CHAPTER 1

General Introduction

1

Introduction

Thyroid hormone (TH) plays an important role in normal growth, development, and metabolic homeostasis. This is emphasized by severe growth retardation and neurodevelopmental impairment in patients with prolonged untreated congenital hypothyroidism or cretinism (1-4). Adults with hypothyroidism develop symptoms such as cold intolerance, constipation, fatigue, weight gain, bradycardia and depression (5). In contrast, heat intolerance, weight loss, and tachycardia are observed in hyperthyroid patients, illustrating the strong influence of TH in human metabolism (6,7).

TH is synthesized in the thyroid gland and released to the circulation under a tight regulation by the hypothalamic-pituitary-thyroid (HPT) axis. Two major forms of TH are produced, namely 3,3',5,5'-tetraiodothyronine or thyroxine (T4), and 3,3',5-triiodothyronine (T3). Both T3 and T4 are transported across the plasma membrane by multiple thyroid hormone transporters such as monocarboxylate transporter 8 (MCT8). Transcriptional gene regulation (genomic actions) is the principal action of TH, which is mediated by binding of TH to its nuclear thyroid hormone receptors (TRs). Since T3 binds to TRs with a high affinity, it is regarded as the biologically active TH. T4, despite being the most abundant in the circulation, is considered as a prohormone because of its lower biological potency. T4 is converted to T3 in peripheral tissues by outer ring deiodination (ORD) by the type 1 and type 2 deiodinase enzymes (DIO1 and DIO2).

Thyroid hormone synthesis and regulation

Thyroid hormone production

TH is produced by the thyroid gland. The process starts by active transport of iodide (I^-) into thyroid follicular cells via the Na^+/I^- symporter (NIS; SLC5A5) at the basolateral membrane. Intracellular I^- is then delivered into the follicular lumen using a transporter at the apical membrane, possibly Pendrin (PDS; SLC26A4). Next, I^- is oxidized by the membrane-bound thyroperoxidase (TPO) enzyme, which requires the presence of H_2O_2 generated by the dual oxidase enzyme DUOX2 and its specific maturation factor DUOXA2. Oxidized iodide is incorporated (organified) into tyrosyl residue of thyroglobulin (TG), a large glycoprotein that serves as a matrix for TH synthesis, to create two iodinated forms, namely, mono- and diiodotyrosine (MIT and DIT). TPO also catalyzes the coupling of MIT and DIT, and of two DIT molecules to form T3 and T4, respectively. The iodinated TG is subsequently internalized back into the follicular cells by micropinocytosis and endocytosis. After TG is hydrolysed in lysosomes, T3 and T4 are released into the circulation by transporters, including the TH transporter MCT8. MIT and DIT are deiodinated in the cytosol of thyroid follicular cells by the iodotyrosine dehalogenase (DEHAL1) enzyme to recycle iodine for further TH synthesis (Figure

1) (8-10). Genetic defects in each step of TH synthesis result in thyroid dyshormonogenesis, which causes approximately 15% of all cases of primary congenital hypothyroidism (11,12).

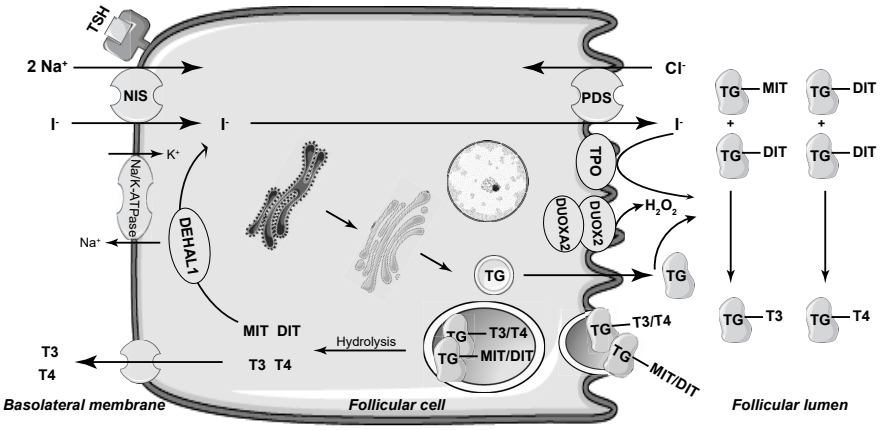

Figure 1. Steps in thyroid hormone synthesis.

