We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Negative Regulation of the Thyrotropin β Gene by Thyroid Hormone

Shigekazu Sasaki, Akio Matsushita and Hirotoshi Nakamura
*Second Division of Internal Medicine
 Hamamatsu University School of Medicine
 Japan*

1. Introduction

Thyroid hormone (T3 and T4) is secreted from the thyroid gland, and is known to reduce the level of serum thyrotropin (thyroid-stimulating hormone, TSH) in the pituitary gland (Sarapura et al., 2002; Shupnik et al., 1989) (Fig. 1A). This is a typical example of negative feedback between the pituitary and endocrine organs, and is a key component of thyroid hormone homeostasis. TSH is one of the peptide hormones generated in the anterior pituitary, and is a heterodimer composed of an α chain (α -glycoprotein subunit, α GSU) and a β chain (TSH β) (Shupnik et al., 1989). While α GSU is common to follicle stimulating hormone (FSH), luteinizing hormone (LH) and chorionic gonadotropin (CG), TSH β is specific to TSH alone. Although the concentration of serum T4 is much higher than that of T3, T4 is converted to T3 by deiodinase (Dio) in the TSH-producing cells (thyrotrophs) of the pituitary (Christoffolete et al., 2006), and T3 exhibits biological activity as a thyroid hormone (Gereben et al., 2008). T3 inhibits expression of both *TSH β* and *α GSU* at the transcriptional level (Shupnik et al., 1989). The magnitude of T3-induced repression of the *TSH β* gene is greater than that of *α GSU*. Here, we provide an overview of the molecular mechanisms involved in T3-induced negative regulation of the *TSH β* gene and its related genes.

2. Structure of T3 receptors (TRs)

T3 receptor (TR) belongs to the nuclear hormone receptor (NHR) superfamily, and is a ligand-dependent transcription factor (Cheng et al., 2010). TR is encoded by two separate alleles; *TR α* and *TR β* . Through alternative splicing, the *TR α* gene generates TR α 1 and TR α 2, while the *TR β* gene generates TR β 1 and TR β 2 (Fig. 2). While TR α 1, TR β 1 and TR β 2 have T3-binding capacity, TR α 2 does not bind T3. Hence, TR α 1, TR β 1 and TR β 2 are thought to be the functional TRs. TR β 2 is expressed in limited organs including pituitary, hypothalamus and retina, while TR α 1 and TR β 1 are ubiquitously expressed (Cheng et al., 2010). As in the case of other NHRs, TR consists of an N-terminal region (NTD), a central DNA binding domain (DBD), a hinge region and a C-terminal ligand binding domain (LBD) (Fig. 2).

Fig. 1. Negative feedback loop in the hypothalamus-pituitary-thyroid axis and negative regulation of *TSHβ* and *αGSU*. A. The secretion of TSH (a heterodimer of *TSHβ* and *αGSU* subunits) in the anterior pituitary and TRH in the hypothalamus is inhibited by thyroid hormones (T3 and T4). β , *TSHβ* chain. α , *αGSU* chain. TRH, thyrotropin releasing hormone. Synthesis of TRH in hypothalamus is also negatively regulated by T3. B. In patients resistant to thyroid hormone (RTH), a negative feedback loop is impaired due to a defect in T3 receptor (TR) β . This finding provides the evidence for the involvement of TR β in the negative regulation of the *TSHβ* and *αGSU* genes. Because of increased secretion of TSH, diffuse goiters are often found in the patient with RTH.

Fig. 2. Schematic representations of TR isoforms. TR consists of an N-terminal region (NTD, A/B domain), a central DNA-binding domain (DBD, C domain), a hinge region (D domain) and a C-terminal ligand binding domain (LBD, E/F domain). The numbers within the box represent the amino acid homology (%). While TR α 1, TR β 1 and TR β 2 have T3-binding capacity, TR α 2 does not bind T3. The P and D boxes are required for the recognition of half-site sequences (typically, AGGTCA) and the number of spacing nucleotides, respectively. The T and A boxes are involved in dimer formation and polarity of TR-RXR heterodimers on positive TRE (pTRE). P, Pbox. D, D box. T, Tbox. A, A box. RTH, three hot spots where mutations are frequently found in patients with RTH. AF-2, activation function 2.

3. Mechanism of positive regulation by T3

TR activates or inhibits the transcription of its target genes in a T3-dependent manner, and the molecular mechanism of T3-dependent activation (positive regulation) has been elucidated (Cheng et al., 2010) (Fig3A). Because findings in molecular mechanisms of positive regulation by T3 have greatly influenced the studies of negative regulation, it is necessary to outline the mechanism of T3-dependent positive regulation (Fig. 3A) before describing the negative regulation of the TSH β gene (Fig. 3B). In the positive regulation, TR heterodimerizes with retinoid X receptor (RXR) at the T3-responsive element (TRE) of the gene, the transcription of which is positively regulated by T3-bound TR (T3/TR) (Cheng et al., 2010). In the absence of T3, TR-RXR heterodimers interact with co-repressors, including nuclear receptor co-repressor (NCoR) or silencing mediator for retinoid and thyroid hormone receptors (SMRT). These co-repressors recruit histone deacetylase (HDAC), which represses the transcription of the target genes. This repressive effect by unliganded TR is referred to as “silencing” and is thought to play an important role in the clinical symptoms in hypothyroidism (Astapova et al., 2008; Astapova et al., 2011). Upon T3 binding, the TR-RXR heterodimers release NCoR or SMRT and then recruit p160 family cofactors including

steroid receptor coactivator-1 (SRC-1). The TR-RXR-p160 complex also recruits an additional coactivator, CBP/p300 (Chen et al., 1999; Glass and Rosenfeld, 2000; Huang et al., 2003). Both the p160 family and CBP/p300 have intrinsic histone acetyltransferase (HAT) activity and modify chromatin structure, resulting in the transactivation of the target genes (Fig. 3A).

Fig. 3. Schematic representation of T3-dependent transactivation (positive regulation) (A) and transrepression (negative regulation) of the *TSHβ* gene (B). A. TR heterodimerizes with retinoid X receptor (RXR) on the T3-responsive element (TRE) of the gene, the transcription of which is positively regulated by T3-bound TR (T3/TR). Arrow, half-site sequence (typically, AGGTCA). 4xN, random four nucleotides for spacing. B. An nTRE (GGGTCA) has been postulated in the region immediately downstream to the transcription start site (TSS) of the *TSHβ* gene. In contrast to the mechanism for positive regulation, the molecular mechanism of negative regulation has been controversial (see text).

As shown in Fig. 3A, a typical TRE has a unique configuration designated as direct repeat 4 (DR4), in which the random four base pairs (spacer) are incorporated into tandem repeats of a hexameric half-site (Cheng et al., 2010; Umesono et al., 1991). Both the half-site sequence (typically AGGTCA) and the number of spacer nucleotides determine the specificity for DNA recognition by the TR-RXR heterodimer. Analogous to TR-RXR heterodimer binding at DR4, RXR heterodimerizes with vitamin D3 receptor (VDR) on direct repeats of half-sites spaced with 3 nucleotides (DR3) and it functions as a heterodimer partner for retinoic acid receptor (RAR) on direct repeats spaced with 5 nucleotides (DR5) (Cheng et al., 2010; Glass and Rosenfeld, 2000). In the studies of

positive regulation by T₃, monkey kidney-derived CV1 cells (Jensen et al., 1964) have been often used (Naar et al., 1991; Tillman et al., 1993; Umesono et al., 1991) because they possess endogenous RXR but not TR.

4. Involvement of TR in negative regulation of the *TSH β* gene: Syndrome of thyroid hormone resistance (RTH)

Although the molecular mechanism in negative regulation of the *TSH β* gene has been disputed (Lazar, 2003; Shupnik, 2000; Weitzel, 2008), it is apparent that TR plays a crucial role in it. Syndrome of resistance to thyroid hormone (RTH) is characterized by a reduced tissue response to T₃ (Fig. 1B). The majority of patients with RTH have mutations in the LBD of the *TR β* gene, of which amino acid sequence is shared by TR β 1 and TR β 2 proteins (Refetoff et al., 1993) (Fig. 2). These mutant TR β 1s and TR β 2s have defects in their T₃-binding capacity but have intact DBDs capable of recognizing the TRE (Fig. 3A). Thus, they bind to the TRE and constitutively interact with NCoR or SMRT, even in the presence of T₃, resulting in silencing. In the majority of patients with RTH, inheritance is usually autosomal dominant, and mutant *TR β* is thought to interfere with T₃-induced activation by wild-type TR bound to the TRE (dominant negative effect). Of note, patients with RTH also exhibit elevated secretion of TSH (syndrome of inappropriate secretion of TSH, SITSH) (Refetoff et al., 1993) (Fig. 1B). This finding provides evidence for the involvement of *TR β* in the negative regulation of the *TSH β* and *aGSU* genes. However, the mechanism downstream to TR has been unknown (Lazar, 2003; Shupnik, 2000; Weitzel, 2008). With regard to the mechanism of negative regulation of *TSH β* and *aGSU*, the central question has been whether TR directly interacts and recognizes the DNA sequence of the *TSH β* promoter, as identified for the TRE in positive regulation. Some theories indicate the direct binding of TR with DNA, while others favor models that are independent of a direct binding with DNA.

5. Direct binding of TR with DNA: Negative TRE (nTRE) hypothesis

Following the identification of the role of the TRE in the positive regulation of genes (hereafter, positive TRE, pTRE), some researchers have postulated a so-called negative TRE (nTRE) in the *TSH β* (Fig. 3B) and *aGSU* genes (Chin et al., 1993; Shupnik, 2000; Wondisford et al., 1989). The observation that serum TSH levels increase in hypothyroidism led to the idea that unliganded TR may be the transcriptional activator for *TSH β* (Fig. 3B, upper panel) and *aGSU*. If unliganded TR is the transcriptional activator on the nTRE of these genes, one may be able to identify the nTRE as the sequence required for the transcriptional activation by unliganded TR. Based on this hypothesis, deletion analysis of the *TSH β* promoter was performed using human kidney-derived 293 cells (Wondisford et al., 1989) and it was reported that the transcriptional activity of this promoter was abolished after deletion of a short DNA sequence immediately downstream to the transcription start site (TSS) (Wondisford et al., 1989) (Fig. 4). This sequence (GGGTCA) has been postulated as the nTRE because it has homology with the consensus sequence of a half-site (AGGTCA). The nTRE hypothesis has been regarded as one of the principal models to explain the molecular mechanism of negative regulation of the *TSH β* gene (Chin et al., 1993; Cohen and Wondisford, 2005), and has been regarded as a potential mechanism of T₃-dependent negative regulation of other genes (Edwards et al., 1994; Kim et al., 2005; Lin et al., 2000; Santos et al., 2006; Wright et al., 1999). However, this raised several questions, as discussed below.

Fig. 4. Schematic representation of the *TSH β* promoter. Pit1-US, functional Pit1-binding site. GATA-TRE, GATA-responsive element. SR, suppressor region. Pit1-like, the sequence similar to Pit1-binding site. TATA. TATA box. An nTRE (GGGTCA) has been postulated immediately downstream to the TSS.

5.1 Does TR heterodimerize with RXR on the nTRE?

While RXR is the obligate heterodimer partner for TR recognition of the pTRE (Fig. 3A), the involvement of RXR with the nTRE has not been determined (Fig. 3B). Although the nTRE sequence appears to be a single half-site, there remains the possibility that its flanking sequences may function as another half-site (Fig. 3B). However, if TR heterodimerizes with RXR on the nTRE, this configuration cannot be discriminated from that present on the pTRE (Fig. 3A), which may be functioning as a T3-dependent transcriptional activator but not an inhibitor. Previous results of reporter assays examining the effect of RXR overexpression on the *TSH β* promoter have been controversial. Cohen et al. (Cohen et al., 1995) and Hallenbeck et al. (Hallenbeck et al., 1993) reported that RXR may antagonize inhibition of the *TSH β* gene by T3/TR, while Safer et al. (Safer et al., 1997) reported that the requirement for RXR is different between TR β 1 and TR β 2. Nagaya et al. (Nagaya and Jameson, 1993) demonstrated that mutant TR β 1 (L428R) which is unable to dimerize with RXR failed to mediate T3-induced inhibition of the *aGSU* promoter, while Takeda et al. (Takeda et al., 1997) reported that overexpression of RXR had no effect on this promoter. According to Laflamme et al. (Laflamme et al., 2002) RXR enhances the negative regulation of the *TSH β* gene by T3, and this effect is mediated by the RXR-LBD and but not the DBD, suggesting that RXR may act as a cofactor.

It was reported that the ligand for RXR (rexinoids) has inhibitory effect on *TSH β* expression (Sherman et al., 1999). However, subsequent analysis revealed that the signaling pathway is mediated via a nt. -200/-149 region of the mouse *TSH β* gene, which is different from the reported nTRE (GGGTCA at nt. +1/+6) (Sharma et al., 2006). While T3 inhibits the synthesis of thyrotropin releasing hormone (TRH) in hypothalamus (Fig 1A) and a nTRE is postulated in this gene (Hollenberg et al., 1995), rexinoids has no effect on its production (Sherman et al., 1999). It was reported that the transcription of the *TSH β* gene is repressed in the CV1 cells treated with retinoic acid (RA) (Breen et al., 1995); however, its precise mechanism is unknown. Although all three RXRs, α , β and γ , are expressed in TSHoma cells, TtT97 (Sharma et al., 2006), double immunostaining studies of the hypothyroid rat pituitary using an antibodies against pituitary hormones and RXR suggest that RXR γ is predominantly expressed in thyrotrophs (Sugawara et al., 1995). Barros et al. (Barros et al., 1998) reported

that no alteration in the serum level of TSH, T3 or T4 was observed in RXR α $(-/+)$ mice or RXR γ $(-/-)$ mice. The authors suggested that ablation of RXRs has little effect on the negative regulation of the *TSH β* gene. Currently, there are few rationales to confirm the recognition of the nTRE by TR-RXR heterodimers.

