

THE MECHANISM OF ACTION OF THYROID HORMONES

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■ **Abstract** Thyroid hormone is essential for normal development, differentiation, and metabolic balance. Thyroid hormone action is mediated by multiple thyroid hormone receptor isoforms derived from two distinct genes. The thyroid hormone receptors belong to a nuclear receptor superfamily that also includes receptors for other small lipophilic hormones. Thyroid hormone receptors function by binding to specific thyroid hormone-responsive sequences in promoters of target genes and by regulating transcription. Thyroid hormone receptors often form heterodimers with retinoid X receptors. Heterodimerization is regulated through distinct mechanisms that together determine the specificity and flexibility of the sequence recognition. Amino-terminal regions appear to modulate thyroid hormone receptor function in an isoform-dependent manner. Unliganded thyroid hormone receptor represses transcription through recruitment of a corepressor complex, which also includes Sin3A and histone deacetylase. Ligand binding alters the conformation of the thyroid hormone receptor in such a way as to release the corepressor complex and recruit a coactivator complex that includes multiple histone acetyltransferases, including a steroid receptor family coactivator, *p300*/CREB-binding protein-associated factor (PCAF), and CREB binding protein (CBP). The existence of histone-modifying activities in the transcriptional regulatory complexes indicates an important role of chromatin structure. Stoichiometric, structural, and sequence-specific rules for coregulator interaction are beginning to be understood, as are aspects of the tissue specificity of hormone action. Moreover, knockout studies suggest that the products of two thyroid hormone receptor genes mediate distinct functions *in vivo*. The increased understanding of the structure and function of thyroid hormone receptors and their interacting proteins has markedly clarified the molecular mechanisms of thyroid hormone action.

INTRODUCTION

Thyroid hormone (T₃), produced by the thyroid gland, plays an important role in development, differentiation, and metabolism (1). The lack of T₃ in early human development results in growth disturbances and severe mental retardation,

a disease called cretinism (2). Later in life, T₃ plays an important role in metabolic balance (3). T₃ action is mediated by nuclear T₃ receptors (TRs) that can bind T₃ with high affinity (1). TRs belong to the nuclear receptor superfamily that also includes the receptors for retinoids, vitamin D, fatty acids, and prostaglandins, as well as “orphan receptors” with no identified ligands (4–7). TR is encoded by two separate genes, designated TR α and TR β , located in different chromosomes (17 and 3, respectively, in humans). Alternative splicing from each gene generates multiple TR isoforms, including TR α 1, TR α 2, and TR α 3 from the TR α gene and TR β 1 and TR β 2 from the TR β gene (reviewed in 1). Like other nuclear receptors, TRs have modular structures with six regions (A–F) and three functional domains (Figure 1).

The main function of a TR as a transcription factor is to regulate target gene expression directly through DNA response elements. The T₃ response element (TRE) is composed of repeated DNA sequences with different configurations (8–11). Although TRs can bind to TREs as monomers or homodimers, the major form of TR bound to the TRE is the heterodimer with retinoid X receptor (RXR) (12–18). An important property of TRs is their ability to bind TREs constitutively independent of ligand occupancy (1, 4–6). Unliganded TR generally represses basal transcription. Ligand binding triggers a conformational change in the TR, resulting in activated transcription of its target gene. In the past few years, great progress in biochemical, functional, and structural studies has clarified the molecular mechanism of TR action. The purpose of this review is to summarize the current knowledge with emphasis on transcriptional regulation by multiple cofactor complexes.

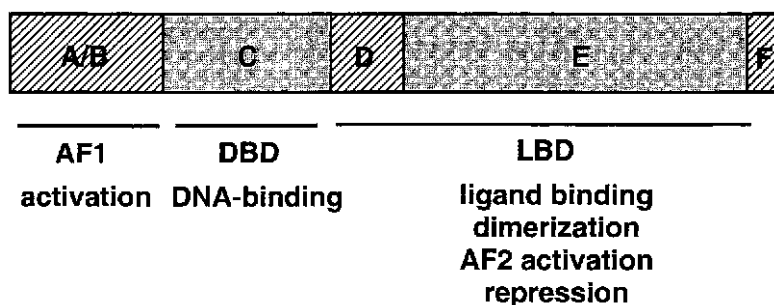


Figure 1 Modular structure of thyroid hormone receptor. Nuclear receptors, including TR, can be divided into six regions (A–F) with three functional domains: AF1, activation function 1 (A and B regions); DBD, DNA-binding domain (C region); LBD, ligand-binding domain (D, E, and F regions).

DNA-BINDING: SPECIFICITY AND FLEXIBILITY

The ability to bind specific sequences in target genes is crucial for TR function. The consensus sequence recognized by nuclear receptors often contains a hexamer AGGTCA known as the half site. Functional and efficient binding requires two of the half-site sequences with different configurations (19, 20). TR—as well as retinoic acid receptor (RAR), vitamin D receptor (VDR), and peroxisome proliferator-activated receptor (PPAR)—predominantly bind DNA response elements as heterodimers with RXRs (12–18). Heterodimer formation is thought to enhance DNA-binding affinity as well as provide target gene specificity, determined by the spacing between two half sites (5, 7). Accordingly, TR/RXR activates through the DR4 element (two half sites in one orientation spaced by four base pairs), whereas VDR/RXR and RAR/RXR activate through DR3 and DR5, respectively (the so-called 3–4–5 rule) (21). TR can also bind to other configurations of the two repeats, such as palindrome TRE and everted repeats (10, 22).

