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Chromatin remodeling and developmental gene regulation by thyroid hormone receptor

Review Article

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Summary

Thyroid horruone (TH) receptors (TRs) are dual function transcription factors. They activate or repress transcription in the presence or absence of TH, respectively. Using the *Xenopus laevis* oocyte as an in vivo system to assemble TH target promoters into chromatin under conditions mimicking somatic cells, we have shown that transcriptional repression by unilganded TR involves histone deacetylase while transcriptional activation by TH-bound TR leads to chromatin disruption. Using *Xenopus laevis* development as a developmental model, we have demonstrated that TR is constitutively bound to its target genes in chromatin. Transcriptional activation induced by TH is accompanied by the release of at least one histone deacetylase and increase in local histone acetylation. These studies together with the developmental expression profiles of TR genes suggest that TH-induced changes in chromatin remodeling play an important role in the dual functions of TR in frog development: gene repression in premetamorphic tadpoles when TH is absent and gene activadon during metamorphosis, a process induced by the endogenously synthesized TH.

I. Introduction

Thyroid hormone (TH) plays important roles during development (Shi, 1999) In humans, TH tectable in the embryonic plasma by 6 months rises to high levels around birth (Tata, 1997) is postembryonic period, extensive tissue and organogenesis take place. TH deficiency during human development leads to developmental, such as mental retardation, short stature, and in the e form, cretinism (Hetzel, 1989; Shi, l999)Likewise, TH is critical for amphibian development. In fact, anurans depend upon TH to develop into adult, frogs (Dodd and Dodd, 1976; Shi, 1999) endogenous synthesis of TH leads to the f giant tadpoles that cannot metamorphose ion of exogenous TH to premetamorphic tadpoles causes precocious metamorphosis. Furthermore, most, if not all, organs are genetically predetermined to undergo specific changes and these changes are organ autonomous. Such properties have made anuran metamorphosis one of the best-studied postemhryonic developmental process at morphological, cellular, and biochemical levels and paved way for current molecular investigations of the underlying mechanisms. Here we summarize some recent advances from studies in *Xenopus laevis.*

II. Chromatin remodeling by TRs

The biological effects of TH are mostly, if not entirely, mediated by thyroid hormone receptors (TRs) TRs belong to the superfamily of nuclear hormone receptors, with two subfamilies of TRs in vertebrates, TRα and TRβ. TR can be divided roughly into 5 domains, A/B, C, D, E, and F, respectively, from the amino- to carboxyl-terminus (Krust et al, 1986) The DNA binding

domain (domain C) is located in the amino half of the protein and is the most highly conserved domain among different receptors of the supeifamily. The large ligand binding domain (domain F) is in the carboxyl half of the protein and is conserved among TRs in different species. The other domains vary in sizes and sequences among different nuclear receptors. The N-terminal A/B domain is highly variable in sequence and length, the shortest being the TRs in *Xenopus laevis* (Yaoita et al, 1990) At least in some TRs, this domain contains a transactivation function (AF), although its role in amphibian TRs is unclear. Another transactivation function domain is the AF-2 domain, which is located at the very C-terminus (F domain and part of the E domain)

TH can both up- and down-regulate gene expression in target tissues or cells. The vast majority of the known TH response genes are up-regulated by the hormone and most studies of receptor function have been on these up-regulated genes. The discussions here focus only on the mechanisms for this class of genes.

Transcriptional activation by TH requires the binding of TRs, most likely as heterodimers with RXRs (9-cis retinoic acid receptors), to TREs (TII response elements) present in the regulatory regions of the TH-response genes. The binding of TREs by TR/RXR heterodimers is, however, independent of TH both in solution and in chromatin (Perlman et al, 1982; Wong et al, 1995) In the absence of TH, TR/RXR represses transcription of target promoters, while in the presence of TH, TR/RXR enhances transcription from these same promoters (Fondell et al, 1993; Tsai and O'Malley, 1994; Wong et al, 1995)

A. Chromatin disruption by liganded TR/RXR

Most of the functional studies of hormone receptors have been carried out *in vitro* or by transient transfection experiments in tissue culture cells. However, genomic DNA in eukaiyotic cells is associated with histones and other nuclear proteins and assembled into chromatin. Thus, to understand the mechanism of TR action, it is important to use properly chromatinized templates.

