The role of thyroid hormone receptor DNA binding in negative thyroid hormone-mediated gene transcription

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Abstract

Thyroid hormone 3,3′,5-tri-iodothyronine (T₃) regulates gene expression in a positive and negative manner. Here, we analyzed the regulation of a positively (mitochondrial glycerol-3-phosphate dehydrogenase) and negatively T₃-regulated target gene (TSHα). Thyroid hormone receptor (TR) activates mGPDH but not TSH promoter fragments in a mammalian one-hybrid assay. Furthermore, we investigated functional consequences of targeting TR to DNA independent of its own DNA-binding domain (DBD). Using a chimeric fusion protein of the DBD of yeast transcription factor Gal4 with TR, we demonstrated a positive regulation of gene transcription in response to T₃. T₃-mediated activation of this chimeric protein is further increased after an introduction of point mutations within the DBD of TR. Moreover, we investigated the capacity of TR to negatively regulate gene transcription on a DNA-tethered cofactor platform. A direct binding of TR to DNA via its own DBD is dispensable in this assay. We investigated functional consequences of point mutations affecting different domains of TR. Our data indicate that the DBD of TR plays a key role in direct DNA binding on positively but not on negatively T₃-regulated target genes. Nevertheless, the DBD is involved in mediating negative gene regulation independent of its capacity to bind DNA.

Journal of Molecular Endocrinology (2008) 41, 25–34

Introduction

Thyroid hormone (3,3′,5-tri-iodothyronine; T₃) is an essential regulator of brain development, cell differentiation, and metabolic balance (Yen 2001). Thyroid hormone regulates gene expression via binding to thyroid hormone receptors (TRs) that belong to the large family of nuclear receptors. As with other members of this superfamily, TR contains an N-terminal activation domain (AF-1), a DNA-binding domain (DBD) followed by the hinge domain. In the C-terminal part of the protein, the ligand-binding domain (LBD) undergoes a conformational change upon hormone binding and the activation domain AF-2 has been located. TRs can bind to thyroid hormone-response elements (TREs) either as a homodimer or as a heterodimer with retinoid X receptor (RXR). The TR interacts with cofactors that mediate the T₃ signaling to the basal transcriptional machinery and activates or represses gene transcription (Zhang & Lazar 2000). Gene expression analysis indicated that the majority of T₃-regulated genes are positively regulated; however, a significant portion of target genes is negatively regulated (Feng et al. 2000, Miller et al. 2001, Weitzel et al. 2001a, 2003, Flores-Morales et al. 2002).

On positively T₃-regulated genes, unliganded TR suppresses basal gene transcriptional activity by interacting with corepressors such as nuclear receptor corepressor (NCoR) and by silencing mediator of retinoid and thyroid hormone receptors (SMRT). Corepressors are associated with histone deacetylases, which modify chromatin into a compact and transcriptionally silent structure. Binding of hormone to the receptor (liganded TR) leads to conformational changes of the TR, which releases the corepressor and recruits the coactivators such as steroid receptor coactivator 1 (SRC-1 or NCoA1). Coactivators possess or recruit proteins with histone acetyltransferase activity (HAT), which remodels chromatin into an open structure allowing for transcription (reviewed by Rosenfeld et al. 2006).

While the mechanism of positive regulation by T₃ is well understood, no single mechanism that explains the negative regulation has been elucidated. In principle, there are three major models to explain the mechanism of negatively regulated gene expression (reviewed by Lazar 2003). The first model suggests a direct binding of TR to DNA at ‘negative TREs’. Due to the specific composition of this DNA-response element, a liganded TR recruits a corepressor complex whereas an
unliganded TR recruits a coactivator complex. Thus, the functional readout on a negative TRE is diametrically opposed to the readout on a positive TRE. DNA sequences close to the transcriptional start site (the so-called z-boxes) have been suggested to be the binding sites for TR within the TRH, thyroid-stimulating hormone β (TSHβ), and neccd gene promoters (Sasaki et al. 1999, Satoh et al. 1999, Shibusawa et al. 2003a, Nygard et al. 2006). In a second model, TR inhibits the function of another DNA-binding protein in a ligand-dependent manner. The T₃-mediated inhibition of the activity of GHF-1/Pit-1, Sp1, GAF, JTF, and TBP are examples that direct binding of TR to DNA may not be required (Sanchez-Pacheco et al. 1995, Kim et al. 2004, 2005, Nakano et al. 2004, Villa et al. 2004). Again, gene expression rates of target genes are negatively regulated in the presence of T₃ whereas an activation of gene expression is observed in the absence of T₃; however, the exact (ligand-dependent) molecular mechanism remains to be elucidated. Finally, a third model suggests that TR does not bind to DNA directly but rather sequesters cofactors from other transcription factor–cofactor complexes (Tagami et al. 1999). According to this model, a release of coactivators by liganded TR leads to a repression of target gene expression. In a reciprocal mechanism, unliganded TR sequesters corepressors from DNA-bound transcription factor complexes thus leading to an activation of target gene expression.

