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Review

Thyroid hormones and their receptors in the regulation of cell proliferation

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In the present work, we have reviewed data showing that triiodothyronine and its nuclear receptors modify expression of different genes/proteins involved in cell cycle control beginning from growth factors (such as EGF and TGF-β), to cell surface receptors (EGFR), as well as proteins acting at the cell membrane (Ras), various transcription factors (c-Fos, c-Myc, E2F1), cyclins, Cip/Kip family of cdk2 inhibitors, and p53 inhibitor Mdm2 (Table 1). We have shown how TRs are also able to modify the fate of a cell, thanks to their ability to form complexes with other transcription factors such as p53 — a key regulator of apoptosis and proliferation. Available data show that the function of thyroid hormones and of their receptors on cell proliferation is not homogenous. In fact, it strongly depends on the cell type, its developmental state (progenitor or differentiated), its patho-physiological state (normal or tumor cell), and the so-called 'cellular context'. Therefore, it is not possible to uniformly recommend T3 treatment or T3 depletion to stop or initiate proliferation of all cell types. Instead, a very individual and careful action should be considered.

Keywords: proliferation, triiodothyronine (T3), thyroid hormone nuclear receptors (TRs), mitogens, oncoproteins

INTRODUCTION

Thyroid hormones regulate cellular metabolic activity and co-regulate cell proliferation, apoptosis, and differentiation. Their crucial role in embryonic development, especially of the central nervous system and musculoskeletal system, is well established (Williams *et al*., 1998; Morreale de Escobar *et al*., 2004; Bernal, 2005). There is a growing body of evidence that disturbed triiodothyronine (T3)-dependent control of cellular processes plays a role in tumorigenesis (Yen & Cheng, 2003; Cheng, 2004; 2005). However, the data regarding T3 influence on proliferation is scattered in different publications and no concise summary on this subject has been published. In this article we present the current state of knowledge regarding confirmed and potential mechanisms by which thyroid hormones might influence cell proliferation. First, we offer a short description of the cell cycle with a special emphasis on the control of the progression from G_1 to S phase, and then we present the principles of T3 action at the molecular level. Finally, we present the genes and proteins involved in the cell cycle control that are under the influence of T3, and based on the available data we describe proposed mechanisms of their regulation by this hormone.

THE CELL CYCLE AND ITS MAJOR REGULATORS AND EXECUTORS

The cycle of the somatic cell consists of mitosis (M), gap 1 (G_1), DNA synthesis (S), and gap 2

Abbreviations: ATF, activation transcription factor; CRE, cyclic AMP response element; CREB, CRE-binding protein; EGF, epidermal growth factor; EGFR, EGF receptor; RB, retinoblastoma protein; RXR, retinoid X receptor; SRE, serum response element; TR, thyroid hormone nuclear receptor; TREs, thyroid hormone response elements; T3, triiodothyronine; T4, thyroxine.

Table 1. The summary of the influence of triiodothyronine treatment on the regulation of the genes/proteins involved in proliferation control

Proliferation activaion		Proliferation inhibition	
Activation	Inhibition	Activation	Inhibition
1. *mdm2*, direct, *via* two positive TREs	1. *P21*, by unknown mechanism, in astrocytoma cells	1. *TGF-β*, by unknown mechanism, in HepG2 cells	1. *E2F1*, direct, *via* negative TRE
2. *Cyclin D1*, by unknown mechanism, in pituitary growth hormone-producing GC cells, hepatocytes, cardiomyocytes		2. *P27*, by unknown mechanism; extended $p27^{Kip1}$ protein half-life in neuroblastoma N2a-β cells; accumulation of $p27^{Kip1}$ protein in Sertoli cells, by unknown mechanism	2. *MYC*, direct, *via* negative TRE and interaction with CTCF suppressor
3. *c-Fos* and *FosB*, by unknown mechanism, in Schwann cells		3. *P21*, by unknown mechanism, in hepatoma cells; accumulation of $p21^{Cip1}$ protein in Sertoli cells, by unknown mechanism	3. *Cyclin D1*, indirect, by interaction with transcriptional activity of CRE-binding proteins in neuroblastoma N2a-β cells
4. *EGF*, by unknown mechanism, in mouse submandibular gland cells			4. *c-Fos*, by interference with AP-1 function or by transcriptional interference with AP-1 and SRE binding sites
5. *EGFR*, by unknown mechanism, in proximal tubule cells of the kidney			
6. p53 protein accumulation, by unknown mechanism, in T47D human breast ductal carcinoma cell line			