We have made use of the ability of *Xenopus* oocyte to assemble exogenous DNA into chromatin (Almouzni et al, 1990) to investigate the mechanism of TR action. When single-stranded plasmid DNA is injected into a frog oocyte nucleus, it is quickly replicated (1-2 hr) and assembled into chromatin in a replication-coupled chromatin assembly pathway, mimicking the chromatin assembly process in somatic cells. The resulting template often produces low level of transcriptional activity. In contrast, when double-stranded promoter-containing plasmid DNA is injected into an oocyte nucleus, it is chromatinized more slowly (5-6 hr) with less well defined nucleosome arrays such that the transcnption from the promoter is often at high levels, Thus, by using different forms of promoter-containing plasmid DNA, it is possible to study the transcriptional regulation under different chromatin conditions. *Xenopus* oncytes have little endogenous TR to affect the transcription of a TRE-containing promoter (Wong and Shi, 1995) However, when exogenous *Xenopus* TRs and RXRs are co-introduced into the oocytes by injecting their mRNA into the cytoplasm, they can repress the transcription from both single-stranded and double-stranded DNA containing a TRE (**Figure 1**) (Wong and Shi, 1995; Wong et al, 1995; Hsia et al, 2000) On the other hand, maximal regulation by TH occurs when the single-stranded DNA is used. This is mainly due to more effective repression of the promoter by unliganded TR/RXR during replication-coupled chromatin assembly process (Wong et al, 1995) We have used two independent assays to investigate the effects of TR/RXR on chromatin structure (Wong et al, 1997a) These are the plasmid DNA supercoiling assay for measuring nucleosomal density andlor DNA wrapping conformation in the plasmid minichromosome, and the micrococcal nuclease digestion assay for determining the nucleosomal array stmcture of the plasmid minichromosome**.**

Both assays have shown that the binding of TR/RXR alone deacetyla has little effect on the gross chromatin structure. On the by unlig other hand, the addition of TH to TR/RXR-containing (**Figure 1**), templates causes the disruption of the ordered chromatin. Furthermore, this chromatin disruption occurs even when transcription elongation is blocked. Thus, TH-bound leads TR/RXR heterodimers can disrupt chromatin structure through an active process, although the nature of the disruption is yet unclear.

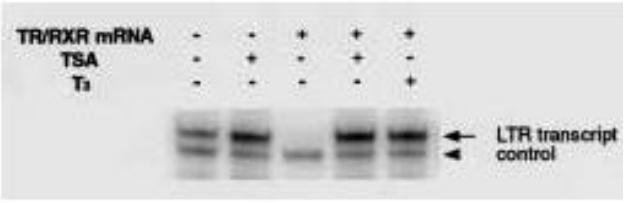

Figure 1. Histone deacetylases is involved in transcnptional repression by TR. A double-stranded plasmid (pHL10) containing HIV- 1 promoter, which is regulated by TH (Hsia et al, 2000), was microinjected into frog oocytes with or without prior injection of TRβ/RXRα mRNAs. The injected oncytes were treated with or without 5 ng/ml TSA or 50 nM T_3 as indicated and the promoter activity was analyzed by primer extension. Note that the addition of TSA activated the promoter slightly. The presence of unliganded TR/RXR repressed the promoter activity. The addition of either T_3 or TSA reversed the inhibition and further activated the promoter, supporting a role of histone deacetylase in the repression by unliganded TR/RXR. The plasmid pCMV-CAT containing a cytomegatovirus promoter driving the expression of CAT reporter gene was used as an internal control (Kass et al, 1997)

B. Regulation of histone acetylation levels through histone acetyltransferases and deacetylases

Both transcriptional repression by unliganded TRs and activation by TH-bound TRs involve TR-interacting cofactors (Chen and Li, 1998; McKenna et al, 1999; Xu et al, 1999; Rachez and Freedman, 2000) Many such factors have been isolated based on their ability to interact c with TRs in the presence or absence of T_3 or under both conditions. The corepressors bind preferentially or exclusively to unliganded TR while the coactivators generally require TH for binding to TR.

Interestingly, the corepressors appear to form multimeric complexes containing histone deacetylases while many coactivators themselves are histone acetyltransferases or acetylases (McKenna et al, 1999; Xu et al, 1999; Burke and Baniabmad, 2000; Hu and Lazar, 2000; Urnov et al, 2000) Our studies have suggested the existence of multiple corepressor complexes, both with and without histone deacetylase activity, in the frog oocyte (Jones et al, unpublished data) This raises the possibility that histone acetylation status may play a role ii transcriptional regulation by TR/RXR.