5.2 Does TR bind to the nTRE as a monomer?

Gel shift assays indicate that TR monomers bind with the nTRE and that this interaction is abolished by RXR (Cohen et al., 1995). However, the hypothesis that TR binds with the nTRE as a monomer (Fig. 3B) also raises other questions which are difficult to answer. First, for the direct recognition of DNA sequence by DNA-binding transcription factors including NHRs, formation of a homo- or heterodimer is usually required. As expected, gel shift assays revealed that TR-monomer binding with single half-sites (i.e. nTRE) is much weaker than that of TR-RXR heterodimer binding to DR4 (Cohen et al., 1995). It should be noted that TSH synthesis in severe hypothyroidism is dramatically high (Fisher et al., 2000). Given that unliganded TR may maintain the basal activity of the *TSH β* promoter in hypothyroidism, it is difficult to imagine that such a weak binding of TR monomer with the nTRE can achieve this high level of transcriptional activity. Second, there is ligand/NHR selectivity, i.e., the negative regulation of the *TSH β* gene is clinically specific to T3 and partially estrogen (E2), but not other NHR ligands (Cohen and Wondisford, 2005). While, in positive regulation, the number of spacing nucleotides between half-sites is a critical factor in determining receptor specificity, it is unknown as to how TR selectively recognizes the nTRE DNA sequence in the *TSH β* gene. Finally, it is not easy to explain why TR monomers on a single half-site can exhibit reverse functions, i.e. recruitment of co-activators in the absence of T3 and association with co-repressors in the presence of T3 (Fig. 3B). We proposed previously a model where TR is able to bind with reported nTRE only in the presence of T3 (Sasaki et al., 1999). Although the nTRE in the *TSH β* gene was originally defined on the basis of the experiments with non-pituitary 293 cells (Wondisford et al., 1989), we and other investigators suggested the possibility that an unknown thyrotroph-specific factor may switch T3/TR on the nTRE from a transcriptional activator to an inhibitor (Sasaki et al., 1999; Shupnik, 2000; Wondisford et al., 1993). However, its existence has not been confirmed because of the limited number of cultured thyrotroph cell lines available (Ooi et al., 2004; Sarapura et al., 2002).

6. Models that do not postulate direct DNA binding of TR

The following hypotheses proposed models for T3-induced negative regulation without the involvement of direct DNA binding of TR.

6.1 NCoR or SMRT

There are many studies with regard to the involvement of NCoR or SMRT in the negative regulation by T3. It was reported that co-expression of NCoR and SMRT may enhance basal stimulation of the *TSH β* , *aGSU* and *prepro-TRH* promoters in a TR-dependent manner (Tagami et al., 1997). With regard to the T3-induced negative regulation of the Rous sarcoma virus-derived 5' truncated terminal repeat (RSV-LTR) (Berghagen et al., 2002) and the rat *CD44* gene (Kim et al., 2005), it was reported that NCoR and SMRT may function as transcriptional co-activators in the transcriptional regulation of these genes. According to

Tagami et al. (Tagami et al., 1999), unliganded TR in solution may squelch NCoR or SMRT from the transcription factor on the target promoter. Upon T3 binding, TR may release these co-repressors, resulting in their association with the DNA-binding transcription factor, which maintains the basal activity of the promoter of the target gene. However, this notion was tempered by following questions. First, the mechanism involved in the association of NCoR or SMRT with the target promoters is unknown. Second, it was undetermined whether the majority of NCoR and SMRT are sequestered by the relatively limited amount of intracellular TR (5000 to 10000 molecule/cell) (Oppenheimer et al., 1974). Third, NCoR and SMRT also interact with unliganded RAR and peroxisome proliferator-activated receptors (PPARs) (Nofsinger et al., 2008; Suzuki et al., 2010). It is unlikely that these NHRs also inhibit *TSH β* or *α GSU* expression in the presence of cognate ligands. Finally, it was reported that the negative regulation by T3 is mediated by the mutant TR β , AHT, of which interaction with co-repressors is impaired (Nakano et al., 2004). Using an experimental model similar to the mammalian two-hybrid assay, Wulf et al. (Wulf et al., 2008) also demonstrated that negative regulation by T3/TR is possible via the interaction of TR with a co-repressor. However, their experimental setting was completely artificial.

Amino acid sequence of TR β 2-NTD is unique and has low homology with that of TR β 1 or TR α 1 (Fig. 2). The N-terminal domain of TR β 2 is known to neutralize the silencing activity of co-repressors in the context of TR-RXR heterodimers on the pTRE (Hollenberg et al., 1996; Yang et al., 1999), and the NTD of TR β 2 may have some role in the negative regulation of the *prepro-TRH* gene (Guissouma et al., 2002). However, the physiological relevance in the negative regulation of *TSH β* gene is unknown. Of note, *in vitro* experiments showed that not only TR β 2 but also TR β 1 and TR α 1 can inhibit the transcription of the *TSH β* gene in a T3 dependent manner (Nakano et al., 2004). The findings in TR β /TR α -double knockout mice (Gothe et al., 1999) indicates that TR α 1 is partially involved in the negative regulation of *TSH β* gene although its expression level is less than that of TR β 2 (see below).

The amino acid sequence required for the interaction of NCoR and SMRT with unliganded TR has been identified. Based on this information, mice harboring mutant SMRT (SMRTmRID) (Nofsinger et al., 2008) or mutant NCoR (NCoRAID) (Astapova et al., 2011), which results in defective interaction with unliganded TR, were established. Importantly, the negative regulation of the *TSH β* gene by T3 was not impaired in knock-in mice with SMRTmRID or NCoRAID, although the latter exhibited a reduced amount of TR protein in the pituitary gland and reduced sensitivity for TSH by the thyroid gland. Likewise, TRH expression in the hypothalamus was not affected in NCoRAID-knock-in mice (Astapova et al., 2011). This is supported by an *in vivo* study, which showed that the overexpression of these co-repressors is incompatible with physiological regulation of TRH (Becker et al., 2001). As in the cases of the *TSH β* and *α GSU* genes, mRNA expression of the myosin heavy chain β subunit (*MHC β*) gene in the heart is also repressed by T3/TR (Gupta, 2007). In NCoRAID mice, T3-induced inhibition of *MHC β* is maintained (Astapova et al., 2011). Moreover, Astapova et al. (Astapova et al., 2008) established a liver-specific NCoRAID knock-in mouse. According to them, of 326 genes that are negatively regulated by T3 in the liver, only 3 genes were repressed in hypothyroid conditions, suggesting little effect of NCoR on the majority of the negatively regulated T3-target genes. While SMRTmRID- or NCoRAID-knock-in mice survive, the global deletion of both the *NCoR* and *SMRT* genes are embryonic lethal (Jepsen et al., 2007). These findings suggest that NCoR and SMRT have important roles other than interaction with liganded NHRs. For example, they interact with

p53, Myc, MyoD Ptx1 and Foxo1 (Nofsinger et al., 2008). Thus, it is unlikely that T3-binding with TR affects the transcriptional regulation of all of the genes regulated by NCoR or SMRT *in vivo*.

6.2 CBP/p300 and p160 family

cAMP-response-element-binding protein-binding protein (CBP)/p300 is required for transactivation by multiple DNA-binding transcription factors including NF κ B and AP-1, and functions as a coactivator for liganded NHRs (Kamei et al., 1996). As a model for the ligand-dependent inhibition by NHRs, it was proposed that liganded NHRs may attenuate the transactivation by DNA-binding transcription factors via interference of CBP/p300 function (Kamei et al., 1996). However, subsequent studies reported that inhibition by liganded NHR is not rescued by overexpression of these co-activators (De Bosscher et al., 1997; De Bosscher et al., 2001; Wu et al., 2004). It was also suggested that the CBP/p300-interacting surface on the NHR-LBD may be different from that required for T3-dependent inhibition (Saatcioglu et al., 1997; Valentine et al., 2000).

Although inappropriate overexpression of TSH is reported in SRC1-deficient mice (Takeuchi et al., 2002), it is difficult to determine what adaptive processes have occurred during pituitary development in SRC1^{-/-} mice since ablation of the SRC-1 gene also affects the expression of other p160 family members and TRs (Sadow et al., 2003; Xu et al., 1998). While p160 proteins are known to interact with multiple NHRs other than TR in a ligand-dependent manner, inhibition of the *TSH β* gene or the *aGSU* gene is specific to T3, and partially estrogen (Cohen and Wondisford, 2005). In some patients with RTH, there are no mutations of the *TR β* or *TR α* gene (non-TR RTH) (Refetoff and Dumitrescu, 2007; Refetoff et al., 1993). Although defects in cofactors that may mediate the negative regulation of the *TSH β* gene have been postulated in these cases, linkage analyses with polymorphic markers showed that the involvement of SRC-1, NCoR, SMRT or RXR γ is unlikely (Reutrakul et al., 2000).

6.3 Protein-protein interaction of TR with DNA-binding transcription factors: Tethering model

In vitro binding assays, including gel shift assays, are limited in that the amount of TR and/or RXR used may not always reflect the *in vivo* situation. To overcome this problem, Shibusawa et al. (Shibusawa et al., 2003a; Shibusawa et al., 2003b) established mice in which TR β is unable to bind DNA due to a mutation in its DBD. They reported that the negative regulation of the *TSH β* gene is relieved in these mice. One may assume that this result provides evidence for the direct binding of TR β with the nTRE because, in positive regulation, the pTRE is recognized by the TR-DBD. However, the function of the DBD of NHR is not limited to DNA recognition. It is known that the DBD also interacts with other DNA-binding transcription factors including NF κ B (De Bosscher et al., 2003; Kalaitzidis and Gilmore, 2005; Kalkhoven et al., 1996; Ray and Prefontaine, 1994; Scheinman et al., 1995; Stein and Yang, 1995; Tao et al., 2001; Wissink et al., 1997), AP-1 (typically Jun/Fos heterodimers) (De Bosscher et al., 2001; Heck et al., 1994; Lopez et al., 1993; Schule et al., 1990; Webb et al., 1995), Nur77 (Martens et al., 2005) and GATA family transcription factors (Clabby et al., 2003; Matsushita et al., 2007), resulting in their inhibition by NHRs in a ligand-dependent fashion.

This kind of ligand-induced repression via protein-protein interactions is referred to as the "tethering mechanism" (De Bosscher et al., 2003; Herrlich, 2001; Nissen and Yamamoto, 2000; Pfahl, 1993). Thus, while mutation of the TR β DBD abrogates the negative regulation of the *TSH β* gene by T3, it does not always imply a direct interaction of the TR with DNA. Of note, there are ligand/receptor specificities in the repression by liganded NHRs via the tethering mechanism (Caldenhoven et al., 1995; Liden et al., 1997; Matsushita et al., 2007). Moreover, dimer formation is not always required for ligand-dependent inhibition via the tethering mechanism. A mutant glucocorticoid receptor (GR), A458T, is known to have a defect in dimer formation and therefore in glucocorticoid-responsive element-dependent transactivation. It was reported that functions that require cross-talk with other transcription factors, such as transrepression of the AP-1-driven genes, remain intact in this mutant GR (Herrlich, 2001; Reichardt et al., 1998). This raises again the question whether heterodimer formation of TR with RXR is necessary for the negative regulation of the *TSH β* gene by T3.

7. Other possible mechanisms

Although T3 treatment is known to reduce the stability of *TSH β* mRNA (Krane et al., 1991), the role of TR has not been clarified, and the involvement of similar mechanisms in the regulation of the α GSU mRNA have not been reported (Staton et al., 2000). A mechanism operating via anti-sense RNA was proposed to be involved in negative regulation of the *MHC β* gene by T3 (Danzi and Klein, 2005; Haddad et al., 2003). In rat chromosome 15, the *MHC β* gene is located upstream to the *MHC α* gene, which has a classic pTRE. It was reported that, in a T3-dependent manner, TR-RXR heterodimers at pTRE of the *MHC α* promoter may activate not only *MHC α* transcription but also synthesis of anti-sense RNA against the *MHC β* gene, resulting in the antagonism of *MHC β* expression. However, this kind of mechanism has not been reported in other negatively regulated genes including the *TSH β* or the α GSU genes.

8. Artificial negative regulation by T3/TR

There have been at least two technical problems that have hindered the elucidation of the mechanism of negative regulation by T3/T.

8.1 pUC/pBR322-derived AP-1 site

As shown above, liganded NHRs, including T3/TR and liganded GR, inhibit the transcriptional activity of AP-1 via the tethering mechanism. Unexpectedly, a functional AP-1 site was identified in nt. 1/138 of pUC-derived plasmids and its activity is repressed by T3/TR (Lopez et al., 1993). More than 2000 plasmid constructs bearing the sequence identical to nt. 1/138 in the pUC18/19 vector were detected in the BLAST database. Interestingly, our computer search revealed that this site is also included in the pBR322 vector (Yanisch-Perron et al., 1985). In early molecular biology studies, both vectors were often utilized in "home-made plasmids". Unfortunately, this AP-1 site was contaminated in some of the plasmids used for the analysis of *TSH β* negative regulation by T3 (Hallenbeck et al., 1993; Wondisford et al., 1989).

8.2 Firefly luciferase gene

The firefly luciferase assay has been utilized in a variety of analyses of transcriptional regulation, including negative regulation of the *TSH β* and the *α GSU* genes due to its advantage over the CAT assay (Misawa et al.). However, at least in CV1 cells (Tillman et al., 1993), JEG3 cells (Maia et al., 1996) and Hepa1-6 mouse hepatoma cells (Chan et al., 2008), firefly luciferase cDNA has been reported to function as a transcriptional regulatory sequence that mediates artificial negative regulation by T3/TR. The length of firefly luciferase cDNA (1653 bp) is much longer than that of the CAT gene (657 bp). A computer search predicts more than 250 potential sites for DNA-binding transcription factors in firefly luciferase cDNA (Liu and Brent, 2008). Misawa et al. (Misawa et al.) recently found that firefly luciferase cDNA behaves as a transcriptional enhancer that can be stimulated by the protein kinase C activator, phorbol 12-O-tetradecanoate-13-acetate (TPA), and that this activity is inhibited by T3/TR in CV1 and JEG3 cells. The cDNA sequences of modified firefly luciferase (luc+) (Annicotte et al., 2001; Paguio et al., 2005) and conventional Renilla luciferase (RL) (Ho and Strauss, 2004; Osborne and Tonissen, 2002; Zhuang et al., 2001) also harbor numerous short sequences that can be recognized by a variety of transcription factors. Modified luciferase genes, including hRLuc (Zhuang et al., 2001) and Luc2 (Paguio et al., 2005), may be more reliable than firefly luciferase (Misawa et al.), presumably because the majority of predicted transcription factor binding sites were mutated.

8.3 Problems with artificial negative regulation in the identification of the nTREs by reporter assays

When a strong promoter is fused to firefly luciferase cDNA, the activity of this DNA sequence as a transcriptional regulatory element can be negligible. Nonetheless, one should remember that sequential deletion or mutation of the promoter sequence often reduces its transcriptional activity. Once the activity of the promoter becomes lower than that of the activity via the firefly luciferase gene, the overall activity of the reporter gene may represent that of the firefly luciferase cDNA, which can be artificially inhibited by T3/TR. For example, a deletion analysis to identify a nTRE in the *α GSU* gene was also carried out using the firefly luciferase reporter system in JEG3 cells (Madison et al., 1993). However, nTRE was not identified because T3-induced negative regulation was detected even a promoter that only has a TATA box and a TSS. There is the possibility that sequential deletion of DNA might reduce the transcription activity of the *α GSU* promoter, thereby permitting the firefly luciferase cDNA to function as a transcriptional regulatory element, resulting in artificial suppression by T3/TR. Using the luciferase reporter gene, it was previously proposed that the nTRE of the *TSH β* gene may have a direct repeat configuration without spacing nucleotides (DR0) (Naar et al., 1991). The authors reported that a reduction in spacing nucleotide number may convert DR4 from a pTRE to a nTRE. Unexpectedly, this was not reproduced by the CAT-based reporter system (private communication from Dr. Kazuhiko Umesono). According to Tillman et al. (Tillman et al., 1993), deletion of spacing nucleotides might destroy the T3-dependent activation and allowed artificial repression by T3/TR via firefly luciferase cDNA.