The DNA-binding domain is the most conserved region of the nuclear receptor superfamily. Structures of DNA-DNA-binding domain complexes have been determined for the glucocorticoid receptor (23), estrogen receptor (24), and Rev-Erb (25), as well as TR/RXR heterodimer (26). Two zinc-containing modules in the DNA-binding domain mediate the specific sequence recognition and confer spacing specificity. A DNA recognition helix (P box) in the carboxyl terminus of the first zinc finger mediates the half-site sequence recognition by directly contacting the major groove nucleotides. It is important that the P box can distinguish a single base change in the half site used by two subfamilies of the receptors (AGGACA for the glucocorticoid receptor versus AGGTCA for the estrogen and thyroid receptors) (21). In addition to the major groove contact, several members of the nuclear receptor family make additional minor groove contact through the carboxyl-terminal extension downstream of the second zinc-containing module, as shown in the crystal structures of TR/RXR bound to DR4 repeats (26). This carboxyl-terminal extension recognizes two additional nucleotides T, (A/G) at the 5' of the hexamer and thus enhances the monomer binding affinity to the octamer (27). The structural basis for the spacing recognition results from the steric constraints imposed by a specific and weak interaction between the D box from the RXR DNA-binding domain (5' position) and the T box from the DNA-binding domain of a receptor at 3' position (7). This interaction also provides a weak dimerization interface (28–31). There is a strict binding polarity of TR/RXR heterodimer on DR4 such that RXR occupies the upstream half site and TR occupies the downstream half site (26, 32).

A stronger heterodimerization surface is located in the ligand-binding domain (33–36). Both dimerization surfaces contribute to the heterodimerization of RXR with TR, although DNA binding is required only for heterodimerization via the DNA-binding domain (37). In the crystal structure of the ligand-binding domain of unliganded RXR, the ligand-binding domain, a hydrophobic patch composed

mainly of helix 9 and 10, is proposed to mediate the dimerization (38). This region includes the ninth heptad hydrophobic repeat that has been suggested by mutational analysis to correspond to the potential heterodimerization surface (32, 36, 39). Cooperative DNA-binding requires ligand-binding domain-mediated heterodimerization. By contrast, the DNA-binding domain heterodimerized surface restricts binding to specific half-site spacing (37). Thus, the two heterodimerized surfaces are not functionally redundant, but rather differentially determine the DNA-binding specificity as well as the flexibility. The flexibility of the hinge region allows permissive binding to these TREs with different half-site orientations, including inverted and everted repeats in addition to the directly repeated sites. These binding interactions are highly dependent on the presence of the ligand-binding domain dimerization surface (40, 41). This dependence suggests that flexible and cooperative TR/RXR binding to multiple TREs may require TR and RXR to form a solution heterodimer as the first step (37, 41).

TR α 2 is an alternative splicing product of the TR α gene that lacks an intact ninth heptad and acquires a unique carboxyl-terminal region leading to an inability to bind T3 (1). Dominant negative activity of TR α 2 requires DNA binding (42, 43). Loss of the ligand-binding domain dimerization surface abolishes the interaction with RXR in solution and impairs the ability of TR α 2 to form heterodimers on some DR4 sites (41, 44). Unlike TR α 1, which forms stable heterodimers equally well on four DR4 sites, TR α 2 preferentially forms stable heterodimers on a subset of DR4 that contains two additional T(A/G) nucleotides before the downstream hexamer and is the optimal site for TR monomer binding. This difference is probably the consequence of additional contact with the A box of the DNA-binding domain (41).

Thus distinct mechanisms are used by TR isoforms to regulate the heterodimerization and DNA-binding activity. The unique carboxyl-terminal region of TR α 2 can be phosphorylated *in vivo* and *in vitro* (45). Phosphorylation greatly reduces the monomeric binding affinity of TR α 2 and impairs the heterodimer formation on permissive DNA-binding sites (45). The increased dominant-negative action of a nonphosphorylatable form of TR α 2 suggests that phosphorylation may provide a means to regulate TR function (45).

UNIQUE FUNCTIONS OF AF1 DOMAIN: MODULATION OF THYROID HORMONE RECEPTOR ACTIVITY

The amino-terminal regions are least conserved among nuclear receptor sequences. This domain is highly divergent between TR α and TR β isoforms, which suggests differential roles in transcriptional regulation. In addition, alternative splicing of the TR β gene generates two isoforms, TR β 1 and TR β 2, with completely different amino-terminal domains (1). The amino-terminal domain is not required for T3-dependent transcriptional activation by rat TR β 1 (46), which suggests that the amino-terminal domain might modulate—rather than be essen-

tial for—TR function. The amino terminus of TR β 2 contains two distinct trans-activation regions that are important for the unique transcriptional properties of this isoform (47, 48).