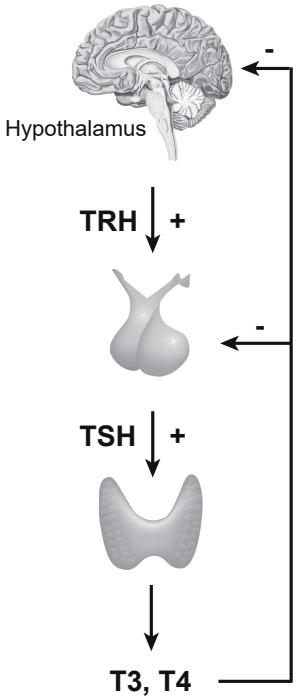

Figure 2. The hypothalamic-pituitary-thyroid axis.

Hypothalamic-Pituitary-Thyroid (HPT) axis

Thyroid hormone synthesis is stimulated by thyroid stimulating hormone (TSH; thyrotropin), a glycoprotein which is released from the anterior pituitary gland. TSH binds to the TSH receptor (TSHR), a G-protein coupled receptor at thyroid follicular cells, to promote multiple steps of TH synthesis, including iodide trapping in the thyroid gland, iodotyrosine and thyroglobulin synthesis, and thyroid hormone release. The concentration of TSH is controlled by thyrotropin-releasing hormone (TRH), produced in the TRH neurons in the paraventricular nucleus of the hypothalamus (13). High concentrations of TH can suppress the production of both TRH and TSH (Figure 2). Animal studies have shown that local conversion of T4 into T3 by the DIO2 in tanycytes, specialized glial cells lining the third ventricle, plays a crucial role in TRH suppression by TH (14,15). Local DIO2 in folliculostellate cells, agranular cells in the human anterior pituitary, converts T4 to T3 and transports T3 to thyrotrophs in a paracrine manner for TSH suppression (16,17). Via this negative feedback mechanism, circulating TH concentrations are maintained within the normal range.

Thyroid hormone transport

To facilitate its genomic action, TH has to enter the cells and bind to TRs. It was previously believed that TH entered the cells by passive diffusion because of its lipophilic property that would allow easy passage of TH through the phospholipid bilayers of the cell membrane. However, later evidence suggested that T3 and T4 are taken up into the cells by transporter proteins located at the plasma membrane of the cells. To date, several TH transporters have been identified, including the iodothyronine-specific transporters monocarboxylate transporters MCT8 (SLC16A2) (18,19) and MCT10 (SLC16A10 or TAT1) (20), the organic anion transporting polypeptide (OATP) family, especially OATP1C1 (SLCO1C1) (21), the Na^+-taurocholate co-transporting polypeptide (NTCP; SLC10A1) (22), and the L-type amino acid transporters LAT1 (SLC7A5) and LAT2 (SLC7A8) (23).

The importance of TH transport across the cell membrane is illustrated by the identification of patients carrying mutations in the TH transporters. The Allan-Herndon-Dudley syndrome (AHDS) was first described in 1944 in a large family with X-linked psychomotor retardation (24). Inactivating mutations in MCT8 were subsequently identified as a cause of this genetic syndrome (25,26). The clinical phenotype of AHDS includes cognitive impairment, intellectual disability, and central hypotonia. Thyroid function tests (TFTs) show low free and total T4, low reverse T3 (rT3), high free and total T3, increased T3/T4 and T3/rT3 ratio, and normal to mildly elevated TSH concentrations (18,27). Recently, Strømme et al. reported a homozygous missense mutation in OATP1C1 as a cause of developmental regression, progressive dementia, spastic diplegia, and cold intolerance in a 15-year-old girl with normal TFTs (28). Since OATP1C1 is important for TH transportation across the blood-brain barrier and into glia and neuronal cells in the brain, loss of the OATP1C1 function likely leads to brain-specific hypothyroidism and neurodegeneration.