To get insight into the molecular mechanism of negatively T₃-mediated gene expression, we investigated the activity of various heterologous TR fusion proteins. Our data indicate that T₃ treatment of DNA-bound TR leads to an activation of gene expression. Furthermore, liganded TR has the ability to block gene transcription that is mediated by DNA-tethered cofactors. Finally, the investigation of point mutants indicated that the DBD of TR is important for interference with the transcriptional machinery independent of its function to bind DNA. Our data suggest that direct binding of TR to DNA via its endogenous DBD is dispensable for negative T₃-mediated gene transcription.

### Materials and methods

#### Isolation and characterization of DNA sequences

A human promoter fragment from −802 to +22 of TSHα was amplified by PCR using the primers WL390 (5'-tctaaagcagttctctacgg-3') and WL391 (5'-ctttagtacctcagtaactg-3') and human genomic DNA as template. The resulting PCR fragments were ligated into a) pGL3-basic (Promega) upstream of the firefly luciferase and b) pRL-MA (Promega) upstream of the thymidine kinase (TK) promoter driving the expression of the Renilla luciferase. The promoter fragment B(−316/+109) of rat mGPDH in pGL3-basic has been described previously (Weitzel et al. 2001b) and was further subcloned into pRL-MA. All clones were confirmed by sequencing.

#### Cell experiments

Human hepatocarcinoma HepG2 and human embryonic kidney HEK293 cells were cultured under standard conditions in Dulbecco’s Modified Eagle’s Medium (DMEM) plus Glutamax (Invitrogen) and 10% complete or stripped fetal calf serum as described (Weitzel et al. 2001b, 2003). Rat pituitary GC cells (a gift of Danielle Gourdji, Paris, France) were cultured in a 1:1 mixture of DMEM and Ham’s F12 media plus 10% fetal calf serum. Transient transfection experiments were performed using a modified calcium phosphate technique described previously. In brief, for each 9·6 cm² dish, 2 µg of promoter containing pGL3-basic or pRL-MA luciferase reporter plasmid (for constructs, see above) were mixed with 0·2 µg chicken TRz1 in pSG5 (TR), 0·2 µg human RXRβ in pSG5 (RXR), and stimulated with 100 nM tri-iodothyronine (Sigma). Optionally, 2 µg full-length human SRC-1α in pSG5 (a gift of Malcolm Parker, London, UK) or full-length mouse NCoR in pCMX (a gift of Ingolf Bach, Hamburg, Germany) were additionally added.

For mammalian one-hybrid analyses, 0·4 µg TR-VP16 (full-length TRz1) or VP16 alone in pAASV (gifts of Tetsuya Tagami, Kyoto, Japan) was mixed with 2 µg luciferase reporter plasmid.

For mammalian two-hybrid analyses, 0·4 µg of a fusion protein of the Gal4 DBD with full-length cTRz1 (Gal4-TR), Gal4 fused with NCoR (a gift of Tetsuya Tagami, Kyoto, Japan) was mixed with 2 µg luciferase reporter plasmid containing five copies of the upstream activator sequence (UAS) upstream of the luciferase gene (5× UAS-Luc). Optionally, 0·4 µg expression plasmids for TR, NCoR, or SRC-1 (see above) were added. Various point mutations within the TR gene were introduced using the QuickChange site-directed mutagenesis system (Stratagene, Amsterdam, The Netherlands) according to the manufacturer’s recommendations. All clones were confirmed by sequencing.