The function of the amino-terminal domain appears to be mediated through interaction with basal transcriptional factors, especially with transcription factor IIB (49). Indeed, a sequence of 10 amino acids in chicken TR α 1 amino-terminal domain, which contains a cluster of 5 basic amino acids, mediates interaction with transcription factor IIB, and the interaction correlates with its requirement for full transcriptional activation (49). At least two separate serine phosphorylation sites are present in the chicken amino-terminal domain (50, 51). Only one of the sites, ²⁸Ser-Ser-Glu-Cys-Leu-Val-Lys, is retained in the P75-gag-v-ErbA protein (52). v-ErbA is a mutant form of the chick TR α that has been corrupted by the avian erythroblastosis virus (53, 54). Activation of either protein kinase C or cAMP-dependent protein kinase greatly enhances the phosphorylation of this site in both p46c-ErbA and P75gag-v-ErbA in vivo (51). In addition, phosphorylation through this site appears to be necessary for the oncogenic properties of v-ErbA protein. Mutation of two serine residues to alanine or treatment with kinase inhibitors dramatically impairs oncogenic transformation. In contrast, conversion of two serines to threonines, which can still be phosphorylated by protein kinase A, retains v-ErbA oncogenic activity (52). Protein kinase A phosphorylation on this site dramatically reduced monomeric DNA-binding affinity (55). It is interesting that the cluster of five basic amino acids, ²³Lys-Arg-Lys-Arg-Lys²⁷, is located immediately before these two serine residues. These five basic residues appear to be involved in the modulation of multiple TR activities (56). Conversion of these residues to ²³Thr-Ile-Thr-Ile-Thr²⁷ abolishes transcription factor IIB interaction, significantly decreases T3-dependent transcriptional activation, and inhibits monomeric DNA binding (56). The similar effect of protein kinase A phosphorylation might result from neutralizing the positive charges of the basic residues by introducing a negatively charged phosphate group. This effect also suggests a potential link between the amino-terminal region and the v-ErbA oncogenic activity.

In addition to modulating ligand-dependent activation function, the amino-terminal domain has also modulates ligand-independent interaction with corepressors (57). Unlike TR α 1 or TR β 1, TR β 2 fails to repress transcription in the absence of hormone. Consistent with this, it appears that the nuclear coreceptor (N-CoR) does not function for TR β 2 (57). Both TR β 1 and TR β 2 isoforms, however, interact with corepressors equally well on DNA. This interaction suggests that the amino-terminal domain of TR β 2 can inactivate or mask the corepressor function through other unknown mechanisms (57).

MECHANISMS OF TRANSCRIPTIONAL REGULATION

As a transcriptional factor, a key function of the TR is to regulate the target gene expression in response to multiple signaling pathways. Tremendous effort and progress have recently been made in understanding the molecular mechanism of

nuclear receptor action. Unlike steroid receptors, TR—as well as RAR, RXR, and PPAR—constitutively bind to DNA response elements in the absence and presence of the ligand. Unliganded TR represses the basal transcription. Ligand binding causes derepression and enhances transcriptional activation. Thus the biological significance of repression is to turn off target genes in the absence of hormone and to increase the magnitude of transcriptional activation by hormone ligand. A group of cofactor proteins (coactivators and corepressors) mediate repression and activation. Cofactors alone cannot bind DNA but instead they directly interact with DNA-bound nuclear receptors, as a result of which they are recruited to the proximity of the target gene promoter region and affect the rate of transcription.

A higher level of transcriptional regulation is provided by a change of chromatin structure. Open chromatin (euchromatin) is thought to facilitate the assembly of basal transcriptional machinery and increase the transcription rate. In contrast, a highly condensed chromatin (heterochromatin) blocks the entry of TATA-binding protein and leads to transcriptional repression. Chromatin structure can be greatly affected by acetylation of histones in the nucleosome octamer. Hyperacetylation of histones loosens the interaction between DNA and nucleosome by reducing the net positive charge. Conversely, histone deacetylation opposes the structural change of nucleosomes brought by histone acetylation. Both histone acetyltransferase and histone deacetylase activities are functionally associated with coactivators and corepressors, respectively, thus providing an enzymatic link to the activation and repression by nuclear receptors (Figure 2).

Activation Is Mediated by Multiple Coactivator Proteins

Transcriptional activation of liganded nuclear receptors, including TR, is mediated by coactivator proteins that associate with nuclear receptors in a ligand-dependent manner. Ectopic expression of coactivators leads to potentiation of the ligand-dependent transactivation function, and most of the coactivators contain activation domains. Early evidence for the existence of coactivators came from observations of ligand-dependent transcriptional interference, or squelching, such that cotransfection of one activated nuclear receptor reduces the transcriptional activity of another (58–61). Squelching occurs away from DNA and involves competition by the squelching receptor for binding to one or more limiting proteins required for transcriptional activity.