Thyroid hormone metabolism: deiodination

As mentioned previously, T4 is the dominant form of TH that is secreted from the thyroid gland and is subsequently converted into the active form T3 at peripheral tissues. The deiodinase enzymes, a subfamily of three selenoproteins capable of removing an iodine atom from the inner (tyrosyl) or outer (phenolic) ring of TH, mediate this conversion (Figure 3). The DIO1, expressed in the liver, kidney, and thyroid gland, can deiodinate both the inner and outer ring of T4 to produce T3 and rT3, respectively. The DIO2, which is ubiquitously expressed in brain, pituitary, retina, brown adipose tissue, innate immune cells, and skeletal muscle, is only able to deiodinate the outer ring (5') of TH. The major role of DIO2 is therefore the conversion of T4 to T3 as well as the control of local tissue T3 concentration. The DIO3 is a TH-inactivating enzyme, as it can only deiodinate the iodine atom from the inner ring of T4 and T3. DIO3 is mainly expressed in fetal tissue and plays a crucial role in embryogenesis. It is also expressed in retina, neurons, pituitary gland, and various type of tumors, such as hemangioma, glioma and gliosarcoma, basal cell carcinoma, pituitary adenoma, and papillary thyroid carcinoma (29-32). Both DIO2 and DIO3 work antagonistically to control intracellular T3 concentrations. DIO2 converts T4 to T3 and therefore increases TH signaling, whereas DIO3 inactivates T4 and T3 and decreases TH signaling (33).

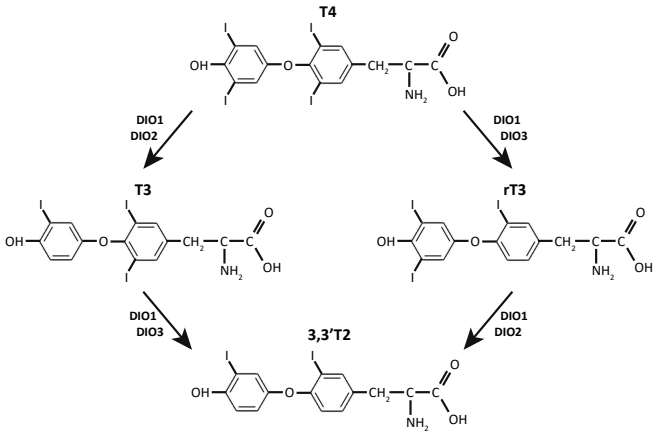

Figure 3. TH metabolism by deiodinase enzymes.

Thyroid hormone receptors and its genomic actions

Genomic actions of thyroid hormone are initiated by binding of T3 to TRs. TRs either homodimerize with the other TR or, more preferably, heterodimerize with retinoid X receptor (RXR) and bind to thyroid hormone response elements (TREs) in the promoter region of TH

target genes to regulate gene expression. In the absence of T3, the TRs recruit corepressor proteins that modify the chromatin structure, resulting in transcriptional repression of genes that are under positive control by TH. In the presence of T3, TRs then release the corepressors and recruit coactivators to induce gene transcriptional activation.

Multiple thyroid hormone isoforms

TRs are members of the nuclear receptor superfamily. Similar to other nuclear receptors, TRs consist of multiple functional domains, including an amino-terminal A/B domain, a central DNA-binding domain (DBD), a hinge region, and a carboxy-terminal ligand binding domain (LBD). There are multiple TR isoforms, generated from two different genes; however, only three functional isoforms have been described that are capable of binding T3 and controlling nuclear gene transcription, namely TRα1, TRβ1, and TRβ2 (Figure 4) (34-36). The structure of these three isoforms is highly homologous. TRα1 is encoded by *THRA* gene on chromosome 17. The alternative splice variant TRα2 is encoded by the same gene but has no T3-binding ability because of differences in length and amino acid composition in the C-terminal region. The *THRB* gene, located on chromosome 3, encodes two T3-binding TR isoforms, TRβ1, and TRβ2, which share high sequence homology in both DBD and LBD but differ in the length and amino acid sequences in the N-terminal A/B domain.