The DNA mixtures in 125 mM CaCl₂, 140 mM NaCl, 1·5 mM Na₂HPO₄, and 25 mM HEPES (pH 7·2) were applied to the culture medium containing ~7×10⁵ cells/well. The cells were harvested after a 24-h incubation, luciferase activity was determined as described previously (Weitzel et al. 2001b, 2003) and normalized to the total protein concentration of the samples, which was determined by the Bradford method.
method (Bio-Rad). Luciferase measurements were carried out in duplicate, and each construct was tested in at least three independent transfection experiments with two to three culture dishes per experiment ± s.d.

Western blot

For immunological detection of TR or TR fusion proteins, equal amounts of transfected cell samples (50 μg protein per lane; see above) were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) by electroblotting. Membranes were incubated with a 1:1000 dilution of rabbit anti-TRα1 antibody (no. H0204 Santa Cruz Biotechnology, Santa Cruz, CA, USA) as described previously (Weitzel et al. 2001b, 2003). Antibody binding was detected by carrying out secondary antibody incubation using peroxidase-conjugated antibodies (Dianova, Hamburg, Germany) diluted 1:10 000. Secondary antibody was detected using the ECL system according to the manufacturer’s recommendation (Amersham Pharmacia Biotech).

Results

In order to gain insight into the molecular mechanism of T3-regulated genes, we investigated the promoter of TSHz. In the pituitary, TSH is negatively regulated by T3 as part of the hypothalamus–pituitary–thyroid negative feedback axis. As an example of a positively regulated gene, we investigated the regulatory element of mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) that contains a well-characterized positive TRE. The regulatory elements of mGPDH and TSHz were cloned upstream of firefly luciferase, Renilla luciferase, or chloramphenicol reporter vectors and transfected into different cell lines. The reporter gene activity was measured after the stimulation of T3 or without stimulation and additional cotransfection of TR and RXR expression constructs to maintain appropriate concentrations of functionally active nuclear receptors. As shown in Fig. 1A and B, mGPDH promoter-driven reporter constructs led to a positive regulation of gene expression. A positive regulation of gene expression has been observed for transfection of a firefly luciferase as well as for transfection of Renilla luciferase reporter constructs in HepG2 cells (Fig. 1A and B) but has also been observed for a chloramphenicol reporter construct and after cotransfection in HEK293 and pituitary GC cells (data not shown).

The investigation of TSHz promoter demonstrated a negative regulation. Again, the firefly luciferase and Renilla luciferase reporter constructs were cotransfected into cells and stimulated with or without T3. As shown in Fig. 1C and D, TSH promoter-driven reporter constructs were negatively regulated after the stimulation by T3. This has similarly been observed using the chloramphenicol reporter construct and different cell types for transfection. Of note, a similar readout has been maintained using rat pituitary GC cells, which might be a better cell system to investigate TSH regulation (Supplementary Figure 2, see Supplementary data in the online version of the Journal of Molecular Endocrinology at http://jme.endocrinology-journals.org/content/vol40/). However, since GC cells contain endogenous functionally active TRs (which maintain reporter gene activities independent of exogenous TR cotransfection (Sanchez-Pacheco et al. 1995, Miller et al. 2001)), we excluded this cell line for further investigation since a manipulation of TR by point mutations is impeded in this cell system (see below).

We next investigated the regulatory elements of mGPDH and TSHz in a mammalian one-hybrid assay using a fusion protein of full-length TRz1 with the viral activator domain VP16 (TR-VP16). A binding of TR-VP16 to the respective promoter regions via DBD of TR directly improves reporter gene activity independent of T3 stimulation. As shown in Fig. 2, TR-VP16 activates the mGPDH construct but failed to activate the TSH construct. Differences in gene activation are not due to differences in expression levels of TR-VP16 (Fig. 2, inset). This indicates that TR is able to bind and activate the mGPDH promoter but failed to activate the TSH promoter under identical experimental conditions. Of note, the investigated promoter fragment is identical to those showing a negative gene regulation in Fig. 1C and D.