The first evidence for the existence of such limiting factors came from a biochemical approach with the ligand-binding domain of estrogen receptors (62). Two estrogen receptor-associated proteins (ERAP), ERAP-140 and ERAP-160, were found to associate with the glutathione *S*-transferase/estrogen receptor in the presence—but not in the absence—of estradiol or diethylstilbestrol. Mutational analysis indicated that this ligand-dependent interaction correlates with the ability to activate transcription. Moreover, the estrogen antagonist 4-hydroxy-tamoxifen (4-OHT) and the pure anti-estrogen ICI 164384 are unable to promote

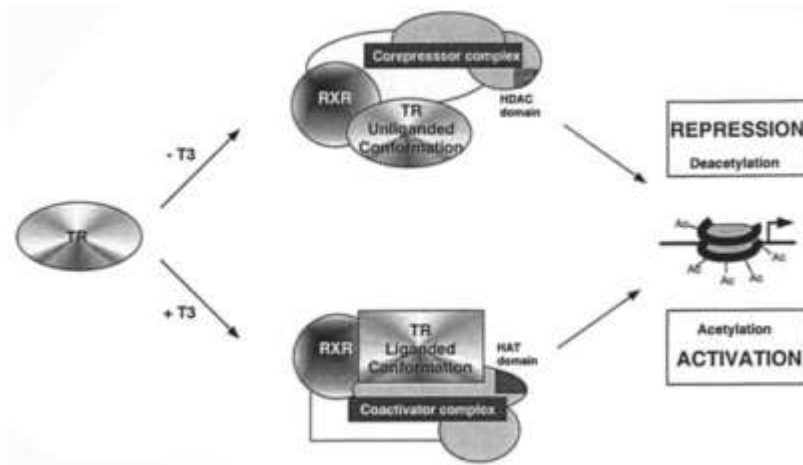


Figure 2 Model for activation and repression by thyroid hormone receptor. In the absence of T3, TR/RXR recruits a corepressor complex that has histone deacetylase (HDAC) activity. In the presence of T3, TR/T3/RXR releases the corepressor complex and recruits a coactivator complex that has histone acetyltransferase (HAT) activity. Enzymatic modification of nucleosomes by HDAC or HAT activity results in a closed or open chromatin structure that leads to transcriptional repression or activation.

the association of the estrogen receptor with ERAP. These data provide the evidence that ERAPs are estrogen coactivators. Similar biochemical approaches were used to characterize a glucocorticoid receptor coactivator (63). The 160-kDa forms of these proteins have included the first cloned coactivator, steroid receptor coactivator-1 (SRC-1).

SRC-1 was the first nuclear receptor coactivator to be cloned and characterized (64). SRC-1 associates with the progesterone receptor in the presence of agonist, but not in the presence of antagonist. In addition, SRC-1 enhances the ligand-dependent transcriptional activity of all the steroid receptors tested, including progesterone receptor, estrogen receptor, and glucocorticoid receptor. SRC-1 can reverse the ligand-dependent transcriptional interference of the progesterone receptor by cotransfected human estrogen receptor in the presence of the ligand estradiol, indicating that SRC-1 is a limiting cofactor recruited by both the liganded progesterone and estrogen receptors. SRC-1 also enhances the activity of liganded TR, RXR, and HNF-4 (65, 66). Furthermore, an amino-terminal-truncated SRC-1 containing the receptor-interacting domain exhibits strong dominant-negative activity on liganded progesterone and thyroid receptors. These results establish SRC-1 as a general common coactivator not only for receptors of this class. An important role of SRC-1 in T3 action is revealed by the fact that the mice without functional SRC-1 are T3 resistant (67).

Other members of the SRC family include TIF2/GRIP1 (68–74) and pCIP/ACTR/AIB1/TRAM1/RAC3 (75–81). In yeast cells, these coactivators dramatically enhance the transcriptional activity of nuclear receptors, including TR (82, 83). TIF2 is the human homolog of mouse GRIP1 protein. A novel fusion protein between MOZ and TIF2, which resulted from chromosomal translocation *inv(8)(p11q13)*, associates with a distinct subtype of acute myeloid leukemia (84, 85). This subtype is usually associated with the *t(8;16)(p11; p13)*, a translocation between *MOZ* and *CBP* genes (86). MOZ-TIF2 retains the histone acetyltransferase domain and also the CBP-binding domain of TIF2. The apparently identical phenotypes observed in both cases are likely caused by recruitment of CBP by the TIF2 part of the fusion protein. AIB1, which was also identified by its ability to bind and activate TR (79), is amplified in ~10% of breast cancers and highly expressed in 60% of cases. Altered expression of AIB1 may contribute to steroid-dependent cancers (78, 79).

SRC-1 contains two intrinsic transferable activation domains, AD1 and AD2, which are needed to achieve maximum activity of steroid receptors (87). SRC-1 is capable of interacting not only with the carboxyl-terminal ligand-binding domain of the nuclear receptor, but also with the amino-terminal domain (87). Thus SRC-1 appears to be involved in both ligand-dependent AF2 and ligand-independent AF1 transactivation functions (87). In addition, in experiments with isolated AF1 (ABCD) and AF2 (EF) fragments, SRC-1 potentiates the functional interaction between AF1 and AF2 regions of the estrogen receptor in the presence of ligand E2, consistent with the role of SRC-1 on the full-length receptor (88, 89). Thus one of the roles of SRC-1 in estrogen receptor function, and potentially in that of other members of the nuclear receptor superfamily, is to act as an adaptor protein and promote the cross-talk between amino-terminal AF1 and carboxyl-terminal AF2 to achieve maximum synergistic activity. SRC-1 possesses intrinsic histone acetyltransferase activity through its carboxyl-terminal region and also interacts with another histone acetyltransferase, p300/CREB-binding protein-associated factor (90–93).