Whereas firefly luciferase assay has broad linearity, careful interpretation and appropriate control are necessary in particular when the promoter activity before T3 addition is reduced in the course of deletion or mutation analysis (Misawa et al.). Although several nTREs in different genes have been suggested in the vicinity of TSSs, few of these reports excluded the possibility of T3-mediated artificial negative regulation by a reporter plasmid backbone.

9. TR β 2 is the main mediator for the negative regulation of the TSH β gene by T3

There is one family with RTH in which the TR β allele was globally deleted (Takeda et al., 1992). Elevated synthesis of TSH, i.e. SITSH, was found in a homozygote in this family, presumably due to the defect in TR signaling in the thyrotrophs. Likewise, mice deficient for the TR β gene exhibit increased expression of TSH β and *aGSU* in thyrotrophs (Forrest et al., 1996). These findings imply the involvement of TR β in the negative regulation of these genes. Moreover, Abel et al. (Abel et al., 1999) reported that TR β 2-null mice develop a similar degree of central resistance to T3 similarly to TR β -null mice, suggesting that, among TR β 1, TR β 2 and TR α 1, TR β 2 is the main mediator for the inhibition of the TSH β gene. This notion is compatible with our findings that the expression of TR β 2 is much higher than TR α 1 or TR β 1 (Nakano et al., 2004) in the thyrotroph cell line, T α T1 (Yusta et al., 1998). The fact that no resistance to T3 is observed in mice deficient for the TR α gene (Fraichard et al., 1997; Wikstrom et al., 1998) indicates that TR α 1 in thyrotrophs has a limited role in the negative feedback of the TSH β gene by T3. It should be noted, however, that serum TSH levels in TR β /TR α -double knockout mice (Gothe et al., 1999) is higher than TR β -null mice (Forrest et al., 1996). Hence, the negative regulation of the TSH β gene is partially mediated by TR α 1 *in vivo*.

10. What is the mechanism that maintains the basal transcriptional activity of the TSH β gene before T3 addition?

It is apparent that the negative regulation of the TSH β gene by T3/TR can be observed only when its promoter is activated prior to T3 addition. However, previous studies have paid little attention to the mechanism of activation because some of the hypotheses mentioned above regard the basal transcriptional activity of the TSH β gene to be maintained by unliganded TR. In addition, due to the limitation of the cell lines that recapitulate the thyrotroph phenotype (Ooi et al., 2004; Sarapura et al., 2002), the nTRE of the TSH β gene has been studied using either kidney-derived 293 cells (Wondisford et al., 1989), COS cells (Carr et al., 1992) or somatotroph-derived GH3 cells (Sasaki et al., 1999). Of note, even in the presence of overexpressed TR, the magnitude of the basal transcriptional activity of the TSH β gene prior to T3 addition is extremely low in these cell lines (Sasaki et al., 1999; Wondisford et al., 1989), and is almost negligible compared with that observed in the presence of the thyrotroph-specific transcription factors, Pit1 and GATA2 (Nakano et al., 2004) (see below).

11. Unliganded TR per se is not a transcriptional activator for the TSH β gene

Because negative regulation has been regarded as the mirror image of the positive regulation, unliganded TR was thought to be a transcriptional activator (Wondisford et al., 1989). If this were the case, TSH β and *aGSU* expression would be reduced in mice lacking the TR gene irrespective of serum T3 and T4 levels. However, as described above, their expression was not reduced but rather was increased in mice deficient for the TR β gene (Abel et al., 1999; Forrest et al., 1996; Weiss et al., 1997) or both TR α and TR β genes (Gothe et al., 1999). In the family with RTH, in which the TR β allele is globally deleted (Takeda et al., 1992), elevation of serum TSH was also found in the homozygotes of this family. This again

suggests that unliganded TR β is not necessary for the activity of the *TSH β* promoter in human and that the *TSH β* gene is activated by factors other than unliganded TR.

12. GATA2 and Pit1 maintain basal *TSH β* expression in thyrotrophs

It is known that a pituitary-specific transcription factor, Pit1 (Fig 5A), plays a critical role in *TSH β* expression since its mutation causes combined pituitary hormone deficiency (CPHD), where the syntheses of TSH β , prolactin and growth hormone are crippled or abolished (Cohen and Radovick, 2002).

Fig. 5. Schematic representations of Pit1 (A) and GATA1, 2 and 3 (B). A. CBP, amino acid sequence interacting with CBP. POU_S, POU specific domain. POU_H. POU homeodomain. GATA, amino acid sequence interacting with GATA2. B. Structure of GATA1, 2, 3, GATA2-NZ and GATA2-ZC (see text). The numbers within the box represent the amino acid homology (%).

Promoter analysis of the *TSH β* gene in TSHoma cells, TtT97, revealed that nt. -269 from the TSS is sufficient for thyrotroph-specific expression of this gene (Wood et al., 1990). As shown in Fig. 4, a functional Pit1-binding site is included in this region (Haugen et al., 1993) and was designated as Pit1-US (Kashiwabara et al., 2009). Interestingly, comparison of the pattern of DNA foot printing using nuclear extracts from TtT97 cells and that from GH3 cells revealed that this promoter region also has binding sites for the transcription factor, GATA2 (Fig 4 and 5B) (Gordon et al., 1997), which was originally identified to be involved in the gene regulation of a hematopoietic cell lineage (Shimizu and Yamamoto, 2005). Indeed, there are two GATA-responsive elements (GATA-REs) immediately downstream of the Pit1-US (Gordon et al., 1997). Subsequent analysis with various transgenic mice revealed that co-expression of Pit1 and GATA2 is crucial for the differentiation of thyrotrophs (Dasen et al., 1999).

13. GATA2, not Pit1, is the true activator that drives the promoter activity of *TSH β*

Kashiwabara et al. (Kashiwabara et al., 2009) reported that the co-operation of Pit1 with GATA2 is strictly determined by the number of nucleotides between the Pit1-US and GATA-REs (Fig 4), and suggested the possibility that a configuration of the Pit1-US and GATA-REs may be critical for the recruitment of CBP/p300 (Fig 6).

Fig. 6. Molecular mechanism of the transcriptional regulation of the *TSH β* gene. A configuration of the Pit1-US and GATA-REs may be critical for the recruitment of CBP/p300. T3/TR represses the GATA2-dependent activation of the *TSH β* promoter via a tethering mechanism. Pit1 binds with the Pit1-like element in SR and competes with SR binding protein (SRBP), resulting in protection of GATA2 functionality from inhibition by SR (de-repression). TRH-R, TRH receptor, FOG2, friend of GATA 2.

The authors also noticed that a 30bp region downstream from the GATA-REs is highly conserved among rat, mice and humans, and includes a sequence similar to the Pit1-binding site (Pit1-like element, Fig. 4). This sequence was designated the suppressor region (SR) because its deletion increased the transactivation by GATA2 and Pit1. Interestingly, deletion of the SR enabled GATA2 to transactivate the *TSH β* gene without Pit1. Detailed analysis revealed that Pit1 binds with the Pit1-like element in the SR (Fig. 4) and competes with binding of SR binding protein (SRBP), resulting in protection of the GATA2 function from inhibition by SRBP (Fig 6). Thus, cooperation of Pit1 with GATA2 is not synergistic, but Pit1 protects GATA2 from inhibition by SR (de-repression). These findings not only provide an insight as to why *TSH β* expression is restricted in thyrotrophs where Pit1 and GATA2 co-exist, but also imply that the true activator that drives the *TSH β* promoter activity is GATA2 but not Pit1 (Kashiwabara et al., 2009).

14. Negative regulation of the *TSH β* gene is not the mirror image of positive regulation

Gordon et al. (Gordon et al., 1997) reported that the *TSH β* gene can be activated by Pit1 and GATA2 in CV1 cells. Because CV1 cells are kidney derived and has been utilized in the studies of positive regulation by T3/TR (Naar et al., 1991; Tillman et al., 1993; Umesono et al., 1991), Nakano et al. (Nakano et al., 2004) tested whether negative regulation of *TSH β* by T3 may be simulated when TR is co-expressed with Pit1 and GATA2 in this cell line. They employed the CAT-based reporter gene, *TSH β* -CAT (Sasaki et al., 1999), in which the pUC/pBR322-derived AP-1 element (Lopez et al., 1993) was deleted. Using this experimental system, Nakano et al. (Nakano et al., 2004) found the following results. First, T3-induced inhibition of the *TSH β* gene was readily observed in CV1 cells transfected with Pit1, GATA2 and TR β 2. This implies that T3-induced negative regulation of the *TSH β* gene does not require so-called thyrotroph specific factors except for Pit1, GATA2 and TR. Second, T3-induced inhibition was also detected with all three functional TRs, TR β 1, TR β 2 and TR α 1, with TR β 2 exhibiting the most potent T3-dependent inhibition among them. This observation again supports the notion that TR β 2 is the principal TR that mediates negative regulation of the *TSH β* gene (Abel et al., 1999). Third, without Pit1 or GATA2, unliganded TR did not transactivate the *TSH β* promoter at all. This implies that unliganded TR alone is not the transcriptional activator. This notion is in line with the results of data from TR β -knockout mice (Forrest et al., 1996) and TR β /TR α -double knockout mice (Gothe et al., 1999). Therefore, in negative regulation of the *TSH β* gene, T3/TR is a transcriptional repressor, but unliganded TR per se is not an activator. This is in contrast with positive regulation (Fig. 3A), where the T3-target genes are activated by T3/TR while it is repressed by unliganded TR (silencing). These findings run counter to the hypothesis that the negative regulation of this gene may be a mirror image of its positive regulation (Wondisford et al., 1989).

15. T3/TR represses GATA2-dependent activation of the *TSH β* promoter via the tethering mechanism

The putative nTRE was defined by analysis of the *TSH β* promoter in the absence of T3 (Wondisford et al., 1989). Because this was based on the hypothesis that unliganded TR

may be a transcriptional activator, Matsushita et al. (Matsushita et al., 2007) re-evaluated the function of nTRE by deletion analysis of this promoter (Fig. 4) in the presence of Pit1, GATA2 and TR. Unexpectedly, we found that repression of the *TSH β* promoter by T3/TR β 2 was maintained after the nTRE is completely deleted or mutated. Thus, the reported nTRE (Fig. 4) is dispensable for T3/TR-dependent inhibition. Moreover, repression by T3/TR was also observed even in a deletion construct that has only Pit1-US and two GATA-REs. These findings suggest that direct DNA binding of TR is unnecessary and that the mechanism for T3-dependent inhibition may be mediated by the crosstalk of T3/TR with Pit1 or GATA2 (Matsushita et al., 2007).

As mentioned above, the true activator that drives the *TSH β* promoter is GATA2 but not Pit1 (Kashiwabara et al., 2009) and the deletion of SR enables GATA2 to transactivate the *TSH β* promoter without Pit1. Using the reporter gene lacking for SR, Matsushita et al. (Matsushita et al., 2007) found that T3/TR β 2 inhibits the transactivation by GATA2 alone. Thus, GATA2 is thought to be the target of inhibition by T3/TR (Fig. 6). This notion is supported by the observation that T3/TR β 2 inhibits GATA2-induced activation of the *aGSU* promoter and the endothelin-1 (*ET-1*) promoter, both of which are known to bear a functional GATA-RE (Jorgensen et al., 2004; Steger et al., 1994; Dorfman et al., 1992). In addition, T3/TR inhibited the *CD34* gene-derived GATA-RE fused to a heterologous thymidine kinase promoter (Matsushita et al., 2007). Co-immunoprecipitation experiments and GST-pull down assays demonstrated that the DBD of TR β 2 interacts with the Zn finger domain of GATA2 *in vivo* in a T3-independent manner. Thus, the TR-DBD is involved in protein-protein interactions with GATA2 but not in direct binding of DNA (Matsushita et al., 2007). These results indicate that negative regulation of the *TSH β* gene is mediated by tethering of T3/TR by GATA2 (Fig. 6).

16. Ligand/receptor specificity in negative regulation of the *TSH β* gene

As discussed above, ligand/receptor specificity has been reported in ligand-dependent inhibition via the tethering mechanism (Caldenhoven et al., 1995; Liden et al., 1997). Matsushita et al. (Matsushita et al., 2007) found that GATA2-induced activity of the *TSH β* promoter was specifically inhibited by T3/TR but not by RA/RAR or vitamins D3 (VD3)/VDR. This may reflect ligand selectivity *in vivo* in negative regulation of the *TSH β* gene (Cohen and Wondisford, 2005). Of note, it is known that estrogen (E2) inhibits expression of the *TSH β* gene (Cohen and Wondisford, 2005) at the transcriptional level, although its magnitude is smaller than that by T3. E2 is also known to reduce expression of the *aGSU* gene (Chaidarun et al., 1994; Shupnik et al., 1988), the promoter of which has a functional GATA-RE (Jorgensen et al., 2004; Steger et al., 1994). In agreement, the serum level of TSH in women has a tendency to elevate after the menopause (Nagayama et al., 2008). To explore the molecular mechanism underlying inhibition by E2, Nagayama et al. (Nagayama et al., 2008) tested the effect of E2-bound ER α (E2/ER α) using CV1 cells cotransfected with Pit1 and GATA2, and found that E2/ER α significantly inhibits activity of the *TSH β* promoter. As predicted, the magnitude of inhibition by E2/ER α was approximately half of that by T3/TR β 2. They also found that E2/ER α directly interacts with GATA2, as shown for GATA1. Testosterone was reported to have the effect similar to estrogen, presumably due to the conversion of testosterone to estrogen (Ahlquist et al.,

1987). This may explain why elevation of serum TSH level is also found in aged men (Surks and Hollowell, 2007).

17. The role of GATA2 and TR in TRH signaling in thyrotrophs

TRH is processed from prepro-TRH and secreted from the hypothalamus (Fig. 1A). TRH signaling not only stimulates TSH secretion but also enhances expression of *TSH β* and *aGSU* (Franklyn et al., 1986; Shupnik et al., 1986). To clarify the role of TRH-induced transactivation and T3/TR mediated inhibition in the hypothalamic-pituitary-thyroid (H-T-P) axis, various *in vivo* studies, including genetic ablation of these genes, have been performed (Forrest et al., 1996; Friedrichsen et al., 2004; Gothe et al., 1999; Mittag et al., 2009; Nikrodhanond et al., 2006; Shibusawa et al., 2000). Although the *in vivo* evidence observed in these experiments is definitely important, the experimental system using cultured cells has the advantage that the effect of individual hormone can be analyzed in detail without influence by a negative feedback loop.

