Novel aspects of T_3 actions on GH and TSH synthesis and secretion: physiological implications

P Bargi-Souza*, F Goulart-Silva* and M T Nunes

Department of Physiology and Biophysics of the Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil
*(P Bargi-Souza and F Goulart-Silva contributed equally to this work)

Correspondence should be addressed to M T Nunes
Email
mtnunes@icb.usp.br

Abstract

Thyroid hormones (THs) classically regulate the gene expression by transcriptional mechanisms. In pituitary, the encoding genes for growth hormone (GH) and thyroid-stimulating hormone (TSH) are examples of genes regulated by triiodothyronine (T_3) in a positive and negative way, respectively. Recent studies have shown a rapid adjustment of GH and TSH synthesis/secretion induced by T_3 posttranscriptional actions. In somatotrophs, T_3 promotes an increase in *Gh* mRNA content, poly(A) tail length and binding to the ribosome, associated with a rearrangement of actin cytoskeleton. In thyrotrophs, T_3 reduces *Tshb* mRNA content, poly(A) tail length and its association with the ribosome. In parallel, it promotes a redistribution of TSH secretory granules to more distal regions of the cell periphery, indicating a rapid effect of T_3 inhibition of TSH secretion. T_3 was shown to affect the content of tubulin and the polymerization of actin and tubulin cytoskeletons in the whole anterior pituitary gland, and to increase intracellular alpha (CGA) content. This review summarizes genomic and non-genomic/posttranscriptional actions of TH on the regulation of several steps of GH and TSH synthesis and secretion. These distinct mechanisms induced by T_3 can occur simultaneously, even though non-genomic effects are promptly elicited and precede the genomic actions, coexisting in a functional network within the cells.

Key Words
- triiodothyronine
- GH
- TSH
- non-genomic actions
- secretion

Journal of Molecular Endocrinology
(2017) **59**, R167–R178

Journal of Molecular Endocrinology

Nuclear thyroid receptors and the gene expression regulation

Most of the well-characterized actions of thyroid hormones (THs) are mediated by thyroid hormone receptors (THRs), the alpha (THRA) and beta (THRB) receptors, which belong to the superfamily of nuclear receptors. THRs act as transcriptional factors interacting with specific sequences of the DNA called thyroid hormone response elements (TREs) and promoting the activation or inhibition of target gene transcription. THRs can also indirectly bind to DNA through proteins such as AP1 (jun/fos) complexes, influencing the gene expression (Flamant *et al.* 2017). The presence of THRA and THRB isoforms in humans, mice, rats, chickens and *Xenopus laevis* was described and indicates that this mechanism is conserved across a variety of species (Tata & Widnell 1966, Samuels & Tsai 1973, Sap *et al.* 1986, Weinberger *et al.* 1986, Yaoita *et al.* 1990).

The THRs are encoded by *Thra* and *Thrb* genes. The heterogeneous mRNAs are subjected to alternative splicing or alternative use of promoters generating nine

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

different transcripts: *Thra1*, *Thra2*, *Thra3*, *ThrΔa1*, *ThrΔa2*, *Thrb1*, *Thrb2*, *Thrb3* (found only in the kidney, liver and lung of rats) and *ThrΔb* (O'Shea & Williams 2002, Ortiga-Carvalho *et al*. 2014). The expression of these transcripts changes during the development and cellular differentiation processes, being also transcriptionally and posttranscriptionally regulated. The THRs are differentially expressed in most tissues. *Thrb2* is highly expressed in the pituitary, paraventricular, ventromedial and arcuate nuclei of hypothalamus, inner ear and in some regions of the brain during development, while *Thrb1* mRNA is mainly detected in the brain, skeletal muscle, liver and kidney of rats (Lazar 1993, Ortiga-Carvalho *et al*. 2016). Mutations in THR isoforms are associated with different phenotypes according to tissue distribution of the mutated receptor (Mendoza & Hollenberg 2017).

THRs bind to TREs under the form of homodimers or heterodimers, mainly with retinoid acid receptors and retinoid X receptors (RXRs) that also belong to the superfamily of nuclear receptors. The THRs interact with TH, coactivators or corepressors modulating the gene transcription (Shupnik 2000, Yen 2001, Cheng *et al*. 2010).

In the anterior pituitary gland, THs downregulate the expression of genes that encode CGA (glycoprotein hormones alpha chain) and TSHB subunits of thyroid-stimulating hormone (TSH) (Franklyn *et al*. 1988) and upregulate *Gh* transcription (Crew & Spindler 1986, Koenig *et al*. 1987, Lavin *et al*. 1988). The THRB2 isoform participates in the positive and negative regulations of *Gh* and *Tshb* expressions, respectively (Abel *et al*. 1999, Barra *et al*. 2004). In humans, mutations in THRB isoforms led to the classic form of resistance to thyroid hormone (RTHB) characterized by high TH levels and normal or slightly elevated serum TSH concentration, due to the resistance of pituitary and hypothalamus to respond to the negative feedback triggered by TH (Forrest *et al*. 1996, Refetoff *et al*. 2014). Mild-to-moderate growth retardation and delayed bone maturation have also been reported in RTHB (Refetoff *et al*. 1993, Kaneshige *et al*. 2000).

Hypothyroidism induced by thyroidectomy in male rats increases the expression of both TSH subunits (Bargi-Souza *et al*. 2013, 2015), while the expression of GH, which is involved in linear growth and intermediary metabolism modulation, is markedly reduced (Hervas *et al*. 1975, Volpato & Nunes 1994). On the other hand, studies performed in hyperthyroid rodents and in T_3-treated TtT97 cells, a thyrotrophic tumor-transplantable cell line, have shown a marked increase in *Gh* mRNA content and a significant reduction in *Cga* and *Tshb* contents (Shupnik *et al*. 1985, Chin *et al*. 1993, Volpato & Nunes 1994, Shupnik 2000, Bargi-Souza *et al*. 2013, 2015).

Transcriptional regulation of *Gh* by T_3

During the 1980s, several studies carried out in rats demonstrated that the *Gh* expression is positively regulated by THs (Crew & Spindler 1986, Larsen *et al*. 1986, Koenig *et al*. 1987, Lavin *et al*. 1988). The increase in the *Gh* transcription rate triggered by T_3 requires the interaction of the THR/T_3 complex with sequences located close to the transcription start site (TSS) of *Gh* and the presence of specific transcriptional factors expressed in somatotrophs, since the insertion of *Gh* promoter in fibroblast or kidney cells did not evoke *Gh* transcription in response to T_3 (Larsen *et al*. 1986, Koenig *et al*. 1987).

Studies on the mutational analysis of the TRE of the rat *Gh* gene (r*Gh*) and other positively T_3-regulated genes suggest that (G/A)GGT(C/G)A is a putative consensus hexamer half-site sequence (Flamant *et al*. 2017). Changes in the orientation, spacing and number of half-sites of TRE were also observed (Yen 2001, Ortiga-Carvalho *et al*. 2016). In the rGh promoter, the sequences could be oriented as palindromes, or direct repeats separated by 4 nucleotides (Yen 2001, Cheng *et al*. 2010).

In the case of THs positively regulated genes, such as *Gh*, the THRB2 heterodimerizes mainly with the RXRs and in the absence of the ligand, the THR/RXR complex is linked to the DNA, repressing the transcriptional process through the interaction with corepressors such as NCoR1 (nuclear receptor corepressor-1) and the SMRT (silencing mediator for retinoid and thyroid receptor), a process that involves the alteration of histone deacetylases, such as HDAC1 or HDAC3 (Harvey & Williams 2002). Numerous corepressors interact with THRs suggesting a high level of complexity in the basal suppression process in the absence of THs.

The presence of ligand promotes a conformational change in THRB2 that results in the dissociation of corepressors (CoR) and recruitment of coactivators (CoA) such as the nuclear receptor coactivator 1 (NCoA-1 or SRC-1) establishing a new complex that leads to histone acetylation or methylation, which, in turn, changes the interaction between RNA polymerase and other transcriptional factors inducing the gene transcription (Ortiga-Carvalho *et al*. 2014, Mendoza & Hollenberg 2017). It is worth mentioning that T_3-mediated activation of rat *Gh* gene expression presents a synergism between transcriptional factor Pit-1 (POU domain, class 1, transcription factor 1) and T_3,

Journal of Molecular Endocrinology

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

increasing the *Gh* mRNA content (Schaufele *et al*. 1992, Sinha & Yen 2000, Yen 2001).