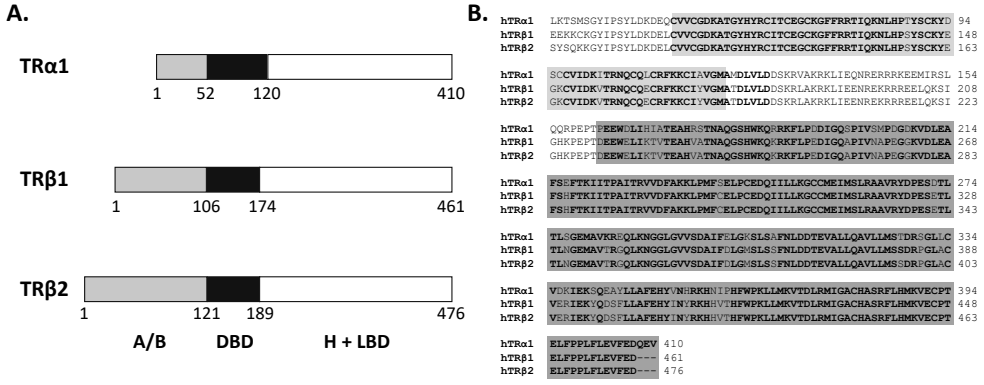

Figure 4. (A) Three TR isoforms that are capable of binding T3 and controlling gene transcription. TRα1 is encoded by *THRA* gene on chromosome 17. TRβ1 and TRβ2 are encoded by *THRB* gene on chromosome 3. (B) Comparison of the sequences shows a high sequence homology (bold) of the DBD (light grey) and LBD (dark grey) of the three TR isoforms. [A/B, A/B domain; DBD, DNA-binding domain; H, hinge region; LBD, ligand binding domain] (Adapted from Langlois MF et al. 1997, Hahm JB et al. 2013, and Wagner RL et al. 1995 (37-39))

The expression of TR isoforms varies among tissues. TRα1 is predominantly expressed in the central nervous system, bone, cardiac tissue, gastrointestinal tract, and skeletal muscle while the non-T3 binding isoform TRα2 is more wildly expressed throughout the whole body. TRβ1 is principally expressed in the liver, kidney and thyroid gland, whereas

TRβ2 is predominantly expressed in the retina, cochlea, as well as the hypothalamus and pituitary gland, where it plays a crucial role in the HPT axis regulation (34,35).

Isoform-dependent functions of TRs

To date, it is unclear whether the different TR isoforms have specific functions or share a similar role in gene transcriptional regulation. Although the properties of all TR isoforms are quite similar *in vitro*, *in vivo* studies show clear differences in the consequences of mutations in the different receptors (35,40). TRα gene knock-out mice ($TR\alpha^{0/0}$) have growth retardation, intestinal malformation, delayed bone maturation, bradycardia, abnormal cardiac contractility, and hypothermia (41). In contrast, TRβ gene knock-out mice ($TR\beta^{-/-}$), in which both TRβ1 and β2 are absent, have normal growth, but HPT axis dysfunction, hearing loss, and abnormal retinal development (42-44). These findings suggest differences in intrinsic properties and/or cell-specific effects of the receptor isoforms. In addition, the phenotypes of these knock-out mice are matched by the phenotypes of patients with resistance to thyroid hormone (RTH) due to mutations of *THRA* gene which is different from the phenotype of patients carrying mutations of the *THRB* gene.

It is generally assumed that differences in tissue distribution of the TRs predominantly dictate the isoform-specific functions. However, there is evidence suggesting that even in cell types where two TR isoforms are available, TRs regulate gene transcription in an isoform-specific manner. For instance, in brown adipose tissue (BAT) where both TRα1 and TRβ1 are expressed (45,46), TRα1 is responsible for norepinephrine-induced BAT thermogenesis whereas TRβ1 mediated expression of mitochondrial uncoupling protein 1 (UCP1), a mitochondrial membrane protein that plays a crucial role in BAT thermogenesis (47). Data from TR knock-out mice also showed that DIO1 expression have differences in tissue and isoform regulation. The expression is solely regulated by TRβ in the kidney but requires both TR isoforms in the liver (48). Furthermore, co-expression of both TRα1 and TRβ1 is also observed in Purkinje neurons, in which cell differentiation is T3-dependent. Only *Thra* knock-out, but not *Thrb* knock-out, altered *in vitro* differentiation of Purkinje neurons, suggesting an TRα1-specific effect (49). However, both *in vitro* studies performed in other cell types and *in vivo* studies are needed to confirm these findings.

Ligand of TRs: T3 and beyond

In 1952, it was recognized that T3 has a higher biological potency than T4 (50-53). This fundamental discovery led to the clinical concept that T3 is the biologically active hormone. Crystal structures showed that the LBD of TRs consists of 12 α-helices (H1-H12) that fold into a hydrophobic-core pocket. T3 can be tightly accommodated in this pocket and induce conformational changes of the TR, especially at the location of H12, to enclose the ligand-binding pocket (54,55). This conformational rearrangement induces corepressor protein dissociation and allows coactivator protein association, which is essential for gene transcriptional activation.