Histone acetylation has long been implicated influence gene expression (Allfrey et al, 1964; Wolffe 1986; Struhl, 1998) Histone acetylation occurs at lysine residues on the amino-terminal tails of the histor leading to the neutralization of the positive charges histone tails and reduced affinity toward DNA (Hon al., 1993) Although we have failed to detect any gross changes in chromatin structure under conditions exp to alter histone acetylation levels of plasmid minichromosome (Wong et al, 1998), alteratic histone acetylation levels will likely chang nucleosomal conformation and chromatin access thus influencing transcription.

Indeed, our studies in the oocyte have provided evidence for a role of histone acetylation in promoter activation (**Figure 1**) (Wong et al, 1998; Hsia et al, 2000) First, addition of a specific inhibitor of deacetylase, TSA (trichostatin A), can reverse the repression by unliganded TR/RXR, mimicking the addition of TH (**Figure 1**), indicating the involvement of histone deacetylase in the repression by TR/RXR. Conversely, overexpression of the catalytic subunit of a frog histone deacetylase complex (Rpd3) leads to transcriptional repression of a TH-inducible promoter. This deacetylase-induced repression can be reversed by either TR/RXR in the presence of TH or TSA (Wong et al, 1998)

C. A model for gene regulation by TR/RXR

Although the studies so far are supportive of an important role for histone acetylation in transcriptional activation, other pathways are likely involved. First, we have shown that transcriptional activation by liganded TR/RXR leads to chromatin disruption but over-expression or blocking the function of histone deacetylases has no such effect despite dramatic influences on transcription. In addition, many cofactors can interact with the transcriptional machinery directly (Burke and Baniahmad, 2000; Hu and Lazar, 2000; Rachez and Freedman, 2000) Finally, at least one coactivator complex, the DRIP/TRAP complex, has no histone acetyltransferase activity but can activate transcription from chromatin templates (Rachez and Freedman, 2000) Thus, transcriptional regulation by TR/RXR is likely to involve a complex, multi-step, multi-component process. A potential model for TR/RXR function is outlined in **Figure 2**. Tn the absence of TH, TR/RXR recruits a corepressor and its associated deacetylase complex to the promoter, leading to histone deacetylation and transcriptional repression. Upon TH binding, the corepressor complex is dissociated and one or more coactivator complexes are recruited to the promoter. This recruitment may lead to increased histone acetylation (Utley et al, 1998; Sachs and Shi, 2000), chromatin disruption, and transcriptional activation.

III. Dual function of TRS in frog development

Four TR genes, two TRα and two TRβ genes, are present in *Xenopus laevis* (**Figure 3**) (Yaoita et al, 1990) The total dependence of anuran metamorphosis on TR offers an opportunity to study TR/RXR function during development. Expectedly, both TRα and TRβ genes are highly expressed luring metamorphosis in *Xenopus* (Yaoita and Brown, 1990; Shi, 1999) In addition, RXR genes are also expressed during metamorphosis (Wong and Shi, 1995) More importantly, the expression of both TR and RXR genes correlates temporally w metamorphosis of individual organs. Thus, high levels of both TR and RXR mRNAs are present in the limb during early sages of metamorphosis (Stage 54-58) when limb morphogenesis takes place. Subsequently as limb undergoes growth with little morphological changes, both TR and RXR genes are down regulated. On the other hand, both TR and RXR genes are upregulated toward the end of metamorphosis (after stage 60), which corresponds to the period of tail resorption. Such correlation argues that TR/RXR heterodimers are indeed the mediators of the controlling effects of TII on metamorphosis in all organs (Shi et al, 1996)

Interestingly, TRα and TRβ genes are differentially regulated dunng development (**Figure 3**) (Yaoita and Brown, 1990) The TRα genes have little expression prior to metamorphosis and are themselves direct TH-response genes (Ranjan et al, 1994; Machuca et al, 1995) Their expression is upregulated by the rising concentration of endogenous TH during metamorphosis (**Figure 3**) In contrast, the TRα genes are activated shortly after the completion of embryogenesis and their mRNAs reach high levels by stage 45 when tadpole feeding begins (**Figure 3**)