17.1 TRH signaling-promoted *TSH β* expression is mediated by GATA2

Although serum TSH levels are reduced in patient with CPHD who have mutations in the *Pit1* gene (Cohen and Radovick, 2002), the involvement of *Pit1* downstream of TRH signaling has been controversial (Ohba et al., 2011; Steinfelder et al., 1992a; Steinfelder et al., 1992b). Unfortunately, previous analyses have been performed without consideration of GATA2. Interestingly, the increase in *TSH β* expression in hypothyroidism was impaired in mice with pituitary-specific ablation of the GATA2 gene (Charles et al., 2006). Because TRH synthesis in the hypothalamus is expected to increased in hypothyroidism (Fig. 1A) (Abel et al., 2001; Kakucska et al., 1992), this finding suggested the involvement of GATA2 in the TRH signaling pathway.

Using CV1, GH3 and T α T1 cells, Ohba et al. (Ohba et al., 2011) recently reported that TRH signaling potentiates GATA2/*Pit1*-induced transcriptional activity of the *TSH β* gene. Additionally, experiments with a *TSH β* promoter that lacks SR revealed that GATA2 but not *Pit1* is the target of TRH signaling (Fig. 6). Similar results were obtained with GATA2-induced activation of the *aGSU* and *ET-1* promoters. It is known that the signal from TRH receptor activates protein kinase C (PKC) (Gershengorn and Osman, 1996; Sun et al., 2003). The PKC pathway is also known to enhance DNA binding of GATA2 with GATA-RE in the *aGSU* (Fowkes et al., 2002) and the *V-CAM1* promoters (Minami et al., 2003). Gel shift assays also suggested that DNA binding of GATA2 with the *TSH β* promoter is facilitated by the TRH/PKC pathway (Ohba et al., 2011). Thus, GATA-REs seem to be the point of convergence for both activation and inhibition signals controlling *TSH β* transcription. Although it has been postulated that TRH signaling in transactivation of the *TSH β* gene may be mediated by unliganded TR on nTRE (Wondisford et al., 1993), Ohba et al. (Ohba et al., 2011) showed that unliganded TR without *Pit1* or GATA2 failed to mediate the stimulating effect by TRH on this promoter and that reported nTRE (Fig. 4) is dispensable for activation of the TRH-induced transcription.

17.2 TRH-dependent activation vs. T3/TR-induced repression

The *in vitro* data demonstrated by Ohba et al. (Ohba et al., 2011) correlate well with the *in vivo* findings (Fig. 7).

Fig. 7. Schematic representation of transcriptional regulation of the *TSHβ* gene by TRH signaling and T3/TR. With support by Pit1, GATA2 maintains the basal transcription of the *TSHβ* gene and mediates TRH/TRH-R1 signaling in hypothyroidism, while unliganded TR alone is not a transcriptional activator. Inhibition by T3/TR is dominant over activation by GATA2 even in the presence of TRH signaling. The release of T3/TR-induced repression (a) is more crucial for *TSHβ* expression than TRH signaling (b) since the inhibition by T3/TR is dominant over the stimulation by TRH (c). DNA binding of GATA2 with the *TSHβ* promoter is facilitated by the TRH pathway (b).

First, they showed that as long as T3 is at low concentrations or absent, expression of the *TSHβ* gene is maintained by Pit1 and GATA2 without stimulation by TRH signaling (Fig. 7(a)) (Gordon et al., 1997; Kashiwabara et al., 2009; Ohba et al., 2011). In agreement with this, the signaling and the number of TSHβ-positive cells in the pituitary of TRH-deficient mice were comparable with those of wild-type mice (Shibusawa et al., 2000). Second, given that unliganded TR is not a transcriptional activator, elevation of *TSHβ* expression in hypothyroidism should depend on TRH signaling but not on unliganded TR (Fig. 7(b)). Nikrodhanond et al. (Nikrodhanond et al., 2006) compared *TSHβ* expression in wild-type, TRH-, TRβ- and TRH/TRβ-double knockout mice and found that, in hypothyroidism, TSH expression predominantly depends on TRH signaling but not by unliganded TRβ. Since the authors regarded unliganded TRβ as the stimulator for the *TSHβ* gene, they mentioned that their findings were unexpected. However, their results are in agreement with the notion that unliganded TR is not the activator or mediator for the *TSHβ* gene in the absence or presence of TRH signaling. Finally, our data suggested that, in *TSHβ* transcription, the inhibitory effect by T3/TR is dominant over the TRH-induced stimulation (Fig. 7(c)) (Ohba et al., 2011). In accordance with this, an earlier study with human subjects indicated that continuous injection of TRH cannot release the inhibition of serum TSH in thyrotoxicosis (Chan et al.,

1979). Taking advantage of the fact that the Pax8-null mouse is an excellent animal model for congenital hypothyroidism (Friedrichsen et al., 2004), Mittag et al. (Mittag et al., 2009) demonstrated that thyrotroph differentiation in Pax8/TRH-R double-knockout mice is comparable with that in the hypothyroidism of mice homologous for a Pax8-null allele. Their results support the notion that release of T3/TR-induced inhibition (Fig. 7(a) is more critical for *TSH β* expression than TRH signaling (Fig. 7(b)) because inhibition by T3/TR is dominant over stimulation by TRH (Fig. 7(c)).

18. Mechanism of T3/TR interference with GATA2 transactivating function

Negative regulation of the *TSH β* gene is expected to provide an excellent experimental model to study transcriptional regulation since this promoter is activated by TRH signaling and repressed by T3/TR (Fig. 6). An important next step would be to investigate how T3/TR interferes with the transactivation function of GATA2. As pointed out above, the involvement of RXRs, TR-related coactivator (p160) or co-repressors (NCoR, SMRT) has been controversial. We favor another possibility; that TR may regulate the function of GATA2-related cofactors in a T3-dependent manner.

In pituitary-specific GATA2-null mice (Charles et al., 2006), the defect in *TSH β* expression was partial and GATA3 expression was increased. Thus, GATA3 may be able to compensate for the reduction in GATA2 expression and there may be functional redundancy between GATA2 and GATA3. Amino acid homology between the Zn-finger domains of GATA1, GATA2 and GATA3 is well conserved (Fig. 5B) and plays a pivotal role in DNA recognition as well as cofactor interaction (Bates et al., 2008; Shimizu and Yamamoto, 2005). Consistent with this, our *in vitro* results show that GATA1, GATA2 and GATA3 have the capacity to mediate cooperation with Pit1 (Kashiwabara et al., 2009), TRH signaling-induced transactivation (Ohba et al., 2011) and inhibition by T3/TR (Matsushita et al., 2007). All these properties were also observed in the deletion mutant of GATA2 that lacks an N-terminal domain (GATA2-ZC) or a C-terminal domain (GATA2-NZ) (Fig. 5B). These findings suggest a critical role of the Zn-finger domain in GATA2 (GATA2-Zf) in *TSH β* gene regulation. Besides CBP/p300, TR-associated protein (TRAP) 220/MED1 and Friend of GATA (FOG) 1 or 2 are known to interact with this domain. Of course, there is the possibility that other unknown factors may play a critical role in negative regulation by T3/TR and that there is interplay among various histone modifications to achieve local control of *TSH β* gene transcription. Although the chromatin immunoprecipitation (ChIP) assay is expected to provide important information, the amount of endogenous GATA2 in T α T1 cells may not be sufficient for this approach (Ohba et al., 2011).

18.1 TRAP220/MED1 and Mediator complex

TRAP220/MED1 is a constituent of the Mediator complex that directly regulates the function of RNA polymerase II (Pol II) (Fig. 6) (Chadick and Asturias, 2005). The following findings indicate the involvement of TRAP220/MED1 in transactivation by the GATAs (Fig. 6). First, *in vitro* experiments show that TRAP220/MED1 interacts with GATA2-Zf (Gordon et al., 2006). Second, homozygous TRAP220/MED1-null mice are embryonic lethal due to an abnormality in cardiac function and its phenotype is reminiscent of that observed in mice deficient for the GATA family transcription factors (Crawford et al., 2002). Third, expression

of the *TSH β* gene is reduced in heterozygous TRAP220/MED-knockout mice (Ito et al., 2000), suggesting that TRAP220/MED1 is required for expression of the *TSH β* gene, which is GATA2-dependent. Interestingly, TRAP220/MED1 possesses two LXXLL motives, which functions as an interface for interaction with the TR-LBD in a T3-dependent manner. Mutant TRAP220/MED1, which only has two LXXLL motives but lacks other transactivation domains, is reported to function as a dominant negative inhibitor against wild-type TRAP220/MED1 in T3-dependent positive regulation via TR-RXR heterodimers on the pTRE (Yuan and Gambie, 2000). Matsushita et al. (Matsushita et al., 2007) found that mutant MED1/TRAP220 also attenuates T3-induced inhibition of the *TSH β* gene. It was reported that mutant TRAP220/MED1 specifically interferes with the activity of wild-type TRAP220/MED1 but not other LXXLL-type co-activators including the p160 family and CBP/p300 (Acevedo and Kraus, 2003). Thus, there is the possibility that TR may regulate the activity of a complex containing GATA2 and TRAP220/MED1 in a T3-dependent fashion. Given that inhibition by T3/TR targets the final step of GATA2-induced transactivation, i.e. TRAP220/MED1-Pol II complex, repression might occur downstream of or at the same point as TRH-induced activation. This may account for the findings that inhibition by T3/TR is dominant over TRH-induced activation of the *TSH β* gene (Mittag et al., 2009; Ohba et al., 2011). *In vivo* experiments with mouse embryonic fibroblasts showed that the requirement of TRAP220/MED1 may be specific to TR, but not to RAR or VDR (Ito et al., 2000). Intriguingly, the binding of different activators triggers a specific conformational change in the Mediator complex, which may have a critical role in the regulation of Pol II (Chadick and Asturias, 2005).

18.2 FOG2 and chromatin-remodeling factors

The co-repressor, friend of GATA (FOG) 1 or 2 (Cantor and Orkin, 2005) may be involved in T3/TR-induced negative regulation via GATA2. FOG1 and FOG2 interact with the N-terminal Zn finger of the GATAs and recruit chromatin-remodeling factors (Fig. 6) (Hong et al., 2005; Roche et al., 2008; Rodriguez et al., 2005). In addition, FOG2 is expressed in non-hematopoietic tissues and interacts with TR β (Rouf et al., 2008) and other NHRs (Clabby et al., 2003; Huggins et al., 2001). Matsushita et al. (Matsushita et al., 2007) generated a mutant GATA2 (C295A) which is predicted to have impaired interaction with the FOGs. Although the basal transcriptional activity of this mutant was also reduced (by approximately half) compared with wild-type GATA2, inhibition by T3/TR (fold repression) was significantly relieved in mutant GATA2.

19. Molecular mechanism of SITSH in RTH

As described above, patients with RTH exhibit SITSH (Refetoff et al., 1993). The mutant TR β found in RTH patients is supposed to interact with GATA2 because it has an intact DBD, which is the interface for the Zn-finger domain of GATA2. Nakano et al. (Nakano et al., 2004) tested whether mutant TR β 2s identified in RTH patients exhibit a dominant negative effect on the negative regulation of the *TSH β* gene using CV1 cells cotransfected with Pit1, GATA2 and wild-type TR β 2. As predicted, mutant TR β 2s blunted the T3-induced inhibition of the *TSH β* gene by wild-type TR β 2. Although these findings are likely to be the result of dominant-negative interference of wild-type TR β function by mutant TR β , further studies are required with regard to its molecular mechanism. Following questions also remain. First,

in patients with non-TR RTH (see above), no genetic abnormalities in the *TR β* or *TR α* genes have been identified. Although linkage analysis of these patients showed no relation with NCoR, SMRT, SRC-1 or RXR γ (Reutrakul et al., 2000), an understanding of the transcriptional control mechanisms underlying non-TR RTH may provide an insight into the molecular basis of negative regulation of the *TSH β* gene. Second, RTH is clinically classified as a generalized resistance to thyroid hormone (GRTH) and resistance of the pituitary to thyroid hormone (PRTH) (Refetoff et al., 1993). Patients with PRTH possess mutations similar or identical to those found in GRTH; however, PRTH patients display greater resistance to thyroid hormone in thyrotrophs compared to peripheral tissues, resulting in thyrotoxicosis. Currently, the mechanism of pituitary-dominant resistance in PRTH is unknown (Nakano et al., 2004).

20. Mechanism of logarithmic alterations in serum TSH by linear changes of T3/T4

TSH synthesis in the pituitary is dramatically altered by subtle changes in serum T3 and T4. Indeed, linear changes in the concentration of serum T4 and T3 correspond to logarithmic changes in serum TSH (Fekete and Lechan, 2007; Fisher et al., 2000; Kakucska et al., 1992). Such a sensitive alteration of *TSH β* expression may be necessary for thyroid hormone homeostasis (Fig. 1A) because TSH signaling is thought to be one of the most critical determinants of T3 and T4 synthesis in the thyroid gland. In other words, serum TSH level has been regarded as most sensitive clinical marker for thyroid gland function. Indeed, SITSH is an important indicator for RTH.

With regard to the molecular mechanism of logarithmic changes in serum TSH, there are the following possibilities. First, T3/TR negatively regulates not only *TSH β* and *aGSU* but also the prepro-TRH gene (Fig. 1A). Thus, the dual inhibitory mechanism at the hypothalamus and the pituitary may be important for the non-linear change of serum TSH level. Second, Pit1 expression may be negatively regulated by T3 (Sanchez-Pacheco et al., 1995). Because this was found in a somatotroph cell line, GH3 (Ooi et al., 2004), which lacks endogenous GATA2 (Gordon et al., 1997), an unknown pituitary factor may be involved in this inhibition. Third, expression of GATA2 in thyrotrophs may also be negatively regulated by T3. It was reported that there are two promoters in the *GATA2* gene, and that the distal one contains a GATA-RE (Kobayashi-Osaki et al., 2005). Therefore, there may be a positive feedback mechanism in the expression of *GATA2* and this mechanism is thought to be crucial in the differentiation of hematopoietic cell lineages. There is the possibility that, in thyrotrophs, T3/TR may interfere with the transactivation function of GATA2 not only on the *TSH β* promoter but also on the *GATA2* promoter. In addition, GATA2 protein may be quickly degraded by the ubiquitin system (its half life is approximately 30 min) (Minegishi et al., 2005). This may also contribute the drastic regulation of serum TSH level. Finally, there is the possibility that T3-induced inhibition of prepro-TRH expression in the hypothalamus may also be logarithmic (Fekete and Lechan, 2007; Kakucska et al., 1992). Although an nTRE was postulated in the prepro-TRH promoter (Hollenberg et al., 1995), the molecular mechanism of logarithmic inhibition of prepro-TRH expression by T3 is unknown.

The diagnoses of both subclinical hypo- and subclinical hyper-thyroidism also depend on the sensitive change in serum TSH level. Although serum free T3 and T4 levels are within normal range, subclinical hypo- or hyperthyroidism influence lipid metabolism (Walsh et

al., 2005b) and cardiovascular function (Walsh et al., 2005a). In their pathogeneses, there may be the abnormality in the transcription of the gene which is negatively regulated by T3 as robustly as the *TSH β* gene.

