# CELLULAR ACTION OF THYROID HORMONE

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**Revised 14 February 2018**

## ABSTRACT

Thyroid hormones (THs) regulate growth, development, metabolism. This chapter aims to provide a comprehensive overview of the molecular and cellular mechanism(s) for intracellular signaling by TH. At the cellular level, THs bind to thyroid hormone receptors (TRs) that are members of the nuclear hormone receptor family. TRs act as ligand-activated transcription factors that bind to their cognate thyroid hormone response elements (TREs) in the promoters of target genes. TRs regulate gene transcription by employing TR-interacting protein complexes containing coactivators (CoAs) or corepressors (CoRs). Coactivator and corepressor complexes include histone modifying enzymes known as histone acetyltransferases (HATs) or histone deacetylases (HDACs), respectively, that induce epigenetic changes in the chromatin structure of target gene promoters to enhance or repress the transcriptional efficiency of RNA polymerase on TH-responsive genes. TRs also can mediate transcriptional effects indirectly by binding to other transcription factors or activating cell signaling cascades. Additionally, emerging evidence suggests that THs may bind to cell membrane proteins other than TRs to activate cell signaling pathways. The important role of TH on metabolism, has spawned interest the pharmacological use of TH and its analogs/metabolites for the treatment of metabolic diseases such as hypercholestronemia, hypertriglycerimia obesity, and non-alcoholic fatty liver disease (NAFLD).

## INTRODUCTION

The thyroid hormones (THs, thyroxine ($T_4$) and triiodothyronine ($T_3$)) have important effects on development, growth, and metabolism (1-3). Some of the most prominent effects of TH occur during fetal development and early childhood. In humans, the early developmental role of TH is illustrated by the distinctive clinical features of cretinism observed in iodine-deficient areas. In childhood, lack of TH can cause delayed growth. However, in this latter case, many of the effects of TH may be metabolic rather than developmental, as growth is restored rapidly after the institution of TH treatment. In adults, the primary effects of THs are manifested by alterations in metabolism. These effects include changes in oxygen consumption, protein, carbohydrate, lipid, and vitamin metabolism. The clinical features of hypothyroidism and hyperthyroidism emphasize the pleiotropic effects of these hormones on many different pathways and target organs.

At the clinical level, identification of quantitative markers of TH action has been difficult (4). At the extreme ends of the clinical spectrum, which extends from hypothyroidism to hyperthyroidism, the diagnosis of a thyroid abnormality is usually apparent. Clinical suspicion of a thyroid abnormality can be confirmed using laboratory tests for THs and thyroid stimulating hormone (TSH). However, more subtle forms of thyroid dysfunction, such as subclinical hypothyroidism or hyperthyroidism, pose a greater challenge. Although the level of circulating TSH provides a sensitive and quantitative indicator of TH action at the level of the hypothalamic-pituitary axis, there are few reliable peripheral or intracellular markers of TH action (5,6). The effect of TH on basal metabolism has been re-evaluated using measurements of resting energy expenditure (REE). In hypothyroid patients taking varying levels of TH replacement, there is a strong inverse correlation between REE and the TSH level (6). Nevertheless, TSH remains the most sensitive and useful clinical indicator of TH action. As discussed below, tissue-selective metabolism of THs, and variable tissue sensitivity to their effects, underscores the need to develop additional markers of TH activity in peripheral tissues.

Since the initial description of TH effects on metabolic rate more than 100 years ago (7), many theories have been proposed to explain its mechanism of hormone action. The proposed models include: uncoupling oxidative phosphorylation, stimulation of energy expenditure by the activation of Na+-K+ ATPase activity, and direct modulation of TH transporters and enzymes in the plasma membrane and mitochondria (8). Recently, there has been increasing evidence for non-genomic actions (see later under non-genomic actions of TH) (8); however, the major effects of TH occur via nuclear receptors that mediate changes in gene expression.

In 1966, Tata proposed that TH increased gene expression with attendant increases in protein synthesis and enzyme activity (9). In 1972, high affinity nuclear binding sites for TH were documented (Kd approximately 10-10 M for $T_3$) (10,11). The receptor-binding affinity of various THs and analogues correlated with their biologic potencies, consistent with the view that most biologic effects are mediated via the nuclear receptor (11– 14). Over the past 25 years, there has been a dramatic surge of new information on TH action resulting from the cloning of the TH receptors (15,16), the identification of regulatory DNA elements in TH responsive genes (1-3), the generation of TR isoform knockout mice (17,18), and the discovery and phenotype characterization of patients with mutations in TRα and TRβ (5,19,20). In this chapter, we will focus on our current understanding of nuclear TH receptor action.

## BINDING OF THs TO NUCLEAR RECEPTORS

In many respects, $T_4$ can be regarded as a prohormone for the more potent hormone, $T_3$. Most of the TH bound to receptors is in the form of $T_3$, either secreted into the circulation by the thyroid gland or derived from $T_4$ to $T_3$ conversion by 5' monodeiodinases (see Chapter 3C). There are three distinct deiodinases- type I, type II, and type III (21,22). The distribution and regulation of these enzymes can have important effects on TH action. For example, Type II deiodinase has high affinity for $T_4$ (Kd in the nanomolar range) and is found primarily in the pituitary gland, brain, and brown fat where conversion of $T_4$ to $T_3$ modulates the intracellular concentration of $T_3$. Thus, tissues that contain type II deiodinase can respond differently to a given

circulating concentration of $T_4$ (by intracellular conversion to $T_3$) than organs that only can respond to $T_3$ (23,24). Additionally, it appears that both type 1 and type II deiodinase regulate the circulating $T_4$ and $T_3$ levels (25). Recently, MCT8, OATP-1, and System L amino acid transporters have been identified as TH transporters which regulate $T_4$ and $T_3$ uptake into cells (26,27). Mutations in the former have been involved in a number of syndromes of x-linked mental retardation and neurologic deterioration (27,28).

$T_3$ binds to its receptors with approximately 10 fold higher affinity than $T_4$. The dissociation constants for liver nuclear receptors measured in vitro are $2 \times 10^{-9}$ M for $T_4$ and $2 \times 10^{-10}$ M for $T_3$ (1,2). Nuclear receptors are approximately 75% saturated with TH in brain and pituitary and 50% saturated with TH in liver and kidney. It is notable that the extent of TH receptor occupancy varies in different tissues, providing a mechanism for alterations in circulating TH levels to alter receptor activity. In contrast to the related steroid hormone receptors, TRs are mostly nuclear both in the absence and presence of TH (1,2,30). In fact, TH receptors are tightly associated with chromatin (1-3,30), consistent with their proposed role as DNA-binding proteins that regulate gene expression.

## CLONING, STRUCTURE, AND EXPRESSION OF TH RECEPTORS

### Cloning of TRs

TH receptors (TRs) were first cloned in 1986 and belong to the nuclear hormone receptor superfamily that includes the glucocorticoid, estrogen, progesterone, androgen, aldosterone, vitamin D, retinoic acid (RARs), retinoid X (RXRs) and "orphan" (unknown ligand and/or DNA target) receptors (1,2,15,16,30-33). TRs are the cellular homologs of v-erbA, a viral oncogene product involved in chick erythroblastosis. TRs are encoded at two genomic loci (α and β) located on human chromosomes 17 and 3, respectively and their gene products result in two major isoforms, TRα and TRβ .

### Structure of TRs

Like other members of the nuclear receptor superfamily, the TRs have a central DNA-binding domain (DBD) and a carboxy-terminal ligand-binding domain (LBD) (Figure 3d-1). The two major TR isoforms have high amino acid sequence homology in their respective DBDs and LBDs. Dimerization domains also are found in both DBDs and LBDs. The amino-terminal regions are more variable between TRα and TRβ (1-3), and contain ligand-independent activation domains. In contrast, multiple sub-regions are located in the LBD for ligand-dependent transcriptional activation and basal repression of target genes.

