acetylases (HATs) (McKenna et al., 1999; Xu et al., 1999; Burke and Baniahmad, 2000; Hu and Lazar, 2000; Urnov et al., 2000). Both corepressors and coactivators form multimeric complexes with other proteins and at least in the case of an SRC-1 complex, an RNA (Chen et al., 1997; Heinzel et al., 1997; Nagy et al., 1997; McKenna et al., 1998; Dressel et al., 1999; Ito et al., 1999; Lanz et al., 1999; Rachez et al., 1999; Ryu et al., 1999; Guenther et al., 2000; Li et al., 2000b; Jones et al., 2001).

Corepressor complexes

Our own studies suggest that at least three distinct complexes containing the TR-binding corepressor N-CoR exist in the egg of anuran *Xenopus laevis* (Jones et al., 2001). Although the identities of most of the composite polypeptides are yet to be determined, biochemical analyses indicate that two of the complexes have HDAC activities while the third one does not. Western blot analysis and co-immunoprecipitation studies have revealed that one of the HDAC complexes contain the corepressor Sin3, HDAC1 (Rpd3), and RbAp48 (Retinoblastoma A associated protein). The existence of such an N-CoR complex is in agreement with earlier studies showing that 1) N-CoR can interact with Sin3 and its associated HDAC1/2 (Alland et al., 1997; Laherty et al., 1997), and 2) RbAp48 associates with the Sin3/HDAC complex (Hassig et al., 1997).

Similar to the frog egg, Hela cells contain at least two N-CoR complexes, one containing Sin3 and HDAC1 and the other does not (Underhill et al., 2000). In addition, single step affinity purification approaches to isolate HDAC complexes in vivo have shown that the related corepressors N-CoR and SMRT are both associated with several different HDACs (Huang et al., 2000; Li et al., 2000b), supporting the existence of multiple N-CoR/HDAC complexes in HeLa cells. However, the compositions of various HDAC complexes are not entirely consistent with each other, possibly due to the use of different purification procedures and/or the existence of multiple HDAC complexes containing N-CoR or SMRT with the abundance and stability of the complexes varying with different growth conditions. Currently, it is unclear whether the N-CoR complexes in Hela cells are identical or related to the ones isolated from the frog egg.

The majority of the SMRT protein in HeLa cells are present in a core complex with HDAC3 and a WD40 family protein TBL1 (Guenther et al., 2000; Li et al., 2000b). Interestingly, most polypeptides in various complexes are distinct to either N-CoR or SMRT complexes. Although N-CoR also forms a complex with HDAC3 and TBL1, no SMRT-containing complexes have been found to have Sin3 and HDAC1. Thus, it is quite likely that N-CoR and SMRT may use different HDAC complexes to mediate transcriptional repression despite their structural and sequence similarities.

Histone acetylation in gene regulation by TR function

The ability of TR to interact with HDAC-containing corepressor complexes in the absence of TH and HAT-containing coactivator complexes in the presence of TH suggest that histone acetylation plays a role in TH-dependent gene regulation. Indeed, recent studies have shown that the HAT activity of coactivators is required for gene activation by nuclear receptors, including TRs (Chen et al., 1999; Li et al., 1999, 2000a) and ligand-induced transcription is accompanied by an increase in histone acetylation specifically at the hormone regulated promoters (Chen et al., 1999; Sachs and Shi, 2000). Furthermore, our studies with the frog oocyte model system have shown that unliganded TR represses target

gene expression and this repression can be relieved by the addition of trichostatin A (TSA), a specific inhibitor of HDACs (Wong et al., 1998). Conversely, the repression induced by the overexpression of *Xenopus* HDAC1 (Rpd3) at a TH-inducible promoter can be reversed by either the expression of TR/RXR in the presence of TH or the addition of TSA (Wong et al., 1998). These and other studies suggest a dual function model for TR (Fig. 1). In the absence of TH, TR/RXR recruits a HDAC-corepressor complex to the promoter, leading to histone deacetylation and transcriptional repression. Upon TH binding, the corepressor complex is dissociated and a HAT-coactivator complex is recruited to the promoter. This leads to increased histone acetylation and gene activation.