Follow up studies suggest that T4 can also bind to TRs with a lower binding affinity (10-30 fold) than that of T3 (56-58). The T4-bound WT TRβ1 crystal structure revealed that the ligand-binding pocket of TRβ1 could accommodate both T3 and T4, although the H11-H12 loop is more loosely packed in the presence of T4 than T3 (57). However, the molecular and structural mechanisms underlying the higher affinity of T3 than T4 have not been investigated in detail. In addition, the precise role of T4 as a prohormone and the possibility that T4 might function directly as an active hormone in at least specific cellular contexts remains inconclusive.

In addition to T3 and T4, there are naturally occurring TH metabolites that can also bind to TRs, such as 3,3',5-triiodothyroacitic acid (Triac), the T3-derivative containing an acetic acid group. Evidence indicates that Triac binds to TRα1 with a similar affinity as T3 and binds to TRβ1 and TRβ2 with a 3- to 6-fold higher affinity than T3 (59). Therefore, Triac is considered as a TRβ-selective agonist which can be used as a treatment option in a certain condition such as RTHβ and AHDS (60). However, since the concentration of Triac in human circulation is approximately 50-fold lower than that of T3, the physiological role of this TH derivative is yet unclear (60,61).

Over the past decades, numerous TH analogs have been synthesized in order to create novel therapeutic agents for certain conditions, for instance, hyperlipidemia, obesity, and non-alcoholic fatty liver diseases (NAFLD) (62-66). These analogs can bind to TRs with differences in isoform specificity. However, most of them are designed as more specific for the TRβ isoforms to minimize the TRα-dependent cardiac side effects. A list with examples of TH analogs is summarized in Table1.

Table 1. TH analogs

Compound	Isoform specificity	Potential benefit(s)
DITPA	Non-selective	↓ cholesterol and triglyceride levels, ↑ cardiac output (without significant increase in heart rate)
GC-1 (Sorbetirome)	TRβ	↓ cholesterol levels, ↓ hepatic steatosis, ↑ liver regeneration
GC24	TRβ	↓ triglyceride levels
KB-141	TRβ	↓ triglyceride levels, ↓ body weight
KB-2115 (Eprotirome)*	TRβ	↓ cholesterol and triglyceride levels, ↓ hepatic steatosis, ↑ hepatocyte proliferation
MB07811	TRβ	↓ cholesterol and triglyceride levels, ↓ hepatic steatosis
MGL3196	TRβ	↓ triglyceride levels, ↓ hepatic steatosis

*Evidence shows adverse effects on cartilage and drug-induced liver toxicity (elevated AST, ALT, and gamma glutamyltranspeptidase).

Multilevel regulation of TR transcriptional activation

Multiple configurations of thyroid hormone response elements

To regulate gene transcription, both unliganded and liganded TRs (in combination with other TR or RXRs) bind to the TREs using the DBD. The TREs consist of two consensus hexanucleotide half-sites [(A/G)GGT(C/A/G)A] that can be arranged as a direct repeat (DR), inverted repeat (IR), and everted repeat (ER) (Figure 5) (35). The space between the two half-sites varies, depending on the orientation of the half-site. There is evidence showing that the orientation of TREs determines the dimerization pattern of TRs (67,68). In addition, *in vitro* studies of TRβ1 mutations show a differential effect on different TREs of some mutants (69,70), suggesting a TRE-specific transcriptional impairment. However, ChIP-seq analyses show that the DR4-TRE is the most common TR binding site identified at the promoter regions of TH target genes (71-74). Therefore, the exact role of different TRE configurations on transcriptional gene regulation is still doubtful.