The interaction between TR β and SRC-1 requires integrity of the carboxyl-terminal amphipathic helix (65, 94). This helix contains a consensus sequence $\phi\phi XE\phi\phi$ (ϕ represents a hydrophobic amino acid, X can be any amino acid, E is glutamic acid), also called the AF2 motif. Deletion of the six AF2 amino acids (amino acids 451–456) of TR β abolishes the ligand-dependent association with SRC-1 in vitro and the coactivation function in vivo (65).

Physiologically, aberrant coactivator recruitment is a feature of an autosomal inherited disease, T3 resistance (RTH) (95–97). RTH is caused by mutations in the TR β gene and is characterized by elevated serum-free T3 levels, increased levels of thyroid-stimulating hormone (TSH), and variable peripheral refractoriness to hormone action (96, 98). TR β mutants from RTH patients exhibit impaired SRC-1 recruitment, with variable T3 binding affinity (94, 95). In some mutants, SRC-1 recruitment is disproportionately impaired compared with altered T3 affinity, which suggests that the inability of mutant TRs to interact with coactivators

such as SRC-1 might be a determinant of dominant-negative activity (94, 97). Three clusters of RTH natural TR β mutations (amino acids 310–353, 429–461, and 234–282) correspond to three boundaries of the ligand-binding cavity and to the receptor-coactivator surface composed of helices 3, 4, 5, and 12 (95, 99, 100). In addition to influencing coactivator recruitment, corepressors also appear to play an important role in RTH syndrome (see below).

Coactivators of the SRC family also associate with p300/CBP (69, 75, 76, 101). CBP was originally identified by its interaction and coactivation function for phosphorylated CREB (102, 103). p300 is a functional homolog of CBP (104, 105). p300/CBP is involved in nuclear receptor signaling (106–108) in that it directly interacts with nuclear receptors and potentiates their transcriptional activation function *in vivo* (106–108). p300/CBP also interacts with and mediates the activation of other transcriptional regulation factors, including AP-1 (109), p53 (110), STAT proteins (109, 111), NF- κ B (112), C/EBP (113), and NF-E2 (114). Thus p300/CBP is a regulator of multiple signaling pathways (115).

Originally identified as a CBP-associated factor (93), PCAF has intrinsic histone deacetylase activity (93) and is a nuclear receptor coactivator (92). PCAF differs from other coactivators because it interacts with the DNA-binding domain of nuclear receptors and because the interaction is ligand independent (92). However, the ligand-independent association with corepressors inhibits the PCAF recruitment in the absence of the ligand and therefore may confer ligand dependency of PCAF association *in vivo* (92). PCAF also interacts with SRC family coactivators (76, 90, 116). Thus, PCAF, p300/CBP, and SRC family coactivators may form a multiprotein complex to mediate transactivation functions of diverse transcriptional factors. Microinjection studies suggest that different transcription factors have distinct requirements for coactivation complex formation (116). For example, TR activation function, but not STAT-1 function, is blocked by microinjection of PCAF antibody. Whereas PCAF is required for both nuclear receptor and CREB functions, the histone acetyltransferase activity of PCAF is indispensable for nuclear receptor function but is not essential for CREB function (116). In contrast, the histone acetyltransferase activity of CBP is required for CREB but not for nuclear receptor function (116).

In addition to the histone acetyltransferase-containing coactivators, a separate coactivator complex used by TR (117), VDR (118), and other classes of transcription factors (119) has also been identified. For TR this complex has been referred to as the TRAP (117). This complex does not appear to contain histone acetyltransferase activity and is transcriptionally active even in the absence of chromatin. It contains multiple polypeptides that are shared with a more general transcriptional regulatory complex referred to as the mediator complex SMCC (120, 121). The relative roles of the histone acetyltransferase and TRAP complexes are a subject of intense investigation. The roles of other TR coactivators, including TRIP230 (122) and p120 (123), are also unclear. A motif of LXXLL (Leu-XX-Leu-Leu; X is any amino acid) is present in most of the coactivators identified so far, including SRC-1, TIF2, and p300/CBP (124). This motif is

necessary and sufficient to mediate the ligand-dependent interaction with nuclear receptors (124). It is interesting that the LXXLL motif is also involved in the interaction between CBP and SRC family coactivators (125). SRC family coactivators contain three LXXLL motifs (termed the NR box) in the central region of the proteins. These NR boxes are not functionally redundant. In contrast, other usages of NR boxes are receptor specific (125). TR interaction requires both the second and third NR boxes and correct spacing between them. It appears that the specificity of NR box usage is determined by the carboxyl-terminal sequences to the LXXLL motif (125). These carboxyl-terminal residues can make differential contacts with helices 1 and 3 (or 3') of nuclear receptors (125). Thus the presence of multiple NR boxes in the coactivator protein provides the specificity and flexibility for the assembly of nuclear receptor-coactivator complexes that serve diverse biological functions.