It is important to point out that histone acetylation is only one of several possible mechanisms by which TR can regulation gene expression. First, TR and corepressors can interact with basal transcription factors and thus may inhibit transcription independent of their ability to recruit deacetylases. Second, there are coactivator complexes that do not have HAT activity but can interact with the basal transcriptional machinery directly (Rachez and Freedman, 2000; Ito and Roeder, 2001). Third, histone acetyltransferases can also acetylate proteins other than histones, e.g., basal transcription factors (Imhof et al., 1997) or other transcriptional regulators such as p53 (Gu and Roeder, 1997). Thus, HATs may also affect transcription independent of histone acetylation. Finally, extensive chromatin disruption independent histone acetylation is induced by TR in the presence of TH (Wong et al., 1997). Thus, transcriptional regulation by TR is likely to be much more complex than that portrayed in Fig. 1. TH-induced activation may involve sequential or concurrent recruitments of different complexes to modify chromatin structure, thus allowing the access of transcriptional machinery to the promoter and to facilitate subsequent activation of the promoter.

Regulation of HIV-1 LTR by TR

The LTR controls the transcription of the HIV-1 genome. The important regulatory regions of the LTR are located between -454 and +184, where +1 is the transcription start site. This region contains the TATA box and binding sites for several host transcription factors such as Sp1 and NFkB, etc. (Fig. 2A) (Vaishnav and Wong-Staal, 1991; Pereira et al., 2000). Two bindings sites for TR are present within the LTR. They overlap with the bindings sites for Sp1 and NFkB, respectively (Fig. 2A) (Desai-Yajnik and Samuels, 1993; Rahman et al., 1995; Xu et al., 1996; Hsia et al., 2001). These TREs diverge considerably from the consensus TRE made of two direct repeats of AGGTCA separated by 4 bp. Consistently, TR/RXR heterodimers bind to these TREs with much lower affinities than to a consensus TRE (Fig. 2B), with the TRE at the Sp1 binding sites (Sp1-TRE) as the stronger one for TR/RXR than the one at the NFkB binding sites, i.e., NFkB-TRE (Hsia et al., 2001). Interestingly, TR by itself binds with similar affinities to the HIV-1 Sp1-TRE and a consensus TRE (Fig. 2B). In addition, TR binds to the Sp1-TRE with equal or slightly higher affinity than TR/RXR (Fig. 2B). Thus, while TR/RXR may be the preferred form to bind to a consensus TRE, both TR and TR/RXR may function similarly at the HIV LTR promoter.

Regulation of the LTR-1 by TR in the context of chromatin in vivo

The ability of TR to bind to the LTR in vitro suggests that LTR is regulated by TH. This was first demonstrated in transient transfection studies by several