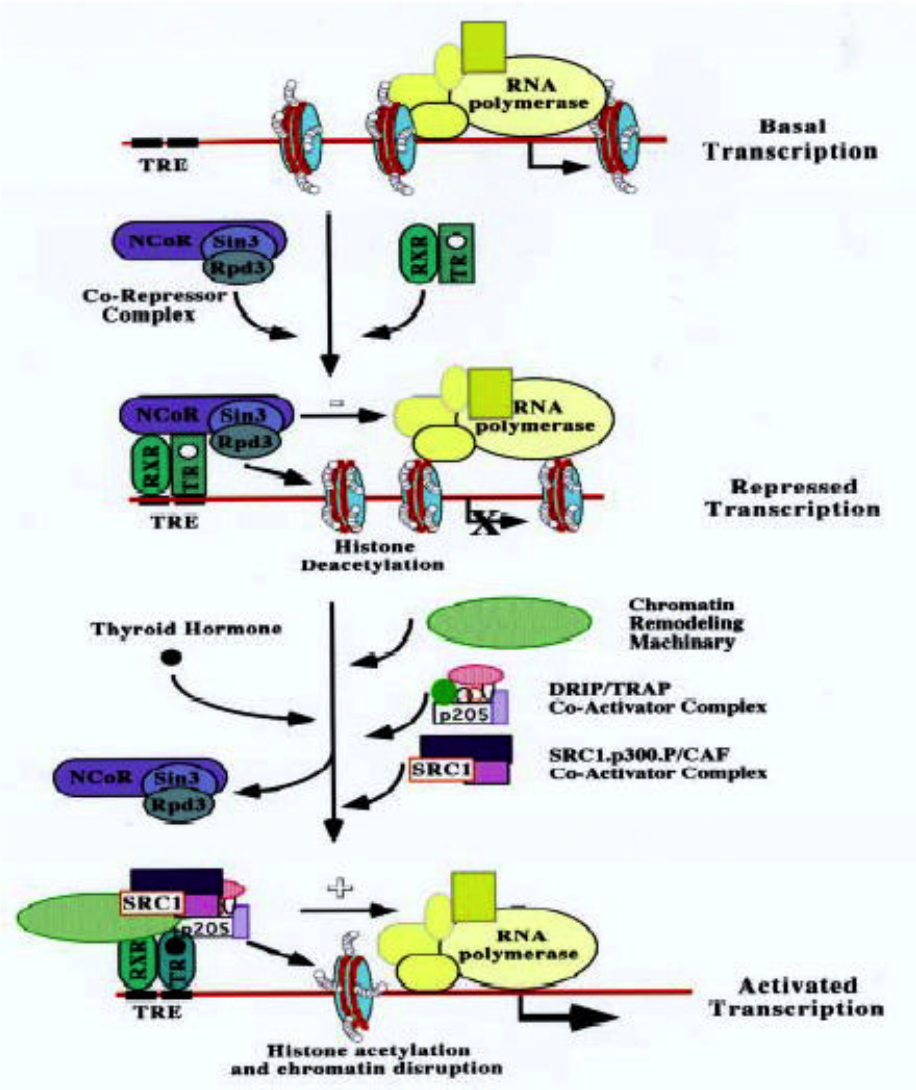

Figure 2. A model for transcriptional regulation by TRs. TR functions as a heterodimer with RXR. In the absence of TH, the heterodimer represses gene transcription through the recruitment of a corepressor complex containing the corepressor such as N-CoR, Sin3A and histone deacetylase such as Rpd3. This leads to histone deacetylation and transcriptional repression. When TH is present, the corepressor complex is released and a coactivator complex containing coactivators such as SRC-l, CBP/p300, and P/CAF, and/or the DRIP/TRAP coactivator complex is recruited. The DRIP/TRAP complex may contact RNA polymerase directly to activate gene transcription. On the other hand, the SRC-l, CBP/p300, and P/CAF complexes may function through chromatin modification as they possess histone acetylase activity. In addition, transcriptional activation is associated with chromatin disruption, which may be due to the recruitment of chromatin remodeling machinery by TR/RXR.