(G_2) phases (Fig. 1). The cell responds to both mitotic and antiproliferative signals from the end of mitosis until it reaches restriction point 'of no return' (R) at the end of G_1 phase. In response to mitogenic signals cyclins D1, D2, and D3 (Sherr, 1993; Sherr & Roberts, 2004) form complexes with cyclin-dependent kinases cdk4 or cdk6 (Matsushime *et al.*, 1992; Meyerson & Harlow, 1994). While cdk4 and cdk6 are quite stable, cyclins D are short-lived proteins, and their synthesis and activity are dependent on the persistent presence of mitogens. The cyclin D-cdk4, -cdk6 complexes initiate the phosphorylation of retinoblastoma protein (RB) (Harbour *et al.*, 1999) bound to E2F transcription factors (Attwooll *et al.*, 2004). Upon RB phosphorylation the activatory members of E2F family are released from RB–E2F complexes and activate a number of target genes including *cyclin E*, *cyclin A* and other genes (Geng *et al.*, 1996; Helin *et al.*, 1998) necessary for cell cycle progression from the G_1 to the S phase (Wu *et al.*, 2001). Cyclin E forms a complex with cdk2 which completes RB phosphorylation. Once the cell enters S phase, cyclin E is degraded and the released cdk2 forms a complex with cyclin A.

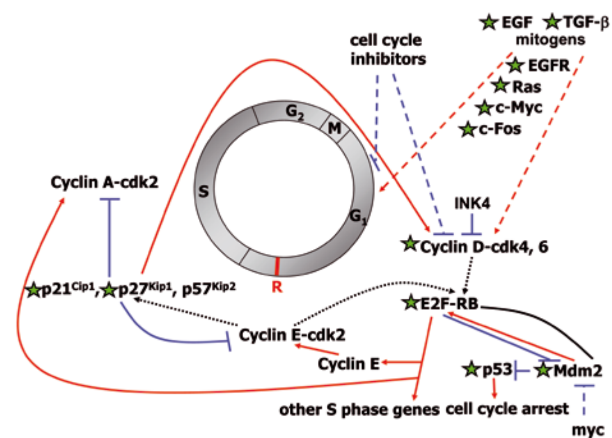

Figure 1. Simplified scheme of the cell cycle and selected proteins involved in the control of the cycle.
Red arrows, activation; blue lines, inhibition; black dotted arrows, phosphorylation; green star, place of triiodothyronine/thyroid hormone nuclear receptors action; r, restriction point.

The action of cdk2 but not of cdk4 and cdk6 is inhibited by Cip/Kip family members (that includes $p21^{Cip1}$, $p27^{Kip1}$, $p57^{Kip2}$, among others). The presence of Cip/Kip family members is also required for the assembly of cyclin D-cdk4, -cdk6 complexes, while absence of free Cip/Kip proteins is necessary for cyclin E-cdk2 activation and cell cycle progression through the G_1 phase. In addition, cyclin E-cdk2 phosphorylates $p27^{Kip1}$, targeting it for ubiquitination and degradation (Sherr, 2000; Vidal & Koff, 2000). Other cdk inhibitors are proteins belonging to INK4 family that oppose the activity of cyclin D-dependent kinases. INK4 proteins sequester cdk4 and cdk6 facilitating the release of Cip/Kip proteins that then inhibit cyclin E-cdk2 complexes leading to cell

cycle arrest (Sherr & Roberts, 1999; Vidal & Koff, 2000).

THE PRINCIPLES OF THYROID HORMONE ACTION AT THE MOLECULAR LEVEL

Triiodothyronine (T3), the most active form of thyroid hormone is mainly produced by extra-thyroidal tissues *via* deiodination of the outer ring of thyroxine (T4) (Bianco *et al*., 2002). Even though thyroid hormones can act *via* non-genomic mechanisms (Bassett *et al*., 2003), they exert their function mostly by interacting with thyroid hormone nuclear receptors (TRs). TRs are transcription factors that regulate activity of T3-target genes in a ligand-dependent manner. They are protein products of *TRA* and *TRB* genes that, in humans, are located on chromosome 17 and 3, respectively. Alternative splicing and the use of alternative promoters result in the production of a number of receptor isoforms including TRα1 and TRα2, as well as TRβ1 and TRβ2 main isoforms (Cheng, 2000; Weiss & Ramos, 2004). TR isoforms, although present in almost all human tissues, are differentially expressed (Sakurai *et al*., 1989; Shahrara *et al*., 1999).