In a next series of experiments, we investigated a fusion protein of full-length TRα1 with the DBD of the yeast protein Gal4. This experimental setting directs TR to DNA via the Gal4 fusion partner without the utilization of the endogenous DNA-binding capacity of TR. For this assay, we transfected a luciferase reporter under the control of the UAS of yeast together with a fusion protein of Gal4-DBD plus TR (Gal4-TR) into cells and stimulated these with T3. Using a TR wild-type fusion construct, we observed a 138-fold induction of gene expression after an administration of T3 (Fig. 3A). This dramatic activation might be explained by the presence of five repeats of UAS upstream of the luciferase reporter. Since Gal4 is able to form homodimers, this would theoretically allow promoter occupancy of up to ten Gal4-TR fusion proteins per reporter construct.

Interestingly, introduction of point mutations within the zinc finger DBD of TR (defective DNA-binding capacity of TR; mutations C71S and C89S) further increased the T3-mediated activation (>500-fold activation). By contrast, previously described point mutations within the hinge domain (P158R), the LBD (C253K; impaired CoR binding), or activation domain
AF-2 (E401A; impaired CoA binding; Nakano et al. 2004) of TR reduced the potential to activate gene expression in response to T3 (Fig. 3A). The differences in activation were not due to alterations in protein expression rates as detected by western blot analysis (Fig. 3B). These observations indicate that i) forced binding of TR to DNA leads to a positive regulation of gene expression in response to T3, ii) the DBD of TR has (besides DNA binding) an additional function since mutation of this region further increases T3-mediated

Figure 1 Regulation of (A and B) mGPDH and (C and D) TSHα promoter gene fragments by thyroid hormone. Rat mGPDH promoter fragment from −316 to +109 was ligated into a pGL vector upstream of the firefly luciferase (A) or into a pRL vector upstream of the Renilla luciferase (B) and transfected into HepG2 cells. Human TSHα promoter fragment from −802 to +22 was similarly ligated into firefly luciferase (C) and Renilla luciferase (D) reporter vectors respectively. For T3 induction experiments, expression vectors of TR and RXR were cotransfected and stimulated with 100 nM T3. Promoter activities are presented as percentage of unstimulated activities, normalized to total protein concentrations of the cell extract ± S.D. Each construct was tested in at least three independent transfections with three culture dishes per experiment.
activation, and iii) both coactivator and corepressor binding sites of TR are necessary for full T₃-mediated activation.

Coactivators (like SRC-1) and corepressors (like NCoR) do not bind to DNA directly but rather via DNA-bound transcription factors. Cofactors build a platform for further protein–protein interactions integrating various enzymatic activities into chromatin structure in order to modulate gene transcription as a functional readout. We therefore selected an assay that directs the cofactors to DNA via a Gal4-DBD fusion protein. Since cofactors contain different interaction

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**Figure 2** TR binds to mGPDH but not TSH promoter sequences in a mammalian one-hybrid assay.

A chimeric protein of wild-type TRα1 with the viral activator domain VP16 (TR-VP16) or the VP16 domain alone (VP16) plus promoter–reporter vectors for mGPDH and TSHα were transfected into HepG2 cells. A schematic of the mammalian one-hybrid assay is shown on the right-hand side. Similar expression levels of TR-VP16 proteins have been assayed by western blot analysis (below right). Promoter activities are presented as percentage of VP16 alone activities ± S.D.

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**Figure 3** DNA-bound TR activates gene transcription in response to T₃. (A) A chimeric protein of Gal4 DNA-binding domain with chicken TRα1 (Gal4-TR; wild type and mutants thereof) and a reporter vector containing five binding sites for Gal4 (5× UAS) upstream of the TK promoter were transfected and stimulated with 100 nM T₃. Point mutations within the DBD of TRα1 (C71S and C89S) increased T₃-mediated gene transcription whereas those within the hinge domain (P158R), LBD (C253K), or activation domain 2 (E401A) reduced T₃-mediated gene transcription. Promoter activities are presented as percentage of unstimulated activities ± S.D. (B) Similar expression levels of Gal4-TR fusion proteins were monitored using an anti-TR antibody by western blot analysis.
domains (e.g., endogenous HAT activity in the C-terminal part of SRC-1), we omitted these sequences within the fusion proteins. For investigation, we used the following constructs: Gal4-SRC-1 (LXXLL, amino acids 493–1007) and Gal4-SRC-1 (q-rich, amino acids 1050–1185) or corepressor NCoR (Gal4-NCoR, amino acids 1564–2444), expression plasmids for TR-VP16 (wild-type or DBD mutants C71S, C89S or E69G,G70S (‘GS mutant’)) and 5× UAS reporter vector were transfected and stimulated with 100 nM T₃. Promoter activities are presented as percentage of unstimulated (Gal4-SRC-1) or stimulated (Gal4-NCoR) activities ± s.d.