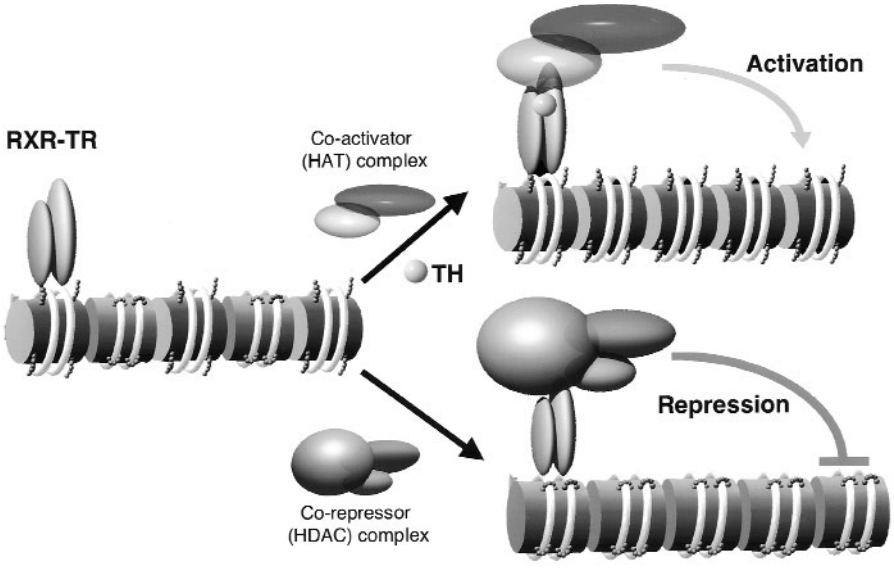
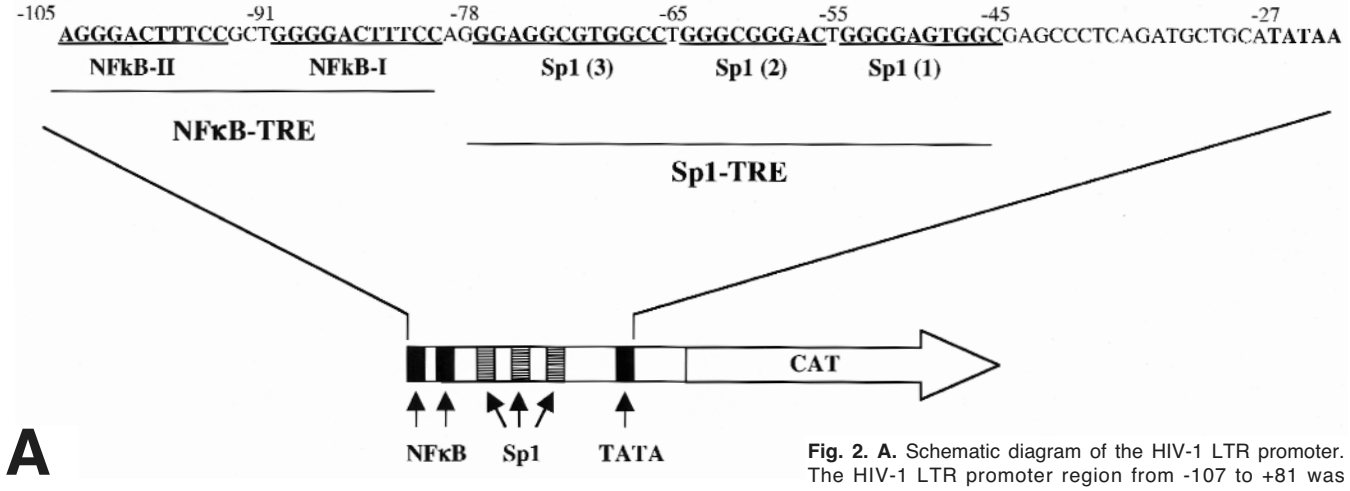


Fig. 1. A model for transcriptional regulation by TR. TR functions as a heterodimer with RXR. In the absence of TH, the heterodimer represses gene transcription through the recruitment of an HDAC complex containing a TR-interacting corepressor such as N-CoR or SMRT. This leads to histone deacetylation and transcriptional repression. TH binding causes the dissociation of the corepressor complex and recruitment of a coactivator complex with HAT activity. This results in an increase in histone acetylation and transcriptional activation. Note that the existence of coactivators lacking HAT activity and ability of liganded TR to disruption chromatin suggest that the actual mechanisms may be more complicated (see text).

groups. Surprisingly, Rahman et al. (1995) reported that unliganded TR activated the LTR while the addition of TH reversed this effect, which contradicted the findings by two other groups (Desai-Yajnik and Samuels, 1993; Xu et al., 1996). Possible explanations include the use of different model systems and/or the LTR was not properly chromatinized in transiently transfected cells. In addition, it is also possible that TR may regulate the LTR indirectly by regulating the expression of other cellular genes since it was not shown if TR was bound to the LTR in vivo.