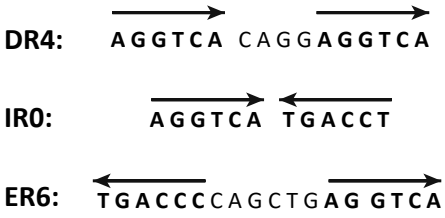

Figure 5. Consensus TRE half-site and three main TRE configurations. [DR4, direct repeat; IR0, inverted repeat; ER6, everted repeat] (Adapted from Cheng SY et al. 2010 (35))

TR-RXR heterodimerization

As mentioned previously, TRs either form homodimers or heterodimers with RXRs to regulate gene expression. However, heterodimerization with RXR is the primary form of TR-dimerization for both TRα1 and TRβ1 isoforms. The heterodimerization dramatically increases the binding of TRs to TREs, and the T3-induced transcriptional activation (35). TRs bind to RXRs via a highly conserved ninth heptad region in H11 of the TR LBD (Leu367-Leu374 of TRα1 and Leu421-Lue428 of TRβ1) (75). Mutations in this region concomitantly decrease heterodimerization and receptor transactivation (34) Although TRs mainly form heterodimers, previous studies indicate that TRβ1 may have a greater tendency than TRα1 to form homodimers on several TREs, suggesting that these two isoforms may have different dimerization potentials (34)

TR-coregulatory protein interactions

To control target gene expression, TRs associate with many coregulatory proteins. These proteins modify the histone core of nucleosomes by acetylation, methylation, and ubiquitination, all of which lead to a change of the chromatin structure (chromatin remodeling) and accessibility of target genes (73,76). In case of genes that are positively regulated by TH, unliganded TRs recruit corepressor proteins to repress gene transcription, while liganded TRs induce coactivator recruitment and consequently stimulate gene transcription. This process is called the “classic bimodal switch model” of TR action (Figure 6) (77).

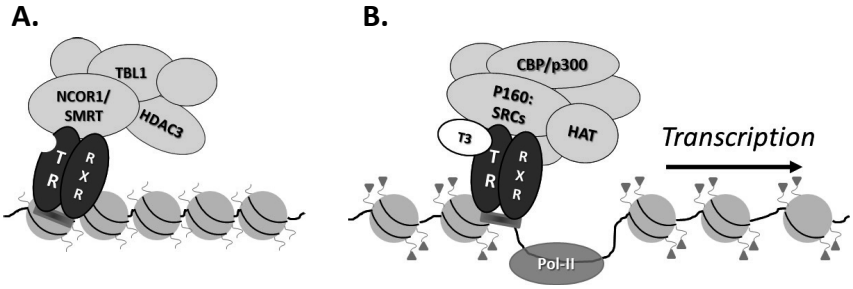

Figure 6. Classic bimodal switch model of TR action. (A) Unliganded-TR heterodimerizes with RXR and recruits corepressor proteins, leading to nucleosome packing. (B) Liganded-TR, in combination with RXR, recruits coactivator proteins, including histone acetyltransferase (HAT) that acetylate (triangles) neighboring histones. This process unpacks the nucleosomes and allows critical enzymes such as RNA-polymerase II (Pol-II) to approach the target gene and initiate gene transcription.

The most well-known TR corepressors are NCoR (nuclear receptor corepressor) and its homolog, SMRT (silencing mediator of retinoid and thyroid hormone receptors). These proteins bind to the corepressor interacting sites in the C-terminal region of TRs and recruit other nuclear proteins such as transducing-like protein (TBL1 or TBL1R) and histone deacetylase 3 (HDAC3) to form large corepressor complexes (78-80). By removing the acetyl group from histones, HDAC3 creates nucleosome compaction, thereby inhibiting the binding of RNA polymerase II which results in suppression of target gene transcription.

Binding of TH to TRs causes a conformational change in H12 of the TR-LBD, in a way that favors dissociation of the corepressors from and association of the coactivators with the TRs. The main TR-binding coactivators are steroid hormone receptor co-activator 1, 2, and 3 (SRC-1, -2, and -3) (81,82). SRCs interact with coactivator interaction sites of TRs by using the LXXLL motif (NR box) located in the central part of the SRC molecule. After binding to the TRs, two activating domains (ADs) located in the C-terminal region of SRC recruit chromatin-

modifying coregulatory complexes such as CBP/p300 processing histone acetyltransferase (HAT), which results in chromatin accessibility and activates gene transcription.

Apart from well-known corepressors and coactivators, other nuclear proteins have also been reported as coregulators for TRs. For instance, Hairless (83,84), Alien (85,86), RIP-140 (87), and Jab1 (88) were identified as corepressors, whereas nuclear receptor-interacting factor 3 (NRIF3), as known as integrin subunit beta 3 binding protein (ITG3BP), was identified as a coactivator for TRs (89,90). This evidence highlights the complexity of gene transcriptional regulation by TRs. In addition, the expression of many nuclear receptor coregulatory proteins could be tissue-dependent (40,91-93), and some patterns of coregulatory protein recruitment could be isoform-specific (38,91,94). These mechanisms could further explain the various transcriptional regulations of TR in different tissues.

Mutation of TRs: Resistance to thyroid hormone

Resistance to TH (RTH) is a syndrome of reduced sensitivity to TH of target tissues, which was firstly described in 1967 (95). Mutations of the gene encoding TRβ *(THRB)* were subsequently identified as a cause of this disease (96). Since then, the term RTH has become synonymous with this condition. In 2012, mutations of the gene encoding TRα *(THRA)* were identified (97,98), thereby extending the spectrum of RTH. Today, RTH includes all syndromes resulting from dysfunction in TH transport, deiodination and receptor dysfunction (99,100). However, in this thesis, we mainly focus on RTH caused by mutations of the TRs (RTHα and RTHβ respectively).

RTHβ

Mutations in the LBD of TRβ1 and TRβ2 lead to RTHβ. Common biochemical characteristic includes high serum T3 and T4 concentrations with normal or slightly increased TSH level. However, the clinical presentation varies between patients. This is partly dependent on the severity of hormonal resistance, but there is also large variation between different family members with the same mutation. Goiter is the main clinical finding that prompts patients to seek for medical investigations (100,101). Tachycardia, short stature, and attention deficit disorders can also be part of the clinical presentation in affected individuals because of the effect of high THs in TRα predominant tissues such as heart, brain, and bone. The incidence of RTHβ is approximately 1:40,000 live births (102,103).

RTHβ is usually inherited in an autosomal dominant fashion (100). Single nucleotide substitutions leading to amino acid replacement are more common than frameshift or nonsense mutations that result in premature protein truncations (104-106). To date, over 160 different mutations have been identified as a cause of RTHβ in more than 350 families (101,107). These mutations are located in three CpG rich hotspots (cluster 1; codon 426-461, cluster 2;

310-353, and cluster 3; codon 234-282) that are prone to mutate in the LBD and the hinge region of TRβ (100). Therefore, mutations commonly impair affinity for TH and consequently reduce transcriptional activity of the receptor. However, mutations that impair TR dimerization or interaction with nuclear co-regulatory proteins have also been reported (70,108-115).

RTHα

Because of a high degree homology between TRα and TRβ (overall 80% of amino acids in their LBD are identical), it was anticipated for many years that mutations in TRα would also be able to cause RTH. Therefore, many knock-in and knock-out mouse models were generated to predict the clinical phenotype of RTHα (116-119). All of the mutated mice showed impaired growth and bone development but near normal TFTs, complicating the easy identification of RTHα patients. As a consequence, mutations in *THRA* as a cause of RTHα had not been identified until 2012 (97,98), probably due to this lack of an obvious thyroid function abnormality.

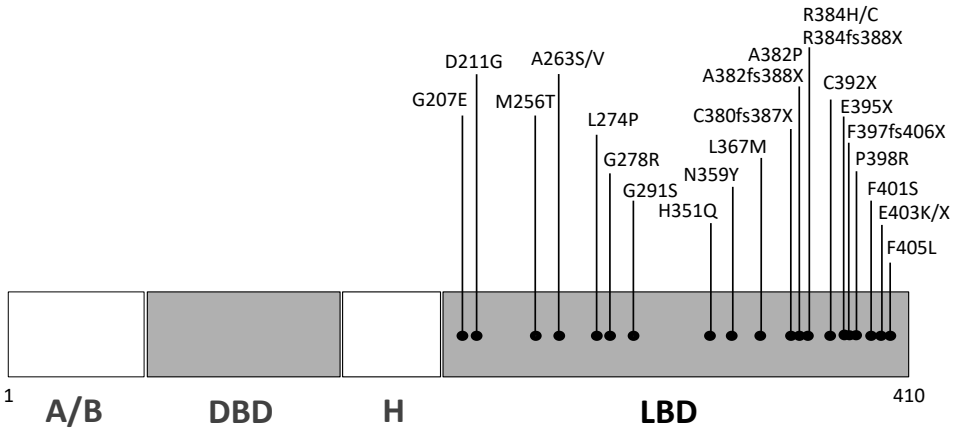

Figure 7. Localization of 25 TRα1 mutations identified in RTHα patients. [A/B, A/B domain; DBD, DNA-binding domain; H, hinge region; LBD, ligand binding domain]

The clinical phenotype of RTHα patients is distinct from RTHβ and includes growth retardation, macrocephaly, constipation, intellectual disability, autistic spectrum disorder, and anemia. Their TFTs are typically characterized by high to high-normal (F)T3, low to low-normal (F)T4, low rT3 and normal TSH concentrations, resulting in markedly increased (F)T3/(F)T4 and (F)T3/rT3 ratios. To date, 25 mutations (in a total of 40 patients) have been reported as a cause of RTHα, all of which are located in the LBD of TRα1 and impair T3 binding affinity (Figure 7). These mutations can be categorized into two groups based on the type of mutation.

The first group consists of truncating mutations caused by frameshift or nonsense mutations that lead to premature stop codons and shorten the length of the LBD (97,98,120-124). This structural alteration abolishes T3 affinity and T3-induced transcriptional activity of TRα1. The second group consists of missense mutations that result in single amino acid substitutions in the LBD (121,122,125-133). These mutant receptors can still bind T3 but with a lower affinity than the WT receptor.

Diverse functional impairment of TR mutants and phenotype variability

In RTHβ, patients who carry different mutations commonly have differences in phenotype severity as well as thyroid dysfunction. Interestingly, these differences are also found between individuals that express the same mutation (100,101). This same variety in clinical phenotype is also observed in RTHα patients. In general, patients with truncating mutations that completely abolish T3 binding affinity have a more severe phenotype than patients with missense mutations that have residual T3 binding (128). However, there are differences within each group and even between patients carrying the same mutation. For instance, in a large RTHα family carrying A263S mutation, the severity of constipation, macrocephaly, delay development, and anaemia, are diverse between affected members (121). So far, the underlying molecular mechanism to explain this observation has not been clearly revealed.

Since mutations in TRα and TRβ are mainly located in the LBD and affect T3 binding affinity of the receptors, it could be anticipated that the severity of T3 binding impairment by the different mutants correlates with the degree of transcriptional impairment and the severity of the clinical phenotype. However, it has been demonstrated in RTHβ that differences in T3 binding do not solely explain the diversely impaired transcriptional activation of the mutants. Some TRβ mutants have severe transcriptional impairment despite only mild disturbances in T3 binding affinity. *In vitro* studies showed that these mutants either impair dimerization (69,108,109,111,134) or TR-cofactor interaction (70,112-115,135). In addition, a small group of TRβ mutants impairs transcriptional activation only when associated with certain TRE configuration (70). These findings may partly explain the phenotype variability in RTHβ patients. Since RTHα has recently been identified, additional patients and studies to explore the differences in RTHα are needed.

Outline of the thesis

In this thesis, we focus on the complexity of the genomic actions of TH. In **chapter 2**, we describe a novel mutation, TRβ1-L341V, as a cause of RTHβ and emphasize the crucial role of the Leu341 in TRβ function. In **chapter 3**, we unravel the molecular and structural mechanism underlying the differences in biological activity of T3 and T4, prompted by the identification of a novel TRα1-M256T and previously reported TRβ1-M310T mutations in RTHα and β patients, respectively. In **chapter 4**, we investigate the factors that contribute to the differential impaired transcriptional activity of seven TRα missense mutations, four of which are derived from RTHα patients. In **chapter 5**, we study the difference in neurocognitive impairment of RTHα patients carrying various truncating mutations by evaluating the pattern of gene expression of stably expressed WT or mutant TRα1 in a human neuronal cell line (SH-SY5Y). In **chapter 6**, we explore the pattern of nuclear coregulatory protein recruitment of TRs using interactome analysis. **Chapter 6a** focuses on the cell-type specific coregulatory protein recruitment of TRα1 by performing the experiments in human liver and neuronal cell lines (HepG2 vs. SH-SY5Y). **Chapter 6b** focuses on the isoform-dependent (TRα1 vs. TRβ1) coregulatory protein recruitment. In **chapter 7**, we discuss the findings presented in this thesis combining with the currently available literature and the possible implications of these studies.

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