21. Other genes negatively regulated by T3/TR

Microarray analyses revealed that approximately 30 to 50% of T3-target genes are negatively regulated (Feng et al., 2000; Weitzel, 2008). Therefore, elucidation of negative regulation by T3 is thought to be the next frontier.

21.1 Reported nTREs in other T3-negatively regulated genes

In addition to the *TSH β* gene, negative regulation by T3/TR has been reported in the genes for α GSU (Chatterjee et al., 1989; Pennathur et al., 1993), *MHC β* (Edwards et al., 1994; Wright et al., 1999), *prepro-TRH* (Hollenberg et al., 1995; Satoh et al., 1996), RSV-LTR (Saatcioglu et al., 1993), rat Na, K-ATPase α 3 subunit (Chin et al., 1998), Nm23-H1 (Lin et al., 2000), phospholamban (PBL) (Belakavadi et al., 2010), rat CD44 (Kim et al., 2005), superoxide dismutase-1 (Santos et al., 2006), deiodinase type 2 (*Dio2*) (Christoffolete et al., 2006) and β -amyloid precursor protein (Villa et al., 2004). In some of these genes, the existence of single half-sites homologous to the *TSH β* nTRE have also been postulated (Chatterjee et al., 1989; Chin et al., 1998; Edwards et al., 1994; Hollenberg et al., 1995; Kim et al., 2005; Lin et al., 2000; Pennathur et al., 1993; Saatcioglu et al., 1993; Santos et al., 2006; Villa et al., 2004; Wright et al., 1999). However, there are few experimental studies that show the molecular mechanism by which these putative nTREs reverse the function of T3/TR from transcriptional activator to repressor. In *TR β* - and/or *TR α 1*-deficient mice, the expression of *α GSU* (Forrest et al., 1996; Gothe et al., 1999) in the pituitary, *prepro-TRH* in the hypothalamus (Abel et al., 2001; Dupre et al., 2004) and *MHC β* in the heart (Mansen et al., 2001) are maintained, suggesting that, as in the case of the *TSH β* gene, the basal activities of these genes are also maintained by a transcriptional activator other than unliganded TR. Thus, existence of nTREs in these genes should also be reconsidered.

21.2 Possible involvement of the tethering mechanism

If T3-dependent inhibition of these genes occurs via a tethering mechanism between a DNA-binding transcription factor and T3/TR, identification of such a transcription factor may provide an insight into the molecular mechanism of T3-induced inhibition. As discussed above, a functional GATA-RE in the *α GSU* promoter may be the target of suppression by T3/TR. GATA-REs are also predicted in the promoters of *Dio2* (Dentice et al., 2003), *MHC β* (Hasegawa et al., 1997; Morimoto et al., 1999) and *PBL* (Belakavadi et al., 2010). *Dio* activity in the thyrotrophs regulates the intracellular concentration of T3, which is the determinant of negative regulation of the *TSH β* gene (Escobar-Morreale et al., 1996). Although *Dio1* and *Dio2* are expressed in thyrotrophs, the inhibitory effect on TSH by T3 was relieved in mice deficient for *Dio2* but not *Dio1* (St Germain et al., 2005), indicating the crucial role of *Dio2* in the regulation of T3 concentration in the thyrotroph. Further studies may clarify the role of the predicted GATA-RE in the *Dio2* promoter (Dentice et al., 2003) and the complexity of the T3 sensing mechanism in regulation of the *TSH β* gene (Christoffolete et al., 2006). Although it was previously reported that the GATA-RE in the *MHC β* gene plays a role in its transcriptional activity (Hasegawa et al., 1997; Morimoto et al., 1999), other investigators suggested that it may not be functional (Vyas et al., 1999). Another study of the *MHC β*

promoter using rat neonatal cardiomyocytes suggests that the M-CAT site in this promoter is critical for its expression (Flink et al., 1992). M-CAT is the recognition site for the TEF family of transcription factors (Yoshida, 2008). TEF family transcription factors are the major target of $\alpha 1$ adrenaline signaling (Chen et al., 2004), which is known to mimic the cardiac phenotype seen in heart failure (Yoshida, 2008). Consistently, overexpression of TEF-1 in vivo exhibits a phenotype similar to that of chronic heart failure (Tsika et al., 2010). Our preliminary data suggests that T3/TR inhibits TEF-dependent transactivation of the *MHC β* gene.

22. Negative regulation by liganded NHRs other than TR

A tethering mechanism has been reported in genes that are negatively regulated by liganded NHRs other than T3/TR. For example, the proopiomelanocortin (POMC) gene is activated by a transcription factor, Nur77, which is also the mediator of corticotropin-releasing hormone (CRH) signaling (Maira et al., 2003). Liganded GR interferes with this activity via a tethering mechanism (Martens et al., 2005). Moreover, a recent report suggested the involvement of chromatin remodeling factors in inhibition of the POMC gene by liganded GR (Bilodeau et al., 2006). Expression of parathyroid hormone (PTH) is inhibited by liganded VDR. PTH expression is maintained in the mice deficient for the VDR gene (Kim et al., 2007), suggesting that unliganded VDR is not the transcriptional activator for the PTH gene. A DNA-binding transcription factor, VDR interacting repressor (VDIR), binds with the promoter region of the *PTH* gene and activates its transcription (Kim et al., 2007). It was reported that VDR associates with VDIR (Kim et al., 2007; Murayama et al., 2004), resulting in VD3-dependent inhibition. A tethering mechanism between liganded VDR and VDIR also plays a role in negative regulation of human $1\alpha(\text{OH})\text{ase}$ (CYP27B1) expression by VD3 (Murayama et al., 2004). In this scenario, a chromatin remodeling factor complex (Kitagawa et al., 2003), and a DNA methylation-related proteins (Kim et al., 2009) may play crucial roles.

23. Conclusion

Negative feedback is the key component in homeostasis of hormones. A typical example is the inhibition of TSH synthesis by T3/TR. Although serum TSH levels are increased in hypothyroidism, observations in TR-knockout mice (Forrest et al., 1996; Gothe et al., 1999), human subjects with a deletion of the *TR β* gene (Takeda et al., 1992) and *in vitro* experiments (Nakano et al., 2004) provide evidence that unliganded TR is not a transcriptional activator (Fig. 7). Moreover, deletion analysis of the *TSH β* gene with co-expression of GATA2 and Pit1 revealed that a putative nTRE (Fig. 4) is dispensable for inhibition by T3/TR (Matsushita et al., 2007). Study of the *TSH β* gene suggests the importance of a transcription factor that maintains the basal transcriptional activity of the promoter before ligand addition (Fig. 7). Identification of the factor required for the basal promoter activity may also be important and the first step in the analysis of other promoters that are repressed by T3/TR or other liganded NHRs. The factor may interact with NHRs. Once such a transcription factor is identified, it will be possible to study negative regulation using cells that express the factor and to carry out reporter analysis with co-transfection of its expression plasmid. For example, it will be possible to compare the mechanisms of positive and negative regulation using same cell line, for instance CV1 (Nakano et al., 2004). Information of the

factor required for the basal promoter activity would be helpful to avoid artificial negative regulation mediated by plasmid backbones (for example a pUC/pBR322-derived AP-1 site or firefly luciferase cDNA). We are only just beginning to unravel some of complexities involved in negative regulation by liganded NHR including T3/TR.

24. References

- Abel, E.D., Ahima, R.S., Boers, M.E., Elmquist, J.K. and Wondisford, F.E. (2001) Critical role for thyroid hormone receptor beta2 in the regulation of paraventricular thyrotropin-releasing hormone neurons. *J Clin Invest*, 107, 1017-1023.
- Abel, E.D., Boers, M.E., Pazos-Moura, C., Moura, E., Kaulbach, H., Zakaria, M., Lowell, B., Radovick, S., Liberman, M.C. and Wondisford, F. (1999) Divergent roles for thyroid hormone receptor beta isoforms in the endocrine axis and auditory system. *J Clin Invest*, 104, 291-300.
- Acevedo, M.L. and Kraus, W.L. (2003) Mediator and p300/CBP-steroid receptor coactivator complexes have distinct roles, but function synergistically, during estrogen receptor alpha-dependent transcription with chromatin templates. *Mol Cell Biol*, 23, 335-348.
- Ahlquist, J.A., Franklyn, J.A., Wood, D.F., Balfour, N.J., Docherty, K., Sheppard, M.C. and Ramsden, D.B. (1987) Hormonal regulation of thyrotrophin synthesis and secretion. *Horm Metab Res Suppl*, 17, 86-89.
- Annicotte, J.S., Schoonjans, K., Haby, C. and Auwerx, J. (2001) An E-box in pGL3 reporter vectors precludes their use for the study of sterol regulatory element-binding proteins. *Biotechniques*, 31, 993-994, 996.
- Astapova, I., Lee, L.J., Morales, C., Tauber, S., Bilban, M. and Hollenberg, A.N. (2008) The nuclear corepressor, NCoR, regulates thyroid hormone action in vivo. *Proc Natl Acad Sci U S A*, 105, 19544-19549.
- Astapova, I., Vella, K.R., Ramadoss, P., Holtz, K.A., Rodwin, B.A., Liao, X.H., Weiss, R.E., Rosenberg, M.A., Rosenzweig, A. and Hollenberg, A.N. (2011) The nuclear receptor corepressor (NCoR) controls thyroid hormone sensitivity and the set point of the hypothalamic-pituitary-thyroid axis. *Mol Endocrinol*, 25, 212-224.
- Barros, A.C., Erway, L.C., Krezel, W., Curran, T., Kastner, P., Chambon, P. and Forrest, D. (1998) Absence of thyroid hormone receptor beta-retinoid X receptor interactions in auditory function and in the pituitary-thyroid axis. *Neuroreport*, 9, 2933-2937.
- Bates, D.L., Chen, Y., Kim, G., Guo, L. and Chen, L. (2008) Crystal structures of multiple GATA zinc fingers bound to DNA reveal new insights into DNA recognition and self-association by GATA. *J Mol Biol*, 381, 1292-1306.
- Becker, N., Seugnet, I., Guissouma, H., Dupre, S.M. and Demeneix, B.A. (2001) Nuclear corepressor and silencing mediator of retinoic and thyroid hormone receptors corepressor expression is incompatible with T(3)-dependent TRH regulation. *Endocrinology*, 142, 5321-5331.
- Belakavadi, M., Saunders, J., Weisleder, N., Raghava, P.S. and Fondell, J.D. (2010) Repression of cardiac phospholamban gene expression is mediated by thyroid hormone receptor- α 1 and involves targeted covalent histone modifications. *Endocrinology*, 151, 2946-2956.

- Berghagen, H., Ragnhildstveit, E., Krogsrud, K., Thuestad, G., Apriletti, J. and Saatcioglu, F. (2002) Corepressor SMRT functions as a coactivator for thyroid hormone receptor T3R α from a negative hormone response element. *J Biol Chem*, 277, 49517-49522.
- Bilodeau, S., Vallette-Kasic, S., Gauthier, Y., Figarella-Branger, D., Brue, T., Berthelet, F., Lacroix, A., Batista, D., Stratakis, C., Hanson, J., Meij, B. and Drouin, J. (2006) Role of Brg1 and HDAC2 in GR trans-repression of the pituitary POMC gene and misexpression in Cushing disease. *Genes Dev*, 20, 2871-2886.
- Breen, J.J., Matsuura, T., Ross, A.C. and Gurr, J.A. (1995) Regulation of thyroid-stimulating hormone beta-subunit and growth hormone messenger ribonucleic acid levels in the rat: effect of vitamin A status. *Endocrinology*, 136, 543-549.
- Caldenhoven, E., Liden, J., Wissink, S., Van de Stolpe, A., Raaijmakers, J., Koenderman, L., Okret, S., Gustafsson, J.A. and Van der Saag, P.T. (1995) Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Mol Endocrinol*, 9, 401-412.
- Cantor, A.B. and Orkin, S.H. (2005) Coregulation of GATA factors by the Friend of GATA (FOG) family of multitype zinc finger proteins. *Semin Cell Dev Biol*, 16, 117-128.
- Carr, F.E., Kaseem, L.L. and Wong, N.C. (1992) Thyroid hormone inhibits thyrotropin gene expression via a position-independent negative L-triiodothyronine-responsive element. *J Biol Chem*, 267, 18689-18694.
- Chadick, J.Z. and Asturias, F.J. (2005) Structure of eukaryotic Mediator complexes. *Trends Biochem Sci*, 30, 264-271.
- Chaidarun, S.S., Eggo, M.C., Sheppard, M.C. and Stewart, P.M. (1994) Expression of epidermal growth factor (EGF), its receptor, and related oncoprotein (erbB-2) in human pituitary tumors and response to EGF in vitro. *Endocrinology*, 135, 2012-2021.
- Chan, I.H., Borowsky, A.D. and Privalsky, M.L. (2008) A cautionary note as to the use of pBi-L and related luciferase/transgenic vectors in the study of thyroid endocrinology. *Thyroid*, 18, 665-666.
- Chan, V., Wang, C. and Yeung, R.T. (1979) Thyrotropin: alpha- and beta-subunits of thyrotropin, and prolactin responses to four-hour constant infusions of thyrotropin-releasing hormone in normal subjects and patients with pituitary-thyroid disorders. *J Clin Endocrinol Metab*, 49, 127-131.
- Charles, M.A., Saunders, T.L., Wood, W.M., Owens, K., Parlow, A.F., Camper, S.A., Ridgway, E.C. and Gordon, D.F. (2006) Pituitary-specific Gata2 knockout: effects on gonadotrope and thyrotrope function. *Mol Endocrinol*, 20, 1366-1377.
- Chatterjee, V.K., Lee, J.K., Rentoumis, A. and Jameson, J.L. (1989) Negative regulation of the thyroid-stimulating hormone alpha gene by thyroid hormone: receptor interaction adjacent to the TATA box. *Proc Natl Acad Sci U S A*, 86, 9114-9118.
- Chen, H., Lin, R.J., Xie, W., Wilpitz, D. and Evans, R.M. (1999) Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. *Cell*, 98, 675-686.
- Chen, H.H., Mullett, S.J. and Stewart, A.F. (2004) Vgl-4, a novel member of the vestigial-like family of transcription cofactors, regulates alpha1-adrenergic activation of gene expression in cardiac myocytes. *J Biol Chem*, 279, 30800-30806.