Repression and the Corepressor—Histone Deacetylase Complex

TRs are located in the nucleus and bound to chromatin in the absence or presence of T3 (1, 4–6, 126). The most important difference between v-erbA and TR α is a 9-amino-acid deletion at the carboxyl terminus that removes the AF2 motif. This change greatly impairs the ligand-binding affinity of v-erbA but has little affect on its DNA binding. As the result, v-erbA acts as a strong dominant-negative inhibitor of c-erbA (127–129). Dominant negative activity requires v-erbA to bind DNA response element (130). Both v-erbA and c-erbA contain a transferable repression domain in the ligand-binding domain region (131). Mutation analysis reveals that two regions, located in the amino terminus (hinge region) and carboxyl terminus of v-erbA ligand-binding domain, are required for the repression function (131). Thus unliganded TR is capable of actively repressing the basal transcription (silencing), in contrast to the transcriptional activation mediated by the liganded TR.

Studies using in vitro transcription systems demonstrate that unliganded TR represses basal transcription in either HeLa nuclear extracts or defined reconstituted systems (132–134). Unliganded TR inhibits the formation of a functional preinitiation complex, probably through direct interaction with TBP and transcription factor IIB, which suggests a possible mechanism of active TR repression (132–134). However, functional assays strongly suggest the existence of a limiting corepressor protein involved in TR repression (135–137).

A major breakthrough has been the cloning of two corepressors, N-CoR/RIP13 (138, 139) and silencing mediator for retinoid and thyroid receptors (SMRT)/TRAC (140–142). N-CoR is a 270-kDa, ubiquitously expressed nuclear protein, identified by yeast two-hybrid screening with the ligand-binding domain of TR as the bait. SMRT was cloned by yeast two-hybrid screening as an RXR-interacting protein. N-CoR and SMRT share significant sequence homology, which

suggests that they are members of a novel corepressor family. They show ligand-independent interaction with RAR and TR *in vitro* and exhibit modular structures, with carboxyl termini as the nuclear receptor-interacting regions and amino termini as the autonomous repression domains. They contain multiple transferable repression domains, which can repress transcription when fused to heterologous Gal4 DBD (138, 141, 143–145). N-CoR also serves as corepressor for other nuclear receptors, including orphan receptors Rev-Erb (146, 147) and COUP-TF (148, 149). Other classes of transcription regulatory complexes, such as POZ domain-containing repressors, also use N-CoR and/or SMRT (150, 151).

A region referred to as the CoR box in the hinge region (helix 1) of TR and RAR is required for the interaction with corepressors N-CoR and SMRT (138, 140, 152, 153). Consistent with this requirement, the hinge region mutation Pro-144-Arg in *v-erbA* abolishes repression and oncogenic transformation activity of *v-erbA* (154–156). N-CoR and SMRT each contain two independent receptor-interacting regions (ID1 and ID2) (146, 157–159). This similarity is likely related to the finding that two ligand-binding domains from two receptors are required for functional interaction with corepressors on DNA (160, 161), which suggests that stoichiometric and steric principles govern corepression function (160).

TR ninth heptad mutants and TR α 2 cannot interact with RXR in solution and are defective in both repression and corepressor recruitment (33, 40, 135, 162). Although RXR is a poor repressor that only minimally interacts with corepressors on its own (40, 138, 142, 159, 163), recruitment of RXR via a heterologous dimerization interface complements repression-defective ninth-heptad TR mutants, which suggests an active role of RXR in repression by TR (40). RXR interaction with apo-TR requires an intact CoR box as well as the ninth heptad, but not AF2; conversely, AF2, but not the CoR box or ninth heptad of TR, is required for RXR interaction in the presence of T3 (40). Differential recognition of apo- and holo-TR by RXR is thus likely to play an important role in corepressor recruitment by TR/RXR heterodimer. TR homodimers also interact with N-CoR and SMRT (158, 160) and thus may also play a role in repression.

Small *Unique Nuclear receptor Co-Repressor* (SUN-CoR) is a 16-kDa nuclear protein identified by yeast two-hybrid screening with Rev-Erb as the bait (164); it shares no homology with N-CoR and SMRT. SUN-CoR contains a transferable repression domain and also interacts with and potentiates repression by TR. SUN-CoR also interacts with N-CoR *in vitro* and *in vivo*, which suggests that it may function as a component of the repression complex involved in repression by TR and orphan receptors. Another TR-interacting molecule, TRUP-1, inhibits activation by TR but not basal transcription on TRE-containing promoters (165).

Transcriptional repression by nuclear receptors varies among different cell types (166), which suggests a potential regulatory mechanism for cell-specific corepressor protein expression. However, the N-CoR and SMRT genes are ubiquitously expressed (138). By yeast two-hybrid screening, mSiah2, a mammalian homolog of the *Drosophila* “seven in absentia” (Sina) protein, was identified as

an N-CoR-interacting protein (167). mSiah2- and Sina-mediated proteasomal regulation have been implicated in proteolysis of other proteins (166, 168, 169). mSiah2 specifically targets N-CoR for degradation, thereby inhibiting repression by Rev-Erb (which specifically relies on N-CoR as a corepressor) and blunting repression by TR. mSiah2 is expressed prominently in germ cells and cells of the nervous system, which explains at least some aspects of cell specificity of N-CoR protein expression and the magnitude of nuclear receptor repression.

What are the downstream targets of the corepressors? Recent studies support the existence of a multiprotein complex, including N-CoR/SMRT, Sin3, and histone deacetylase (170–177). In contrast to histone acetyltransferase activity, which results in an open chromatin environment and gene activation, histone deacetylase contributes to a more compact chromatin structure, excluding the recruitment of basal transcriptional machinery and thereby repressing the basal transcription. This corepressor-histone deacetylase complex thus provides an enzymatic link to the repression by unliganded nuclear receptors.