Therefore, the biological effect of T3 in a given tissue depends on a number of factors: the amount of bioavailable hormone, the levels of different TR isoforms (Cheng, 2000; 2005), and post-transcriptional modifications of TRs (Chen *et al*., 2003; Lin *et al*., 2005), the type of their heterodimerization partners — retinoid X receptors (RXRs) (Rastinejad, 2001; Szanto *et al*., 2004), the interaction with co-repressors and co-activators (Chen & Evans, 1995; Onate *et al*., 1995; McKenna *et al*., 1999; Manteuffel-Cymborowska, 1999; Cheng, 2000; Sadow *et al*., 2003), and on the structure of thyroid hormone response elements (TREs) in the promoters of target genes.

TREs present in T3-activated promoters are known as 'positive' TREs, while TREs present in the promoters inhibited by this hormone are considered 'negative'. Consensus, ideal 'positive' TREs consist of two AGGT(C/A)A hexamers separated by four neutral base pairs (direct repeat TRE-DR4). Such hexamers can also be arranged as inverted palindromes separated by six neutral base pairs (TRE-IP6), or as palindromes with no additional base pairs (TRE-P0) (Schrader & Carlberg, 1994). TRs can bind to DNA both in the absence, and in the presence of T3. TR/RXR heterodimer bound to 'positive' TRE in the absence of T3 recruits a co-repressor complex that contains histone deacetylase, among others. This enzyme removes acetyl groups from certain histones, thus stabilizing chromatin structure and making it inaccessible for transcriptional activators. This results in the inhibition of gene transcription below the basal (T3 and TR-independent) level (Wolffe *et al*., 2000; Zhang & Lazar, 2000; Eckey *et al*., 2003; Moore & Guy, 2005). In the presence of the hormone the structure of TR changes, forcing co-repressors to dissociate, and allowing co-activators to bind. Co-activator complexes contain another chromatin structure-modifying enzyme, histone acetyltransferase, that remodels chromatin, making it accessible for other transcription factors. This causes the increase of gene transcription markedly above the basal level (Wolffe *et a*l., 2000; Zhang & Lazar, 2000; Moore & Guy, 2005).

The CAAAG(T/C) sequence creates a best described 'negative' TRE, a so-called z-element (Carr & Wong, 1994; Sasaki *et al*., 1999; Shibusawa *et al*., 2003). The gene containing the 'negative' TRE within its promoter bound by unliganded TR is activated, while bound by T3–TR complex is inhibited (Chatterjee *et al*., 1989; Carr & Wong, 1994; Radoja *et al*., 1997; Nygard *et al*., 2003).

THE DIVERSE INFLUENCES OF TRIIODOTHYRONINE ON PROLIFERATION

The first clues for a role of thyroid hormones in the regulation of cell proliferation came from the observations, at the beginning of XX Century, of amphibian metamorphosis. Metamorphosis is strictly and exclusively controlled by T3. Three types of changes take place during this process: complete involution of some organs, remodeling of others, and *de novo* development of a new ones. On the cellular level, these changes are caused by a combination of apoptosis and cell proliferation (Su *et al*., 1999).

The result on proliferation of treatment with T3 depends on the cell/tissue type and its developmental state. For example, T3 activates proliferation of hepatocytes after partial hepatectomy and is, in fact, considered a primary liver mitogen (Francavilla *et al*., 1994; Malik *et al*., 2003). It also accelerates wound healing of the skin (Safer *et al*., 2005), induces proliferation of cultured bovine thyroid cells (Di Fulvio *et al*., 2000), of bone marrow pro-B cells (Foster *et al*., 1999), of pancreatic acinar cells (Ohmura *et al*., 1997; Ledda-Columbano *et al*., 2005), and of renal proximal tubular epithelial cells (Ohmura *et al*., 1999). Finally, it increases DNA synthesis in osteoblasts (Kassem *et al*., 1993), and in other cells. On the other hand, T3 treatment blocks proliferation and induces differentiation of oligodendrocyte progenitor cells (Baas *et al*., 1997), of neuroblastoma N2a-β cells (Garcia-Silva *et al*., 2002), and of erythroid progenitors (Bauer *et al*., 1998); it also prevents cycling of postnatal Sertoli cells (Palmero *et al*., 1995; Holsberger *et al*., 2003), as well as of mammary epithelial cells (Gonzales-Sancho *et al*., 2002).