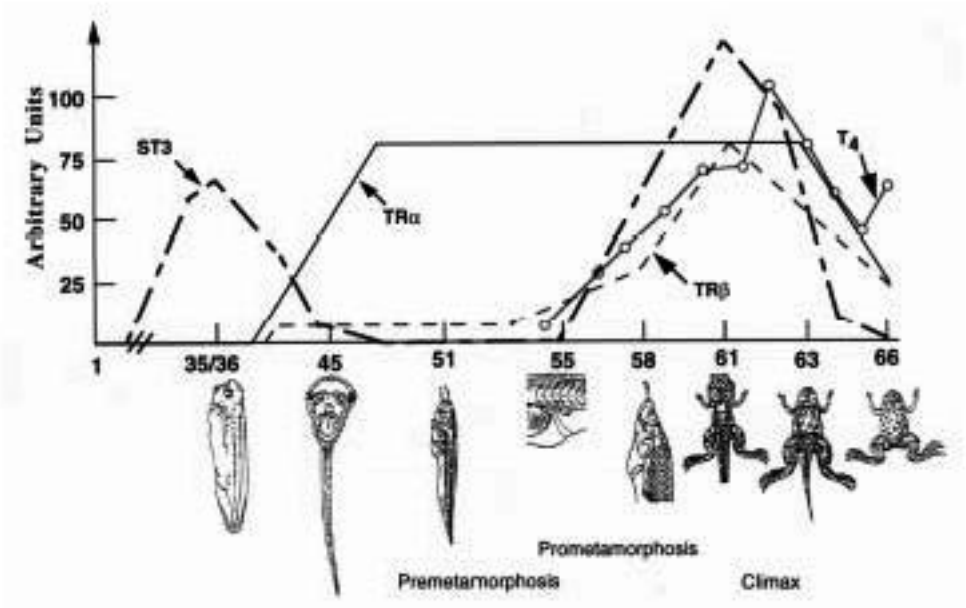

Figure 3. Developmental expression of TR genes suggests dual functions for TR in frog development. The TH-inducible gene stromelysin-3 (ST3) is expressed during late embryogenesis when little TR mRNA is present. As the TRa genes are activated, ST3 is repressed. When endogenous TH levels rise after stage 54 both ST3 and TRβ genes are activated. The TR and RXR mRNA levels are based on (Yaoita and Brown, 1990; Wong and Shi, 1995) The ST3 mRNA levels are based on (Patterton et al, 1995) Thyroid hormone T_4 levels are from (Leloup and Buscaglia, 1977)

The expression profiles together with the ability of TR to both repress and activate TH-inducible genes in the absence and presence of TH, respectively, suggest dual functionsfor TRs during development. In premetamorphic tadpoles, TRs, mainly TRα, act to repress TH-response important also for the gene regulation by TR. Thus, TR/RXR heterodimers function as transcriptional repressors of TH-inducible genes in premetamorphic tadpoles when TR is absent, and as transcriptional activators during metamorphosis when TH is available.

IV. Constitutive DNA-binding and involvement of histone acetylation in developmental gene regulation by TRS

The studies in the frog oocyte and other model systems have provided strong evidence that TR/RXR may regulate gene transcription at least in part by recruiting histone deacetylase or acetylase (acetyltransferase) complexes, depending upon the absence or presence of TH, respectively. To investigate the possible involvement of histone acetylation in gene regulation by TR in vivo, we have treated tadpoles with TH or TSA, a specific drug for blocking histone deacetylases, and analyzed the effect on the expression of TH response genes (Sachs and Shi, 2000) Surprisingly, no detectable upregulation of TH response genes by TSA can be detected in whole animals, although T_3 induces the expression of TH response genes as expected (**Figure 4B**) Since TR-treatment leads to a large array of very different changes in the premetamorphic tadpoles, it is possible that the regulation of TH response genes may be tissue/organ-specific, depending upon the changes in the tissues/organs. Thus, we have chosen the intestine and the tail to investigate the role of histone acetylation further. These two organs are among the few well-characterized organs that undergo extensive remodeling and are known to have the most dramatic upregulation of TH-response genes during metamorphosis. Premetamorphic tadpole intestine consists predominantly of a single tissue, the larval epithelium, which undergoes apoptosis and is replaced by the adult epithelium (Yoshizato, 1989; Shi, 1996) The tail, on the other hand, completely absorbs through an apoptotic pathway (Dodd and Dodd, 1976; Yoshizato, 1989; Shi, 1999) Thus, these two organs offer relatively homogeneous tissues for study tissue specific changes in gene expression and chromatin remodeling. Indeed, our studies on these two organs indicate that TSA induces precocious expression of most TH response genes analyzed, including the only two genes that have been shown to contain TREs (Ranjan et al, 1994; Machuca et al, 1995; Furlow and Brown, 1999), the TRb and TH/bZIP genes **Figure. 4A**) (Sachs and Shi, 2000) On the other hand, TSA had little effect on the expression of TRα genes, which are not direct TH response genes. Thus, these data support the involvement of histone deacetylase in the repression of TH response genes by unliganded TR/RXR.