In addition to the histone deacetylase-dependent mechanism, nuclear receptors and corepressors may also use other histone deacetylase-independent mechanisms to repress transcription (178). Trichostatin A, a specific inhibitor of histone deacetylase, cannot relieve Sin3A-mediated repression (157). N-CoR and SMRT directly interact with transcription factor IIB, TAF_{II}32, and TAF_{II}70 (157, 179). These interactions may interfere with the formation of functional preinitiation complex and thus decrease the rate of initiation. In addition, experiments with coupled chromatin assembly and transcription suggest that apo-TR/RXR heterodimer and histone deacetylase use distinct mechanisms to repress transcription (180). However, histone deacetylase activity is also required for TR/RXR to repress, because trichostatin A can relieve its repression (180). The fact that apo-TR/RXR represses transcription under conditions of incomplete chromatin assembly suggests that deacetylase recruited by TR/RXR may have functions in repression other than facilitating the chromatin assembly (180).

Dysregulation of corepressor function often leads to human disease. Recruitment of corepressors appears to be a prerequisite for the ability of TR β RTH mutants to exhibit strong dominant-negative inhibition (95, 181–184). Consistent with this assumption, all RTH mutants have normal DNA-binding activity (185).

Corepressor interaction is also essential for v-erbA oncogenic activity (140). Recruitment of corepressor-histone deacetylase complex is also linked to different types of myelogenous leukemia, including acute promyelocytic leukemia (153, 186–190) and acute myeloid leukemia (191–193). It is interesting that differential response to retinoic acid treatment for two types of acute promyelocytic leukemia correlates with the ability of retinoic acid to dissociate corepressors from the corresponding oncogenic fusion proteins. Additional contact to corepressor from the POZ-domain-containing PLZF moiety results in constitutive corepressor-histone deacetylase binding that fails to respond to retinoic acid treatment, whereas retinoic acid binding to PML-RAR releases corepressor complex and achieves complete remission.

Chromatin remodeling complexes provide additional levels of regulation of repression (194–200). The chromatin remodeling complex contains histone deacetylase and, in addition, ATPase activity. The coupling of histone deacetylase with remodeling activity may facilitate the assembly of a repressive chromatin structure. However, the role of the chromatin remodeling complex in transcriptional regulation needs more investigation.

STRUCTURAL BIOLOGY OF THE THYROID HORMONE RECEPTOR: ROLE OF THE LIGAND

Crystallographic structures of the ligand-binding domains from several unliganded and liganded nuclear receptors have been solved. These receptors include unliganded RXR (38), liganded TR (100), liganded RAR (201, 202), PPAR (203, 204), an agonist- or antagonist-bound estrogen receptor (205, 206), and a liganded progesterone receptor (207). The ligand-binding domains are composed mostly of α -helices. All the receptors exhibit a common fold with 12 helices arranged in three layers. Instructive information comes from the comparison of unliganded with liganded conformations. Overall, the liganded structure is more compact and stable than the unliganded structure, at least in part because the receptor folds around the ligand. The ligand becomes buried within the interior of the receptor and makes multiple contacts with several helices and β -strands to stabilize the conformation. This arrangement suggests that the ligand plays a structural role in triggering completion of the receptor fold. Likewise, the several hydrophobic cavities in unliganded RXR structures indicate that the fold cannot be completed without the ligand.

Most of the unliganded and liganded conformations can be superimposed except for a few important structural changes, which reveal the role of the ligand in receptor function. The most important change is the repositioning of helix 12, which contains the AF2 motif important in ligand-dependent transactivation. In unliganded RXR, helix 12 extends from the ligand-binding domain core, whereas in liganded TR, helix 12 folds back toward the ligand-binding domain, realigns with helix 3 and helix 4, and contacts the ligand, thereby closing the ligand cavity and generating a surface for coactivator recruitment (mousetrap model) (99, 100). With the release of helix 12, the Ω loop flips over underneath helix 6, carrying along the amino terminus of helix 3 (99, 100) and completing the fold around the ligand (Figure 3).

The repositioning of helix 12 plays an important role in nuclear receptor function. Consistent with the structural model, biochemical studies identified a hydrophobic cleft composed of helices 3–5 and 12 that is crucial for coactivator binding (99, 100). It is interesting that the core AF2 sequence $\phi\phi XE\phi\phi$ has an LXXLL motif (NR box), which is sufficient to bind to the coactivator-interacting surface. In antagonist-bound estrogen receptor, AF2 sterically prevents coactivator bind-