We have taken advantage of the ability of the frog oocyte to replicate and chromatinize exogenous single-stranded (ss) DNA in a process similar to that in normal somatic cells (Almouzni et al., 1990) to study the regulation of the LTR in vivo (Fig. 3A). We microinjected ss DNA containing the LTR promoter into *Xenopus* oocytes that had or had not been pre-injected with the mRNA encoding a TR or mRNAs encoding both a TR and an RXR. Our studies revealed that unliganded TR or TR/RXR repressed the LTR in a chromatin context. The addition of TH reversed this repression and further activated the promoter to a level slightly higher than that observed in the absence of TR/RXR (Fig. 3B) (Hsia et al., 2001). Furthermore, chromatin immunoprecipitation (ChIP) assay using antibodies again the TR or RXR showed that TR was bound to the LTR both in the presence and absence of



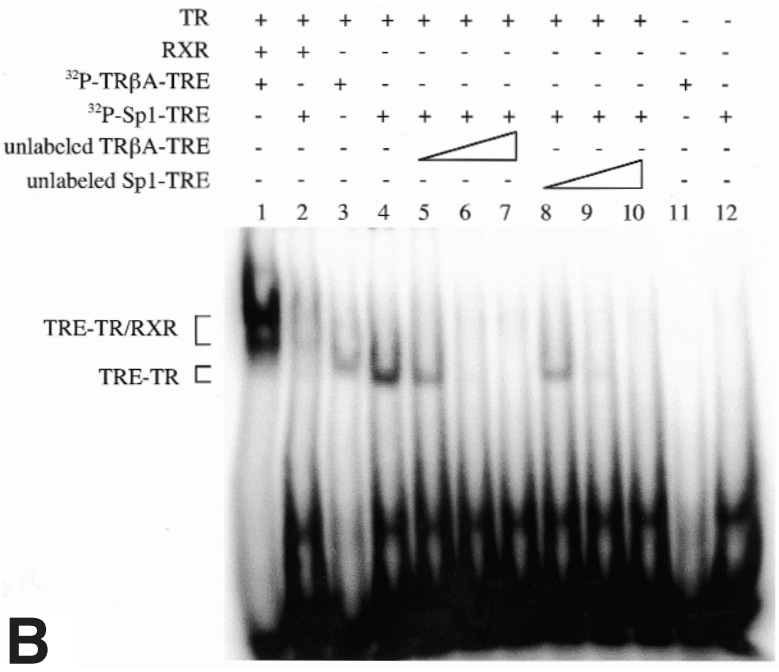


Fig. 2. A. Schematic diagram of the HIV-1 LTR promoter. The HIV-1 LTR promoter region from -107 to +81 was cloned in front of a 300 bp CAT reporter fragment. The promoter fragment included two NFkB sites, three Sp1 sites, and a TATA box. Two TREs are located in the regions containing the NFkB binding sites (NFkB-TRE) and Sp1 binding sties (Sp1-TRE), respectively. The Sp1-TRE is a stronger TRE compared to NFkB-TRE (Hsia et al., 2001). **B.** Both TR and TR/RXR bind to HIV-1 TRE in vitro. TR or TR/RXR overexpressed and purified from *E. coli* was incubated with 32p-labeled double-stranded oligonucleotide corresponding to the Sp1-TRE of the HIV-1 LTR **(A)** in the presence or absence of different concentrations of unlabeled competitor TRE oligonucleotides as indicated and the resulting complexes were analyzed on a native polyacrylamide gel. The TRE of *Xenopus* TRβA gene (TRβA-TRE), a strong, nearly perfect consensus TRE for TR/RXR (Ranjan et al., 1994), was used for comparison in these experiments. Each reaction had 1 ng of labeled TRE and 100 ng TR or TR/RXR in 20 μl. The competitors used were 1X, 2X, and 10X of unlabeled TRβA-TRE or Sp1-TRE for lanes 5-7 or 8-10, respectively. Note that the Sp1-TRE showed a much weaker binding to TR/RXR heterodimer (Lane 2) compared to TRβA-TRE (Lane 1). However, in the absence of RXR, TR bound with similar or slightly higher affinity to Sp1-TRE (Lane 4) compared to TRβA-TRE (Lane 3). Consistently, binding of TR to Sp1-TRE could be competed similarly by both unlabeled Sp1-TRE and TRβA-TRE. In addition, TR/RXR and TR bound to the Sp1-TRE with similar affinities (compared lanes 2 and 4) although TR bound to the TRβA-TRE with much lower affinity than TR/RXR (compared lane 3 and lane 1).