The regulation of GH synthesis and secretion mediated by T_3 differs in non-mammalian species. T_3 increases GH synthesis and secretion in fish, but decreases *Gh* mRNA expression in birds and GH secretion in chickens (Melamed *et al*. 1998). These findings could be related to a differential expression of coactivators and repressors in different species, which affects the response to TH, explaining the diversity of T_3 effects on GH regulation. Currently, the literature and knowledge are relatively poor for reptile and amphibian species.

Negative transcriptional regulation of *Cga* and *Tshb* genes by T_3

The involvement of THRA and THRB isoforms in the control of hypothalamus–pituitary–thyroid (HPT) axis has been evaluated in patients with natural mutations. Clinical observations have shown that in patients with a mutation in the THRA, the HPT axis is minimally or not impaired, while the opposite is observed in patients with mutation of THRB isoform (Ortiga-Carvalho *et al*. 2014).

These clinical observations are supported by studies performed in different models of *Thrb1* and *Thrb2* knockout mice. The lack of all THRB isoforms (*Thrb*$^{-/-}$) led to resistance to thyroid hormone (RTH-b) characterized by the enlargement of the thyroid gland (goiter), increased size and number of follicles, elevated T_4 and T_3 concentrations, normal or elevated serum TSH concentration and increased *Tshb* and *Cga* mRNA expressions due to defects in thyrotrophic response to THs (Forrest *et al*. 1996, O'Shea & Williams 2002, Mendoza & Hollenberg 2017). The generation of THRB2 knockout (*Thrb2*$^{-/-}$) mice provided evidence that this isoform plays a major role in the negative feedback exerted by T_3 in thyrotrophs and in the stimulation of *Gh* mRNA synthesis in somatotrophs (Abel *et al*. 1999, 2003).

The absence of both *Thra1* and *Thra2* in mice led to the development of an impaired thyroid gland and to the reduction of thyroxine (T_4) synthesis (O'Shea & Williams 2002), while the overexpression of THRA1 results in low levels of T_3 and T_4 and normal levels of TSH (Mendoza & Hollenberg 2017). Mice with double knockout for *Thra* and *Thrb* (*Thra*$^{-/-}$ *b*$^{-/-}$) present higher T_4, T_3 and TSH serum concentrations than *Thrb*$^{-/-}$ mice (Weiss *et al*. 1997, Abel *et al*. 1999, Chiamolera *et al*. 2012). In TαT1 cells, the THRB is essential for T_3 inhibition of *Tshb* mRNA expression, while the THRA plays an important role only in the absence of THRB (Chiamolera *et al*. 2012). In humans, some RTH patients with mutations in THRA presented normal or low TSH levels and reduced T_4/T_3 ratio. All these data together suggest that the THRA isoform plays an important role in the negative feedback mechanism and that THRA and THRB are required for the adequate regulation of the HPT axis (Mendoza & Hollenberg 2017).

Although the precise sequence of negative TREs (nTRE) has not been described yet (Ortiga-Carvalho *et al*. 2016), previous studies demonstrated that they appear to be near the TSS of *Cga/CGA* and *Tshb/TSHB* genes of rats, mice and humans (Carr *et al*. 1987, Shupnik 2000). However, recent studies have shown that the negative regulation of human *TSHB* gene exerted by T_3 is independent of nTRE (Matsushita *et al*. 2007, Matsunaga *et al*. 2015).

In the pituitary, the glycoprotein alpha subunit (*CGA*) is expressed in thyrotrophs and gonadotrophs and composes the mature molecule of TSH, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) together with the beta-subunit, which, in turn, guarantees hormonal specificity (Pierce & Parsons 1981).

Regarding the transcriptional effects of triiodothyronine on the *Cga* expression, it is known that in the rat and mouse genomes, there are several hexameric TRE half-site motif regions in the *Cga* gene, supporting the hypothesis that there is a suppression of *Cga* transcription by T_3 (Burnside *et al*. 1989). Studies in the human choriocarcinoma cell line JEG-3, transfected with THR, have shown that T_3 treatment markedly suppresses the *CGA* expression. The presence of a binding site for THR located between −22 and −7 base pairs of TSS of *CGA* gene immediately downstream from the TATA box suggests a possible interference of THs/TATA box with transcriptional factors reducing the synthesis of *CGA* mRNA (Chatterjee *et al*. 1989).

Considering that the *Cga* subunit is negatively regulated by THs (Shupnik & Ridgway 1987, Bargi-Souza *et al*. 2015), it might be expected that during hypothyroidism and hyperthyroidism, significant alterations in gonadotrophin synthesis and secretion would occur since *CGA* is essential for the composition of FSH and LH and that binding between alpha and beta subunits is important for hormonal maturation, biological activity and the recognition of these glycoproteins by specific receptors on gonads (Pierce & Parsons 1981, Bargi-Souza *et al*. 2015). Our recent studies indicate that alterations in the HPT axis strongly affect the hypothalamus–pituitary–gonad axis in rats, which might explain some reproductive disorders observed under hypothyroid and hyperthyroid conditions (Romano *et al*. 2013).

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

Despite the efforts, the precise mechanism implicated on the molecular basis of T_3 negative regulation of *Tshb* and *Cga* remains unknown. Four models were proposed for the negative regulation triggered by T_3 involving the nTREs as follows: (a) the position of nTRE, which prevents the formation of RNA polymerase II complex and consequently the initiation of transcription; (b) the interaction of the THR–TH complex with co-coactivators altering the conformation of the DNA molecule and thus preventing the transcription process; (c) the interaction of THR–TH with transcription inhibitors and (d) the presence of an nTRE inside an enhancer sequence, thus preventing transcription (Chin *et al*. 1993). Moreover, the mutation in the activation domain of THRB abolishing CoA recruitment but preserving normal T_3 binding and CoR interactions has shown that this portion is required for positive and, paradoxically, for negative regulation by TH, increasing the complexity of TH gene expression regulation (Ortiga-Carvalho *et al*. 2005). It is noteworthy to mention that transcriptional alterations occur concomitantly with alterations in GH and TSH secretion in rats, which implies that different mechanisms are triggered for the proper synthesis/secretion regulation of these hormones (Zoeller *et al*. 2007).

The regulation of HPT in non-mammals appears to be very similar to that in mammals. In teleost and amphibians, the activity of HPT is important for the normal development and metamorphosis of the animals and their larvae are sensitive to slight disruption of HPT function (Carr & Patino 2011). In the few fish and amphibians species studied, both T_4 and T_3 decrease TSH secretion by reducing *Tshb* gene transcription (Blanton & Specker 2007). In birds, THs present a shorter half-life than that in mammals but the negative feedback triggered by THs at the pituitary level is similar to that observed in mammals (McNabb 2007).

Non-genomic actions of THs

The non-genomic or posttranscriptional actions of THs are not dependent on the nuclear receptor interaction with TRE, even though the signaling resulting from these mechanisms could lead to the regulation of gene expression (Davis *et al*. 2013, 2016). These actions are rapidly triggered even in the presence of transcription inhibitors (Yusta *et al*. 1998, Lorenzo *et al*. 2002) by TH interactions with THRs coupled to specific enzymes in the cytosol or to other binding sites located on the plasma membrane and organelles such as mitochondria and endoplasmic reticulum (Moeller *et al*. 2006, Davis *et al*. 2008, 2009, Goulart-Silva *et al*. 2011).

There are a substantial number of studies connecting non-genomic actions of THs with complex physiological processes like the traffic of intracellular protein, the basal activity of transporter proteins, the regulation of the half-life of specific mRNAs (Davis *et al*. 2005, Hammes & Davis 2015), the cellular metabolism (Moeller *et al*. 2006), the brain development (Anton *et al*. 1999, Leonard 2008, Cao *et al*. 2009), the cardiac growth (Kenessey & Ojamaa 2005), angiogenesis (Bergh *et al*. 2005) and the osteoblast proliferation (Kalyanaraman *et al*. 2014).

In 2005, a binding site for THs was identified in the extracellular domain of alphaVbeta3 integrin present on the plasma membrane, which has a crucial role in pro-angiogenic actions triggered by THs (Bergh *et al*. 2005, Cody *et al*. 2007, Lin *et al*. 2011). This integrin has two binding sites, S1 and S2, for THs with different affinities for T_4 and T_3. The S1 site only recognizes T_3 and, in the presence of the ligand, activates the Src and PI3K pathways, whereas the S2 site recognizes both T_4 and T_3 with higher affinity for T_4 and activates the ERK1/2 pathway (Davis *et al*. 2011). Parts of TH actions elicited via alphaVbeta3 integrin are blocked by RGD (arginine-glycine-aspartate) peptide which strongly suggests that this TH binding site is located at or near the RGD recognition site on the alphaVbeta3 integrin (Davis *et al*. 2005, Cody *et al*. 2007). It was shown that the presence of RGD peptide prevented the trafficking of THRs from the cytosol to the nucleus induced by T_3 treatment (Cheng *et al*. 2010), pointing out a crucial participation of the alphaVbeta3 integrin in gene transcription regulation.