It has long been known that TR is chromatin-associated in somatic cells (Penman et al, 1982) Furthermore, in the frog oocyte system, we have shown that TR/RXR can bind to TRE both prior to and subsequent of replication-coupled chromatin assembly (Wong and Shi, 1995; Wong et al, 1997a) However, a direct demonstration of TR/RXR binding to the TREs of its target genes is lacking in any developmental system. If TR/RXR indeed functions to repress TH response genes in premetamorphic tadpoles as suggested above, we would expect that they are bound to TREs of endogenous TH response genes independent of TH. To test this possibility, we have made use of the sensitive chromatin irnmunoprecipitation (ChIP) assay using antibodies against TR or RXR (Sachs and Shi, 2000) PCR analysis of the immunoprecipitates for the binding of TR or RXR to the TRE regions of the Xenopus TRb and TH/bZip genes, have demonstrated clearly that both TR and RXR are bound to the TREs in the intestine and tail (**Figure 4C**) (Sachs and Shi, 2000) Furthermore, tbe binding is independent of TH or TSA treatment, in agreement with studies *in vitro* and in the frog oocyte.

The ChIP assay also offers an opportunity to study whether local histone acetylation levels change in response to TH binding to TR/RXR. This has been done by using an antibody against acetylated histone H4 on the two TH response genes (TRb and THIbZip) in *Xenopus laevis* intestine and tail. The results have shown that TH treatment of premetamorphic tadpoles leads to an increase of histone acetylation specifically at the TRE regions of TH response genes (**Figure 5A**) (Sachs and Shi, 2000) without affecting global histone acetylation or the acetylation of chrornatin far away from the TRE (**Figure 5B**) On the other hand, TSA treatment of premetamorphic tadpoles elevates global histone acetylation levels, including the TRE regions of TH response genes. Similarly, ChIP assay using an antibody against the histone deacetylase Rpd3, the only characterized deacetylase in *Xenopts laevis,* demonstrates that Rpd3 is present at the TRE regions of TH response genes and its binding is reduced upon TH treatment of premetamorpbic tadpoles (analyzed in whole animals as Rpd3 was not detectable in prernetamorphic intestine, Sachs and Shi, 2000) Thus, these data together suggest that TR/RXR is bound to TREs assembled into chromatin in vivo. In the absence of TH, TR/RXR recruits histone deacetylase complexes to silence transcnption, at least in the intestine and tail. In the presence of TH, histone deacetylase complexes are released and histone acetylase complexes are likely recruited by TR/RXR, resulting in increased histone acetylation and gene activation.

V. Conclusion

TH regulates a wide range of biological processes across most animal species by influencing gene transcription through TR. The roles of TRα and TRβ in regulating anuran metamorphosis are supported by their temporal and spatial expression profiles during development. Furthermore, these receptors appear to have dual functions depending upon the cell types and developmental stages when they expressed. In premetamorphic tadpoles, they are likely to function as unliganded transcriptional repressors to block the expression of TH response genes that are involved in metamorphosis, thus ensuring a proper period of tadpole growth. When TM becomes available during metamorphosis, it binds to the receptors and converts them into activators to upregulate the TH-inducible genes, thus initiating metamorphosis.

Our studies involving over-expression of TRJRXR in embryos have provided some in vivo evidence that supports the involvement of TR/RXR heterodimers in repressing TM-inducible genes in the absence of TM and in activating them when TM is present. ChIP assays have directly shown that TRs are bound to TREs assembled into chromatin. Furthermore, our data support the model that in the absence of TM, they recruit histone deacetylase complexes to silence transcription at least in some organs/tissues. The binding of TM to chromatin-bound TR leads to local histone acetylation likely due to the release of deacetylase complexes and possible recruitment of acetylase complexes. These findings are also consistent with those from *in vitro* studies and from analyses in the frog oncyte system, where it has been shown that histone acetylation plays an important role in gene regulation by TR and that transcriptional activation by TM leads to additional chromatin remodeling. Thus a model for TR action based on a TM-dependent switch between transcriptional repression and activation involving chromatin remodeling provides one possible molecular mechanism for the dual functions of TRs in development.

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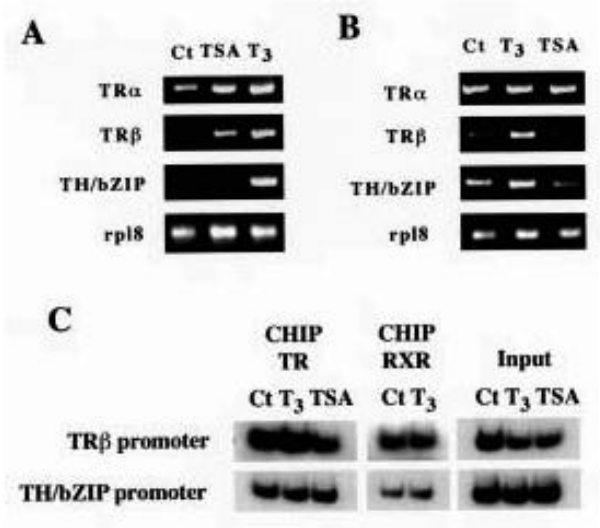

Figure 4. TH and TSA induce transcription of TH response genes in premetasnorphic tadpole intestine. Stage 55 tadpoles were treated for two days with T_3 (10 nM) or TSA (100 nM) **A**) T_3 arid TSA treatments increase TH response gene expression. The intestine was isolated for total RNA extraction. The RNA was used for analysis of TRα, TRβ and TH/bZip mRNA expression by PCR. The expression of ribosomal protein gene rp18 was used as an internal control (Shi and Liang., 1994) Note that TRβ is not a directly TH response gene and is not induced by TH or TSA during the treatment period. **B**) T_3 and TSA treatments do not alter overall mRNA levels of TH response genes in whole ariimals. Total RNA was extracted from whole ariimals arid used for PCR analysis of TRα, TRβ and TH/bZip expression. **C)** TR/RXR binds to TREs in chromatin constitutively. Chromatin from T_3- or TSA-treated stage 55 tadpole intestine was immunoprecipitated with antibodies against TR or RXR arid arialyzed by PCR for the presence of the TRE regions of the two TH response genes in the TR/RXR-bound chromatin fraction. Aliquots of the chromatin prior to immunoprecipitation were used directly in PCR as a DNA control (Input)

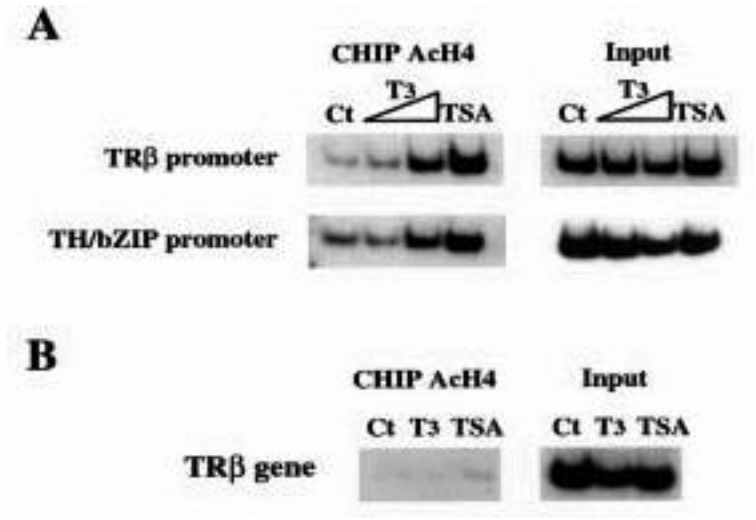

Figure 5. TH treatment increases histone H4 acetylation specifically at the TRE regions of TH response genes in premetamorphic tadpole intestine. Stage 55 tadpoles were treated for two days with T_3 (10 nM) or TSA (100 nM) The intestine was isolated for extraction of the nuclei used for ChIP assay with an antibody against acetylated histone H4. Aliquots of the chromatin prior to immunoprecipitation were used directly in PCR as a DNA control (Input) T_3 and TSA treatment leads to increases in histone H4 acetylation at TH response gene promoters (TRE regions) of both TRβ arid TH/bZIP (A) but not in the transcribed region far from the promoter of TRβ (B)

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