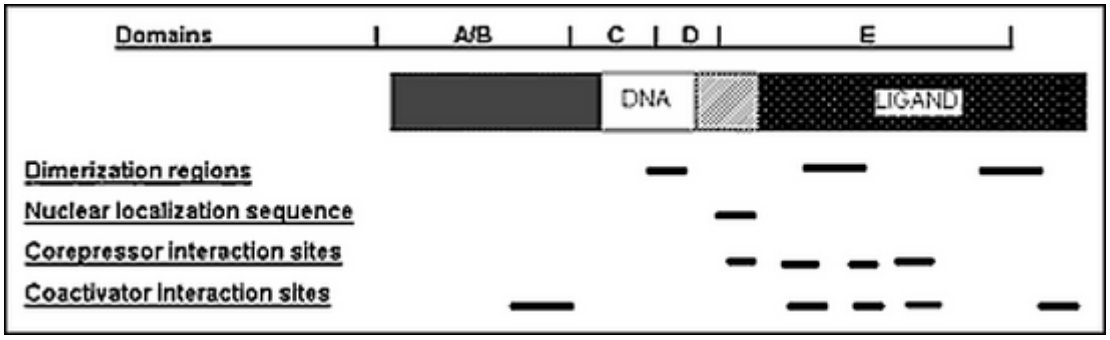


**Figure 1. Functional domains of the TH receptor (TR). The TH receptor (TR) is depicted schematically. The zinc finger DNA-binding domain (DBD) is denoted along with the carboxy terminal ligand-binding domain (LBD). Other functional domains and interaction sites are indicated.**

The DNA-binding domains of the nuclear receptors are comprised of two distinct zinc fingers that are separated by a 15-17 amino acid linker sequence. The crystal structure of the DNA-binding domains of the TH receptor and its heterodimeric partner, RXR has been determined. The two heterodimer partners interact with a direct repeat of the receptor binding site in a head-to-tail manner (34-37).

A small stretch of amino acids at the base of the first finger (referred to as the P-box) dictates the DNA sequence specificity of the receptor (35-39). The P-box sequence of the TH receptor is shared by other receptors that bind to similar or identical DNA recognition sites (AGGTCA). The underlined amino acids in the P-box (EGCKG) of the TH receptor are also found in the retinoic acid receptors, the retinoic acid X receptors, the rev-erbA protein, the vitamin D receptor, and NGFI-B. Of note, the steroid hormone receptors have a different P box sequence and bind as homodimers to a different consensus DNA half-site sequence (AGAACA). The region between the DBD and LBD is called the hinge region and contains the nuclear localization signal, typically a basic amino acid-rich sequence, first described in viral nuclear proteins.

X-ray crystallographic studies of the liganded rat TRα-1 show that TH is embedded in a hydrophobic "pocket" lined by discontinuous stretches of amino acid sequences within the LBD. Additionally, there are several hydrophobic interfaces within the LBD that contribute to the TR homo- and heterodimerization with RXR (39). There are twelve amphipathic helices in the LBD and specific helices among them provide the critical contact surfaces for protein-protein interactions with co-activators and co-repressors (helices 3,5,6,12 and 3,4,5,6, respectively) (40-43). Ligand-binding to TR causes a major conformational change in the LBD, particularly in helix 12. This, in turn, facilitates TR discrimination between co-activators and co-repressors (see below).

**Splicing variants of TRs**

The carboxy-terminal hormone-binding domain of the TRα gene is alternatively-spliced to generate several protein products (Figure 3d-2, below). One variant, referred to as α-2, is identical to TRα-1 through the first 370 amino acids, but then its sequence diverges completely, owing to splicing of alternate exons (44-47). Another splicing variant, referred to as TRvII or α-3, is similar to α-2 except that it lacks the first 39 amino acids found in the unique region of α-2 (45). α -2 cannot bind TH because of the replacement of critical amino acids at the extreme carboxy-terminal end of the protein due to alternative splicing (48), and thus cannot mediate ligand-dependent gene transcription (49– 51). The amino acid replacements in α-2 also alter its dimerization properties and reduce DNA-binding affinity (52-55). The α-2 splicing variant is highly expressed in many tissues such as brain, testis, kidney, and brown fat, but its function remains poorly understood (56). The α-2 isoform has been proposed to be an endogenous inhibitor of TH receptor function as it inhibits TRα and β activity in transient gene expression assays (44,54). The mechanism by which α-2 antagonizes TR action is controversial. Some studies indicate that α-2 competes for active receptor complexes at DNA target sites (57,58). Other studies indicate that α-2 inhibits TR activity independent of DNA-binding (59). It is likely that the inhibitory effects of α-2 involve more than one mechanism. Amino acid substitutions in the carboxy-terminal region of α-2 also prevent its interactions with transcriptional corepressors (see below) (55), and may provide an explanation as to why α-2 is not a more potent inhibitor of TR activity. Additionally, the phosphorylation state of α-2 may modulate its inhibitory activity (60). Given the foregoing features, the TRα-1 and α-2 system represents one of the few examples in mammals whereby multiple mRNAs generated by alternative splicing encode proteins that are antagonistic to each other.

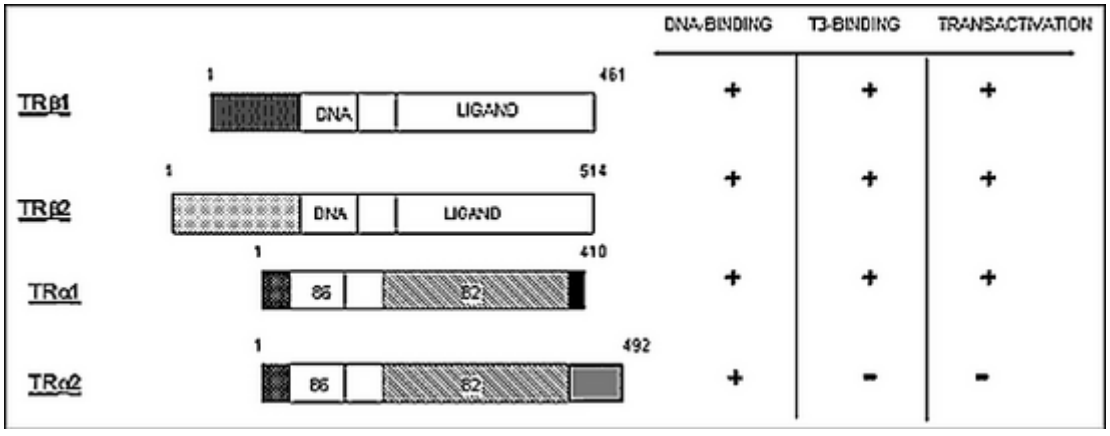


**Figure 2. TH receptor isoforms. The TH receptors (TR) β and α are expressed from separate genes. Each TR gene can be expressed as distinct isoforms, reflecting the use of alternate promoters and exons. The central zinc finger DNA-binding region is indicated and unique domains are shown by distinct patterns of shading. The TRβ-2 isoform, which is expressed predominantly in the pituitary and hypothalamus, contains a unique amino-terminus. The TRα-2 isoform contains unique carboxy-terminal sequences that eliminate hormone binding. The DNA-**

**and $T_3$-binding properties and transcriptional activity of the various isoforms are shown at the right.**

A receptor-like molecule, Rev-erbA, is, surprisingly, encoded on the opposite strand of the TRα gene locus (61, 62). Rev-erbA mRNA contains a 269-nucleotide stretch which is complementary to the α-2 mRNA due to its transcription from the DNA strand opposite of that used to generate TRα-1 and α-2. This protein also is a member of the nuclear hormone receptor superfamily, and is highly expressed in adipocytes and muscle cells. Rev-erbA, contains a DBD that is homologous to the TR DBD. However, Rev-erbA does not bind TH and its putative LBD has minimal homology with other nuclear hormone receptors. Since no cognate ligand has been identified for Rev-erbA, it is categorized as an "orphan nuclear receptor. " It can act as a transcriptional repressor for nuclear hormone receptors and other transcription factors (63-65). Since Rev-erbA shares an exonic segment of the bidirectionally transcribed TRα gene, it is possible that it modulates the expression or splicing of TRα-1 and α-2 (66, 67) as parallel increases in rev-erbA mRNA and TRα-1 mRNA expression occur relative to α2 mRNA expression.

The major variant of the TRβ gene, TRβ-2, has a different amino-terminus than TRβ 1 (Figure 3d-2, above) (68). The distinct amino-terminal region of the TRβ-2 is due to transcription from a tissue-specific promoter. The function of the amino-terminus of the TH receptor is not known, but it likely plays a role in transcriptional control (69,70). The TRβ-1 and TRβ-2 isoforms function similarly in most transient gene expression assays (69, 71), although differences in the transcriptional activities of the TRβ 1 and TRβ 2 isoforms have been noted with respect to certain target genes (69-72). It is likely that tissue-restricted expression of the TRβ-2 isoform contributes to unique patterns of TR expression, which in turn, may modulate target gene regulation.

Recently, short isoforms of TRα and TRβ have been described (73, 74). The novel TRα isoforms arise from translational start sites in the 7th intron and yield shortened TRα 1 and α 2 isoforms that have dominant negative activity on WT TR. Novel short TRβ isoforms arise from alternative splicing of TRβ. It is possible that these isoforms may modulate $T_3$-responsiveness in a tissue- and/or developmental stage-specific manner.