- Cheng, S.Y., Leonard, J.L. and Davis, P.J. (2010) Molecular aspects of thyroid hormone actions. *Endocr Rev*, 31, 139-170.
- Chin, S., Apriletti, J. and Gick, G. (1998) Characterization of a negative thyroid hormone response element in the rat sodium, potassium-adenosine triphosphatase alpha3 gene promoter. *Endocrinology*, 139, 3423-3431.
- Chin, W.W., Carr, F.E., Burnside, J. and Darling, D.S. (1993) Thyroid hormone regulation of thyrotropin gene expression. *Recent Prog Horm Res*, 48, 393-414.
- Christoffolete, M.A., Ribeiro, R., Singru, P., Fekete, C., da Silva, W.S., Gordon, D.F., Huang, S.A., Crescenzi, A., Harney, J.W., Ridgway, E.C., Larsen, P.R., Lechan, R.M. and Bianco, A.C. (2006) Atypical expression of type 2 iodothyronine deiodinase in thyrotrophs explains the thyroxine-mediated pituitary thyrotropin feedback mechanism. *Endocrinology*, 147, 1735-1743.
- Clabby, M.L., Robison, T.A., Quigley, H.F., Wilson, D.B. and Kelly, D.P. (2003) Retinoid X receptor alpha represses GATA-4-mediated transcription via a retinoid-dependent interaction with the cardiac-enriched repressor FOG-2. *J Biol Chem*, 278, 5760-5767.
- Cohen, L.E. and Radovick, S. (2002) Molecular basis of combined pituitary hormone deficiencies. *Endocr Rev*, 23, 431-442.
- Cohen, O., Flynn, T.R. and Wondisford, F.E. (1995) Ligand-dependent antagonism by retinoid X receptors of inhibitory thyroid hormone response elements. *J Biol Chem*, 270, 13899-13905.
- Cohen, R.N. and Wondisford, F.E. (2005) Factors that control thyroid function: Thyrotropin. *Werner and Ingbar's The Thyroid*, Ninth Edition, pp159-197.
- Crawford, S.E., Qi, C., Misra, P., Stellmach, V., Rao, M.S., Engel, J.D., Zhu, Y. and Reddy, J.K. (2002) Defects of the heart, eye, and megakaryocytes in peroxisome proliferator activator receptor-binding protein (PBP) null embryos implicate GATA family of transcription factors. *J Biol Chem*, 277, 3585-3592.
- Danzi, S. and Klein, I. (2005) Posttranscriptional regulation of myosin heavy chain expression in the heart by triiodothyronine. *Am J Physiol Heart Circ Physiol*, 288, H455-460.
- Dasen, J.S., O'Connell, S.M., Flynn, S.E., Treier, M., Gleiberman, A.S., Szeto, D.P., Hooshmand, F., Aggarwal, A.K. and Rosenfeld, M.G. (1999) Reciprocal interactions of Pit1 and GATA2 mediate signaling gradient-induced determination of pituitary cell types. *Cell*, 97, 587-598.
- De Bosscher, K., Schmitz, M.L., Vanden Berghe, W., Plaisance, S., Fiers, W. and Haegeman, G. (1997) Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. *Proc Natl Acad Sci U S A*, 94, 13504-13509.
- De Bosscher, K., Vanden Berghe, W. and Haegeman, G. (2001) Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators. *Mol Endocrinol*, 15, 219-227.
- De Bosscher, K., Vanden Berghe, W. and Haegeman, G. (2003) The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev*, 24, 488-522.
- Dentice, M., Morisco, C., Vitale, M., Rossi, G., Fenzi, G. and Salvatore, D. (2003) The different cardiac expression of the type 2 iodothyronine deiodinase gene between

- human and rat is related to the differential response of the *Dio2* genes to *Nkx-2.5* and *GATA-4* transcription factors. *Mol Endocrinol*, 17, 1508-1521.
- Dorfman, D.M., Wilson, D.B., Bruns, G.A. and Orkin, S.H. (1992) Human transcription factor *GATA-2*. Evidence for regulation of preproendothelin-1 gene expression in endothelial cells. *J Biol Chem*, 267, 1279-1285.
- Dupre, S.M., Guissouma, H., Flamant, F., Seugnet, I., Scanlan, T.S., Baxter, J.D., Samarut, J., Demeneix, B.A. and Becker, N. (2004) Both thyroid hormone receptor (TR)beta 1 and TR beta 2 isoforms contribute to the regulation of hypothalamic thyrotropin-releasing hormone. *Endocrinology*, 145, 2337-2345.
- Edwards, J.G., Bahl, J.J., Flink, I.L., Cheng, S.Y. and Morkin, E. (1994) Thyroid hormone influences beta myosin heavy chain (beta MHC) expression. *Biochem Biophys Res Commun*, 199, 1482-1488.
- Escobar-Morreale, H.F., del Rey, F.E., Obregon, M.J. and de Escobar, G.M. (1996) Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. *Endocrinology*, 137, 2490-2502.
- Fekete, C. and Lechan, R.M. (2007) Negative feedback regulation of hypophysiotropic thyrotropin-releasing hormone (TRH) synthesizing neurons: role of neuronal afferents and type 2 deiodinase. *Front Neuroendocrinol*, 28, 97-114.
- Feng, X., Jiang, Y., Meltzer, P. and Yen, P.M. (2000) Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. *Mol Endocrinol*, 14, 947-955.
- Fisher, D.A., Schoen, E.J., La Franchi, S., Mandel, S.H., Nelson, J.C., Carlton, E.I. and Goshi, J.H. (2000) The hypothalamic-pituitary-thyroid negative feedback control axis in children with treated congenital hypothyroidism. *J Clin Endocrinol Metab*, 85, 2722-2727.
- Flink, I.L., Edwards, J.G., Bahl, J.J., Liew, C.C., Sole, M. and Morkin, E. (1992) Characterization of a strong positive cis-acting element of the human beta-myosin heavy chain gene in fetal rat heart cells. *J Biol Chem*, 267, 9917-9924.
- Forrest, D., Hanebuth, E., Smeyne, R.J., Everds, N., Stewart, C.L., Wehner, J.M. and Curran, T. (1996) Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. *Embo J*, 15, 3006-3015.
- Fowkes, R.C., King, P. and Burrin, J.M. (2002) Regulation of human glycoprotein hormone alpha-subunit gene transcription in LbetaT2 gonadotropes by protein kinase C and extracellular signal-regulated kinase 1/2. *Biol Reprod*, 67, 725-734.
- Fraichard, A., Chassande, O., Plateroti, M., Roux, J.P., Trouillas, J., Dehay, C., Legrand, C., Gauthier, K., Kedingier, M., Malaval, L., Rousset, B. and Samarut, J. (1997) The *T3R* alpha gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. *Embo J*, 16, 4412-4420.
- Franklyn, J.A., Wilson, M., Davis, J.R., Ramsden, D.B., Docherty, K. and Sheppard, M.C. (1986) Demonstration of thyrotrophin beta-subunit messenger RNA in rat pituitary cells in primary culture--evidence for regulation by thyrotrophin-releasing hormone and forskolin. *J Endocrinol*, 111, R1-2.

- Friedrichsen, S., Christ, S., Heuer, H., Schafer, M.K., Parlow, A.F., Visser, T.J. and Bauer, K. (2004) Expression of pituitary hormones in the Pax8-/- mouse model of congenital hypothyroidism. *Endocrinology*, 145, 1276-1283.
- Gereben, B., Zavacki, A.M., Ribich, S., Kim, B.W., Huang, S.A., Simonides, W.S., Zeold, A. and Bianco, A.C. (2008) Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev*, 29, 898-938.
- Gershengorn, M.C. and Osman, R. (1996) Molecular and cellular biology of thyrotropin-releasing hormone receptors. *Physiol Rev*, 76, 175-191.
- Glass, C.K. and Rosenfeld, M.G. (2000) The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev*, 14, 121-141.
- Gordon, D.F., Lewis, S.R., Haugen, B.R., James, R.A., McDermott, M.T., Wood, W.M. and Ridgway, E.C. (1997) Pit-1 and GATA-2 interact and functionally cooperate to activate the thyrotropin beta-subunit promoter. *J Biol Chem*, 272, 24339-24347.
- Gordon, D.F., Tucker, E.A., Tundwal, K., Hall, H., Wood, W.M. and Ridgway, E.C. (2006) MED220/thyroid receptor-associated protein 220 functions as a transcriptional coactivator with Pit-1 and GATA-2 on the thyrotropin-beta promoter in thyrotropes. *Mol Endocrinol*, 20, 1073-1089.
- Gothé, S., Wang, Z., Ng, L., Kindblom, J.M., Barros, A.C., Ohlsson, C., Vennstrom, B. and Forrest, D. (1999) Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. *Genes Dev*, 13, 1329-1341.
- Guissouma, H., Dupre, S.M., Becker, N., Jeannin, E., Seugnet, I., Desvergne, B. and Demeneix, B.A. (2002) Feedback on hypothalamic TRH transcription is dependent on thyroid hormone receptor N terminus. *Mol Endocrinol*, 16, 1652-1666.
- Gupta, M.P. (2007) Factors controlling cardiac myosin-isoform shift during hypertrophy and heart failure. *J Mol Cell Cardiol*, 43, 388-403.
- Haddad, F., Bodell, P.W., Qin, A.X., Giger, J.M. and Baldwin, K.M. (2003) Role of antisense RNA in coordinating cardiac myosin heavy chain gene switching. *J Biol Chem*, 278, 37132-37138.
- Hallenbeck, P.L., Phyllaier, M. and Nikodem, V.M. (1993) Divergent effects of 9-cis-retinoic acid receptor on positive and negative thyroid hormone receptor-dependent gene expression. *J Biol Chem*, 268, 3825-3828.
- Hasegawa, K., Lee, S.J., Jobe, S.M., Markham, B.E. and Kitsis, R.N. (1997) cis-Acting sequences that mediate induction of beta-myosin heavy chain gene expression during left ventricular hypertrophy due to aortic constriction. *Circulation*, 96, 3943-3953.
- Haugen, B.R., Wood, W.M., Gordon, D.F. and Ridgway, E.C. (1993) A thyrotrope-specific variant of Pit-1 transactivates the thyrotropin beta promoter. *J Biol Chem*, 268, 20818-20824.
- Heck, S., Kullmann, M., Gast, A., Ponta, H., Rahmsdorf, H.J., Herrlich, P. and Cato, A.C. (1994) A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *Embo J*, 13, 4087-4095.
- Herrlich, P. (2001) Cross-talk between glucocorticoid receptor and AP-1. *Oncogene*, 20, 2465-2475.

- Ho, C.K. and Strauss, J.F., 3rd. (2004) Activation of the control reporter plasmids pRL-TK and pRL-SV40 by multiple GATA transcription factors can lead to aberrant normalization of transfection efficiency. *BMC Biotechnol*, 4, 10.
- Hollenberg, A.N., Monden, T., Flynn, T.R., Boers, M.E., Cohen, O. and Wondisford, F.E. (1995) The human thyrotropin-releasing hormone gene is regulated by thyroid hormone through two distinct classes of negative thyroid hormone response elements. *Mol Endocrinol*, 9, 540-550.
- Hollenberg, A.N., Monden, T., Madura, J.P., Lee, K. and Wondisford, F.E. (1996) Function of nuclear co-repressor protein on thyroid hormone response elements is regulated by the receptor A/B domain. *J Biol Chem*, 271, 28516-28520.
- Hong, W., Nakazawa, M., Chen, Y.Y., Kori, R., Vakoc, C.R., Rakowski, C. and Blobel, G.A. (2005) FOG-1 recruits the NuRD repressor complex to mediate transcriptional repression by GATA-1. *Embo J*, 24, 2367-2378.
- Huang, Z.Q., Li, J., Sachs, L.M., Cole, P.A. and Wong, J. (2003) A role for cofactor-cofactor and cofactor-histone interactions in targeting p300, SWI/SNF and Mediator for transcription. *Embo J*, 22, 2146-2155.
- Huggins, G.S., Bacani, C.J., Boltax, J., Aikawa, R. and Leiden, J.M. (2001) Friend of GATA 2 physically interacts with chicken ovalbumin upstream promoter-TF2 (COUP-TF2) and COUP-TF3 and represses COUP-TF2-dependent activation of the atrial natriuretic factor promoter. *J Biol Chem*, 276, 28029-28036.
- Ito, M., Yuan, C.X., Okano, H.J., Darnell, R.B. and Roeder, R.G. (2000) Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. *Mol Cell*, 5, 683-693.
- Jensen, F.C., Girardi, A.J., Gilden, R.V. and Koprowski, H. (1964) Infection Of Human And Simian Tissue Cultures With Rous Sarcoma Virus. *Proc Natl Acad Sci U S A*, 52, 53-59.
- Jepsen, K., Solum, D., Zhou, T., McEvilly, R.J., Kim, H.J., Glass, C.K., Hermanson, O. and Rosenfeld, M.G. (2007) SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. *Nature*, 450, 415-419.
- Jorgensen, J.S., Quirk, C.C. and Nilson, J.H. (2004) Multiple and overlapping combinatorial codes orchestrate hormonal responsiveness and dictate cell-specific expression of the genes encoding luteinizing hormone. *Endocr Rev*, 25, 521-542.
- Kakucska, I., Rand, W. and Lechan, R.M. (1992) Thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is dependent upon feedback regulation by both triiodothyronine and thyroxine. *Endocrinology*, 130, 2845-2850.
- Kalaitzidis, D. and Gilmore, T.D. (2005) Transcription factor cross-talk: the estrogen receptor and NF-kappaB. *Trends Endocrinol Metab*, 16, 46-52.
- Kalkhoven, E., Wissink, S., van der Saag, P.T. and van der Burg, B. (1996) Negative interaction between the RelA(p65) subunit of NF-kappaB and the progesterone receptor. *J Biol Chem*, 271, 6217-6224.
- Kamei, Y., Xu, L., Heinzl, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K. and Rosenfeld, M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, 85, 403-414.