TH or RXR while the binding of RXR to the LTR was dependent on the presence of TR (Hsia and Shi, 2002). Thus, our results indicate TR regulates the LTR directly either alone or as a heterodimer with RXR in the context of chromatin. They further suggest that in HIV-1 infected cells, the LTR is repressed by unliganded TR and can be activated by TH since TR is expressed in the cells susceptible to HIV-1 infection, e.g., T-cells and neurons (Bernal and Andersson, 1984; Oppenheimer et al., 1987).

TH-dependent chromatin remodeling at the LTR

It is well documented that transcriptional regulation is often, if not always, accompanied by the remodeling of the chromatin structure (Svaren and Horz, 1993; Lewin, 1994; Kornberg and Lorch, 1995; Wolffe, 1998; Workman and Kingston, 1998). Among the changes in chromatin structure are the disruption of normal nucleosomal array at the promoter, covalent modifications of the histones, and alteration of acceptability of DNA in the nucleosome (Strahl and Allis, 2000; Narlikar et al., 2002). Transcriptional activation of the HIV-1 LTR in the frog oocyte by TH-bound TR is also associated with extensive chromatin remodeling (Hsia and Shi, 2002). Analyses of the minichromosome of the LTR plasmid generated in the frog oocyte by a partial digestion with micrococcal nuclease (MNase), which preferentially cleaves the internucleosomal linker region, show that the LTR minichromosome has regularly spaced nucleosomes in the absence of TH regardless of the presence or absence of TR (Fig. 4A) (Hsia and Shi, 2002). TH binding to TR leads to the disruption of this normal nucleosomal structure as reflected by the smearing of the DNA ladder (Fig. 4A). This conclusion is also supported by the supercoiling assay, which is a measure of the distribution of the plasmid DNA with different numbers of supercoils after deproteinization as determined on a chloroquine-containing gel. As shown in Fig. 4B (Hsia and Shi, 2002), TR binding to the LTR alone does not alter the supercoiling pattern while liganded TR causes a loss of 2-3 negative supercoils. As each nucleosome in its normal configuration generates one negative supercoil after deproteination, it appears that the binding of TR to the LTR leads to changes that are equivalent to the loss of 2-3 nucleosomes. The nature of the chromatin disruption is yet unclear. It may be due to either the loss of nucleosomes from the minichromosome or changes in the conformation of the minichromosome or a combination of both.