**Tissue- and development-specific expression of TRs**

Most studies of TR isoform expression have employed mRNA analyses rather than protein measurement (30). In general, the α and β receptor isoforms are distributed widely and exhibit overlapping patterns of expression (30,75). TRα 1 mRNA is expressed in skeletal and cardiac muscle whereas TRβ-1 mRNA is predominant in liver, kidney, and brain. A-2 mRNA is most prevalent in brain and testis. In contrast, TRβ-2 mRNA has the most tissue-restricted expression, and is present in the anterior pituitary gland, hypothalamus, and cochlea (75-79).

The TRs also are expressed in specific stages during development, and are subject to regulation by hormones and other factors (78, 79). For instance, TRα-1 mRNA is expressed early whereas TRβ 1 mRNA is expressed later during embryonic brain development. In the rat pituitary gland, TH decreases TRβ 2, TRα-1, and α-2 mRNAs while slightly increasing TRβ-1 mRNA. However, in most other tissues, TH decreases TRα-1 and α-2, but not TRβ-1 mRNA. Isoform-specific knockout mice of each of the TR isoforms display distinct phenotypes (17,18). However, lack of significant TR isoform-specific gene expression was observed in cDNA microarrays of hepatic genes in TR isoform knockout mice (80). Given the apparent redundancy in TR isoform function, it is possible the different KO phenotypes may be due to absolute TR expression levels in critical tissues and developmental stages.

## TRANSCRIPTIONAL REGULATION BY TRS

TH receptors bind to TH response elements (TREs) in specific target genes (Figure 3d-3, below). After binding TH, the receptor induces changes in gene expression by either increasing or decreasing the transcriptional activity of target genes. Examples of the target genes that are positively- and negatively-regulated by TH are summarized in Table 1. cDNA microarrays have been employed to study TH regulation of hepatic genes in mice, and led to the identification of a large number of novel target genes (both positively- and negatively-regulated) (81,82). These studies demonstrated that TH affected gene expression in a wide range of cellular pathways and functions, including gluconeogenesis, lipogenesis, insulin signaling, adenylate cyclase signaling, cell proliferation, and apoptosis. Although many of the TH-responsive genes were regulated directly by TRs, others were probably regulated indirectly through intermediate genes. Indirect regulation of TH-mediated transcription is suggested when the time course for induction is slow (hours) and when it is blocked by protein synthesis inhibitors. Although TH acts mainly at the level of transcription, it also can affect mRNA stability, translational efficiency, and miRNA regulation (83,84). Thus, TH acts at multiple levels to alter protein expression.

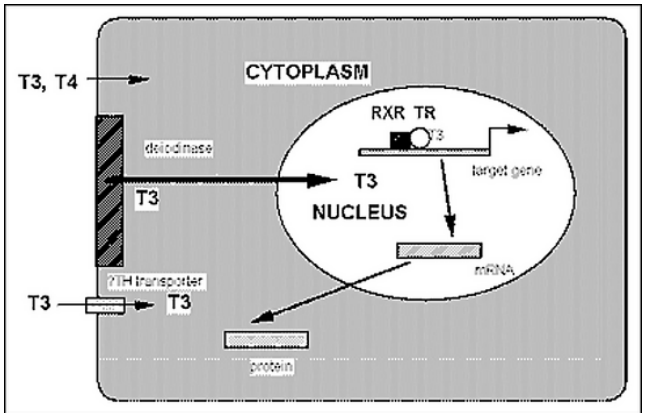


**Figure 3. Mechanism of TH action via its nuclear receptor. TH is transported across plasma membrane and likely diffuses through nuclear membrane to bind to its receptor. The TH receptor (open circle) is localized almost exclusively in the nucleus where it associated with DNA as a homodimer or as a heterodimer with RXR (stippled box). The**

**hormone-activated receptor binds to TH response elements (TREs) to alter rates of gene transcription and consequently levels of mRNA.**

**Table 1. Examples of Genes Positively-regulated by T3.**

1. **Fatty acid synthetase**
2. **Growth hormone**
3. **Lysozyme silencer**
4. **Malic enzyme**
5. **Moloney leukemia virus enhancer**
6. **Myelin basic protein**
7. **Myosin heavy chain α**
8. **Phosphoenolpyruvate carboxykinase**
9. **RC3**
10. **Spot 14 lipogenic enzyme**
11. **Type I 5'-deiodinase**
12. **Uncoupling protein**

**Table 2. Examples of Genes Negatively-regulated by $T_3$.**

1. **Epidermal growth factor receptor**
2. **Myosin heavy chain β**
3. **Prolactin**
4. **Thyroid-stimulating hormone α**
5. **Thyroid-stimulating hormone β**
6. **Thyrotropin-releasing hormone**
7. **Type II 5'-deiodinase**

**TR binding to TH response elements (TREs)**

Detailed analyses of thyroid response elements (TREs) have led to the identification of a canonical TRE half-site sequence (1, 2,85) (Figure 3d-4). The TRE half-site is generally considered to be a hexamer (AGGTCA), but TR binding is optimal with a more extended binding site (37,88,89).

Specifically, the sequence TAAGGTCA is optimal for TR binding and $T_3$-responsiveness. However, inspection of TREs from many different target genes reveals there is a relatively low degree of sequence conservation among these elements. This finding suggests the possibility that naturally-occurring TREs may have diverged from an ideal consensus element during evolution as a means to modulate the degree of TH responsiveness.

TR interactions with DNA are quite different from those observed with steroid receptors, which bind to palindromic DNA sequences as homodimers. Although TR also can bind to certain TREs *in vitro* as a homodimer, it binds preferentially to most TREs as a heterodimer with the retinoid X receptors (RXRs) (1-3,30). The TR-RXR heterodimer binds to half-sites that are arranged in several different configurations. These include palindromic arrangements (head-to-head), direct repeats (head-to-tail), and inverted repeats (tail-to-tail). Most naturally occurring TREs are direct repeats (Figure 3d-4), typically separated by four nucleotides. The ability of TR dimers to bind to TRE's in different configurations suggests a flexible protein structure, or the possibility that distinct protein surfaces are involved in the formation of dimers (34,39,90). Taken together, the specificity and affinity for the TR-RXR heterodimer is primarily determined by sequences within the half-site, the length of the spacer region between the half sites, and the sequence context within the spacer region.

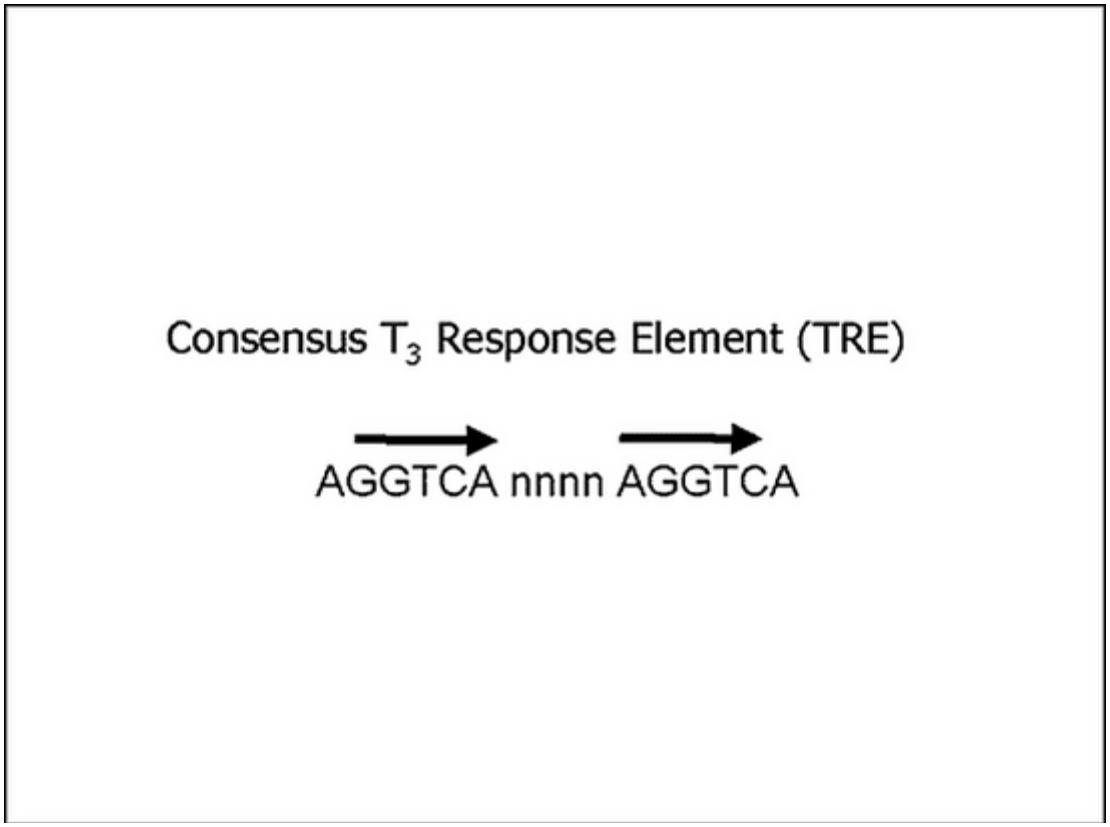


**Figure 4. Consensus thyroid response element (TRE). Studies of TRE's in many different promoters has allowed the derivation of a**