- Kashiwabara, Y., Sasaki, S., Matsushita, A., Nagayama, K., Ohba, K., Iwaki, H., Matsunaga, H., Suzuki, S., Misawa, H., Ishizuka, K., Oki, Y. and Nakamura, H. (2009) Functions of PIT1 in GATA2-dependent transactivation of the thyrotropin beta promoter. *J Mol Endocrinol*, 42, 225-237.
- Kim, M.S., Fujiki, R., Murayama, A., Kitagawa, H., Yamaoka, K., Yamamoto, Y., Mihara, M., Takeyama, K. and Kato, S. (2007) 1 α ,25(OH) $_2$ D $_3$ -induced transrepression by vitamin D receptor through E-box-type elements in the human parathyroid hormone gene promoter. *Mol Endocrinol*, 21, 334-342.
- Kim, M.S., Kondo, T., Takada, I., Youn, M.Y., Yamamoto, Y., Takahashi, S., Matsumoto, T., Fujiyama, S., Shiode, Y., Yamaoka, I., Kitagawa, H., Takeyama, K., Shibuya, H., Ohtake, F. and Kato, S. (2009) DNA demethylation in hormone-induced transcriptional derepression. *Nature*, 461, 1007-1012.
- Kim, S.W., Ho, S.C., Hong, S.J., Kim, K.M., So, E.C., Christoffolete, M. and Harney, J.W. (2005) A novel mechanism of thyroid hormone-dependent negative regulation by thyroid hormone receptor, nuclear receptor corepressor (NCoR), and GAGA-binding factor on the rat cD44 promoter. *J Biol Chem*, 280, 14545-14555.
- Kinugawa, K., Yonekura, K., Ribeiro, R.C., Eto, Y., Aoyagi, T., Baxter, J.D., Camacho, S.A., Bristow, M.R., Long, C.S. and Simpson, P.C. (2001) Regulation of thyroid hormone receptor isoforms in physiological and pathological cardiac hypertrophy. *Circ Res*, 89, 591-598.
- Kitagawa, H., Fujiki, R., Yoshimura, K., Mezaki, Y., Uematsu, Y., Matsui, D., Ogawa, S., Unno, K., Okubo, M., Tokita, A., Nakagawa, T., Ito, T., Ishimi, Y., Nagasawa, H., Matsumoto, T., Yanagisawa, J. and Kato, S. (2003) The chromatin-remodeling complex WINAC targets a nuclear receptor to promoters and is impaired in Williams syndrome. *Cell*, 113, 905-917.
- Kobayashi-Osaki, M., Ohneda, O., Suzuki, N., Minegishi, N., Yokomizo, T., Takahashi, S., Lim, K.C., Engel, J.D. and Yamamoto, M. (2005) GATA motifs regulate early hematopoietic lineage-specific expression of the Gata2 gene. *Mol Cell Biol*, 25, 7005-7020.
- Krane, I.M., Spindel, E.R. and Chin, W.W. (1991) Thyroid hormone decreases the stability and the poly(A) tract length of rat thyrotropin beta-subunit messenger RNA. *Mol Endocrinol*, 5, 469-475.
- Laflamme, L., Hamann, G., Messier, N., Maltais, S. and Langlois, M.F. (2002) RXR acts as a coregulator in the regulation of genes of the hypothalamo-pituitary axis by thyroid hormone receptors. *J Mol Endocrinol*, 29, 61-72.
- Lazar, M.A. (2003) Thyroid hormone action: a binding contract. *J Clin Invest*, 112, 497-499.
- Liden, J., Delaunay, F., Rafter, I., Gustafsson, J. and Okret, S. (1997) A new function for the C-terminal zinc finger of the glucocorticoid receptor. Repression of RelA transactivation. *J Biol Chem*, 272, 21467-21472.
- Lin, K.H., Shieh, H.Y. and Hsu, H.C. (2000) Negative regulation of the antimetastatic gene Nm23-H1 by thyroid hormone receptors. *Endocrinology*, 141, 2540-2547.
- Liu, Y.Y. and Brent, G.A. (2008) Stealth sequences in reporter gene vectors confound studies of T3-regulated negative gene expression. *Thyroid*, 18, 593-595.

- Lopez, G., Schaufele, F., Webb, P., Holloway, J.M., Baxter, J.D. and Kushner, P.J. (1993) Positive and negative modulation of Jun action by thyroid hormone receptor at a unique AP1 site. *Mol Cell Biol*, 13, 3042-3049.
- Madison, L.D., Ahlquist, J.A., Rogers, S.D. and Jameson, J.L. (1993) Negative regulation of the glycoprotein hormone alpha gene promoter by thyroid hormone: mutagenesis of a proximal receptor binding site preserves transcriptional repression. *Mol Cell Endocrinol*, 94, 129-136.
- Maia, A.L., Harney, J.W. and Larsen, P.R. (1996) Is there a negative TRE in the luciferase reporter cDNA? *Thyroid*, 6, 325-328.
- Maira, M., Martens, C., Batsche, E., Gauthier, Y. and Drouin, J. (2003) Dimer-specific potentiation of NGFI-B (Nur77) transcriptional activity by the protein kinase A pathway and AF-1-dependent coactivator recruitment. *Mol Cell Biol*, 23, 763-776.
- Mansen, A., Yu, F., Forrest, D., Larsson, L. and Vennstrom, B. (2001) TRs have common and isoform-specific functions in regulation of the cardiac myosin heavy chain genes. *Mol Endocrinol*, 15, 2106-2114.
- Martens, C., Bilodeau, S., Maira, M., Gauthier, Y. and Drouin, J. (2005) Protein-protein interactions and transcriptional antagonism between the subfamily of NGFI-B/Nur77 orphan nuclear receptors and glucocorticoid receptor. *Mol Endocrinol*, 19, 885-897.
- Matsushita, A., Sasaki, S., Kashiwabara, Y., Nagayama, K., Ohba, K., Iwaki, H., Misawa, H., Ishizuka, K. and Nakamura, H. (2007) Essential role of GATA2 in the negative regulation of thyrotropin beta gene by thyroid hormone and its receptors. *Mol Endocrinol*, 21, 865-884.
- Minami, T., Abid, M.R., Zhang, J., King, G., Kodama, T. and Aird, W.C. (2003) Thrombin stimulation of vascular adhesion molecule-1 in endothelial cells is mediated by protein kinase C (PKC)-delta-NF-kappa B and PKC-zeta-GATA signaling pathways. *J Biol Chem*, 278, 6976-6984.
- Minegishi, N., Suzuki, N., Kawatani, Y., Shimizu, R. and Yamamoto, M. (2005) Rapid turnover of GATA-2 via ubiquitin-proteasome protein degradation pathway. *Genes Cells*, 10, 693-704.
- Misawa, H., Sasaki, S., Matsushita, A., Ohba, K., Iwaki, H., Matsunaga, H., Suzuki, S., Ishizuka, K., Oki, Y. and Nakamura, H. Liganded thyroid hormone receptor inhibits phorbol 12-O-tetradecanoate-13-acetate-induced enhancer activity via firefly luciferase cDNA. in submission.
- Mittag, J., Friedrichsen, S., Strube, A., Heuer, H. and Bauer, K. (2009) Analysis of hypertrophic thyrotrophs in pituitaries of athyroid Pax8^{-/-} mice. *Endocrinology*, 150, 4443-4449.
- Morimoto, T., Hasegawa, K., Kaburagi, S., Kakita, T., Masutani, H., Kitsis, R.N., Matsumori, A. and Sasayama, S. (1999) GATA-5 is involved in leukemia inhibitory factor-responsive transcription of the beta-myosin heavy chain gene in cardiac myocytes. *J Biol Chem*, 274, 12811-12818.
- Murayama, A., Kim, M.S., Yanagisawa, J., Takeyama, K. and Kato, S. (2004) Transrepression by a liganded nuclear receptor via a bHLH activator through co-regulator switching. *Embo J*, 23, 1598-1608.

- Naar, A.M., Boutin, J.M., Lipkin, S.M., Yu, V.C., Holloway, J.M., Glass, C.K. and Rosenfeld, M.G. (1991) The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. *Cell*, 65, 1267-1279.
- Nagaya, T. and Jameson, J.L. (1993) Thyroid hormone receptor dimerization is required for dominant negative inhibition by mutations that cause thyroid hormone resistance. *J Biol Chem*, 268, 15766-15771.
- Nagayama, K., Sasaki, S., Matsushita, A., Ohba, K., Iwaki, H., Matsunaga, H., Suzuki, S., Misawa, H., Ishizuka, K., Oki, Y., Noh, J.Y. and Nakamura, H. (2008) Inhibition of GATA2-dependent transactivation of the TSHbeta gene by ligand-bound estrogen receptor alpha. *J Endocrinol*, 199, 113-125.
- Nakano, K., Matsushita, A., Sasaki, S., Misawa, H., Nishiyama, K., Kashiwabara, Y. and Nakamura, H. (2004) Thyroid-hormone-dependent negative regulation of thyrotropin beta gene by thyroid hormone receptors: study with a new experimental system using CV1 cells. *Biochem J*, 378, 549-557.
- Nikrodhanond, A.A., Ortiga-Carvalho, T.M., Shibusawa, N., Hashimoto, K., Liao, X.H., Refetoff, S., Yamada, M., Mori, M. and Wondisford, F.E. (2006) Dominant role of thyrotropin-releasing hormone in the hypothalamic-pituitary-thyroid axis. *J Biol Chem*, 281, 5000-5007.
- Nissen, R.M. and Yamamoto, K.R. (2000) The glucocorticoid receptor inhibits NFkappaB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev*, 14, 2314-2329.
- Nofsinger, R.R., Li, P., Hong, S.H., Jonker, J.W., Barish, G.D., Ying, H., Cheng, S.Y., Leblanc, M., Xu, W., Pei, L., Kang, Y.J., Nelson, M., Downes, M., Yu, R.T., Olefsky, J.M., Lee, C.H. and Evans, R.M. (2008) SMRT repression of nuclear receptors controls the adipogenic set point and metabolic homeostasis. *Proc Natl Acad Sci U S A*, 105, 20021-20026.
- Ohba, K., Sasaki, S., Matsushita, A., Iwaki, H., Matsunaga, H., Suzuki, S., Ishizuka, K., Misawa, H., Oki, Y. and Nakamura, H. (2011) GATA2 mediates thyrotropin-releasing hormone-induced transcriptional activation of the thyrotropin beta gene. *PLoS One*, in press.
- Ooi, G.T., Tawadros, N. and Escalona, R.M. (2004) Pituitary cell lines and their endocrine applications. *Mol Cell Endocrinol*, 228, 1-21.
- Oppenheimer, J.H., Schwartz, H.L. and Surks, M.I. (1974) Tissue differences in the concentration of triiodothyronine nuclear binding sites in the rat: liver, kidney, pituitary, heart, brain, spleen, and testis. *Endocrinology*, 95, 897-903.
- Osborne, S.A. and Tonissen, K.F. (2002) pRL-TK induction can cause misinterpretation of gene promoter activity. *Biotechniques*, 33, 1240-1242.
- Paguio, A., Almond, B., Fan, F., Stecha, P., Garvin, D., Wood, M. and Wood, K. (2005) pGL4 Vectors: A New Generation of Luciferase Reporter Vectors. *Promega Notes*, 89, 7-10.
- Pennathur, S., Madison, L.D., Kay, T.W. and Jameson, J.L. (1993) Localization of promoter sequences required for thyrotropin-releasing hormone and thyroid hormone responsiveness of the glycoprotein hormone alpha-gene in primary cultures of rat pituitary cells. *Mol Endocrinol*, 7, 797-805.
- Pfahl, M. (1993) Nuclear receptor/ AP-1 interaction. *Endocr Rev*, 14, 651-658.

- Ray, A. and Prefontaine, K.E. (1994) Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappa B and the glucocorticoid receptor. *Proc Natl Acad Sci U S A*, 91, 752-756.
- Refetoff, S. and Dumitrescu, A.M. (2007) Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. *Best Pract Res Clin Endocrinol Metab*, 21, 277-305.
- Refetoff, S., Weiss, R.E. and Usala, S.J. (1993) The syndromes of resistance to thyroid hormone. *Endocr Rev*, 14, 348-399.
- Reichardt, H.M., Kaestner, K.H., Tuckermann, J., Kretz, O., Wessely, O., Bock, R., Gass, P., Schmid, W., Herrlich, P., Angel, P. and Schutz, G. (1998) DNA binding of the glucocorticoid receptor is not essential for survival. *Cell*, 93, 531-541.
- Reutrakul, S., Sadow, P.M., Pannain, S., Pohlenz, J., Carvalho, G.A., Macchia, P.E., Weiss, R.E. and Refetoff, S. (2000) Search for abnormalities of nuclear corepressors, coactivators, and a coregulator in families with resistance to thyroid hormone without mutations in thyroid hormone receptor beta or alpha genes. *J Clin Endocrinol Metab*, 85, 3609-3617.
- Roche, A.E., Bassett, B.J., Samant, S.A., Hong, W., Blobel, G.A. and Svensson, E.C. (2008) The zinc finger and C-terminal domains of MTA proteins are required for FOG-2-mediated transcriptional repression via the NuRD complex. *J Mol Cell Cardiol*, 44, 352-360.
- Rodriguez, P., Bonte, E., Krijgsveld, J., Kolodziej, K.E., Guyot, B., Heck, A.J., Vyas, P., de Boer, E., Grosveld, F. and Strouboulis, J. (2005) GATA-1 forms distinct activating and repressive complexes in erythroid cells. *Embo J*, 24, 2354-2366.
- Rouf, R., Greytak, S., Wooten, E.C., Wu, J., Boltax, J., Picard, M., Svensson, E.C., Dillmann, W.H., Patten, R.D. and Huggins, G.S. (2008) Increased FOG-2 in failing myocardium disrupts thyroid hormone-dependent SERCA2 gene transcription. *Circ Res*, 103, 493-501.
- Saatcioglu, F., Deng, T. and Karin, M. (1993) A novel cis element mediating ligand-independent activation by c-ErbA: implications for hormonal regulation. *Cell*, 75, 1095-1105.
- Saatcioglu, F., Lopez, G., West, B.L., Zandi, E., Feng, W., Lu, H., Esmaili, A., Apriletti, J.W., Kushner, P.J., Baxter, J.D. and Karin, M. (1997) Mutations in the conserved C-terminal sequence in thyroid hormone receptor dissociate hormone-dependent activation from interference with AP-1 activity. *Mol Cell Biol*, 17, 4687-4695.
- Sadow, P.M., Koo, E., Chassande, O., Gauthier, K., Samarut, J., Xu, J., O'Malley, B.W., Seo, H., Murata, Y. and Weiss, R.E. (2003) Thyroid hormone receptor-specific interactions with steroid receptor coactivator-1 in the pituitary. *Mol Endocrinol*, 17, 882-894.
- Safer, J.D., Langlois, M.F., Cohen, R., Monden, T., John-Hope, D., Madura, J., Hollenberg, A.N. and Wondisford, F.E. (1997) Isoform variable action among thyroid hormone receptor mutants provides insight into pituitary resistance to thyroid hormone. *Mol Endocrinol*, 11, 16-26.
- Sanchez-Pacheco, A., Palomino, T. and Aranda, A. (1995) Negative regulation of expression of the pituitary-specific transcription factor GHF-1/Pit-1 by thyroid hormones