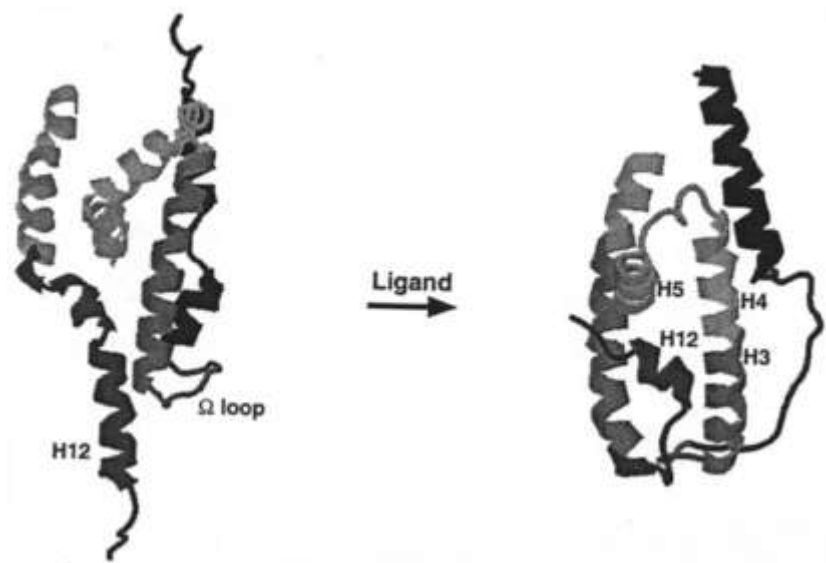


Figure 3 Ligand-induced conformational change of nuclear receptors. Structures that exhibit significant differences are *left*, unliganded RXR structure; *right*, liganded RAR structure. The structure of apo-TR has not been solved, and coordinates of ligand-bound TR are not currently available.

ing by mimicking the NR box to adopt the coactivator position (206). Liganded TR structures also provide important insights into the structural basis of RTH syndrome. RTH mutations essentially map to the boundaries of the ligand cavity, thus explaining the impaired T3 binding for almost all mutants (95, 208, 209).

LIFE WITHOUT THYROID RECEPTOR HORMONE: REDUNDANCY AND SPECIFICITY OF TR α AND TR β

T3 action is mediated by two receptors, TR α and TR β . Despite extensive studies to understand the mechanism of TR action, some *in vivo* functions remain undefined. Overall, the functional proteins encoded by TR α and TR β genes have similar biochemical and functional properties. Both TR α 1 and TR β receptors bind T3 with high affinity. Unliganded receptors are potent repressors, and ligand binding converts them to transcriptional activators. The TR α and TR β genes are coexpressed in some tissues (210–215). This arrangement raises the possibility that they are functionally redundant. Therefore, knockout mice that lack functional TR α and/or TR β genes have been generated to study the differential roles of these genes for mediating T3 action.

TR α 1 has been shown to be especially important for normal cardiac function. TR α 1-specific knockout mice have lower heart rates under normal conditions and after T3 stimulation. They also show prolonged QRS- and QT_{end}-durations in electrocardiograms and have lower body temperatures but appear normal in overall behavior and reproduction (216). In contrast to relatively minor phenotypic abnormalities in TR α 1 knockout mice, TR $\alpha^{-/-}$ mice that are devoid of TR α 1 and TR α 2 become progressively hypothyroid and exhibit growth arrest, which leads to death within 5 weeks after birth (217). This phenotype could be caused by the additional absence of TR α 2 but may also relate to the specific scheme used to delete the TR α gene, which might allow for expression of dominant negative proteins at the TR α locus (217).

TR β is essential for auditory development. TR $\beta^{-/-}$ mice exhibit a permanent deficit in hearing, for which other receptors do not compensate. Because TR β does not control cochlear morphogenesis, TR α or other unidentified receptors may influence morphogenesis, and TR β may mediate functional maturation of the ear (218). Inactivation of TR β leads to hyperthyroxinemia (219), similar to what is observed in RTH, and is consistent with the negative regulation of the TSH β gene by liganded TR β . The elevated level of TSH in TR $\beta^{-/-}$ mice suggests that TR α alone cannot complement deficient TR β function. TR $\alpha^{-/-}$ TR $\beta^{-/-}$ mice have much higher levels of TSH, which suggests that TR α at least partially controls TSH expression (220). Thus partial redundancy may exist for a limited number of functions of TR α and TR β gene products, but generally these products mediate specific functions in a time- and tissue-specific manner. The described double knockout has multiple abnormalities but at present does not appear to completely recapitulate the athyroid phenotype. This condition could be caused by an undiscovered third T3 receptor gene or by nongenomic effects of T3. Another fundamental difference between the absence of ligand and the absence of a receptor is that the absence of T3 locks the TR in a repressed state, whereas genes containing TREs would be in a relatively more active neutral state in the absence of TR. Future and additional studies of double knockouts will undoubtedly clarify the role of each TR gene product in thyroid physiology and will shed great light on the relative roles of repression and activation in TR function.

SUMMARY AND FUTURE DIRECTIONS

Our knowledge of mechanisms underlying T3 action has grown exponentially over the past decade with TR cloning, identification and cloning of the TR heterodimer partner RXR, and the identification of transcriptional coregulators. The challenge for the future is to better understand the underlying particularity of the mechanisms: specific functions of each TR isoform, specific functions of TR homo- and heterodimers, and specific functions for each TR coactivator and corepressor. This understanding will require an expanded view of TR target genes in each target tissue and cell type under different physiological conditions, which

will be possible with the anticipated progress of the mapping of human and mouse genomes and new computational approaches to complex networks of gene regulation. The convergence of these technologies lends great promise to the hope of relating the molecular mechanism of T3 action to thyroid physiology.

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