**"consensus" TRE comprised of a direct repeat of the hexameric sequence, AGGTCA, spaced by four nucleotides (n). Of note, there is considerable diversity in the sequences of half-sites, orientation of half-sites, and bases that form the spacers between half-sites (see text).**

Although TR can interact with a wide variety of other nuclear receptors and transcriptional adaptor proteins (see below), the RXR proteins (α, β, and g) represent its most important heterodimeric partners (1-3). The RXR proteins enhance TR binding to DNA and reduce the rate of receptor dissociation from DNA (91). RXR binds to the 5' sequence and TR binds to the 3' sequence of TREs in which half-sites are arranged as direct repeats (92, 93). The DNA-binding domains interact with the major grooves of the half-sites on the same face of the DNA (34,92,93). The carboxy-terminal end of the TR DNA-binding domain forms an α -helical structure that interacts with the spacer region in the DNA minor groove between the TRE half-sites. Although protein-protein contacts between the RXR and TR DNA-binding domains are important for dimerization, the major sub-regions involved in dimerization reside in the carboxy-terminii of the receptors (34). The dimerization surface of the TR appears to involve residues that lie along the surfaces of helices 10 and 11. $T_3$ binding enhances the formation of TR-RXR heterodimers (94). On the other hand, $T_3$ dissociates TR-TR homodimers (95). These findings raise the possibility that $T_3$ binding might induce disruption of TR homodimers and induce the formation of TR-RXR heterodimers. The RXRs bind a stereoisomer of all trans retinoic acid, 9-cis retinoic acid (96,97). which variably alters transcriptional activity depending on the nature of the TH responsive gene (1,2). Additional studies are required to clarify the functional roles of RXRs and their ligands in TH action and interaction with other transcription factors. Recently, Hollenberg and colleagues ~~recently~~ analyzed the hepatic TRβ cistrome of hyper- and hypothyroid mice using Chip-seq technology (98). They found that the majority of TRβ-1 binding sites were not in the proximal promoter region but in other portions of target genes. Interestingly, by comparing the TR binding sites with previous Chip-seq data for RXRa, they found that some target genes may be regulated by TR homodimers rather TR/RXR heterodimers. Additionally, $T_3$ increased TRβ-1 binding to DNA sites that, in turn, was correlated with $T_3$-induced gene expression. DR-4 and DR-0 motifs were significantly enriched at the DNA binding sites where $T_3$ increased or decreased in TRβ1 binding, and were associated with positive and negative transcriptional regulation by $T_3$. Interestingly, in another study using Chip-chip methodology (99), some TH-regulated genes were identified that had little TR binding, despite the presence of putative TREs suggesting that other mechanisms such as receptor cross-talk, non-genomic effects, or indirect signaling mechanisms (see below) may be involved in regulating these genes.

TRs can have cross-talk with other nuclear hormone receptors owing to their common abilities to heterodimerize with RXRs. TR crosstalk with peroxisome proliferator-activated receptor (PPAR) and LXR signaling via heterodimerization with RXR is a prominent example. PPARg regulates the expression of its target genes by binding to the PPAR response element (direct repeat 1; DR1) as a heterodimer with RXR. Recently, it was shown that TRβ1 competes with PPARg for binding to DR1 as a heterodimer with

RXR *in vitro* and *in vivo* to repress the transcriptional activity of PPARg (100). Since PPARg plays a key role in lipid metabolism, carcinogenesis, and cardiovascular diseases (101.102), this mode of TR may exert some of its effects by crosstalk with PPARs. Recently, cell-based studies indicate that TRβ inhibits the activity of LXR-α transcription activity of the CYP7A1 promoter which shares a common DR-4 element with TR (103). These studies show that TR cross-talk with other nuclear hormone receptor-mediated signaling expands TR effects beyond those target genes directly regulated by TRs (104).

**Basal repression/Transcriptional corepressors**

After binding to DNA, TR alters transcriptional activity by interacting directly or indirectly with a complex array of transcriptional cofactors. These proteins include corepressors (CoRs), coactivators (CoAs), integrators like CREB-binding protein (CBP), and general transcription factors (GTFs) (reviewed in 1-3, 31). Many of these factors have been identified by protein-protein interaction assays such as the yeast two-hybrid and glutathione-s-transferase pull down assays.

In the absence of TH, TR represses basal transcription in proportion to the amount of receptor and the affinity of receptor binding sites in positively-regulated target genes.This phenomenon also is referred to as transcriptional silencing (105-108). (Figure 3d-5, below). The addition of TH reverses basal repression and increases transcriptional activation above basal levels seen in the absence of receptor. Our understanding of the molecular mechanism for basal repression of transcription by unliganded receptor was advanced significantly by the discovery of a family of repressor proteins that bind selectively to unliganded TRs and RARs. This corepressor family includes silencing mediator for retinoid and TH receptors (SMRT) and nuclear receptor corepressor (NCoR) (105,109,110). These corepressors are 270 kD proteins that contain three transferable repression domains and two carboxy-terminal α -helical interaction domains. They are able to mediate basal repression by TR and RAR, as well as orphan members of the nuclear hormone receptor family such as rev-erbAα and chicken ovalbumin upstream transcription factor (COUP-TF). They have little or no interaction with steroid hormone receptors and therefore do not mediate basal repression by these receptors. Another protein, small ubiquitous nuclear co-repressor (SUN-CoR) enhances basal repression by TR and rev-erbA (31). This 16kD protein may form part of a co-repressor complex as it interacts with NCoR.

Within the interaction domains of NCoR and SMRT are consensus LXXI/HIXXXI/L sequences which resemble the LXXLL sequences that enable co-activators to interact with nuclear hormone receptors (40-42) (see below). Interestingly, these motifs allow both corepressors and co-activators to interact with similar amino acid residues on helices 3, 5, and 6 which are part of the ligand-binding pocket of TR. Differences in the length and specific sequences of the co-repressor and co-activator interaction sites coupled with the conformational changes in the LBD upon ligand binding, determine whether corepressor or coactivator binds to TR.

Recently, it has been shown that corepressors can form a complex with other repressors such as Sin 3 and histone deacetylases that are mammalian homologs of well-characterized yeast transcriptional repressors RPD1 and RPD3 (1-3,31). Thus, local histone deacetylation likely plays a critical role in the basal repression by unliganded TR/corepressor complex by maintaining local chromatin structure in a state that decreases basal transcription. Upon $T_3$ binding, TR undergoes a conformational change that dissociates CoRs and recruits an array of coactivators (CoAs). Thus, hormone binding relieves repression and stimulates transcription by altering receptor binding to distinct classes of cofactors. Additionally, DNA-methylation may play a role in basal repression as methyl-CpG-binding proteins can associate with a co-repressor complex containing Sin3 and histone deacetylases (111,112). This repression was relieved by the deacetylase inhibitor, trichostatin A. These findings suggest that two repression processes, DNA methylation and histone deacetylation, may be linked via methyl-CpG-binding proteins.

The fact that TR alters the level of gene transcription in both the absence and presence of $T_3$ has important implications for TH physiology. At low hormone concentrations, such as hypothyroidism, the unliganded receptor is predicted to repress transcription rather than function as an inactive, passive receptor. In some respects, this model is borne out by targeted inactivation of the TRα and TRβ genes. The phenotype of these double knockout mice are, for the most part, much less pronounced than the clinical features of congenital hypothyroidism (113,114). Thus, basal repression of transcription may explain why absence of receptor has less deleterious effects than absence of hormone (80,113,114).