More recently, a THRA of 30 kDa (p30 THRA) was evinced in osteoblasts associated with plasma membrane domains rich in caveolin. T_3 binding to this receptor leads to an increase in intracellular Ca^{++} concentration and subsequent activation of NO-cGMP-PKGII followed by MEK-ERK and PI3K-Akt signaling cascades resulting in increased proliferation and survival of osteoblasts (Kalyanaraman *et al*. 2014). Another non-genomic mechanism of TH actions involves their interaction with nuclear receptors coupled to the regulatory p85 subunit of phosphoinositide 3-kinase (PI3K) in the cytoplasm regulating important proteins associated with glucose uptake, protein synthesis and proteolysis inhibition (Saji & Ringel 2010, Kimura *et al*. 2014). Truncated isoforms of THRs present in the cytoplasm also mediate non-genomic actions of T_4 and reverse T_3 (rT_3) (Cheng *et al*. 2010). The interaction of THRΔA1 with T_4 or rT_3 is associated

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

with the rearrangement of actin cytoskeleton and with the release of laminin, a matrix protein, by astrocytes (Farwell *et al*. 1995). The laminin and the rearrangement of the actin cytoskeleton are related to the induction of neuronal migration and growth of neurites, respectively (Farwell *et al*. 1995).

Thus, the identification of binding sites of THs at the plasma membrane and cytosol indicates a complex mechanism triggered by THs to establish their biological effects and the elucidation of TH non-genomic actions raises the possibility of therapeutic intervention through pharmacological agent actions in these binding sites.

Non-genomic regulation of GH and TSH synthesis and secretion

Posttranscriptional effects of T_3 on *Gh* synthesis

In the beginning of 1990s, few studies addressed the non-genomic actions of THs and they were restricted to T_4 and rT_3. These studies pointed out the rapid modulation of type 2 deiodinase (DIO2) activity triggered by T_4 and rT_3 in astrocytes, but not by T_3, suggesting that genomic actions were triggered by T_3, while T_4 and other metabolites were responsible for the non-genomic actions (Leonard 2008).

However, our group showed that a single T_3 injection in thyroidectomized (Tx) rats promoted an increase in *Gh* mRNA content in 15 min compared to hypothyroid animals (Volpato & Nunes 1994), and in the molecular weight of *Gh* mRNA characterized by its slow migration on the agarose gel 30 min thereafter. After removing the poly(A) tail, *Gh* mRNA from Tx rats treated with T_3 migrated on the gel to the same height of *Gh* mRNA from untreated Tx rats. In fact, sequencing analyses of *Gh* mRNA from T_3-treated Tx rats revealed an increased number of adenine residues in its poly(A) tail, evincing a posttranscriptional action of T_3 in this step of GH processing (*unpublished data*).

It is worth to highlight that *Gh* mRNA from Tx rats presents longer poly(A) tail making this transcript more stable than that of euthyroid animals (Jones *et al*. 1990, Murphy *et al*. 1992). It appears that in the absence of TH, mechanisms are triggered to ensure minimal GH synthesis since the transcriptional rate of *Gh* gene is reduced under the hypothyroidism condition. Furthermore, it has also been shown that suppression of GH synthesis and secretion induced by IGF-I were abolished in hypothyroidism (Melmed & Yamashita 1986), which reinforces that other mechanisms operate to regulate the GH expression in Tx animals.

In our study, T_3 further induced a rapid increase in *Gh* mRNA poly(A) tail length, ensuring more stability for *Gh* transcripts (*unpublished data*), and GH labeling at the perinuclear region of somatotrophs of Tx rats suggesting that GH synthesis was stimulated by T_3 (Silva *et al*. 2006). Our group has proceeded with the investigations on the molecular mechanisms involved in the rapid actions of T_3 on *Gh* mRNA stability and we have also studied the involvement of the cytoskeleton in T_3 non-genomic actions, as discussed later.

Rapid and negative posttranscriptional regulation of *Cga* and *Tshb*

Considering the positive effects of T_3 on the posttranscriptional steps of *Gh* synthesis, our group has investigated whether T_3 could trigger rapid effects on *Tshb* mRNA content and poly(A) tail length (Goulart-Silva *et al*. 2011, Bargi-Souza *et al*. 2013).

Using Tx rats, we observed that in hypothyroidism, the poly(A) tail length of *Tshb* mRNA is increased, followed by a significant rise in the translational rate of *Tshb*, besides the expected increase in the transcriptional process (Bargi-Souza *et al*. 2013). Thirty minutes after treatment with T_3, the poly(A) tail length of *Tshb* mRNA of Tx rats was reduced to values similar to those observed in the poly(A) tail length of euthyroid rats and was positively correlated with a marked reduction in the content of *Tshb* in polysome fractions indicating that T_3 rapidly reduced mRNA translation and TSH protein synthesis (Goulart-Silva *et al*. 2011, Bargi-Souza *et al*. 2013).

Previous studies regarding T_3 effects on the *Tshb* mRNA poly(A) tail length have already shown a decrease in its polyadenylation degree in hypothyroid pituitary cell cultured with T_3 for 8 h and this reduction was more pronounced after 24 h (Krane *et al*. 1991, Staton & Leedman 1998). Euthyroid animals treated for 4 h with higher doses of T_3 also presented a reduction of *Tshb* mRNA poly(A) tail length (Krane *et al*. 1991).

In hypothyroidism, we have also observed an increase in the *Cga* mRNA content and in its association with ribosomes, indicating a rise in the translational rate of CGA. Thirty minutes after T_3 administration, the content of *Cga* mRNA seen in the polysome fraction was reduced suggesting that the translation of both alpha and beta subunits is posttranscriptionally modulated by T_3 in hypothyroid animals (Bargi-Souza *et al*. 2015).

In contrast to the results observed for *Tshb*, the poly(A) tail length of *Cga* was not altered under different thyroidal conditions, which led us to hypothesize that

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

T_3 acts through different pathways modulating the elongation of *Tshb* poly(A) tail and the translation of both transcripts. Thus, the regulation of poly(A) tail length by T_3 is specific for *Tshb* and it could be speculated that such specificity would reduce the impact on *Cga* synthesis since this subunit is also important for LH and FSH hormones.

Cytoskeleton as a target of non-genomic actions of T_3: consequences to GH and TSH secretion

The cytoskeleton is directly involved with the mechanical support system of a cell, its shape and the release of molecules stored in secretory granules by exocytosis (Apodaca 2002), being a target of hormones such as T_3 (Banovac & Koren 2000).

The first experiments showing cytoskeletal rearrangement induced by THs were performed in cultured astrocytes. The treatment with T_4 or rT_3 promoted a cytoskeleton reorganization characterized by an increase in polymerized actin content and led to reduction of the DIO2 enzymatic activity in astrocytes (Siegrist-Kaiser *et al*. 1990).

Considering that DIO2 is responsible for the intracellular generation of T_3 from T_4 and that T_3 is an important *Gh* and *Tshb* gene expression modulator (Ortiga-Carvalho *et al*. 2016), our group attempted to investigate the repercussions of hypothyroidism and T_3 effects in the cytoskeleton of pituitary cells and the consequences to GH and TSH secretion (Silva *et al*. 2006, Bargi-Souza *et al*. 2013).

We have observed a marked disarrangement of the cytoskeleton characterized by a deep reduction of actin and tubulin filament staining in anterior pituitary cells of hypothyroid rats and an evident increase in blood vessel diameter (Silva *et al*. 2006, Bargi-Souza *et al*. 2013). Actin and tubulin polymerization leads to the assembly of microfilaments and microtubules, respectively. The latter is involved in the transport of vesicles and granules within the cell and their release to the extracellular milieu (Fokin *et al*. 2014). Thus, changes in the arrangement of the cytoskeleton in pituitary could be associated with alterations in pituitary hormone secretion.

Studies carried out by different research groups have shown a link between the animal thyroid condition and the microtubule polymerization in the central nervous system (Chaudhury *et al*. 1985, Ravindra & Grosvenor 1990, Lorenzo *et al*. 2002, Gutierrez 2012), as well as a reduction in the amount of actin filaments in astrocyte cytoskeleton (Siegrist-Kaiser *et al*. 1990) in the absence of T_4. The results obtained in the anterior pituitary of hypothyroid rats showed a disarrangement of actin and tubulin filaments, which reinforces the importance of THs in the cytoskeleton integrity of cells.

The repercussions on pituitary hormone secretion were then evaluated by immunohistochemistry. The results showed a decrease in actin filament content (F-actin) in GH immunoreactive cells of hypothyroid rats in which GH granules were predominantly observed at the cell periphery. Thus, cytoskeleton disarrangement in somatotrophs could contribute to the well-established reduction of GH secretion observed in hypothyroidism (Silva *et al*. 2006). However, 30 min after T_3 administration of hypothyroid rats, an increase was observed in the F-actin content mainly near the plasma membrane, characterizing a rapid cytoskeleton rearrangement, even though the total actin protein remained unchanged, which led us to suppose that T_3 only affected actin rearrangement (Silva *et al*. 2006). In parallel, there was a remarkable increase in GH labeling at the perinuclear region and a decrease in GH staining at the cell periphery, strongly indicating that GH was released, which was confirmed by a rapid increase in liver *Igf1* mRNA expression in these animals (Silva *et al*. 2010).

Furthermore, a possible relationship between T_3 effects on the cytoskeleton and the repercussions in GH synthesis cannot be discarded. In fact, the cytoskeleton enables the polysome to anchor to it. Polysomes correspond to the functional units of the translational machinery and they arrest the mRNAs to initiate the translation mechanism. Thus, anchoring polysomes to the cytoskeleton also means attaching and distributing mRNAs to specific cell regions, where they will be translated (Campbell & Hesketh 1996, Veyrune *et al*. 1996, Czaplinski & Singer 2006, Coulon *et al*. 2013). In this sense, the cytoskeleton may be involved in the increased GH synthesis observed after hypothyroid rats had been acutely treated with T_3 and in the marked GH staining at the somatotroph perinuclear region. In the hypothyroid state, immunostaining for GH was almost absent near the nucleus due to the reduced transcriptional rate of the *Gh* gene (Crew & Spindler 1986, Larsen *et al*. 1986, Lavin *et al*. 1988, Samuels *et al*. 1988).

In addition to the changes observed for the actin cytoskeleton, T_3 also promoted a microtubule rearrangement in pituitary as well as an increase in tubulin content and a rapid reduction of the pituitary blood vessel diameter, which, in turn, could alter the dynamics of pituitary blood flow (Bargi-Souza *et al*. 2013). Considering

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

the role of microtubules in hormone secretion, our findings strengthen the important role of THs in the secretion of pituitary hormones consequently affecting metabolism, growth and organic system functions, considering the central position of pituitary in the control of other endocrine glands' activity.

Different from the somatotrophs, no colocalization of TSH and F-actin or microtubule labeling was observed. In fact, despite F-actin and microtubule disarrangement in the pituitary in hypothyroidism, TSH secretion is highly increased. Indeed, the immunohistochemistry and electron microscopy analysis of hypothyroid rat pituitaries showed strong TSH staining near and inside the pituitary blood vessels which is in accordance with the high serum TSH concentration detected in the hypothyroid animals (Bargi-Souza *et al*. 2013).

In contrast, T_3 promptly reduced TSH labeling inside blood vessels and promoted a rapid redistribution of TSH granules in thyrotrophs, increasing their abundance in the whole cytoplasm and reducing their presence in regions close to the plasma membrane. These findings indicate a rapid T_3 effect inhibiting TSH secretion (Bargi-Souza *et al*. 2013).

Recently, studies from our group have shown that the CGA subunit content and secretion are also regulated by T_3 (Bargi-Souza *et al*. 2015). In hypothyroidism, the CGA amount in the pituitary is decreased and, considering only thyrotrophs, this reduction was even more evident. Moreover, T_3 acute administration in physiological and supraphysiological doses promoted a rapid increase in pituitary CGA intracellular content which is highly suggestive of a blockage in CGA secretion, since the *Cga* mRNA content in polysomal fractions was rapidly reduced, suggesting a reduction in CGA protein synthesis (Bargi-Souza *et al*. 2015).

Indeed, studies carried out by our group in anterior pituitary primary culture showed a reduction of CGA content in the cell culture medium after acute T_3 treatment, reinforcing the inhibitory T_3 action on CGA secretion (Bargi-Souza *et al*. 2015). These data together with the observation that acute T_3 administration reduces the *Cga* mRNA translation rate strengthen the evidence that T_3 acts non-genomically reducing the synthesis and secretion of CGA, which might also impact LH and FSH synthesis and secretion, as suggested (Krassas & Pontikides 2004, Romano *et al*. 2013). Hence, the increase in pituitary TSHB and CGA contents acutely triggered by T_3 strongly indicates a rapid inhibition of TSH secretion. In this sense, studies carried out in GH4C1 pituitary cells shows that T_3 increases the activity of the Potassium Voltage-Gated Channel (KCNH2) by non-genomic mechanisms that involve the PI3 kinase pathway activation, antagonizing the thyrotropin-releasing hormone effects on PRL secretion (Storey *et al*. 2006).

Interaction between cytoskeleton and mRNAs

Transcripts anchored to the cytoskeleton may be positioned in specific regions of the cell to be translated where the new protein may exert its function. In fact, the actin transcript has been shown to be attached to cytoskeleton and addressed to cell periphery to be translated to monomeric actin (G-actin), which could be polymerized to F-actin (Bassell *et al*. 1998, Eom *et al*. 2003).

The mRNA interaction with the cytoskeleton may alter transcript stability, which, in turn, affects protein synthesis efficiency (Kosmidou *et al*. 2007, Challa & Stefanovic 2011). T_3 increases the *Gh* transcript stability by adding adenine residues to the 3′end of its poly(A) tail and the F-actin content; both alterations could contribute to increased GH protein synthesis. On the other hand, there are reports showing that some mRNAs become more stable after cytoskeleton disarrangement as it occurs with proinflammatory cytokine mRNAs, expressed on airway epithelial cells, whose translation rate is increased under this condition (van den Berg *et al*. 2006). Based on these findings, we can infer that actin and microtubule disorganizations, both observed in the hypothyroid rat pituitary, could differentially regulate GH and TSH synthesis and secretion.

As mentioned earlier, mRNAs are anchored to the cytoskeleton through interaction with specific proteins. EF1 alpha is one of these proteins, and it is worth mentioning that it has an important role in the protein synthesis elongation phase by transporting the aminoacyl-tRNA to the ribosome via a GTP-dependent mechanism (Greganova *et al*. 2011).

As pointed out, in hypothyroid states, the actin cytoskeleton of pituitary cells is disarranged. Besides that, we observed a reduction of both EF1 alpha and *Gh* mRNA in the F-actin fraction of hypothyroid rat pituitaries (Silva *et al*. 2010), which indicates a reduction of GH synthesis. In fact, the polysome profile analysis of hypothyroid rat pituitary showed a decrease in *Gh* mRNA amount in polysomes. Even though there is a low rate of *Gh* gene transcription in hypothyroid states, we cannot exclude the contribution of posttranscriptional mechanisms

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

to this event since EF1 alpha and *Gh* mRNA binding to F-actin cytoskeleton was shown to be reduced (Silva *et al.* 2010). In fact, T_3 acutely administered to hypothyroid rats rapidly increased the EF1 alpha and *Gh* mRNA amount in the F-actin cytoskeleton, the *Gh* mRNA content in the polysome fraction and promoted cytoskeleton rearrangement (Silva *et al.* 2006, 2010). These events may favor the increase in GH synthesis, and could explain the increase in GH labeling in the perinuclear region of somatotrophs, both observed in pituitary of hypothyroid rats acutely treated with T_3.

Final considerations

In summary, in parallel to the transcriptional regulation of *Gh, Tshb* and *Cga* genes triggered by triiodothyronine which was for a long period considered the unique mechanism for regulation of GH and TSH synthesis and consequently secretion, T_3 acts non-genomically on thyrotrophs and somatotrophs regulating the polyadenylation of *Gh* and *Tshb* mRNAs, and GH and TSH translation/synthesis and secretion. These findings significantly increase the comprehension on novel aspects of the negative feedback mechanism exerted by T_3 on thyrotrophs, as well as add new elements to the understanding of T_3 induction of *Gh* gene expression and secretion which are processes that are considered to be mainly regulated by growth hormone–releasing hormone. These non-genomic actions of T_3 on GH and TSH synthesis/secretion may have an important physiological relevance for the optimal control of the rate of GH and TSH basal secretion, minute by minute. Figure 1 summarizes the alterations observed in GH and TSH expressions under hypothyroid conditions and the non-genomic actions triggered by T_3 modulating GH and TSH synthesis/secretion.

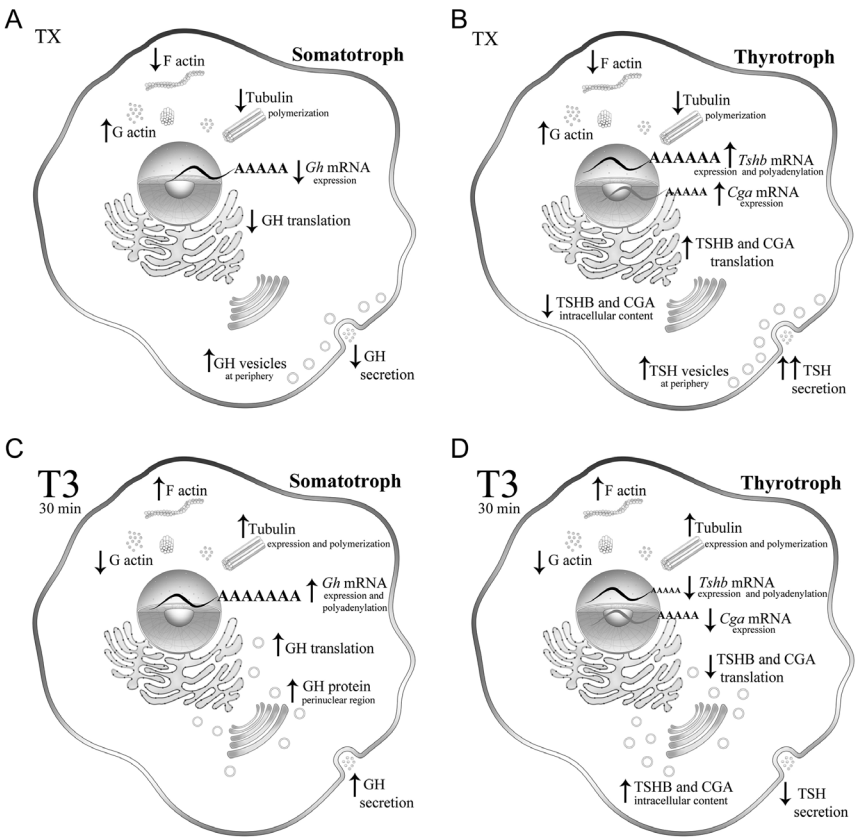

Figure 1
Illustration of GH (A) and TSH (B) synthesis and secretion in hypothyroid states and summary of the non-genomic actions of triiodothyronine (T_3) on somatotrophs (C) and thyrotrophs (D). The actin and tubulin cytoskeleton of the whole pituitary is disarranged in thyroidectomized (Tx) rats (A and B) and are rapidly rearranged by T_3, which increases F-actin and alpha tubulin expression and microtubule polymerization (C and D). In hypothyroidism, *Gh* mRNA content and translation rates are reduced in somatotrophs. GH content is decreased and found in scarce vesicles close to the plasma membrane (A). In thyrotrophs (B), *Tshb* and *Cga* mRNA contents are increased, as well as *Tshb* poly(A) tail length. *Tshb* and *Cga* mRNA translation rates are increased under hypothyroid conditions, while intracellular TSHB and CGA protein contents are reduced due to the elevated TSH secretion rate evidenced by the increased serum TSH concentration and the increased TSHB labeling at the periphery of thyrotrophs and inside the blood vessels. T_3 acutely (30 min) increases *Gh* mRNA content and polyadenylation raising the efficiency of *Gh* translation. The enhanced labeling of GH in the cytoplasm mainly in the perinuclear region and the increased hepatic *Igf1* mRNA content are strong indicators of increased GH synthesis (C) and secretion. In thyrotrophs (D), the contents of both *Tshb* and *Cga* mRNAs are rapidly reduced by T_3, as well as the *Tshb* mRNA poly(A) tail length. The translation rates of both *Tshb* and *Cga* mRNA are promptly reduced and TSHB and CGA protein contents are increased in response to a rapid inhibition of TSH secretion triggered by T_3 through non-genomic mechanisms. Thus, both genomic and non-genomic actions of T_3 positively and negatively modulate the synthesis and secretion of GH and TSH, respectively.

Journal of Molecular Endocrinology

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

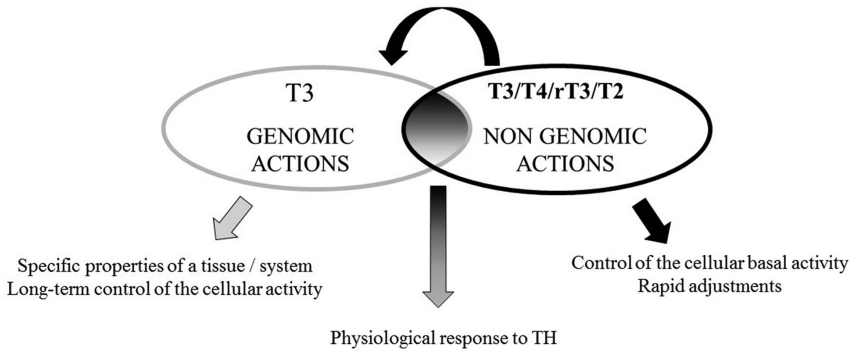

Figure 2
Physiological response to thyroid hormone (TH) actions. The non-genomic actions of TH are triggered by triiodothyronine (T_3), thyroxine (T_4), rT_3 and diiodothyronine (T_2). These actions have shown to affect the expression and activity of many target genes and proteins inducing rapid (few minutes) adjustments in cellular basal activity by activating different intracellular signaling pathways. In parallel to non-genomic actions, TH genomic actions, which are mainly triggered by T_3, promote long-term control of the cellular activity increasing or reducing the transcription rate of T_3 target genes. Cellular responses to genomic actions are late compared to non-genomic actions since they depend on the changes in the transcriptional machinery activity as well as on specific transcriptional factors. The final cellular physiological response to TH is a result of a complex mechanism involving both genomic and non-genomic actions. This figure also highlights the overlap of genomic and non-genomic actions of T_3.

It is worth mentioning that the non-genomic actions elicited by T_3 precede and are in line with its transcriptional actions on both genes since they lead to a positive and negative regulation of GH and TSH, respectively. Other genes/proteins that are targeted by T_3 also present an overlap of non-genomic and genomic mechanisms. The T_4–αVβ3 interaction promotes αVβ3 internalization (Lin *et al*. 2013) and the αV monomer forms a complex with MAPK-p300 inside the cell that binds to the *Thrb1* promoter increasing its transcriptional rate (Hammes & Davis 2015). The increased amount of THRB1, in turn, increases the response to genomic actions of T_3 mediated by this isoform (Hammes & Davis 2015).

Thus, non-genomic actions are responsible for rapid adjustments of cellular basal activity, whereas in parallel, genomic actions promote long-term control of cellular activity, being responsible for the specific properties of a tissue/system (Hammes & Davis 2015). In this sense, the physiological response to TH is the result of both genomic and non-genomic actions, as illustrated in Figure 2.

The understanding of non-genomic molecular pathways activated by T_3 would provide new insights into the physiological role of TH at a cellular level enabling the development of new analogs that at certain degree could compensate the lack of proper TH regulation observed in THR mutation disorders or specific antagonists to avoid tumor cell proliferation and angiogenesis, triggered by non-genomic actions of T_4 through interaction with αVβ3 integrin at the plasma membrane (Hammes & Davis 2015).

It is noteworthy to mention that the literature on the non-genomic actions of TH is recent and there is still much to be investigated. Moreover, some actions are observed in a period of time of only a few seconds to minutes, which represents a limitation for clinical studies. The development of tools to explore the non-genomic actions in endocrine and metabolic disorders is also required. The use of microarray, RNA-Seq, proteomics and functional assays would also be useful to characterize/describe new non-genomic actions triggered by THs. Due to all these reasons, clinical applications of non-genomic actions are still unclear.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as hindering the impartiality of this review.

Funding
This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP: 2006/61713-0 and 2013/05629-4) to M T N. M T N is the recipient of a Conselho Nacional de Pesquisa e Desenvolvimento (CNPq) fellowship and P B-S is the recipient of a FAPESP fellowship (2009/17813-9, 2012/06643-8 and 2014/25888-7).

Acknowledgements
The authors would like to thank Isidro Moisés Cervantes Galicia for the cell illustration model.

References

Abel ED, Kaulbach HC, Campos-Barros A, Ahima RS, Boers ME, Hashimoto K, Forrest D & Wondisford FE 1999 Novel insight from transgenic mice into thyroid hormone resistance and the regulation

Published by Bioscientifica Ltd.

of thyrotropin. *Journal of Clinical Investigation* **103** 271–279. (doi:10.1172/JCI5205)

Abel ED, Moura EG, Ahima RS, Campos-Barros A, Pazos-Moura CC, Boers ME, Kaulbach HC, Forrest D & Wondisford FE 2003 Dominant inhibition of thyroid hormone action selectively in the pituitary of thyroid hormone receptor-beta null mice abolishes the regulation of thyrotropin by thyroid hormone. *Molecular Endocrinology* **17** 1767–1776. (doi:10.1210/me.2003-0109)

Anton ES, Kreidberg JA & Rakic P 1999 Distinct functions of alpha3 and alpha(v) integrin receptors in neuronal migration and laminar organization of the cerebral cortex. *Neuron* **22** 277–289. (doi:10.1016/S0896-6273(00)81089-2)

Apodaca G 2002 Modulation of membrane traffic by mechanical stimuli. *American Journal of Physiology: Renal Physiology* **282** F179–F190.

Banovac K & Koren E 2000 Triiodothyronine stimulates the release of membrane-bound alkaline phosphatase in osteoblastic cells. *Calcified Tissue International* **67** 460–465. (doi:10.1007/s002230001171)

Bargi-Souza P, Romano RM, Salgado Rde M, Goulart-Silva F, Brunetto EL, Tenorio Zorn TM & Nunes MT 2013 Triiodothyronine rapidly alters the TSH content and the secretory granules distribution in male rat thyrotrophs by a cytoskeleton rearrangement-independent mechanism. *Endocrinology* **154** 4908–4918. (doi:10.1210/en.2013-1508)

Bargi-Souza P, Romano RM, Goulart-Silva F, Brunetto EL & Nunes MT 2015 T3 rapidly regulates several steps of alpha subunit glycoprotein (CGA) synthesis and secretion in the pituitary of male rats: potential repercussions on TSH, FSH and LH secretion. *Molecular and Cellular Endocrinology* **409** 73–81. (doi:10.1016/j.mce.2015.04.002)

Barra GB, Velasco LF, Pessanha RP, Campos AM, Moura FN, Dias SM, Polikarpov I, Ribeiro RC, Simeoni LA & Neves FA 2004 Molecular mechanism of thyroid hormone action. *Arquivos Brasileiros de Endocrinologia and Metabologia* **48** 25–39. (doi:10.1590/S0004-27302004000100005)

Bassell GJ, Zhang H, Byrd AL, Femino AM, Singer RH, Taneja KL, Lifshitz LM, Herman IM & Kosik KS 1998 Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. *Journal of Neuroscience* **18** 251–265.

Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S & Davis PJ 2005 Integrin alphaVbeta3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* **146** 2864–2871. (doi:10.1210/en.2005-0102)

van den Berg A, Freitas J, Keles F, Snoek M, van Marle J, Jansen HM & Lutter R 2006 Cytoskeletal architecture differentially controls post-transcriptional processing of IL-6 and IL-8 mRNA in airway epithelial-like cells. *Experimental Cell Research* **312** 1496–1506. (doi:10.1016/j.yexcr.2006.01.010)

Blanton ML & Specker JL 2007 The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. *Critical Reviews in Toxicology* **37** 97–115. (doi:10.1080/10408440601123529)

Burnside J, Darling DS, Carr FE & Chin WW 1989 Thyroid hormone regulation of the rat glycoprotein hormone alpha-subunit gene promoter activity. *Journal of Biological Chemistry* **264** 6886–6891.

Campbell GP & Hesketh JE 1996 Distribution of glutathione peroxidase mRNAs between free and cytoskeletal-bound polysomes in H4 hepatoma cells. *Biochemical Society Transactions* **24** 189S. (doi:10.1042/bst024189s)

Cao X, Kambe F, Yamauchi M & Seo H 2009 Thyroid-hormone-dependent activation of the phosphoinositide 3-kinase/Akt cascade requires Src and enhances neuronal survival. *Biochemical Journal* **424** 201–209. (doi:10.1042/BJ20090643)

Carr JA & Patino R 2011 The hypothalamus-pituitary-thyroid axis in teleosts and amphibians: endocrine disruption and its consequences to natural populations. *General and Comparative Endocrinology* **170** 299–312. (doi:10.1016/j.ygcen.2010.06.001)

Carr FE, Need LR & Chin WW 1987 Isolation and characterization of the rat thyrotropin beta-subunit gene. Differential regulation of two transcriptional start sites by thyroid hormone. *Journal of Biological Chemistry* **262** 981–987.

Challa AA & Stefanovic B 2011 A novel role of vimentin filaments: binding and stabilization of collagen mRNAs. *Molecular and Cellular Biology* **31** 3773–3789. (doi:10.1128/MCB.05263-11)

Chatterjee VK, Lee JK, Rentoumis A & Jameson JL 1989 Negative regulation of the thyroid-stimulating hormone alpha gene by thyroid hormone: receptor interaction adjacent to the TATA box. *PNAS* **86** 9114–9118. (doi:10.1073/pnas.86.23.9114)

Chaudhury S, Chatterjee D & Sarkar PK 1985 Induction of brain tubulin by triiodothyronine: dual effect of the hormone on the synthesis and turnover of the protein. *Brain Research* **339** 191–194. (doi:10.1016/0006-8993(85)90644-4)

Cheng SY, Leonard JL & Davis PJ 2010 Molecular aspects of thyroid hormone actions. *Endocrine Reviews* **31** 139–170. (doi:10.1210/er.2009-0007)

Chiamolera MI, Sidhaye AR, Matsumoto S, He Q, Hashimoto K, Ortiga-Carvalho TM & Wondisford FE 2012 Fundamentally distinct roles of thyroid hormone receptor isoforms in a thyrotroph cell line are due to differential DNA binding. *Molecular Endocrinology* **26** 926–939. (doi:10.1210/me.2011-1290)

Chin WW, Carr FE, Burnside J & Darling DS 1993 Thyroid hormone regulation of thyrotropin gene expression. *Recent Progress in Hormone Research* **48** 393–414.

Cody V, Davis PJ & Davis FB 2007 Molecular modeling of the thyroid hormone interactions with alpha v beta 3 integrin. *Steroids* **72** 165–170. (doi:10.1016/j.steroids.2006.11.008)

Coulon A, Chow CC, Singer RH & Larson DR 2013 Eukaryotic transcriptional dynamics: from single molecules to cell populations. *Nature Reviews Genetics* **14** 572–584. (doi:10.1038/nrg3484)

Crew MD & Spindler SR 1986 Thyroid hormone regulation of the transfected rat growth hormone promoter. *Journal of Biological Chemistry* **261** 5018–5022.

Czaplinski K & Singer RH 2006 Pathways for mRNA localization in the cytoplasm. *Trends in Biochemical Sciences* **31** 687–693. (doi:10.1016/j.tibs.2006.10.007)

Davis PJ, Davis FB & Cody V 2005 Membrane receptors mediating thyroid hormone action. *Trends in Endocrinology and Metabolism* **16** 429–435. (doi:10.1016/j.tem.2005.09.007)

Davis PJ, Leonard JL & Davis FB 2008 Mechanisms of nongenomic actions of thyroid hormone. *Frontiers in Neuroendocrinology* **29** 211–218. (doi:10.1016/j.yfrne.2007.09.003)

Davis PJ, Davis FB, Lin HY, Mousa SA, Zhou M & Luidens MK 2009 Translational implications of nongenomic actions of thyroid hormone initiated at its integrin receptor. *American Journal of Physiology: Endocrinology and Metabolism* **297** E1238–E1246. (doi:10.1152/ajpendo.00480.2009)

Davis PJ, Davis FB, Mousa SA, Luidens MK & Lin HY 2011 Membrane receptor for thyroid hormone: physiologic and pharmacologic implications. *Annual Review of Pharmacology and Toxicology* **51** 99–115. (doi:10.1146/annurev-pharmtox-010510-100512)

Davis PJ, Lin HY, Tang HY, Davis FB & Mousa SA 2013 Adjunctive input to the nuclear thyroid hormone receptor from the cell surface receptor for the hormone. *Thyroid* **23** 1503–1509. (doi:10.1089/thy.2013.0280)

Davis PJ, Goglia F & Leonard JL 2016 Nongenomic actions of thyroid hormone. *Nature Reviews Endocrinology* **12** 111–121. (doi:10.1038/nrendo.2015.205)

Eom T, Antar LN, Singer RH & Bassell GJ 2003 Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *Journal of Neuroscience* **23** 10433–10444.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

Farwell AP, Tranter MP & Leonard JL 1995 Thyroxine-dependent regulation of integrin-laminin interactions in astrocytes. *Endocrinology* **136** 3909–3915. (doi:10.1210/endo.136.9.7649099)

Flamant F, Cheng SY, Hollenberg AN, Moeller LC, Samarut J, Wondisford FE, Yen PM & Refetoff S 2017 Thyroid hormone signaling pathways. Time for a more precise nomenclature. *Endocrinology* **158** 2052–2057 (doi:10.1210/en.2017-00250)

Fokin AI, Brodsky IB, Burakov AV & Nadezhdina ES 2014 Interaction of early secretory pathway and Golgi membranes with microtubules and microtubule motors. *Biochemistry* **79** 879–893. (doi:10.1134/S0006297914090053)

Forrest D, Hanebuth E, Smeyne RJ, Everds N, Stewart CL, Wehner JM & Curran T 1996 Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. *EMBO Journal* **15** 3006–3015.

Franklyn JA, Wood DF, Balfour NJ & Sheppard MC 1988 Effect of triiodothyronine treatment on thyrotrophin beta- and alpha-messenger RNAs in the pituitary of the euthyroid rat. *Molecular and Cellular Endocrinology* **60** 1–5. (doi:10.1016/0303-7207(88)90113-X)

Goulart-Silva F, de Souza PB & Nunes MT 2011 T3 rapidly modulates TSHbeta mRNA stability and translational rate in the pituitary of hypothyroid rats. *Molecular and Cellular Endocrinology* **332** 277–282. (doi:10.1016/j.mce.2010.11.005)

Greganova E, Altmann M & Butikofer P 2011 Unique modifications of translation elongation factors. *FEBS Journal* **278** 2613–2624. (doi:10.1111/j.1742-4658.2011.08199.x)

Gutierrez LM 2012 New insights into the role of the cortical cytoskeleton in exocytosis from neuroendocrine cells. *International Review of Cell and Molecular Biology* **295** 109–137. (doi:10.1016/B978-0-12-394306-4.00009-5)

Hammes SR & Davis PJ 2015 Overlapping nongenomic and genomic actions of thyroid hormone and steroids. *Best Practice and Research Clinical Endocrinology and Metabolism* **29** 581–593. (doi:10.1016/j.beem.2015.04.001)

Harvey CB & Williams GR 2002 Mechanism of thyroid hormone action. *Thyroid* **12** 441–446. (doi:10.1089/105072502760143791)

Hervas F, Morreale de Escobar G & Escobar Del Rey F 1975 Rapid effects of single small doses of L-thyroxine and triiodo-L-thyronine on growth hormone, as studied in the rat by radioimmunoassay. *Endocrinology* **97** 91–101. (doi:10.1210/endo-97-1-91)

Jones PM, Burrin JM, Ghatei MA, O'Halloran DJ, Legon S & Bloom SR 1990 The influence of thyroid hormone status on the hypothalamo-hypophyseal growth hormone axis. *Endocrinology* **126** 1374–1379. (doi:10.1210/endo-126-3-1374)

Kalyanaraman H, Schwappacher R, Joshua J, Zhuang S, Scott BT, Klos M, Casteel DE, Frangos JA, Dillmann W, Boss GR, *et al*. 2014 Nongenomic thyroid hormone signaling occurs through a plasma membrane-localized receptor. *Science Signaling* **7** ra48. (doi:10.1126/scisignal.2004911)

Kaneshige M, Kaneshige K, Zhu X, Dace A, Garrett L, Carter TA, Kazlauskaite R, Pankratz DG, Wynshaw-Boris A, Refetoff S, *et al*. 2000 Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone. *PNAS* **97** 13209–13214. (doi:10.1073/pnas.230285997)

Kenessey A & Ojamaa K 2005 Ligand-mediated decrease of thyroid hormone receptor-alpha1 in cardiomyocytes by proteosome-dependent degradation and altered mRNA stability. *American Journal of Physiology: Heart and Circulatory Physiology* **288** H813–H821. (doi:110.1152/ajpheart.00804.2004)

Kimura K, Cheng XW, Inoue A, Hu L, Koike T & Kuzuya M 2014 beta-Hydroxy-beta-methylbutyrate facilitates PI3K/Akt-dependent mammalian target of rapamycin and FoxO1/3a phosphorylations and alleviates tumor necrosis factor alpha/interferon gamma-induced MuRF-1 expression in C2C12 cells. *Nutrition Research* **34** 368–374. (doi:10.1016/j.nutres.2014.02.003)

Koenig RJ, Brent GA, Warne RL, Larsen PR & Moore DD 1987 Thyroid hormone receptor binds to a site in the rat growth hormone promoter required for induction by thyroid hormone. *PNAS* **84** 5670–5674. (doi:10.1073/pnas.84.16.5670)

Kosmidou I, Moore JP, Weber M & Searles CD 2007 Statin treatment and 3′ polyadenylation of eNOS mRNA. *Arteriosclerosis, Thrombosis, and Vascular Biology* **27** 2642–2649. (doi:10.1161/ATVBAHA.107.154492)

Krane IM, Spindel ER & Chin WW 1991 Thyroid hormone decreases the stability and the poly(A) tract length of rat thyrotropin beta-subunit messenger RNA. *Molecular Endocrinology* **5** 469–475. (doi:10.1210/mend-5-4-469)

Krassas GE & Pontikides N 2004 Male reproductive function in relation with thyroid alterations. *Best Practice and Research Clinical Endocrinology and Metabolism* **18** 183–195. (doi:10.1016/j.beem.2004.03.003)

Larsen PR, Harney JW & Moore DD 1986 Sequences required for cell-type specific thyroid hormone regulation of rat growth hormone promoter activity. *Journal of Biological Chemistry* **261** 14373–14376.

Lavin TN, Baxter JD & Horita S 1988 The thyroid hormone receptor binds to multiple domains of the rat growth hormone 5'-flanking sequence. *Journal of Biological Chemistry* **263** 9418–9426.

Lazar MA 1993 Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocrine Reviews* **14** 184–193. (doi:10.1210/edrv-14-2-184)

Leonard JL 2008 Non-genomic actions of thyroid hormone in brain development. *Steroids* **73** 1008–1012. (doi:10.1016/j.steroids.2007.12.016)

Lin HY, Davis FB, Luidens MK, Mousa SA, Cao JH, Zhou M & Davis PJ 2011 Molecular basis for certain neuroprotective effects of thyroid hormone. *Frontiers in Molecular Neuroscience* **4** 29. (doi:10.3389/fnmol.2011.00029)

Lin HY, Su YF, Hsieh MT, Lin S, Meng R, London D, Lin C, Tang HY, Hwang J, Davis FB, *et al*. 2013 Nuclear monomeric integrin alphav in cancer cells is a coactivator regulated by thyroid hormone. *FASEB Journal* **27** 3209–3216. (doi:10.1096/fj.12-227132)

Lorenzo PI, Menard C, Miller FD & Bernal J 2002 Thyroid hormone-dependent regulation of Talpha1 alpha-tubulin during brain development. *Molecular and Cellular Neuroscience* **19** 333–343. (doi:10.1006/mcne.2001.1087)

Matsunaga H, Sasaki S, Suzuki S, Matsushita A, Nakamura H, Nakamura HM, Hirahara N, Kuroda G, Iwaki H, Ohba K, *et al*. 2015 Essential role of GATA2 in the negative regulation of type 2 deiodinase gene by liganded thyroid hormone receptor beta2 in thyrotroph. *PLoS ONE* **10** e0142400. (doi:10.1371/journal.pone.0142400)

Matsushita A, Sasaki S, Kashiwabara Y, Nagayama K, Ohba K, Iwaki H, Misawa H, Ishizuka K & Nakamura H 2007 Essential role of GATA2 in the negative regulation of thyrotropin beta gene by thyroid hormone and its receptors. *Molecular Endocrinology* **21** 865–884. (doi:10.1210/me.2006-0208)

McNabb FM 2007 The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. *Critical Reviews in Toxicology* **37** 163–193. (doi:10.1080/10408440601123552)

Melamed P, Rosenfeld H, Elizur A & Yaron Z 1998 Endocrine regulation of gonadotropin and growth hormone gene transcription in fish. *Comparative Biochemistry and Physiology: Part C, Pharmacology, Toxicology and Endocrinology* **119** 325–338. (doi:10.1016/S0305-0491(97)00357-X)

Melmed S & Yamashita S 1986 Insulin-like growth factor-I action on hypothyroid rat pituitary cells: suppression of triiodothyronine-induced growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* **118** 1483–1490. (doi:10.1210/endo-118-4-1483)

Mendoza A & Hollenberg AN 2017 New insights into thyroid hormone action. *Pharmacology and Therapeutics* **173** 135–145. (doi:10.1016/j.pharmthera.2017.02.012)

Moeller LC, Cao X, Dumitrescu AM, Seo H & Refetoff S 2006 Thyroid hormone mediated changes in gene expression can be initiated by

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.

cytosolic action of the thyroid hormone receptor beta through the phosphatidylinositol 3-kinase pathway. *Nuclear Receptor Signaling* **4** e020. (doi:10.1621/nrs.04020)

Murphy D, Pardy K, Seah V & Carter D 1992 Posttranscriptional regulation of rat growth hormone gene expression: increased message stability and nuclear polyadenylation accompany thyroid hormone depletion. *Molecular and Cellular Biology* **12** 2624–2632. (doi:10.1128/MCB.12.6.2624)

O'Shea PJ & Williams GR 2002 Insight into the physiological actions of thyroid hormone receptors from genetically modified mice. *Journal of Endocrinology* **175** 553–570.