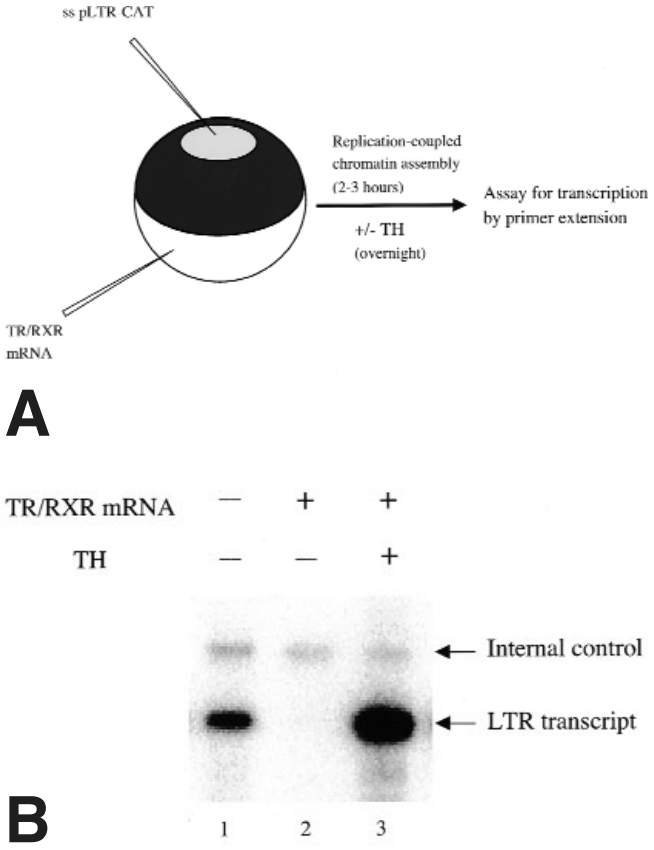


Fig. 3. A. Schematic diagram for analysis of promoter function in the frog oocyte. The single stranded (ss) promoter construct pLTR-CAT (Fig. 2A) is injected into the nucleus while the mRNA for TR or TR/RXR is preinjected into the cytoplasm. The ss DNA is replicated and assembled into chromatin concurrently in the presence of overexpressed TR/RXR within 2-3 hr, mimicking that in somatic cells. After overnight incubation in the presence or absence of TH, the oocyte was harvested for transcriptional analysis by primer extension. **B.** TR/RXR represses the LTR in the absence of TH but activates it when TH is present. The experiment was done as described in A. Similar result was obtained when only TR was overexpressed in the oocyte (Hsia and Shi, 2002).

In addition to the gross structural changes, the ability of TR to recruit HDAC or HAT complexes suggests that histone acetylation may change in response to TH. ChIP assays with antibodies against acetylated histones H3 and H4, the corepressors N-CoR and SMRT, and the HDAC Rpd3 indicate that unliganded TR binding to the LTR minichromosome in the frog oocyte recruits the corepressors and Rpd3 to the LTR (Fig. 5) (Hsia and Shi, 2002). This is accompanied by deacetylation of both H3 and H4 at the LTR (Fig. 5) (Hsia and Shi, 2002). The addition of TH dissociates the corepressors and restores histone acetylation at the LTR. The role of histone acetylation in the TR-regulation of the LTR is further supported by the ability of the specific HDAC inhibitor trichostatin A (TSA) to activate the LTR repressed by unliganded TR (Hsia and Shi, 2002).

Conclusions

A number of studies have implicated a role of TH in the development and progression of AIDS and ARC by regulating the HIV-1 transcription from the LTR. The frog oocyte system has allowed for the first time to demonstrate that TR binds to the LTR in a chromatin context in vivo. The binding of unliganded TR to the

LTR either by itself (monomer or homodimer) or as a heterodimer with RXR recruits corepressors and at least one HDAC, leading to histone deacetylation and transcriptional repression. Liganded TR, on the other hand, causes chromatin disruption and histone acetylation, leading to transcriptional activation. These findings are consistent with the mechanisms of TR action based on studies of other TH-inducible promoters and indicate an important role for chromatin in LTR activity. The involvement of chromatin remodeling in LTR regulation is unlikely to be limited to TR. In fact, histone acetylation has been shown to alter DNA accessibility to nucleases and enhance the activity of the HIV-1 LTR assembled into chromatin in vitro in the absence of TR and RXR (Sheridan et al., 1997; Steger et al., 1998). A future challenge is to determine how the various processes such as gross chromatin reorganization/disruption, histone acetylation, and interactions with the transcriptional machinery, are integrated to effect the transcription from the LTR. An understanding of how various host and viral factors cooperate at the LTR in the chromatin context in vivo will be valuable in helping to design effective approaches to inhibit or reduce the transcription from the LTR under different physiological conditions, thus

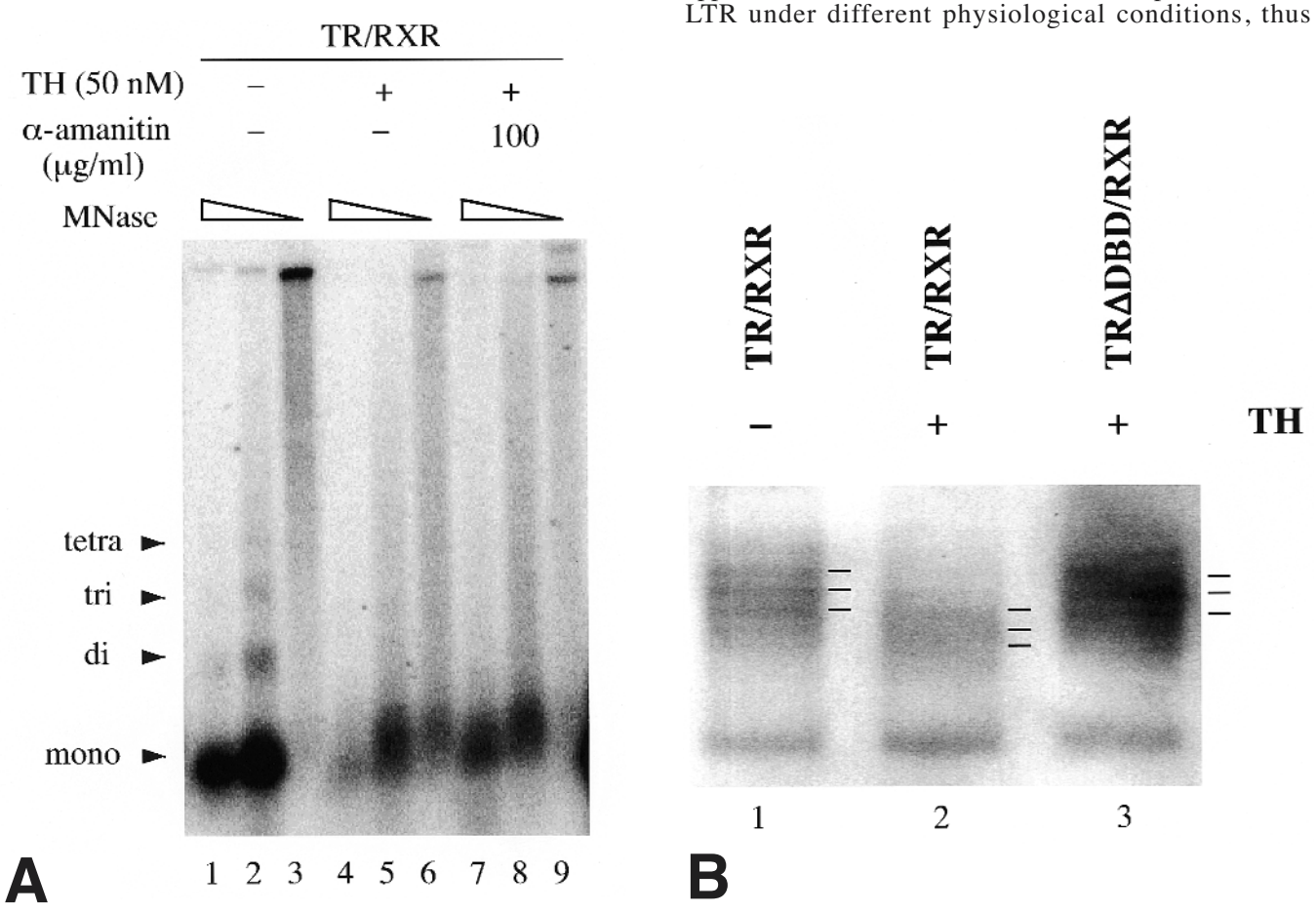


Fig. 4. Liganded TR disrupts chromatin at the LTR independent of transcriptional elongation. **A.** Micrococal nuclease (MNase) digestion assay reveals that liganded TR disrupts the ordered nucleosome array on the LTR plasmid. Occytes were injected and treated as in Fig. 3A except in the presence or absence of 100 μg/ml α-amanitin. After overnight incubation, the oocytes were harvested for MNase digestion assay with increasing amounts MNase (0.16, 0.8, and 4 units, respectively). The digested DNA was purified and analyzed by Southern blot analysis with a labeled LTR probe. Note that in the absence of TH (lanes 1-3) or TR/RXR (not shown), an ordered nucleosomal array was present on the LTR plasmid as indicated by the presence of the mono-, di-, tri-nucleosome bands, etc. In the presence of TH and TR/RXR, this ordered structure was disrupted as indicated by the presence of a smear instead of discrete oligonucleosomal bands (lanes 4-6). Blocking transcriptional elongation with α-amanitin had no effect on the TH-induced chromatin disruption (lanes 7-9). **B.** DNA topology analysis demonstrates that changes in LTR chromatin structure induced by liganded TR/RXR requires direct binding to the LTR. The oocytes were injected with the LTR plasmid and mRNAs encoding RXR and TR or a mutant TR lacking the DNA binding domain (TRΔDBD) (Puzianowsak-Kuznicka et al., 1997). After overnight incubation in the presence or absence