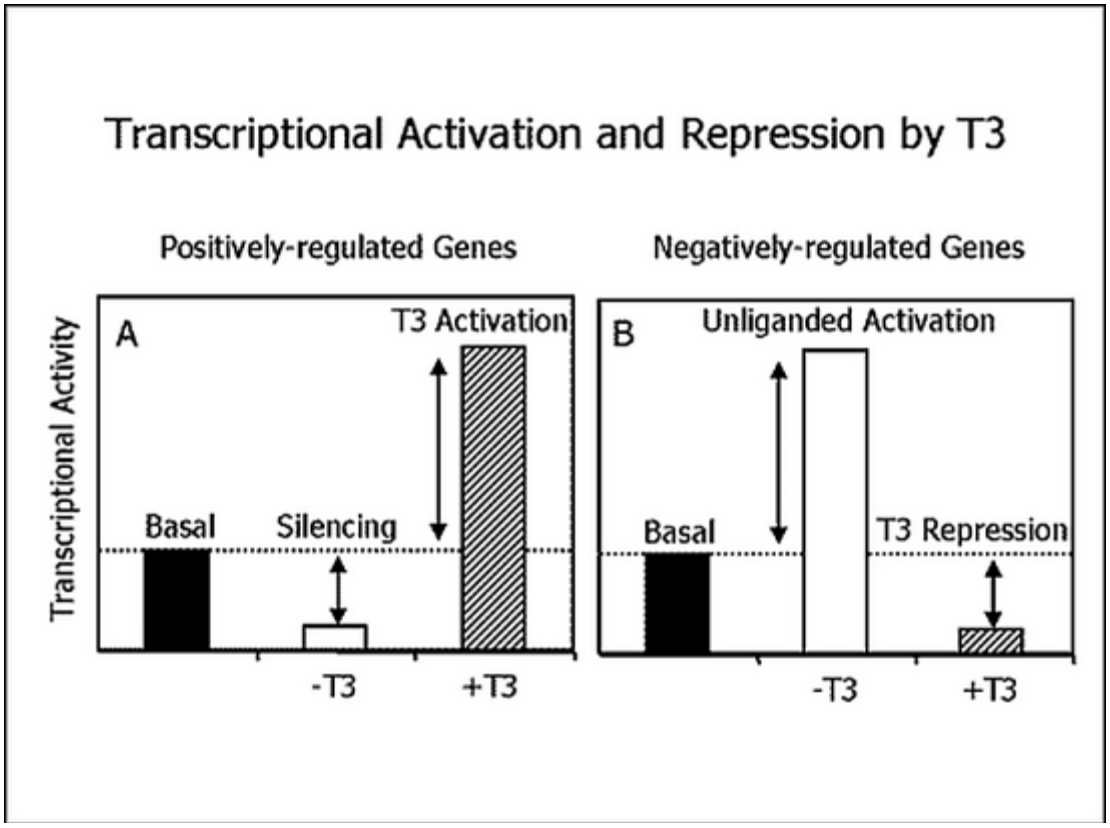


**Figure 5. TH receptor-mediated transcriptional silencing and activation. (A) Positively regulated genes. In the absence of hormone, the unliganded TH receptor represses or "silences" transcription in a process that involves TR interactions with a corepressor complex. Binding of $T_3$ releases corepressors, relieving silencing and inducing the recruitment of coactivators that mediate transcriptional stimulation. (B) Negatively regulated genes. In the absence of hormone, the unliganded receptor activates transcription in a process that involves corepressors. Addition of TH dissociates corepressors and recruits coactivators. In the case of negatively regulated genes, this $T_3$-mediated exchange of corepressors and coactivators inhibits transcription.**

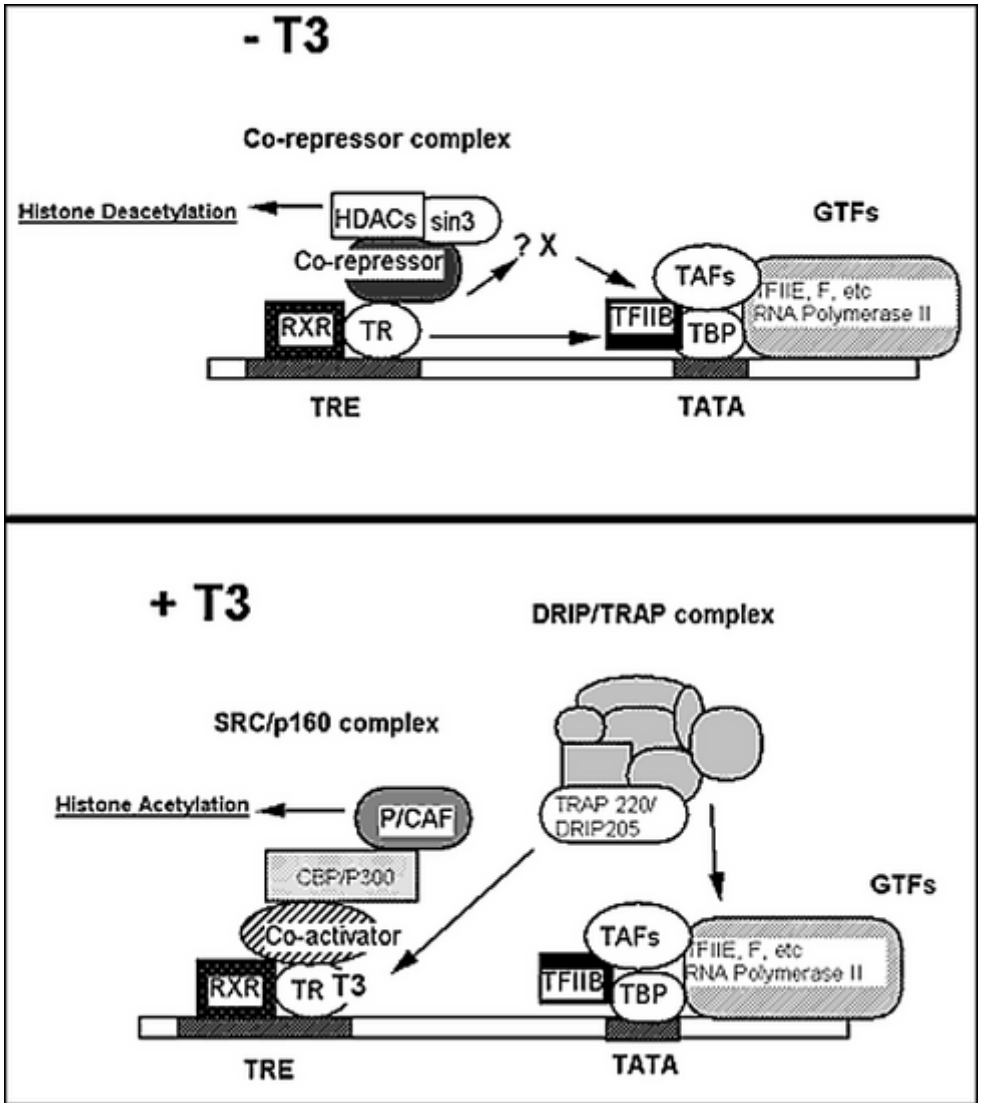


**Figure 6. Role of corepressors and coactivators in the control of $T_3$-regulated genes. In the absence of $T_3$, the RXR-TR heterodimer recruits corepressors (CoR), which in turn, assemble additional components of a repressor complex that includes histone deacetylase (HDAC). Deacetylation of histones induce transcriptional repression. In the presence of $T_3$, the corepressor complex dissociates and coactivators (CoA) bind to TR. The coactivator complex can include steroid receptor co-activators (SRCs)/p160, CREB-binding protein (CBP), p300/CBP associated factor (P/CAF), and proteins with histone acetyltransferase (HAT) activity. Vitamin D receptor interacting protein/TR associated protein (DRIP/TRAP) complex can also interact with liganded TR, and may cycle with SRC/p160 complex. The general transcription factors (GTFs) are also indicated.**

### Transcriptional activation/Coactivators

A large and growing number of co-factors have been shown to interact with liganded nuclear hormone receptors and enhance their transcriptional activation. These include: steroid receptor coactivator 1 (SRC1);

SRC2/transcriptional intermediary factor 2 (TIF2) / glucocorticoid receptor interacting protein 1 (GRIP1); SRC3/ amplified in breast cancer 1 (AIB1)/ receptor associated coactivator 3 (RAC3)/ p300/CBP cointegrator associated protein (p/CIP)/ nuclear receptor coactivator (ACTR)/ thyroid receptor activator molecule 1 (TRAM 1); peroxisome proliferator activated protein binding protein (PBP); TR accesory proteins (TRAPs) /vitamin D receptor interacting proteins (DRIPs); p300/CBP associated factor (p/CAF), and cAMP response element binding protein (CREB) binding protein (CBP)/ p300. among others (reviewed elsewhere (1-3,31).

At present, the precise roles of all these putative coactivators are not known; however, it appears that there are at least two major complexes involved in ligand-dependent transcriptional activation: the steroid receptor co-activator (SRC) complex and the vitamin D receptor interacting protein/TR associated protein (DRIP/TRAP complex) (Fig. 3d-6). SRCs (SRC-1,SRC-2, and SRC-3) are 160 kD proteins that associate with nuclear hormone receptors, including TRs, and enhance their ligand-dependent transcription (115-117). SRCs also interact with the CREB-binding protein (CBP), the co-activator for cAMP-stimulated transcription as well as the related protein, p300, which interacts with the viral co-activator E1A (118-121). Recent studies also have shown that CBP/p300 can interact with PCAF (p300/CBP-associated factor), the mammalian homolog of a yeast transcriptional activator, general control nonrepressed protein 5, GCN5. Like GCN5, PCAF has intrinisic histone acetyltransferase activity (HAT) activity. Both PCAF and CBP interact with TBP associated factors (TAFs) and RNA pol II. Thus, PCAF and CBP possess dual functional roles both as adaptors of nuclear receptors to the basal transcriptional machinery as well as enzymes that can alter chromatin structure by histone acetyl transferase (HAT) activity. SRC-1 and CBP may coordinate with TRs to synergize further the actions of TH, and also allow for the convergence of plasma membrane and nuclear hormone receptor signaling pathways in the cell.

The DRIP/TRAP complex also interacts with liganded VDRs and TRs (122-125). However, none of the subunits are members of the SRC family or their associated proteins. Instead, several DRIP/TRAP components are mammalian homologs of the yeast Mediator complex, which associates with RNA Pol II. Thus, TR recruits DRIP/TRAP complex which, in turn, may recruit or stabilize RNA Pol II holoenzyme via their shared subunits. It is noteworthy that DRIP/TRAP complex does not appear to have intrinisic HAT activity. Recent chromatin immunoprecipitation assays of proteins bound to hormone response elements (HREs), suggest that there may be a sequential, possibly cyclical recruitment, of co-activator complexes to hormone response elements by liganded nuclear hormone receptors (126-129). Studies of co-activator recruitment to TH-regulated genes showed distinct temporal patterns of recruitment. Last, other co-factors such as SW1/Snf and BRG-1 may be involved in early chromatin remodeling before the co-activator complexes are recruited to the TREs (130,131).

### Negative regulation by TRs

In contrast to positively-regulated target genes, negatively-regulated genes can be stimulated in the absence of TH and repressed by TH (Figure 3d-5, above). Regulation of TRH and the TSH α and β -subunit genes have been studied most extensively as models of negatively-regulated genes. From a physiological perspective, negative-regulation of these genes represents a critical aspect of feedback control of the TH axis. The $T_3$-responsive regions of these negatively- regulated genes have been localized to the proximal promoter regions (132-134). However, TR binding to putative TREs in these promoters is relatively weak in comparison to the binding sites in positively-regulated genes.

There are several different potential mechanisms for negative regulation by TH. Negative regulation may involve receptor interference with the actions of other transcription factors or with the basal transcription apparatus (135,136). For instance, TR can inhibit the activity of AP-1, a heterodimeric transcription factor composed of Jun and Fos. $T_3$-mediated repression of the prolactin promoter has been proposed to occur by preventing AP-1 binding (137). The TR also interacts with other classes of transcription factors, including NF-1, Oct-1, Sp-1, p53, Pit-1, CTCF, and GATA (138-144). By binding to these, or other positive transcription factors, the TH receptor may be able to inhibit gene expression by protein-protein interactions. Negative regulation may also occur by TR directly binding to DNA. A negative TRE from the TSHβ gene resides in an exon downstream of the start site of transcription (134) raising the possibility that it occludes the formation of a transcription complex. (Figure 3d-6, above) Additionally, liganded TRs may potentially recruit positive cofactors off DNA (squelching), which in turn, could lead to decreased transcription of target genes.

Transcriptional CoRs and CoAs, or even novel co-factors, may be involved in the control of negatively regulated genes. In contrast to the basal repression by unliganded TR in the case of positively regulated genes, CoRs cause basal activation of the TSH and TRH genes (132-134,145,146). CoAs also play an apparently paradoxical role in $T_3$-dependent repression of negatively regulated genes (146,147). Moreover, both SRC-1 knockout mice and knockin mice which express a TRβ mutant with a mutation in the helix 12 region (that interacts with CoAs) have defective negative regulation of TSH (148,149). Interestingly, histone acetylation can be increased in the $T_3$-mediated negative regulation of TSHα whereas it is decreased in regulation of TSHβ and TRH (150,151).

### Epigenetic modifications by TRs

Transcriptional regulation by TRs is a multistep process involving: (1) association of TRs with regulatory sites in the genome (usually within the targe gene promoters) in the context of chromatin, (2) ligand-dependent recruitment and function of coregulators to modify chromatin and thereby regulating RNA Pol II recruitment to the target genes, and (3) co-valent

modifications of histones to alter chromatin structure, recruit RNA pol II complex, and to mediate transcription. In particular, the site-specific acetylation of histone tails induces local relaxation of chromatin, which enhances the binding of some transcriptional regulators and facilitates the recruitment and functioning of the general transcriptional machinery. Recent studies have demonstarted that thyroid hormone-positively regulated target genes may have distinct patterns of coactivator recruitment and histone acetylation that may enable highly specific regulation (129). However the epigenetic changes associated with negetively regulated gene seems to be much more complex. For instance, histone acetylation of H3K9 and H3K18 sites, two modifications usually associated with transcriptional activation, occur in negative regulation of TSHa promoter. $T_3$ also caused the release of a corepressor complex composed of histone deacetylase 3 (HDAC3), transducin b-like protein 1, and nuclear receptor coprepressor (NCoR)/ silencing mediator for retinoic and thyroid hormone receptor from TSHa promoter in chromatin immunoprecipitation assays. These findings demonstrate the critical role of NCoR/HDAC3 complex in negative regulation of TSHa gene expression and show that similar complexes and overlapping epigenetic modifications can participate in both negative and positive transcriptional regulation (150). Of note, histone deacetylation has been observed in $T_3$-mediated negative regulation of several target genes (150-152). Moreover, abberant histone modification at the TRH and TSHa genes has been implicated in the inappropriate TSH secretion observed in resistance to thyroid hormone (RTH) syndrome (150,151)). Other coregulators may be involved in $T_3$-mediated regulation as RIP140, a coregulator that can decrease transcription by some nuclear hormone receptors, mediated $T_3$ repression of Crabp1 gene via chromatin remodeling during adipocyte differentiation (153). Interestingly, the use of HDAC inhibitors to counteract the effects of basal repression of target genes have restored some transcriptional activity in hypothyroidism associated with RTH syndrome and hypothyroidism (154,155). Although nuclear CoRs play a prominent role in T3 nuclear action (156,157), NCoR-independent signaling may account for basal repression by unliganded TRs for a significant number of target genes (158).

Another mechanism for $T_3$-mediated epigenetic signaling is regulation of small non-coding microRNAs. MicroRNAs act as negative regulators of gene expression by inhibiting the translation or promoting the degradation of target mRNAs. Since individual microRNAs often regulate the expression of multiple target genes with related functions, modulating the expression of a single microRNA can, in principle, influence an entire gene network and thereby modify complex disease phenotypes (159). Thyroid hormone have been shown to regulate the levels of microRNA pair miR-206/miR-133b in human skeletal muscles (160), miR 208a in heart (161), miR21 and miR181d in liver (162,163). These miRNAs regulate important cellular events by TH such as differentiation, contractility, and metabolism. MicroRNAs thus are a novel mechanism for thyroid hormone signaling which may regulate mRNA levels of target genes in which TRs are not recruited to their promoters or directly affect their transcription (164). Last, it recently has been reported that miRNA 27a can modulate the expression of target gene, b-MHC in cardiac myocytes by decreasing TRb mRNA expression (165) and multiple miRNAs may also regulate TRb expression in papillary thyroid cancer (166)

suggesting direction regulation of TR expression may be another mechanism for modulating target gene expression by TH.

### Novel indirect pathways for TH action

It has been assumed that early transcriptional activation of target genes are mediated by direct transcriptional effects by TRs owing to their abilities to bind to TREs and recruit co-activators (167,168). Previous studies have suggested that TH may have non-genomic signaling activation that may result in rapid transcriptional changes (3, see below). However, several groups have shown that TH can activate SIRT-1 activity by a TR-dependent process, that in turn, can lead to deacetylation and activation of transcription factors such as PGC1a and FoxO1a. These findings raise the possibility that TRs can activate some target genes without TREs through activating other transcription factors (169). On the other hand, TRs can interact with SIRT-1 directly so it is possible that it can recruite deacetylase activity that can act on transcription factors as well as modulate transcription through histone modification (170-72).

## THYROID HORMONE RECEPTORS AND CARCINOGENESIS

There are many reports providing evidence that reduced TR expression and/or alterations in TH levels are common events in human cancer (173, 174). These alterations include loss of heterozygosity, gene rearrangements, promoter methylation, aberrant splicing and point mutations (173,175). Tumors, including lung, breast, head and neck, melanoma, renal, uterine, ovarian and testicular tumors, present high frequencies of somatic deletions and mutations in both TR alpha/beta loci (176-178). Aberrant TRs have also been found in more than 70% of human hepatocellular carcinomas The tendency for TR expression to disappear as malignancies progress suggests that TR can act as a tumor suppressor in human cancers; therefore, loss of expression and/or function of this receptor could result in cell transformation and tumor development (179). In fact, TR overexpression in hepatoma cell lines shows repression of various tumor promoting genes such as PTTG1 (180), and activation of anti-tumorogenic TGF-beta. However certain mutant TRs like TRb $^{PV/PV}$ mey even enhance tumor growth by non-genomicaly activating beta-catenin and PI3K pathways (181,182). Last, it recently has been reported that miRNAs can downregulate the expression of dio 1 in renal cell carcinoma, and TRb in papillary thyroid, carcinoma. Clarifying the molecular mechanisms by which TRs influence tumor progression and elucidating the epigenetic modifications of $T_3$ target genes in cancers would perhaps lead to a better understanding of the treatment regime in humans.

## PHENOTYPIC EFFECTS OF TRα AND TRβ KNOCKOUTS AND KNOCKINS (CLINICALLY RECOGNIZED DISORDERS RELATED TO TR DYSFUNCTION ARE DESCRIBED IN CHAPTER 16D)

Recently, targeted gene inactivation or knockout (KO) of TR isoforms, and "knockin" of mutant TRs to their native TR genomic locii have provided new information on the mechanisms of TH action (16,17). The ability to disrupt TR genes by targeted mutagenesis has been particularly challenging given there is more than one gene encoding TRs, multiple splicing variants (TRα-1, α-2, TRβ-1, TRβ-2), and an additional transcript (Rev-erbA) derived from the opposite strand of the TRα gene (12,16,17). Two TRα knockout mouse lines have been generated that display different phenotypes (175, 176). It is likely this difference is due to the different sites in the TRα gene locus used for homologous recombination to generate the knockout mice. The TRα gene is complex as it encodes TRα-1, α-2 (which cannot bind $T_3$), and rev-erbA (generated from the opposite strand encoding TRα) (12, 16, 17). KO mice in which both TRα-1 and α-2 were deleted (TRα $^{-/-}$) had a more severe phenotype with hypothyroidism, intestinal malformation, growth retardation, and early death shortly after weaning (175). $T_3$ injection prevented the early death of pups. KO mice that lacked only TRα-1 (TRα-1$^{-/-}$) had a milder phenotype with decreased body temperature and prolonged QT intervals on electrocardiograms (183). The phenotypic effects of the loss of TRα 1 are relatively mild (184-185). Unexpectedly, there is no evidence of resistance to TH, as occurs with the TRβknockout. Disruption of the TRα-1 causes lower heart rates (19% reduced) and prolonged QRS and QT durations. These cardiac effects persist after hormone replacement. No changes were found in the levels of known TH-responsive genes in the heart (e.g., sarcoplasmic $Ca^{2+}$ ATPase, $Na^{+}$-$K^{+}$ ATPase, β-adrenergic receptors). The bradycardic effect of the TRα-1 knockout may result from alterations in the sympathetic or parasympathetic nervous systems or it could result from an intrinsic defect in cardiac myocytes. The TRα-1-deficient mice also have a 0.5 $^{o}$C reduction in body temperature that is independent of TH levels. The mice have normal amounts of brown adipose tissue.

Samarut and co-workers have reported generation of short TRα isoforms from intronic transcriptional start sites which have dominant negative activity on TR function (73), and it is likely these short TRα isoforms are responsible for the more severe phenotype of the TRα $^{-/-}$ mice. In this connection, TRα KO mice which did not express either TRα-1 and α-2 (TRα$^{o/o}$), had a milder phenotype than TRα $^{-/-}$ mice which expressed only the short TRα isoforms (186). Interestingly, TH stimulation of some target genes was increased, perhaps due to the absence of α-2 which inhibits normal TR-mediated transcription (57,58).

Targeted disruption of the TRβ locus created a mouse deficient in both TRβ-1 and TRβ-2 (16,17). These mice had elevated circulating TSH and $T_4$ levels, thyroid hyperplasia, as well as hearing defects (187,188). These findings are similar to the index patients with resistance to TH who were later shown to have homozygous deletion of TRβ (4,152). Thus, the mouse model appears to faithfully reproduce some of the features seen in humans with resistance to TH who are lack TRβ or express a dominant negative mutant TRβ (189). TRβ-2-selective knockout mice also have been generated and exhibited elevated levels of TH and TSH suggesting TRβ-2 plays the major role in regulating TSH (190). TRβ-2-selective knockout mice also have

abnormal color discrimination and suggest TRβ-2 may play a role in cone development of the retina (191).

The relatively mild phenotypes of the TRα-1 and TRβ KO mice suggest the two isoforms have redundant roles in the transcriptional regulation of many target genes. In this connection, microarray studies of TRα and TRβ KO mice showed similar gene regulation profiles in the absence and presence of $T_3$ in liver (82). Recently, a study employing TRα or TRβ receptor over-expressing cell lines also showed that both these receptor isoforms mostly share a common gene repertoire but with varying degrees of induction or repression of target genes (192).

When both TR isoforms were abolished, the resultant double knockout mice (TRα $1^{-/-}$ TRβ$^{-/-}$) were surprisingly viable (113, 114). Thus, the absence of TRs is compatible with life. These mice had markedly elevated $T_4$, $T_3$, and TSH as well as large goiters. They also showed decreased growth, fertility, heart rate as well as bone density and development. Interestingly comparison of cDNA microarrays of double KO and hypothyroid mice showed only partial overlap of their gene regulation profiles, confirming the observation that the absence of receptor can give a different phenotype than lack of hormone. It is likely that basal transcription occurs even in the absence of receptor whereas basal repression of target genes occurs in the absence of hormone. When the phenotypes of TRa, TRb, and double KO are compared, it is apparent that each isoform may have isoform-specific function, perhaps in part due to different expression patterns of the isoforms as well as gene-specific actions by each isoform (114).

Cheng and colleagues have generated a “knock-in” mouse model in which a mutant TRβ from a patient with RTH (PV) was introduced into the endogenous TRβ gene locus (193). These mice have a phenotype similar to patients with RTH, as the heterozygous mice showed elevated serum $T_4$ and TSH, mild goiter, hypercholesterolemia, impaired weight gain, and abnormal bone development. Homozygous mice had markedly elevated serum $T_4$ and TSH, and a much more severe phenotype than heterozygous mice. Wondisford and colleagues also have generated a “knock-in” mouse that expresses mutant TRβ (194). These mice had abnormal cerebellar development and function, and learning deficits. These latter studies suggest that expression of mutant TRβ under the control of endogenous TRβ promoter produces many of the clinical features of RTH in mice. This same group also recently developed a knock-in of a mutant TRβ that cannot bind DNA. This model should be useful in distinguishing signaling and developmental patterns due to protein-protein interactions of TRs (as well as non-genomic pathways) from those that require TR binding to TREs of target genes (195). Knockin mice harboring a TRa mutant at the same site as the TRβ$^{PV}$ mutant gene decreased white adipose tissue (WAT) and liver mass (196). In contrast, TRβPV markedly induced hepatosteatosis and mass of liver but had little effect on white adipose tissue. The expression of lipogenic genes was decreased in white adipose tissue and liver of TRa$^{PV}$ mice whereas it was increased in liver and normal in TRβ$^{PV}$ mice. A recent study showed that the phenotype of impaired adipogenesis can be restored by

crossing with mice expressing a mutant Ncor1 allele (Ncor1(ΔID) mice) that cannot recruit the TR (197). These findings support the notion that the phenotypes in the $TRa^{PV}$ mutant mice, and perhaps some patients with RTH with mutant TRa, may be due to aberrant repression of target gene (18, 198, 199).

## NONGENOMIC PATHWAYS REGULATED BY TH

There is increasing evidence for non-genomic effects by TH (3) in addition to the transcriptional effects mediated by nuclear TRs. There is continuous shuttling of a small amount of TRs between the cytoplasm and nucleus (200), so non-genomic effects may be mediated by cytoplasmic TRs (see below). Recently, a TRα variant from alternative translation was shown to be palmitoylated and associated with the plasma membrane (201). TH binding to this receptor led to increased intracellular calcium, nitrous oxide, and cyclic guanine monophosphate (cGMP), which in turn activated PKGII, Src, ERK, and Akt signaling pathways. Another recent study suggests that non-DNA-binding TRs that cannot stimulate transcription may have "non-canonical" thyroid hormone signaling to regulate important physiological effects such as serum glucose and triglyceride levels, body temperature, and heart rate. (202). However, it appears that many non-genomic effects by TH are likely mediated by cellular binding proteins other than TRs. Evidence supporting this notion comes from the rapid time course of some TH effects (thus precluding transcription and protein synthesis), utilization of membrane-signaling pathways such as kinases or calmodulin, lack of dependence on the presence of nuclear TRs, and structure-activity correlations by TH analogs that are different than those observed for nuclear TRs (3). Several non-nuclear sites for TH binding have been identified in various cell systems although their functional significances are not well characterized. Some of these include: plasma membrane associated $T_3$ transporters, actin, calcium ATPase, adenylate cyclase, and glucose transporters; an endoplasmic reticulum associated protein, prolyl hydroxylase; and monomeric pyruvate kinase (3, 203-207). A useful guideline that describes transcriptional and non-transcriptional signaling via TR and non-TR mechanisms recently has been published (208)

TH also has profound effects on mitochondrial activity and cellular energy state. A 43 kD protein has been described in mitochondria which also could bind to TREs and could be recognized by antibodies against the TRα ligand-binding domain (209). Recently, it has been shown that TRβ can interact with the p85 subunit of PI3K and activate the PI3K-Akt/PKB signaling cascade; thus, the small subpopulation of cytosolic TRβmay be involved in cell signaling (210). This PI3K activation by $T_3$ leads to both direct and indirect effects on the transcription of several genes involved in glucose metabolism (210, 211,) and provides a mechanism for cross-talk between TH and cell signaling pathways.

Recently, integrin α-Vβ-3, has been identified as a plasma membrane TH-binding site (212). Previously, $T_4$, but not $T_3$, was shown to promote actin polymerization and integrin interaction with laminin in neural cells (213). Additionally, both T4 and $T_3$ activated mitogen-activated protein kinase (MAPK) activity, and led, among other events, to phosphorylation of TRβ (210). Using a chick chorioallantoic membrane (CAM) system, Davis et al. showed that both T4 and $T_3$ stimulated angiogenesis (214). Since integrin α-Vβ-3 is involved in angiogenesis, $T_4$ and $T_3$ binding to it was examined, and $T_4$ was found to bind to integrin α-Vβ-3 with high affinity.

Tetraiodothyroacetic acid (tetrac) and antibodies against laminin blocked $T_4$ binding (213). Moreover, siRNAs against the integrin α-V or β-3 subunits blocked MAPK activation by TH. These findings suggest that TH activates the APK cascade and stimulates angiogenesis via TH binding to integrin α Vβ 3. Additionally, thyroid hormone non-genomically suppresses Src thereby stimulating osteocalcin expression in primary mouse calvarial osteoblasts (215). A direct physical interaction of TRbPV with cellular proteins, namely the regulatory subunit of the phosphatidylinositol 3-kinase (p85alpha), the pituitary tumor transforming gene (PTTG) and beta-catenin, that are critically involved in cell proliferation, motility, migration, angiogenesis and metastasis suggest a novel mode of non-genomic action, whereby mutant TR isoform acts as an oncogene in thyroid carcinogenesis (216).

## TH ANALOGS, METABOLITES, AND ANTAGONISTS

Several tissue-specific and TR isoform-specific compounds have been developed as potential treatments for hypercholesterolemia, obesity, and heart failure. An early prototypical compound was 3,5-dibromo-3-pyridazinone-L-thyronine (L-940901) that bound preferentially to the TRs in the liver over those in the heart (217). Although the relative affinity of this compound for the respective TR isoforms has not been reported, the selective action of L-940901 is likely due to tissue-specific uptake of the compound. Interestingly, mice treated with L-940901 had decreased serum cholesterol levels without cardiotoxicity. Recently, several other TH analogs have been described that have isoform-selective affinity for TRβ compared to TRα (218-220). Since TRs in the liver are approximately 90% TRβ whereas those in the heart are mostly TRα, these isoform-selective compounds may serve as novel agents to lower serum cholesterol with minimal cardiotoxicity.

N-[3,5-dimethyl-4-(4'-hydroxy-3'isopropylphenoxy)-phenyl]-oxamic acid (CGS 23425), 3,5-dimethyl-4(4'-hydroxy-3'-isopropylbenzyl)-phenoxy) acetic acid (GC-1), and 3,5-dichloro-4[(4-hydroxy-3-isoopropylphenoxy)phenyl] acetic acid (KB-141) all have been reported to lower total serum cholesterol and LDL-cholesterol (205-209). CGS 23425 also increases LDL receptor expression in HepG2 cells. Additionally, these compounds can increase serum apoA1 levels; however, the total serum high density lipoprotein (HDL) cholesterol level does not changes or may even decrease. In this connection, GC-1 decreased serum HDL; increased expression of HDL receptor, SR-B1; stimulated the activity of cholesterol 7α hydroxylase; and increased fecal excretion of bile acids in treated mice (221). Thus, GC-1 regulates important

steps in the reverse cholesterol transport pathway (221). Recently, KB141 was shown to be a potential treatment for obesity by decreasing body weight via stimulation of metabolic rate and oxygen consumption (222). The TR agonist MB07811, which is converted to an active metabolite in the liver, has proven to be effective in reducing hepatic steatosis in rodents (223). The TRb-specific compound, KB215 recently was shown to be effective in decreasing LDL cholesterol, apoliprotein B, triglycerides, and Lp(a) in humans (224)when used in combination with statins. These findings suggest that TH analogs may be useful in the treatment of a wide range of metabolic disorders (225).

TH analogs and derivatives also bind specifically to proteins other than TRs, and are involved in non-genomic cell signaling pathways, Thyronamines (3-T1AM, T0AM) are endogenous compounds derived from L-thyroxine or its intermediate metabolites. Activities of intestinal deiodinases and ornithine decarboxylase generate 3-T1AM (226). Significantly, this compound bound poorly to nuclear TRs. T1AM has interesting physiological actions as it produced a rapid drop in body temperature and heart rate when injected intraperitoneally in mice. T1AM also decreased cardiac output in an ex vivo working heart model. Although 3-T1AM have a weak affinity towards classical nuclear TH receptors a number of putative receptors, binding sites, and cellular target molecules mediating actions of 3-T1AM have been proposed. Among those are members of the trace amine associated-receptor family (TAR1), the adrenergic receptor ADRα2a, and the thermosensitive transient receptor potential melastatin 8 channel (226). Preclinical studies using animal models are in progress, and more stable receptor-selective agonistic and antagonistic analogues of 3-T1AM are now being synthesiszed exerting marked cryogenic, metabolic, cardiac and central actions and represents a key lead compound linking endocrine, metabolic, and neuroscience research to advance development of new drugs (226).

TH can increase cardiac performance by increasing cardiac contractility and decreasing systemic vascular resistance (2); however, TH excess also can cause cardiotoxicity. 3,5-diiodothyropropionic acid (DITPA) is a TH-related compound with low metabolic activity and low affinity for nuclear TRs (Kd $10^{-7}$M). DITPA was able to increase cardiac contractility and peripheral circulation without significant effects on heart rate in animal studies (227). Moreover, DITPA improved hemodynamic performance in animal models of congestive heart failure after myocardial infarction. Patients with heart failure treated with DITPA showed significant improvement in systolic cardiac index and systemic vascular resistance in preliminary studies (227). Thus DITPA or similar compounds may represent a novel class of drugs for the treatment of heart failure.

Recently, the naturally occurring analogs, 3,5,3'-triiodothyroacetic (TRIAC) and 3,5,3',5'-tetraiodothyroacetic TETRAC) acids decreased heat-induced albumin fibrillation suggesting they may have a protective effect against amyloid formation (228). Additionally, tetraiodothyroacetic acid (tetrac)

caused radiosensitization of GL261 glioma cells (229). The mechanisms for these effects are not understood at this time but likely involve non-genomic effects as TRIAC and TETRAC have weak binding affinity for TRs. Similarly, 3,5-diiodothyronine ($T_2$) has also shown favorable effects in rodent models of fatty liver diseases (230,231).

## SUMMARY

We have learned much about molecular mechanisms of nuclear TH action during the past 25 years. In particular, the identification and characterization of TRs, their heterodimeric partners, corepressors, coactivators, and TREs, generation of TR knockout mice, and discovering non-genomic pathways have provided new insight into TH action. It is expected that new information will be obtained from microarray and proteomic studies, structural biology approaches, and in vitro transcriptional systems. Such information should provide an even better understanding of the mechanisms of disease caused by abnormal circulating TH and/or altered intracellular TH levels, and provide targets for the development useful therapeutic agents for not only TH-related conditions but also for metabolic derangements such as hypercholesterolemia, non-alcoholic fatty liver disease, and obesity.

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