Figure 5 Non-DNA-bound TR represses gene transcription in response to T₃. A chimeric protein of Gal4 DBD with corepressor NCoR (Gal4-NCoR), 5× UAS reporter vector, and optional expression plasmids for TR± and NCoR were transfected and stimulated with 100 nM T₃. Promoter activities are presented as percentage of unstimulated activities ± s.d.
Gal4-NCoR system mimics the transcriptional response from a negatively T₃-regulated gene.

We next extended the mammalian two-hybrid assay by investigating Gal4-SRC-1 (LXXLL), Gal4-SRC-1 (q-rich), and Gal4-NCoR together with wild-type TR, different TR point mutations, or N-terminal TR deletion mutants (Fig. 6). Cotransfection of wild-type TR together with Gal4-SRC (LXXLL) did not show a significant alteration in response to $T_3$. However, introduction of point mutations within the DBD of TR (mutations C71S and C89S) activated $T_3$-mediated gene expression. By contrast, mutants with cofactor binding defects (mutations P158R, C253K, and E401A) had negligible effects within this assay (Fig. 6A). Deletion of the ligand-independent

Figure 6 The capacity of TR to modulate $T_3$-mediated gene transcription is increased by mutations within the DBD of TR. (A) Gal4-SRC-1 (LXXLL), (B) Gal4-SRC-1 (q-rich), or (C) Gal4-NCoR expression plasmids were transfected together with TR wild type or mutants thereof (DBD mutants: C71S, C89S; hinge domain mutant: P158R; LBD mutant: C253K; AF-2 mutant: E401A; and N-terminal deletion mutants: −50 ($\Delta N50$) and −112 amino acids ($\Delta N112$)) and 5x UAS reporter construct and stimulated with 100 nM $T_3$. Promoter activities are presented as percentage of unstimulated activities ± s.d. Schematic of the experimental design has been depicted in the upper part of the figures.
transactivation domain AF-1 (N-terminal deletion of 50 amino acids, ΔN50) did not show significant differences upon T₃ stimulation; however, additional deletion of AF-1 plus DBD (N-terminal deletion of 112 amino acids, ΔN112) leads to a T₃-dependent activation of gene expression similar to the DBD point mutants (Fig. 6A).

Investigation of Gal4-SRC (q-rich) together with TR wild type showed a down-regulation of gene expression (Fig. 6B). Again, introduction of DBD point mutations C71S and C89S blunted the down-regulation, as already seen for the Gal4-SRC (LXXLL) construct. By contrast, introduction of point mutations within corepressor or coactivator interaction domains (P158R, C253K, and E401A) preserved the T₃-mediated down-regulation. N-terminal deletion mutant ΔN50 and ΔN112 blunted T₃-mediated down-regulation, probably due to an altered interaction between AF-1 and SRC-1 (q-rich; Fig. 6B).

Investigation of Gal4-NCoR together with wild-type TR demonstrated a down-regulation, as already observed in Fig. 5. Introduction of DBD point mutations C71S and C89S completely reversed the negative regulation leading to a T₃-mediated activation of gene expression. By contrast, introduction of point mutations within cofactor interaction domains (P158R, C253K, and E401A) and deletion of AF-1 (ΔN50) preserved the T₃-mediated down-regulation, whereas deletion of AF-1 plus DBD increased T₃-mediated transcription (Fig. 6C). These data indicate that the DBD of TR has (besides DNA binding) an additional repressor function, since DBD point mutations further activate thyroid hormone-mediated gene transcription.