- through interference with promoter enhancer elements. *Mol Cell Biol*, 15, 6322-6330.
- Santos, G.M., Afonso, V., Barra, G.B., Togashi, M., Webb, P., Neves, F.A., Lomri, N. and Lomri, A. (2006) Negative regulation of superoxide dismutase-1 promoter by thyroid hormone. *Mol Pharmacol*, 70, 793-800.
- Sarapura, V.D., Samuels, M.H. and Ridgway, E.C. (2002) Thyroid-stimulating Hormone. The Pituitary, Second Edition, pp172-215.
- Sasaki, S., Lesoon-Wood, L.A., Dey, A., Kuwata, T., Weintraub, B.D., Humphrey, G., Yang, W.M., Seto, E., Yen, P.M., Howard, B.H. and Ozato, K. (1999) Ligand-induced recruitment of a histone deacetylase in the negative-feedback regulation of the thyrotropin beta gene. *Embo J*, 18, 5389-5398.
- Satoh, T., Yamada, M., Iwasaki, T. and Mori, M. (1996) Negative regulation of the gene for the preprothyrotropin-releasing hormone from the mouse by thyroid hormone requires additional factors in conjunction with thyroid hormone receptors. *J Biol Chem*, 271, 27919-27926.
- Scheinman, R.I., Gualberto, A., Jewell, C.M., Cidlowski, J.A. and Baldwin, A.S., Jr. (1995) Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol*, 15, 943-953.
- Schule, R., Rangarajan, P., Kliwer, S., Ransone, L.J., Bolado, J., Yang, N., Verma, I.M. and Evans, R.M. (1990) Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell*, 62, 1217-1226.
- Sharma, V., Hays, W.R., Wood, W.M., Pugazhenth, U., St Germain, D.L., Bianco, A.C., Krezel, W., Chambon, P. and Haugen, B.R. (2006) Effects of rexinoids on thyrotrope function and the hypothalamic-pituitary-thyroid axis. *Endocrinology*, 147, 1438-1451.
- Sherman, S.I., Gopal, J., Haugen, B.R., Chiu, A.C., Whaley, K., Nowlakha, P. and Duvic, M. (1999) Central hypothyroidism associated with retinoid X receptor-selective ligands. *N Engl J Med*, 340, 1075-1079.
- Shibusawa, N., Hashimoto, K., Nikrodhanond, A.A., Liberman, M.C., Applebury, M.L., Liao, X.H., Robbins, J.T., Refetoff, S., Cohen, R.N. and Wondisford, F.E. (2003a) Thyroid hormone action in the absence of thyroid hormone receptor DNA-binding in vivo. *J Clin Invest*, 112, 588-597.
- Shibusawa, N., Hollenberg, A.N. and Wondisford, F.E. (2003b) Thyroid hormone receptor DNA binding is required for both positive and negative gene regulation. *J Biol Chem*, 278, 732-738.
- Shibusawa, N., Yamada, M., Hirato, J., Monden, T., Satoh, T. and Mori, M. (2000) Requirement of thyrotropin-releasing hormone for the postnatal functions of pituitary thyrotrophs: ontogeny study of congenital tertiary hypothyroidism in mice. *Mol Endocrinol*, 14, 137-146.
- Shimizu, R. and Yamamoto, M. (2005) Gene expression regulation and domain function of hematopoietic GATA factors. *Semin Cell Dev Biol*, 16, 129-136.
- Shupnik, M.A. (2000) Thyroid hormone suppression of pituitary hormone gene expression. *Rev Endocr Metab Disord*, 1, 35-42.
- Shupnik, M.A., Gharib, S.D. and Chin, W.W. (1988) Estrogen suppresses rat gonadotropin gene transcription in vivo. *Endocrinology*, 122, 1842-1846.

- Shupnik, M.A., Greenspan, S.L. and Ridgway, E.C. (1986) Transcriptional regulation of thyrotropin subunit genes by thyrotropin-releasing hormone and dopamine in pituitary cell culture. *J Biol Chem*, 261, 12675-12679.
- Shupnik, M.A., Ridgway, E.C. and Chin, W.W. (1989) Molecular biology of thyrotropin. *Endocr Rev*, 10, 459-475.
- St Germain, D.L., Hernandez, A., Schneider, M.J. and Galton, V.A. (2005) Insights into the role of deiodinases from studies of genetically modified animals. *Thyroid*, 15, 905-916.
- Staton, J.M., Thomson, A.M. and Leedman, P.J. (2000) Hormonal regulation of mRNA stability and RNA-protein interactions in the pituitary. *J Mol Endocrinol*, 25, 17-34.
- Steger, D.J., Hecht, J.H. and Mellon, P.L. (1994) GATA-binding proteins regulate the human gonadotropin alpha-subunit gene in the placenta and pituitary gland. *Mol Cell Biol*, 14, 5592-5602.
- Stein, B. and Yang, M.X. (1995) Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-kappa B and C/EBP beta. *Mol Cell Biol*, 15, 4971-4979.
- Steinfelder, H.J., Radovick, S., Mroczynski, M.A., Hauser, P., McClaskey, J.H., Weintraub, B.D. and Wondisford, F.E. (1992a) Role of a pituitary-specific transcription factor (pit-1/GHF-1) or a closely related protein in cAMP regulation of human thyrotropin-beta subunit gene expression. *J Clin Invest*, 89, 409-419.
- Steinfelder, H.J., Radovick, S. and Wondisford, F.E. (1992b) Hormonal regulation of the thyrotropin beta-subunit gene by phosphorylation of the pituitary-specific transcription factor Pit-1. *Proc Natl Acad Sci U S A*, 89, 5942-5945.
- Sugawara, A., Yen, P.M., Qi, Y., Lechan, R.M. and Chin, W.W. (1995) Isoform-specific retinoid-X receptor (RXR) antibodies detect differential expression of RXR proteins in the pituitary gland. *Endocrinology*, 136, 1766-1774.
- Sun, Y., Lu, X. and Gershengorn, M.C. (2003) Thyrotropin-releasing hormone receptors -- similarities and differences. *J Mol Endocrinol*, 30, 87-97.
- Surks, M.I. and Hollowell, J.G. (2007) Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. *J Clin Endocrinol Metab*, 92, 4575-4582.
- Suzuki, S., Sasaki, S., Morita, H., Oki, Y., Turiya, D., Ito, T., Misawa, H., Ishizuka, K. and Nakamura, H. (2010) The role of the amino-terminal domain in the interaction of unliganded peroxisome proliferator-activated receptor gamma-2 with nuclear receptor co-repressor. *J Mol Endocrinol*, 45, 133-145.
- Tagami, T., Madison, L.D., Nagaya, T. and Jameson, J.L. (1997) Nuclear receptor corepressors activate rather than suppress basal transcription of genes that are negatively regulated by thyroid hormone. *Mol Cell Biol*, 17, 2642-2648.
- Tagami, T., Park, Y. and Jameson, J.L. (1999) Mechanisms that mediate negative regulation of the thyroid-stimulating hormone alpha gene by the thyroid hormone receptor. *J Biol Chem*, 274, 22345-22353.
- Takeda, K., Sakurai, A., DeGroot, L.J. and Refetoff, S. (1992) Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor-beta gene. *J Clin Endocrinol Metab*, 74, 49-55.

- Takeda, T., Nagasawa, T., Miyamoto, T., Hashizume, K. and DeGroot, L.J. (1997) The function of retinoid X receptors on negative thyroid hormone response elements. *Mol Cell Endocrinol*, 128, 85-96.
- Takeuchi, Y., Murata, Y., Sadow, P., Hayashi, Y., Seo, H., Xu, J., O'Malley, B.W., Weiss, R.E. and Refetoff, S. (2002) Steroid receptor coactivator-1 deficiency causes variable alterations in the modulation of T(3)-regulated transcription of genes in vivo. *Endocrinology*, 143, 1346-1352.
- Tao, Y., Williams-Skipp, C. and Scheinman, R.I. (2001) Mapping of glucocorticoid receptor DNA binding domain surfaces contributing to transrepression of NF-kappa B and induction of apoptosis. *J Biol Chem*, 276, 2329-2332.
- Tillman, J.B., Crone, D.E., Kim, H.S., Sprung, C.N. and Spindler, S.R. (1993) Promoter independent down-regulation of the firefly luciferase gene by T3 and T3 receptor in CV1 cells. *Mol Cell Endocrinol*, 95, 101-109.
- Trost, S.U., Swanson, E., Gloss, B., Wang-Iverson, D.B., Zhang, H., Volodarsky, T., Grover, G.J., Baxter, J.D., Chiellini, G., Scanlan, T.S. and Dillmann, W.H. (2000) The thyroid hormone receptor-beta-selective agonist GC-1 differentially affects plasma lipids and cardiac activity. *Endocrinology*, 141, 3057-3064.
- Tsika, R.W., Ma, L., Kehat, I., Schramm, C., Simmer, G., Morgan, B., Fine, D.M., Hanft, L.M., McDonald, K.S., Molkentin, J.D., Krenz, M., Yang, S. and Ji, J. (2010) TEAD-1 overexpression in the mouse heart promotes an age-dependent heart dysfunction. *J Biol Chem*, 285, 13721-13735.
- Umesono, K., Murakami, K.K., Thompson, C.C. and Evans, R.M. (1991) Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. *Cell*, 65, 1255-1266.
- Valentine, J.E., Kalkhoven, E., White, R., Hoare, S. and Parker, M.G. (2000) Mutations in the estrogen receptor ligand binding domain discriminate between hormone-dependent transactivation and transrepression. *J Biol Chem*, 275, 25322-25329.
- Villa, A., Santiago, J., Belandia, B. and 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. *Mol Endocrinol*, 18, 863-873.
- Vyas, D.R., McCarthy, J.J. and Tsika, R.W. (1999) Nuclear protein binding at the beta-myosin heavy chain A/T-rich element is enriched following increased skeletal muscle activity. *J Biol Chem*, 274, 30832-30842.
- Walsh, J.P., Bremner, A.P., Bulsara, M.K., O'Leary, P., Leedman, P.J., Feddema, P. and Michelangeli, V. (2005a) Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. *Arch Intern Med*, 165, 2467-2472.
- Walsh, J.P., Bremner, A.P., Bulsara, M.K., O'Leary, P., Leedman, P.J., Feddema, P. and Michelangeli, V. (2005b) Thyroid dysfunction and serum lipids: a community-based study. *Clin Endocrinol (Oxf)*, 63, 670-675.
- Webb, P., Lopez, G.N., Uht, R.M. and Kushner, P.J. (1995) Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. *Mol Endocrinol*, 9, 443-456.

- Weiss, R.E., Forrest, D., Pohlenz, J., Cua, K., Curran, T. and Refetoff, S. (1997) Thyrotropin regulation by thyroid hormone in thyroid hormone receptor beta-deficient mice. *Endocrinology*, 138, 3624-3629.
- Weitzel, J.M. (2008) To bind or not to bind - how to down-regulate target genes by liganded thyroid hormone receptor? *Thyroid Res*, 1, 4.
- Wikstrom, L., Johansson, C., Salto, C., Barlow, C., Campos Barros, A., Baas, F., Forrest, D., Thoren, P. and Vennstrom, B. (1998) Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. *Embo J*, 17, 455-461.
- Wissink, S., van Heerde, E.C., Schmitz, M.L., Kalkhoven, E., van der Burg, B., Baeuerle, P.A. and van der Saag, P.T. (1997) Distinct domains of the RelA NF-kappaB subunit are required for negative cross-talk and direct interaction with the glucocorticoid receptor. *J Biol Chem*, 272, 22278-22284.
- Wondisford, F.E., Farr, E.A., Radovick, S., Steinfeld, H.J., Moates, J.M., McClaskey, J.H. and Weintraub, B.D. (1989) Thyroid hormone inhibition of human thyrotropin beta-subunit gene expression is mediated by a cis-acting element located in the first exon. *J Biol Chem*, 264, 14601-14604.
- Wondisford, F.E., Steinfeld, H.J., Nations, M. and Radovick, S. (1993) AP-1 antagonizes thyroid hormone receptor action on the thyrotropin beta-subunit gene. *J Biol Chem*, 268, 2749-2754.
- Wood, W.M., Ocran, K.W., Kao, M.Y., Gordon, D.F., Alexander, L.M., Gutierrez-Hartmann, A. and Ridgway, E.C. (1990) Protein factors in thyrotropic tumor nuclear extracts bind to a region of the mouse thyrotropin beta-subunit promoter essential for expression in thyrotropes. *Mol Endocrinol*, 4, 1897-1904.
- Wright, C.E., Haddad, F., Qin, A.X., Bodell, P.W. and Baldwin, K.M. (1999) In vivo regulation of beta-MHC gene in rodent heart: role of T3 and evidence for an upstream enhancer. *Am J Physiol*, 276, C883-891.
- Wu, J., Li, Y., Dietz, J. and Lala, D.S. (2004) Repression of p65 transcriptional activation by the glucocorticoid receptor in the absence of receptor-coactivator interactions. *Mol Endocrinol*, 18, 53-62.
- Wulf, A., Wetzel, M.G., Kebenko, M., Kroger, M., Harneit, A., Merz, J. and Weitzel, J.M. (2008) The role of thyroid hormone receptor DNA binding in negative thyroid hormone-mediated gene transcription. *J Mol Endocrinol*, 41, 25-34.
- Xu, J., Qiu, Y., DeMayo, F.J., Tsai, S.Y., Tsai, M.J. and O'Malley, B.W. (1998) Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. *Science*, 279, 1922-1925.
- Yang, Z., Hong, S.H. and Privalsky, M.L. (1999) Transcriptional anti-repression. Thyroid hormone receptor beta-2 recruits SMRT corepressor but interferes with subsequent assembly of a functional corepressor complex. *J Biol Chem*, 274, 37131-37138.
- Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene*, 33, 103-119.
- Yoshida, T. (2008) MCAT elements and the TEF-1 family of transcription factors in muscle development and disease. *Arterioscler Thromb Vasc Biol*, 28, 8-17.
- Yuan, L.W. and Gambee, J.E. (2000) Phosphorylation of p300 at serine 89 by protein kinase C. *J Biol Chem*, 275, 40946-40951.

- Yusta, B., Alarid, E.T., Gordon, D.F., Ridgway, E.C. and Mellon, P.L. (1998) The thyrotropin beta-subunit gene is repressed by thyroid hormone in a novel thyrotrope cell line, mouse T alphaT1 cells. *Endocrinology*, 139, 4476-4482.
- Zhuang, Y., Butler, B., Hawkins, E., Paguio, A., Orr, L., Wood, M. and Wood, K. (2001) New Synthetic Renilla Gene and Assay System Increase Expression, Reliability and Sensitivity. *Promega Notes*, 79, 6-11.

IntechOpen

IntechOpen

Contemporary Aspects of Endocrinology

Edited by Dr. Evanthia Diamanti-Kandarakis

ISBN 978-953-307-357-6

Hard cover, 454 pages

Publisher InTech

Published online 30, November, 2011

Published in print edition November, 2011

This book aims to provide readers with a general as well as an advanced overview of the key trends in endocrine disorders. While covering a variety of topics ranging from thyroid carcinogenesis and pituitary adenomas to adrenal tumors and metabolic bone disease, this book also focuses on more specific issues not yet fully elucidated (e.g. the molecular pathways involved in thyrotropin beta gene regulation or monogenic phosphate balance disorders). Readers of different fields and background will have the opportunity to update their knowledge and more importantly to clarify areas of uncertainty and controversies in several topics of endocrine disorders.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Shigekazu Sasaki, Akio Matsushita and Hirotohi Nakamura (2011). Negative Regulation of the Thyrotropin β Gene by Thyroid Hormone, Contemporary Aspects of Endocrinology, Dr. Evanthia Diamanti-Kandarakis (Ed.), ISBN: 978-953-307-357-6, InTech, Available from: <http://www.intechopen.com/books/contemporary-aspects-of-endocrinology/negative-regulation-of-the-thyrotropin-946-gene-by-thyroid-hormone>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen