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Minireview

3,5,3'–Triiodothyronine Nuclear Receptors and Their Role in the Thyroid Hormone Action

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Thyroid Hormone Receptors

Thyroid hormones are involved in a complex arrangement of physiological and developmental responses in several tissues of higher vertebrates. Thyroxine, the main product of the thyroid gland, circulates in the blood stream being bound either to specific plasma proteins (i.e. thyroxine binding globulin and transthyretin) or non–specifically to albumin. In the target tissue it is transported into the intracellular space by both the diffusion and energy dependent process (KRENNING et al. 1981). In the cytoplasmic space there is a "pool" of thyroid hormones containing the biologically active metabolite of thyroxine–3,5,3'–L–triiodothyronine (T_3) which originates from the monodeiodination of thyroxine. The T_3 enters the cell nucleus probably in the free form where it is specifically bound to nuclear receptors.

The existence of high–affinity and low–capacity nuclear receptors was first demonstrated two decades ago by OPPENHEIMER et al. (1972). Tissues such as the pituitary, liver, kidney, heart and brain were found to contain relatively high number of T_3 receptors, while other tissues such as spleen and testes were found to have lower numbers of T_3 receptors (OPPENHEIMER et al. 1974). **The nuclear T_3 receptors** are thermolabile "acidic" non–histone proteins with a molecular mass of approximately 50 kDa (LATHAM et a. 1976; ICHIKAWA et al. 1989). In the cell nucleus, the T_3 receptors are localized between nucleosomes and close to the linker region of DNA (PERLMAN et al. 1982; ORTIZ–CARO et al. 1989), and they may be easily extracted from cell nuclei at higher ionic strength conditions (DeGROOT and STRAUSSER 1974; TORRESANI and DeGROOT 1975).

The molecule of T_3 receptor molecule reveals one binding site for T_3 as determined by Scatchard plots (SCATCHARD, 1949) with an equilibrium association constant (K_a) equal to 2.0×10^9 l/mol (DeGROOT and TORRESANI 1975). Photoaffinity labelling of thyroid hormone nuclear receptors in GH_1 rat pituitary tumor cells followed by 0.4 mol/l KCl extraction of nuclei and SDS polyacrylamide gel electrophoresis demonstrated the existence of two forms of T_3 receptors, a predominant form with a molecular mass of 47 kDa and a less abundant one of 57 kDa (PASCUAL et al. 1982). These results underscored the findings on marked heterogeneity of T_3 receptors demonstrated later by cloning of multiple thyroid hormone receptor cDNAs. Isoelectric focusing of T_3 receptors from rat liver also indicated the existence of at least four forms of T_3 receptors with different isoelectric points (ICHIKAWA and DeGROOT 1987).

It is well known that the number of nuclear receptors for T_3 in the liver may vary, i.e. it may be reduced by starvation, glucagon treatment or partial hepatectomy (DeGROOT et al. 1977; DILLMANN et al 1978; BRTKO and KNOPP 1988). Decreased number of nuclear T_3

receptors was also confirmed in rat or mouse liver bearing transplantable neoplasms (SURKS et al. 1978).

A remarkable breakthrough in the field of nuclear thyroid hormone receptors came with the discovery of two research groups (WEINBERGER et al., 1986; SAP et al. 1986) showing that the **c–erbA proto–oncogene**, which is a cellular counterpart of the viral verbA oncogene, encodes a high affinity receptor for T_3. Moreover, the above authors demonstrated that the genes coding the T_3 receptors belong to a "superfamily" of regulatory genes responsible for coding receptors for glucocorticoids, mineralocorticoids, estrogen, dihydroxy vitamin D_3, retinoic acid and many other receptors for which the ligand is still unknown, so they are called "orphan receptors". The interaction of the nuclear receptors with specific DNA sequences represents one of the mechanisms by which the transcription of genes is controlled. Fig. 1 illustrates the domain structure of two different T_3 receptor (TR) forms within the nuclear receptor "superfamily" (EVANS 1988).

Four functional domains have been identified in all members of the receptor superfamily: The **A/B domain** is the most variable domain with a stimulatory or inhibitory transcription function responsible for protein – protein interactions between the receptor molecule and other factors participating in the transcriptional process.

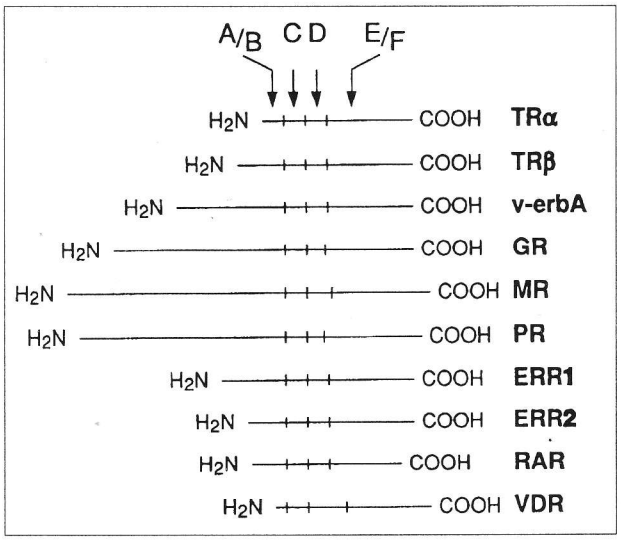

Fig.1 Steroid/thyroid hormone receptor superfamily (TR: thyroid hormone receptor; GR: glucocorticoid receptor; MR: mineralocorticoid receptor; PR: progesterone receptor; ERR: estrogen receptor; RAR: retinoic acid receptor; VDR: dihydroxyvitamin D_3 receptor)

The **C domain** is the highly conserved central cysteine rich DNA–binding region of the receptor molecule containing 66–68 amino acids, including 10–11 cysteines. Cysteine containing repeats within the C domain allow to form the finger–like structures stabilized by coordinate complex formation with a zinc ion, **"zinc fingers"** which enable the binding of receptor molecule to DNA. Fig. 2 illustrates the amino acid sequence and the structure of the DNA binding C domain of the human thyroid hormone receptor as adapted from recent data (UMESONO and EVANS 1989; GLASS and HOLLOWAY 1990). The "zinc finger 1" containing a loop of 13 amino acids is formed by four cysteines chelating one zinc ion. The "zinc finger 2", formed by additional four cysteines and zinc ion and containing a loop of 12 amino acids, is connected with the first one by a "linker" of 17 amino acids. UMESONO and EVANS (1989) localized two non contiguous "boxes" in the DNA binding domain, one proximal "P–box" and one distal "D–box". The "P–box" follows one cysteine and includes the three amino acid highly conserved cluster, containing the E (glutamic acid), G (glycine)–G (glycine). The EG–G amino acid cluster within the first finger was also found in retinoic acid receptors, in the vitamin D_3 receptors as well as in some "orphan" receptors. The "D–box" was found to be located between two cysteines within the second zinc finger, and its amino acid sequence is specific for each of the above nuclear receptors. Both amino acid "boxes" within the C domain of nuclear receptors were found to be important for DNA binding specificity. The "P–box" is critical for identifying the primary nucleotide sequence of the half sites, while the "D–box" is important for the determination of the half–site spacing (UMESONO and EVANS 1989).

The **D domain** represents a highly flexible structure and it plays a role as a "hinge" in the receptor molecule. The carboxy–terminal **E/F domain** contains the hormone binding region. Mutations in this domain reduce the affinity of

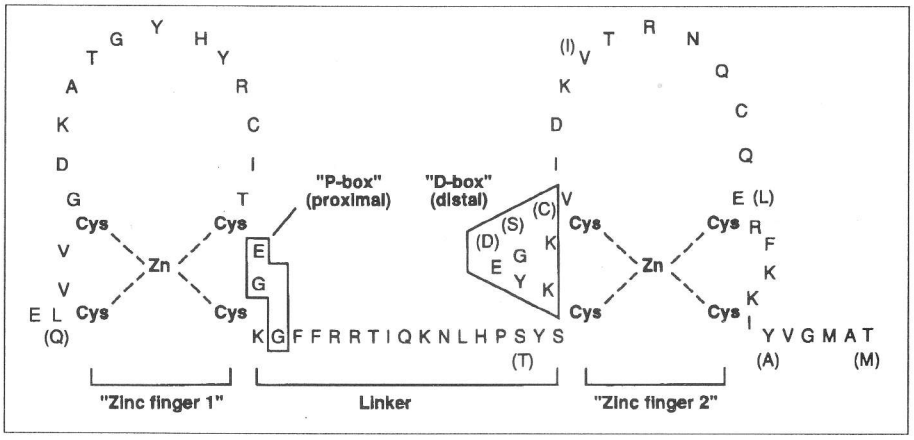

Fig.2 C–domain of the nuclear thyroid hormone receptors. Comparison of the human ß–form with that of the α (Amino acids of the α–form by which the C–domain differs from that of the ß are shown in brackets)

the receptor for the hormone, but have no effect on DNA binding (GIGUERE et al. 1986; GREEN and CHAMBON, 1988). Moreover, the E/F domain was also found to be important for homodimerization or heterodimerization of the TR with other receptor molecules of the steroid/thyroid hormone receptor superfamily (LAZAR et al. 1991).

The landmark findings of the close relationship between the c–erbA proto–oncogene and genes coding the above receptors led to the discovery of **multiple isoforms of thyroid hormone receptor**. The evidence supporting the data on the existence of multiple thyroid hormone receptor, and not species variation was given by THOMPSON et al. (1987) who showed that a novel T_3 receptor gene was mapped to human chromosome 17, distinct from the other T_3 receptor gene which was found on human chromosome 3 (WEINBERGER et al. 1986). Multiple forms of the thyroid hormone receptor molecule have been identified within two years since 1986 (THOMPSON et al. 1987; MITSUHASHI et al. 1988; IZUMO and MAHDAVI, 1988). In general, thyroid hormone receptors are classified into α and ß subtypes based on mapping to human chromosomes and on sequence homology. Gene localization of the thyroid hormone receptor α (TRα) corresponds to human chromosome 17 and the ß–form of thyroid hormone receptor (TRß) to human chromosome 3 (BRADLEY et al. 1989).

Further investigations led to discovery of the carboxy–terminal TRα2 variant of the T_3 receptor which binds to DNA but it fails to bind thyroid hormone (IZUMO and MAHDAVI 1988; MITSUHASHI et al. 1988; LAZAR et al. 1988). Due to alternative splicing, the TRα2 lacks C–terminal 40 amino acids of the thyroid hormone receptor (TRα1) but it contains an additional 120 (human) or 122 (rat and mouse) amino acids with no homology to other known sequences (LAZAR 1993). Moreover, two additional TRα isoforms have also been identified recently, the TRα3 and the Rev–ErbAα (MITSUHASHI et al. 1988; LAZAR et al. 1989). The DNA strand coding for Rev–ErbAα is opposite of that encoding the c–erbA proteins (LAZAR et al. 1990). The TRα2 and TRα3 as well as the Rev–ErbAα are members of the **steroid/thyroid hormone receptors superfamily** in spite of the fact that they do not bind T_3 (Fig. 3). An additional functional thyroid hormone receptor ß, i.e. TRß2 which differs at its amino acid terminus from the previously described TRß (WEINBERGER et al.1986), referred later to as TRß1, was described by HODIN et al. (1989). Although, the TRα1, TRα2, Rev–ErbAα, and TRß1 have been detected in several tissues, the TRß2 was found only in the anterior pituitary gland (HODIN et al. 1989), and in the rat central nervous system (LECHAN et al. 1993). The TRß1 was homogeneously distributed among various tissues, a high concentration being found mainly in the pituitary, brain, liver and kidney (HODIN

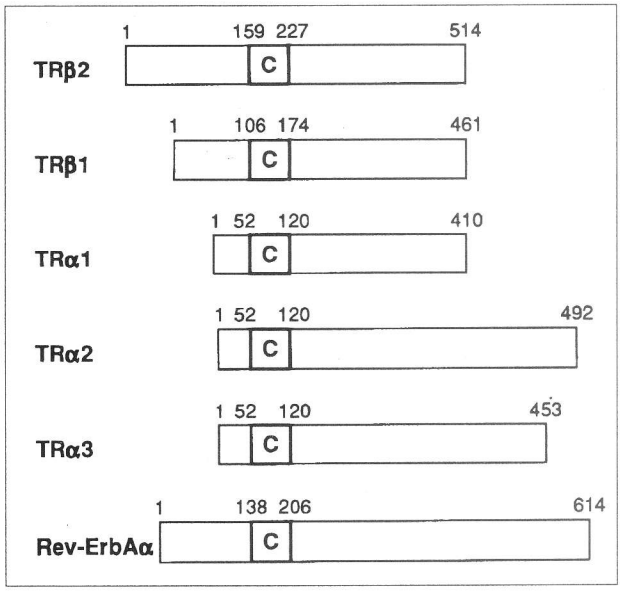

Fig.3 Structures of nuclear thyroid hormone receptors (TRß2, TRß1, TRα1) with their isoforms which fail to bind T_3 (TRα2, TRα3, Rev–ErbAα)

et al. 1990). The TRα1 was found to be in highest abundance in skeletal muscle and brown fat (MITSUHASHI et al. 1988), and the TRα2 was detected in an extremely high abundance in the brain (MITSUHASHI and NIKODEM 1989).

Transcriptional Control Represents a Direct Action of Thyroid Hormone

Multiple levels of gene expression controlled by thyroid hormone, including the rate of the initiation of transcription, messenger RNA stability as well as protein half–life, have been demonstrated. One of the most important properties of T_3–nuclear receptor complex is its ability to stimulate or inhibit gene transcription (GLASS and HOLLOWAY 1990). Thyroid hormone receptors as well as the other members of steroid/thyroid nuclear receptor family regulate gene expression through the binding to short cis–acting DNA sequences referred to as **hormone response elements** (FORMAN et al. 1988; UMESONO et al. 1988; LAZAR 1993). The "T_3–nuclear receptor" complex bound to its corresponding response element subsequently interacts with other components of transcriptional apparatus to increase or decrease the rates of transcription (GLASS et al. 1989). Therefore, the identification of functional hormone responsive elements became crucial for the understanding of the mechanisms by which the thyroid hormone–nuclear receptor complex activates the expression of target genes. The thyroid hormone response elements have been shown to consist of either the palindrome pair of hexameric (AGGTCA) "half–sites" or direct repeats of this sequence spaced by a "gap" containing preferentially four nucleotides (GLASS et al. 1988; WAHLSTRÖM et al. 1992).

Generally, in terms of structure and function of hormone response elements for the members of the steroid/thyroid hormone receptor family they were divided into two groups. As shown in Tab. 1, the first one includes glucocorticoid, progesterone, androgen, and mineralocorticoid receptors and the second one includes thyroid hormone, estrogen, retinoic acid, and vitamin D_3 receptors (BEATO 1989). The thyroid hormone receptor, even in the absence of T_3 is bound to thyroid hormone response elements in target genes. Unliganded form of the thyroid hormone receptor plays a role as a repressor, whereas the T_3 liganded form is an activator of gene transcription (LAZAR 1993).

Research over the past decades allowed to characterize the thyroid hormone dependent effects on the expression of different target genes. One of the most carefully studied processes is the **regulation of rat growth hormone gene transcription by T_3**. Rat growth hormone gene expression is known to be stimulated by glucocorticoids and/or retinoic acid (EVANS et al. 1982; YAFFE and SAMUELS 1984; BEDO et al. 1989; ARANDA et al. 1992; GARCIA–VILLALBA et al. 1993). The 5'flanking region of the rat growth hormone (GH) gene contains a thyroid hormone response element (TRE) located in a region between nucleotides –189 and –164 upstream of the mRNA start site which contains two imperfect direct AGGTCA repeats (–189/–184; –179/–174) and an adjacent region (–172/–167) which is an inverted copy of the above repeats. All the above domains are required for full response to thyroid hormones and retinoic acid (BRENT et al. 1989; GARCIA–VILLALBA et al. 1993). The TRE binds the thy-

Table 1

Nucleotide sequence of "half–sites" of the hormone response elements

Consensus responsive element		HRE 1/2	gap	HRE 1/2
Group 1	GRE	G G T A C A	n n n	T G T T C T
	PRE	G G T A C A	n n n	T G T T C T
	ARE	G G T A C A	n n n	T G T T C T
	MRE	G G T A C A	n n n	T G T T C T
Group 2	TRE/RARE	A G G T C A	*	T G A C C T
	ERE	A G G T C A	n n n	T G A C C T
	RARE	A G G T C A	n n n n	A G G T C A
	VitDRE	A G G T C A	n n n	A G G T C A
	TRE	A G G T C A	n n n n	A G G T C A

* no gap is present

roid hormone receptor which acts as a "ligand inducible transcription factor" (SAMUELS et al. 1988; GLASS and HOLLOWAY 1990; BRENT et al. 1991), and both T_3 and retinoic acid receptors activate the transcription from the rat growth hormone gene via a common response element (UMESONO et al. 1988; GARCIA–VILLALBA et al. 1993). It has been found that the above hexameric nucleotide sequence (AGGTCA) is also well recognized by the retinoic acid receptor (RAR), 9–cis–retinoic acid receptor (RXR) and vitamin D (VDR) receptor. YU et al. (1991) recently identified the retinoic X receptor (RXR), a novel nuclear receptor for retinoids, as a "promiscuous" dimerization partner for the TR, RAR and VDR. Heterodimerization of TR, RAR and VDR with the RXR ("auxiliary protein factor") was found to be important for stable interaction of the above receptors with their cognate responsive elements (BUGGE et al. 1992).

As shown Fig. 4, in rat pituitary cell lines the transcription of the growth hormone gene is increased by both T_3 and retinoic acid (BEDO et al. 1989). The RXR receptor also plays an important role in controlling the activity of the growth hormone gene since it is able to heterodimerize with the T_3 and the retinoic acid receptors and subsequently increase the binding of the complex structure to the TRE as well as to enhance the transcriptional response to T_3 and retinoic acid (YU et al. 1991, ARANDA et al. 1992; GARCIA–VILLALBA et al. 1993). Vitamin D is unable to activate rat growth hormone gene transcription and, moreover, it reduces markedly the response of the rat growth hormone mRNA to T_3 and retinoic acid (GARCIA–VILLALBA et al. unpublished data). The T_3 receptor also binds to estrogen response element but it is transcriptionally inactive on this site (GLASS et al.1988). In general, nuclear receptors are capable to form homodimers or heterodimers and thus yield selective transcriptional responses to the respective hormone(s) (GLASS et al. 1989; YU et al. 1991).

Expression of the growth hormone gene depends on the presence of the pituitary–specific transcription factor GHF–1, a homeodomain protein also known as Pit–1. This protein binds to two *cis*–active elements located from –95 to

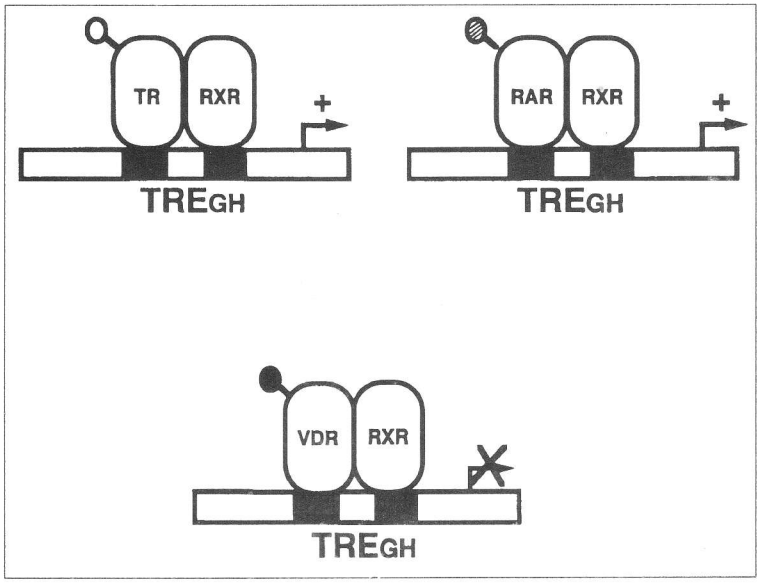

Fig.4 Schematic representation of the ligand–nuclear receptor– DNA complex: Ligand (O T_3; ◍ all trans–retinoic acid; ● dihydroxyvitamin D_3) – heterodimerized forms of nuclear receptors (TR: T_3 receptor; RAR: retinoic acid receptor; VDR: dihydroxyvitamin D_3 receptor; RXR: 9–cis–retinoic acid receptor) – DNA (TRE: thyroid hormone response element; GH: growth hormone gene);
(+) stimulation of transcription

–65 and from –137 to –107 in the rat growth hormone gene promoter (BODNER et al. 1988; INGRAHAM et al. 1988; KARIN et al. 1990). The transcription factor GHF–1, a member of a family of DNA binding proteins, which is only expressed in nuclei from growth hormone and prolactin producing cells has been characterized as a 33 kDa polypeptide (BODNER et al. 1988). This factor was found to be controlled by at least three primary means: a cAMP–activated transcription factor (CREB), positive autoregulation by its own gene product, and transcriptional activation by other pituitary transcription factors (McCORMICK et al. 1990; ARANDA et al. 1992).

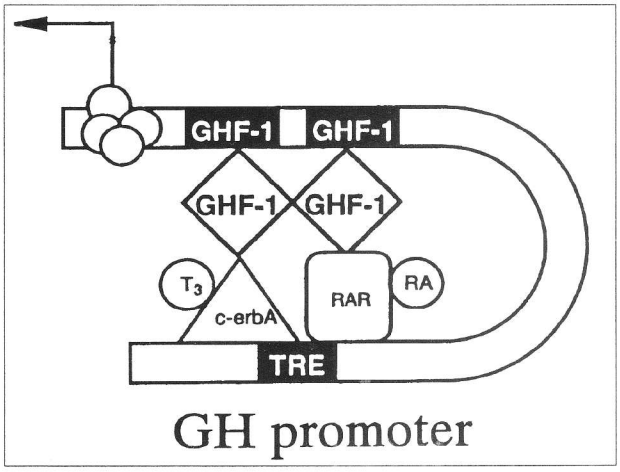

Fig.5 Schematic depiction of the rat growth hormone promoter, showing the known essential components for the expression of the growth hormone gene in rat pituitary nucleus when affected by thyroid hormone or retinoic acid (TRE: thyroid hormone response element; T_3: 3,5,3'–L–triiodo thyronine; c–erbA: nuclear thyroid hormone receptor; RA: all–trans retinoic acid; GHF–1: growth hormone transcription factor 1; **GHF–1**: DNA–binding site for the growth hormone transcription factor 1)

The expression of the growth hormone gene is also regulated by the hypothalamic growth hormone releasing factor (GRF) which stimulates the transcription of both the human and rat growth hormone gene via a cyclic AMP–dependent mechanism (BARINAGA et al. 1983), most likely by increasing the expression of GHF–1. As shown in Fig. 5, the nuclear T_3 and retinoic acid receptors interact with the transcription factor GHF–1 producing a cooperative activation of the growth hormone gene. In the absence of the GHF–1, the growth hormone gene is silent and can not be activated by the nuclear receptors for T_3 and retinoic acid or other transcription factors (ARANDA et al. 1992). According to the recent data the thyroid

hormone was found to decrease GHF–1 gene expression by a novel mechanism that involves transcriptional interference with other regulatory elements of the GHF–1 promoter (SANCHEZ–PACHECO PALOMINO and ARANDA unpublished results).

Transcriptional Control of Other Target Genes Expression by Thyroid Hormone

Studies of other thyroid hormone responsive genes also showed that the interaction of T_3 with nuclear receptors results in a subsequent alteration of mRNA synthesis (OPPENHEIMER et al. 1987; SAMUELS et al. 1987; GLASS and HOLLOWAY 1990). Specific examples include a positive regulation of malic enzyme gene expression (TOWLE et al. 1981), rat liver phosphoenolpyruvate carboxykinase (HARTONG and LAMERS 1986), and rat hepatic α_{2u}–globulin (KURTZ et al. 1976) gene expression by T_3. In addition, the expression of the proteins called Spot–11 and Spot–14 in lipogenic tissues (JUMP et al. 1984; OPPENHEIMER et al. 1987) and the rat cardiac α–myosin heavy chain gene in the heart ventricle (SINHA et al. 1982; IZUMO and MAHDAVI 1988) were also found to be positively regulated by thyroid hormone. Inhibition of transcription by thyroid hormone has been demonstrated for both the α– and ß–subunits of thyroid stimulating hormone (TSH) genes (SHUPNIK et al.1985) as well as for the ß–myosin heavy chain gene in both, the atrium and the ventricle (SINHA et al. 1982; EVERETT et al. 1983). Moreover, thyroid hormone has been demonstrated to regulate the expression of the HMG CoA reductase gene which encodes the enzyme controlling the rate–limiting step in the production of cholesterol, isoprenoids and dolichol compounds (SIMONET and NESS 1988; GLASS and HOLLOWAY 1990), and to stimulate the transcription of the apolipoprotein A–I and A–IV genes (APOSTOLOPOULOUS et al. 1988).

Due to a variety of different responses to T_3 in target tissues of mammals, the further investigation on the molecular and cellular mechanisms of thyroid hormone action mediated by nuclear thyroid hormone receptors and/or other nuclear receptors acting as ligand dependent transcription factors, is highly desirable.

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