Ortiga-Carvalho TM, Shibusawa N, Nikrodhanond A, Oliveira KJ, Machado DS, Liao XH, Cohen RN, Refetoff S & Wondisford FE 2005 Negative regulation by thyroid hormone receptor requires an intact coactivator-binding surface. *Journal of Clinical Investigation* **115** 2517–2523. (doi:10.1172/JCI24109)

Ortiga-Carvalho TM, Sidhaye AR & Wondisford FE 2014 Thyroid hormone receptors and resistance to thyroid hormone disorders. *Nature Reviews Endocrinology* **10** 582–591. (doi:10.1038/nrendo.2014.143)

Ortiga-Carvalho TM, Chiamolera MI, Pazos-Moura CC & Wondisford FE 2016 Hypothalamus-pituitary-thyroid axis. *Comprehensive Physiology* **6** 1387–1428. (doi:10.1002/cphy.c150027)

Pierce JG & Parsons TF 1981 Glycoprotein hormones: structure and function. *Annual Review of Biochemistry* **50** 465–495. (doi:10.1146/annurev.bi.50.070181.002341)

Ravindra R & Grosvenor CE 1990 Involvement of cytoskeleton in polypeptide hormone secretion from the anterior pituitary lobe: a review. *Molecular and Cellular Endocrinology* **71** 165–176. (doi:10.1016/0303-7207(90)90022-Z)

Refetoff S, Weiss RE & Usala SJ 1993 The syndromes of resistance to thyroid hormone. *Endocrine Reviews* **14** 348–399. (doi:10.1210/edrv-14-3-348)

Refetoff S, Bassett JH, Beck-Peccoz P, Bernal J, Brent G, Chatterjee K, De Groot LJ, Dumitrescu AM, Jameson JL, Kopp PA, *et al*. 2014 Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. *Thyroid* **24** 407–409. (doi:10.1089/thy.2013.3393.nomen)

Romano RM, Bargi-Souza P, Brunetto EL, Goulart-Silva F, Avellar MC, Oliveira CA & Nunes MT 2013 Hypothyroidism in adult male rats alters posttranscriptional mechanisms of luteinizing hormone biosynthesis. *Thyroid* **23** 497–505. (doi:10.1089/thy.2011.0514)

Saji M & Ringel MD 2010 The PI3K-Akt-mTOR pathway in initiation and progression of thyroid tumors. *Molecular and Cellular Endocrinology* **321** 20–28. (doi:10.1016/j.mce.2009.10.016)

Samuels HH & Tsai JS 1973 Thyroid hormone action in cell culture: domonstration of nuclear receptors in intact cells and isolated nuclei. *PNAS* **70** 3488–3492. (doi:10.1073/pnas.70.12.3488)

Samuels HH, Forman BM, Horowitz ZD & Ye ZS 1988 Regulation of gene expression by thyroid hormone. *Journal of Clinical Investigation* **81** 957–967. (doi:10.1172/JCI113449)

Sap J, Munoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H & Vennstrom B 1986 The c-erb-A protein is a high-affinity receptor for thyroid hormone. *Nature* **324** 635–640. (doi:10.1038/324635a0)

Schaufele F, West BL & Baxter JD 1992 Synergistic activation of the rat growth hormone promoter by Pit-1 and the thyroid hormone receptor. *Molecular Endocrinology* **6** 656–665. (doi:10.1210/mend.6.4.1584227)

Shupnik MA 2000 Thyroid hormone suppression of pituitary hormone gene expression. *Reviews in Endocrine and Metabolic Disorders* **1** 35–42. (doi:10.1023/A:1010008318961)

Shupnik MA & Ridgway EC 1987 Thyroid hormone control of thyrotropin gene expression in rat anterior pituitary cells. *Endocrinology* **121** 619–624. (doi:10.1210/endo-121-2-619)

Shupnik MA, Chin WW, Habener JF & Ridgway EC 1985 Transcriptional regulation of the thyrotropin subunit genes by thyroid hormone. *Journal of Biological Chemistry* **260** 2900–2903.

Siegrist-Kaiser CA, Juge-Aubry C, Tranter MP, Ekenbarger DM & Leonard JL 1990 Thyroxine-dependent modulation of actin polymerization in cultured astrocytes. A novel, extranuclear action of thyroid hormone. *Journal of Biological Chemistry* **265** 5296–5302.

Silva FG, Giannocco G, Santos MF & Nunes MT 2006 Thyroid hormone induction of actin polymerization in somatotrophs of hypothyroid rats: potential repercussions in growth hormone synthesis and secretion. *Endocrinology* **147** 5777–5785. (doi:10.1210/en.2006-0110)

Silva FG, Giannocco G, Luchessi AD, Curi R & Nunes MT 2010 T3 acutely increases GH mRNA translation rate and GH secretion in hypothyroid rats. *Molecular and Cellular Endocrinology* **317** 1–7. (doi:10.1016/j.mce.2009.12.005)

Sinha R & Yen PM 2000 Cellular action of thyroid hormone. In *Endotext*. Eds LJ De Groot, P Beck-Peccoz, G Chrousos, K Dungan, A Grossman, JM Hershman, C Koch, R McLachlan, M New, R Rebar, *et al*. South Dartmouth, MA, USA: MDText.com, Inc. (available at: https://www.ncbi.nlm.nih.gov/books/NBK285568/)

Staton JM & Leedman PJ 1998 Posttranscriptional regulation of thyrotropin beta-subunit messenger ribonucleic acid by thyroid hormone in murine thyrotrope tumor cells: a conserved mechanism across species. *Endocrinology* **139** 1093–1100. (doi:10.1210/endo.139.3.5799)

Storey NM, Gentile S, Ullah H, Russo A, Muessel M, Erxleben C & Armstrong DL 2006 Rapid signaling at the plasma membrane by a nuclear receptor for thyroid hormone. *PNAS* **103** 5197–5201. (doi:10.1073/pnas.0600089103)

Tata JR & Widnell CC 1966 Ribonucleic acid synthesis during the early action of thyroid hormones. *Biochemical Journal* **98** 604–620. (doi:10.1042/bj0980604)

Veyrune JL, Campbell GP, Wiseman J, Blanchard JM & Hesketh JE 1996 A localisation signal in the 3' untranslated region of c-myc mRNA targets c-myc mRNA and beta-globin reporter sequences to the perinuclear cytoplasm and cytoskeletal-bound polysomes. *Journal of Cell Science* **109** 1185–1194.

Volpato CB & Nunes MT 1994 Role of thyroid hormone in the control of growth hormone gene expression. *Brazilian Journal of Medical and Biological Research* **27** 1269–1272.

Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ & Evans RM 1986 The c-erb-A gene encodes a thyroid hormone receptor. *Nature* **324** 641–646. (doi:10.1038/324641a0)

Weiss RE, Forrest D, Pohlenz J, Cua K, Curran T & Refetoff S 1997 Thyrotropin regulation by thyroid hormone in thyroid hormone receptor beta-deficient mice. *Endocrinology* **138** 3624–3629. (doi:10.1210/endo.138.9.5412)

Yaoita Y, Shi YB & Brown DD 1990 Xenopus laevis alpha and beta thyroid hormone receptors. *PNAS* **87** 7090–7094. (doi:10.1073/pnas.87.18.7090)

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

Yusta B, Alarid ET, Gordon DF, Ridgway EC & Mellon PL 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. (doi:10.1210/endo.139.11.6283)

Zoeller RT, Tan SW & Tyl RW 2007 General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Critical Reviews in Toxicology* **37** 11–53. (doi:10.1080/10408440601123446)

Received in final form 4 September 2017
Accepted 26 September 2017
Accepted preprint published online 26 September 2017

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-17-0068

Published by Bioscientifica Ltd.