**Discussion**

The mechanism of negatively T₃-regulated gene transcription is poorly understood. T₃-mediated positive and negative gene regulation has been observed within the same cell type and must be established by the nature of different DNA elements. This has been exemplified in our experiments using the positively regulated mGPDH promoter and the negatively regulated TSHβ promoter (Fig. 1). The different readout in response to T₃ is independent of the investigated reporter construct and of the investigated cell line (Fig. 1; Supplementary Fig. 2). The regulation of TSH in the pituitary might be additionally regulated by interference of TR with limiting amounts of pituitary-specific transcription factors, such as Pit1 and GATA2 (Sanchez-Pacheco et al. 1995, Nakano et al. 2004). A direct DNA binding of TR has been shown to be dispensable for the negative regulation of the TSHβ gene whereas a critical interaction between the DBD of TR with GATA2 has been reported recently (Matsushita et al. 2007). Positive regulation of mGPDH is accomplished by a well-described TRE that is located close to the transcriptional start site (Weitzel et al. 2001b). Another DNA-binding motif in close vicinity to the transcriptional start site (the so-called z-box) is believed to be responsible for TR binding and negative regulation of the TSHβ and necdin genes (Sasaki et al. 1999, Nygard et al. 2006). However, we could not detect any direct TR-DNA binding to the TSHβ promoter in our cell-based assay (Fig. 2), consistent with the previous data (Tagami et al. 1999). Furthermore, TR binds extremely weakly to ‘negative’ TREs compared with classical ‘positive’ TREs in electrophoretic mobility shift assays (Sasaki et al. 1999, Kim et al. 2005, Hashimoto et al. 2006). Interestingly, most of the suggested binding sites for TR are located close to the transcriptional start site within negatively regulated genes (Sasaki et al. 1999, Villa et al. 2004, Furumoto et al. 2005, Kim et al. 2005, Hashimoto et al. 2006, Nygard et al. 2006, Santos et al. 2006). It remains to be elucidated whether alterations at the transcriptional start site influence the composition of the transcription machinery accounting for the observed negative regulation of gene expression by T₃.

Forced docking of TR to DNA (via a Gal4-DBD-TR fusion protein) results in positive regulation of gene expression in response to T₃ (Fig. 3). Interestingly, introduction of point mutations within the DBD of TR (which is not required for direct DNA binding in this experimental setting) further increased the capacity of this construct to respond to T₃, suggesting a repressor function of DBD that has already been described earlier (Liu et al. 1998). This indicates that the TR-DBD is important not only for DNA binding but also for additional protein–protein interactions (see below; Supplementary Fig. 1, see Supplementary data in the online of version of the Journal of Molecular Endocrinology at http://jme.endocrinology-journals.org/content/vo140/). Not surprisingly, the analysis of point mutations within cofactor binding sites resulted in alleviated responses to T₃, indicating that both coactivator and corepressor binding sites are important for a full functional readout in response to T₃. By contrast, TR without direct binding properties to the reporter construct is able to negatively regulate gene expression in response to T₃ (Fig. 5). Gal4-NCoR, Gal4-SRC (LXXLL), and Gal4-SRC (q-rich) are able to bind to TR as expected (Christiaens et al. 2002, Shibusawa et al. 2003a, Iwasaki et al. 2006) and this interaction is independent of an intact DBD of TR (Fig. 4B).

Using the Gal4-cofactor chimeric constructs, we investigated the consequences of point mutations within TR in T₃-mediated gene expression (Fig. 6). Cotransfection of TR (wild type) together with Gal4-SRC (LXXLL) did not influence T₃-mediated gene expression. However, introduction of point mutations within the DBD of TR increases T₃-mediated gene expression. A similar readout has been observed for the transfection of TR variants together with Gal4-NCoR. Using a TR wild-type construct, we observed a
T$_3$-dependent down-regulation; however, after cotransfection of DBD-binding mutants an increased gene expression in response to T$_3$ has been observed. These data clearly indicate an additional function of the DBD of TR beside its function as DNA-binding site. This function appears to be a repressor function since mutations within the DBD increased gene transcription (Figs 3, 4B and 6).

One function of this modulator appears to be protein–protein interaction. TR has been shown to bind other transcription factors and cofactors via its DBD (Poirier et al. 2005, Matsushita et al. 2007). An interesting interaction is the reported interaction of TR with cAMP response element binding protein (CREB), which is ensured via the DBD of TR (Mendez-Pertuz et al. 2003, Furumoto et al. 2005). Protein–protein interaction leads to an antagonism of CREB-mediated transcription by TR and vice versa. This regulation regime is of particular interest for TSHz and TSHb expressions since both genes are regulated by cAMP (via CREB binding to a CRE site within the promoter sequences) and T$_3$ (Sasaki et al. 1999, Tagami et al. 1999). The DBD of nuclear receptors is the most conserved domain within this family of transcription factors. It is therefore not surprising that mechanisms independent of DNA binding have also been described for DBDs of TR and other nuclear receptors (Reichardt et al. 1998, Poirier et al. 2005). Furthermore, introduction of point mutations within the DBD of TRb, which prevents binding to TRE (GS mutant) conserved some but not all phenotypical alterations in mice compared with conventional TRb knockout animals (Shibusawa et al. 2003b). Since the overall structure of this DBD mutant remains unaffected (Shibusawa et al. 2003a), it is likely that a suggested protein–protein interaction domain remains intact. As also suggested for different members of the nuclear receptor family, these proteins are targets of post-translational modifications, which facilitate their function within the transrepression mechanism (Moeller et al. 2005, Hiroi et al. 2006, Kenessey & Ojamaa 2006, Storey et al. 2006, Wulf et al. 2007). Further investigations should target these questions with special emphasis on the DBD of TR.

Acknowledgements

We are indebted to Ingolf Bach, Malcolm Parker, and Tetsuya Tagami for their kind gifts of plasmid DNA. We would like to thank Hans J Seitz and Josef Köhrle for their continued support during the project and Inga Albers for helpful comments on the manuscript. MK is a recipient of a scholarship of the Studienstiftung des deutschen Volkes. This work was supported by a grant from the Deutsche Forschungsgemeinschaft to J M W. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


Kenessey A & Ojamaa K 2006 Thyroid hormone stimulates protein synthesis in the cardiomyocyte by activating the Akt-mTOR and p70S6K pathways. Journal of Biological Chemistry 281 20666–20672.


Kim SW, Ho SC, Hong SJ, Kim KM, So EC, Christofidele M & Harney JW 2005 A novel mechanism of thyroid hormone-dependent negative regulation by thyroid hormone receptor, nuclear receptor corepressors (NCOr) and GAGA-binding factor on the rat cD44 promoter. Journal of Biological Chemistry 280 14545–14555.


Moeller LC, Dumitrescu AM & Refetoff S 2005 Cytosolic action of thyroid hormone leads to induction of hypoxia-inducible factor-1alpha and glycolytic genes. Journal of Biological Chemistry 280 20666–20672.

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negative regulation of thyrotropin beta gene by thyroid hormone receptors: study with a new experimental system using CV1 cells. Biochemical Journal 378 549–557.


Santos GM, Afonso V, Barra GB, Togashi M, Webb P, Neves FA, Lomri N & Lomri A 2006 Negative regulation of superoxide dismutase-1 promoter by thyroid hormone. Molecular Pharmacology 70 793–800.


Satoh T, Monden T, Ishizuka T, Mitsushashi T, Yamada M & Mori M 1999 DNA binding and interaction with the nuclear receptor co-repressor of thyroid hormone receptor are required for ligand-independent stimulation of the mouse preprothyrotropin-releasing hormone gene. Molecular and Cellular Endocrinology 154 137–149.

Shibusawa N, Hollenberg AN & Wondisford FE 2003a Thyroid hormone receptor DNA binding is required for both positive and negative gene regulation. Journal of Biological Chemistry 278 732–738.


Tagami T, Park Y & Jameson JL 1999 Mechanisms that mediate negative regulation of the thyroid-stimulating hormone alpha gene by the thyroid hormone receptor. Journal of Biological Chemistry 274 22345–22353.

Villa A, Santiago J, Belandia B & Pascual A 2004 A response unit in the first exon of the beta-amyloid precursor protein gene containing thyroid hormone receptor and Sp1 binding sites mediates negative regulation by 3,5,3'-triiodothyronine. Molecular Endocrinology 18 863–873.


Yen PM 2001 Physiological and molecular basis of thyroid hormone action. Physiological Reviews 81 1097–1142.