of TH, the LTR plasmid DNA was isolated for supercoiling assay. After electrophoresis on a chloroquine-containing gel to separate the DNA with different number of negative superhelical turns (the higher the negative superhelical turns, the slower the DNA migrated on the gel), the DNA was detected by Southern blot hybridization with a labeled LTR probe. Note that average number of the negative superhelical turns (see those marked by bars) was reduced by 2-3 when both TR/RXR and TH are present (compare lanes 2 to 1). Deleting the DNA binding domain (TRΔDBD) abolished this disruption (compare lanes 3 to 1).

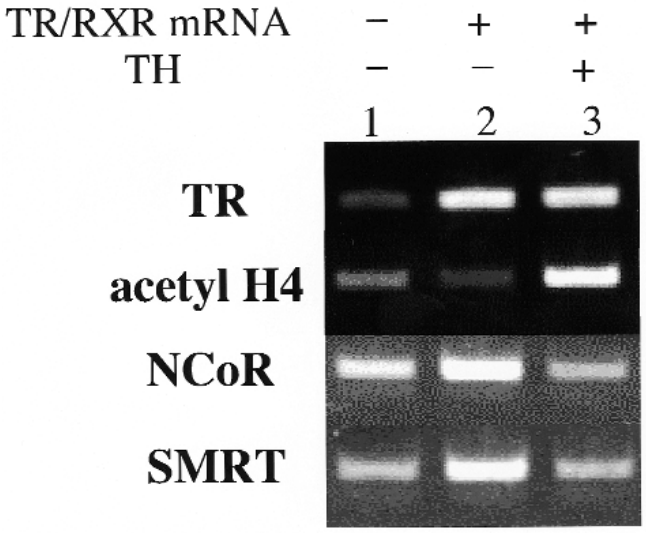


Fig. 5. TH-dependent cofactor recruitment to HIV-1 LTR and changes in histone acetylation. A LTR plasmid was microinjected into oocytes with or without preinjection of mRNAs encoding TR and RXR. After overnight incubation in the presence or absence of TH, ChIP assays were performed for the LTR by using antibodies antibodies against *Xenopus laevis* TR, N-CoR, and SMRT or acetylated histone H4 on the LTR minichromosome. Note that TR binds to the LTR constitutively. In the absence of TH, N-CoR and SMRT are recruited to the LTR, accompanied by histone deacetylation at the LTR (compare lane 2 to Lane 1). TH treatment eliminates this recruitment (compare lane 3 to lanes 2 and 1) and restores histone acetylation. See (Hsia and Shi, 2002) for more details.

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