DIRECT REGULATION BY T3 OF THE GENES ENCODING PROTEINS INVOLVED IN THE CONTROL OF PROLIFERATION

E2F1

E2F1 encodes one of key regulators of proliferation, the E2F1 protein belonging to the family of E2F transcription factors (Attwooll *et al*., 2004). Classic members of E2F family form three groups: transcriptional activators (E2F1-3) that induce S phase entry (Johnson *et al*., 1993; Wu *et al*., 2001), transcriptional suppressors (E2F4-5), and the E2F6 repressor that does not bind pocket proteins (RB, p107 or p130). E2F1 released from the inhibitory complex with RB protein activates transcription of a number of target genes including genes for cyclin E, cyclin A, cdk2, and others (Geng *et al*., 1996; Helin, 1998) that results in cell cycle progression *via* the 'R' restriction point.

It has been shown that treatment of embryonic carcinoma cells and of oligodendrocyte precursor cells with T3 decreases their proliferation rate by arresting cells in the G_1 and S phases, but this effect was dependent on the presence of active TRs. This was accompanied by a rapid downregulation of E2F1 mRNA and of protein levels. A negative TRE resembling those present in *TSH* promoters was found in *E2F1* promoter (–200 to –195 bp relative to transcription start site). Transactivation assays showed that TR bound to this TRE activated transcription in the absence of T3 and repressed it in the presence of the hormone (Nygard *et al*., 2003). The level of *E2F1* promoter activity was dependent on the concentration of T3 (Turowska *et al*., unpublished). In addition, some dominant-negative TR mutants cloned from human cancers (Kamiya *et al*., 2002; Puzianowska-Kuznicka *et al*., 2002) activated *E2F1* promoter both in the absence, and in the presence of T3, suggesting the role for such mutants in the deregulation of cell proliferation in cancer tissues (Turowska *et al*., unpublished).

MYC

c-Myc functions as one of the key downstream effectors of most mitogenic signals (Cole & Nikiforov, 2006). In normal cells, the expression of *MYC* gene is under tight control of growth factor-dependent signals, while in cells with deregulated *MYC* control proliferation often takes place in the absence of external stimuli (Bernard & Eilers, 2006). c-Myc protein is a transcription factor that regulates expression of distinct sets of target genes. Transcriptional activation by c-Myc is mediated by c-Myc/Max dimers binding to CA(C/T)GTG (the E-box) consensus sequence, while transcriptional inhibition is mediated through distinct DNA elements (Amati *et al*., 2001).

Treatment with T3 of neuroblastoma N2a-β cells overexpressing active TRβ1 led to a rapid downregulation of *MYC* gene accompanied by the decrease of the amount of c-Myc protein (Perez-Juste & Aranda, 1999). A negative TRE arranged as an inverted palindrome separated by three neutral base pairs has been found within the first exon of the mouse *c-myc* gene. This TRE lies next to the binding site for CCCTC binding factor (CTCF) that acts as transcriptional repressor. TR bound to its recognition site interacts physically with CTCF both in the absence, and in the presence of T3, interfering with CTCF function. Moreover, the negative TRE lies within the region of RNA polymerase pausing and release. This suggests the involvement of TR bound to its own recognition site in premature termination of transcription from *c-myc* gene (Perez-Juste *et al*., 2000), a hypothesis which was indeed confirmed (Garcia-Silva *et al*., 2002). The regulation of *c-myc* gene might require the presence of different TR isoforms, at least in some cell populations. For example, a liganded TRα but not TRβ is required for such regulation in brain stem cells present in subventricular zone of the brain and for the migration of neuroblasts out of the stem cell niche (Lemkine *et al*., 2005).

Mdm2

Mdm2 is the best known inhibitor of p53. The binding of Mdm2 to p53 causes rapid p53 degradation by a proteasome-mediated process (Kubbutat *et al*., 1997) and inhibition of p53-mediated transactivation (Momand *et al*., 1992). This results in the reversal of p53-mediated cell cycle arrest (Chen *et al*., 1996). It is noteworthy that Mdm2 also interacts with other proteins involved in cell cycle regulation such as RB inhibiting its cell cycle-regulatory activity (Xiao *et al*., 1995), and with E2F1 stimulating its transcriptional activity (Martin *et al*., 1995). It also regulates transcriptional activity of *cyclin A* promoter (Leveillard & Wasylyk, 1997). Therefore, Mdm2 not only releases a proliferative block by silencing p53, but also stimulates progression from the G_1 to the S phase of the cell cycle by inhibiting RB activity and by stimulating E2F1.

Murine *mdm2* gene is activated by p53 that binds to its recognition site within the first intron of this gene. Two functionally active TREs were found within the same sequence (Zauberman *et al*., 1995; Qi *et al*., 1999). T3 treatment of TR-expressing GH-4Cl cells resulted in activation of endogenous *mdm2* *via* these TREs (Qi *et al*., 1999).

INDIRECT REGULATION OF THE GENES ENCODING PROTEINS INVOLVED IN PROLIFERATION CONTROL BY T3

cyclin D1

As mentioned before, *cyclin D1* gene encodes the regulatory subunit of a holoenzyme that phosphorylates and inactivates the RB protein and promotes progression through the G_1 phase of the cell cycle. However, recent analyses of knock-out mice lacking cyclins D revealed that these proteins are absolutely required for proliferation only in selected cell types (Kozar & Sicinski, 2005). In addition, cyclin D1 associates with, and regulates activity of, transcription factors, coactivators, corepressors, and chromatin remodeling proteins. It also regulates cellular metabolism and migration (Fu *et al*., 2004.).

The first observations regarding the influence of T3 on cyclin D1 levels were made in neuroblastoma N2a-β cells overexpressing TRβ1. Incubation of these cells with T3 resulted in a rapid decrease of cyclin D1 mRNA and protein levels (Perez-Juste & Aranda, 1999). Similar effects were observed in mammary epithelial cells (Gonzalez-Sancho *et al*., 2002). Insights into the molecular mechanism of T3 action on *cyclin D1* transcription were provided by experiments performed on N2a-β cells. It has been shown that no classic TRE was present in *cyclin D1* promoter. However, when the cyclic AMP response element (CRE) was deleted, T3–TR-mediated repression was completely abolished suggesting that it was executed *via* the binding to CRE-binding proteins CREB (CRE-binding protein) or ATF-2 (activation transcription factor 2). Since no change in the abundance of these proteins was detected, most possibly T3–TR complex altered not their expression, but their transactivation activity (Garcia-Silva & Aranda, 2004).

It is noteworthy that the opposite was observed in rat pituitary growth hormone-producing GC cells where T3 treatment caused shortening of G_1 phase of the cell cycle accompanied by the increase of mRNA and protein levels of cyclin D1, cyclin E, and cdk2. In addition, kinase activities associated with cyclins D1 and E increased 4-fold upon T3 treatment leading to the increase of RB phosphorylation (Barrera-Hernandez *et al*., 1999). A very fast increase of cyclin D1 mRNA and protein levels upon T3 administration accompanied by accelerated DNA synthesis and a 20-fold increase in mitotic activity was also observed in hepatocytes (Pibiri *et al*., 2001) and in cardiomyocytes of adult rats, where it was accompanied by translocation of cyclin D1 into the nuclei (Ledda-Columbano *et al*., 2006). No molecular explanation of this phenomenon was presented.

c-Fos

The members of the Fos protein family might be divided in two groups, according to their ability to transform rodent fibroblasts: transforming (c-Fos and FosB) and non-transforming (Fra-1 and Fra-2) proteins. c-Fos and FosB contain multiple transactivation modules in their N- and C-terminal parts (Tulchinsky, 2000). They form dimers with Jun or ATF proteins, and as AP-1 transcription factor, regulate gene expression in response to cytokines, growth factors, stress signals, as well as to oncogenic stimuli. Contribution of AP-1 to cell fate in response to a given stimulus depends on the relative abundance of AP-1 subunits, the composition of AP-1 dimers, the cell type, and the cellular microenvironment (Hess *et al*., 2004).

In the presence of a physiological amount of T3 (1 nM) c-Fos mRNA and protein levels are reduced. Transactivation assays with reporter vector containing *c-Fos* promoter showed that promoter activity decreased by 50–60% after 8 h of T3 treatment. This effect was magnified in the presence of overexpressed TRα in a ligand dependent manner. It is most likely that this effect was due to transcriptional interference of liganded TR with AP-1 transcription activator whose recognition site is present in *c-Fos* promoter (Perez *et al*., 1993). This hypothesis was confirmed by the same authors who showed that the repression of *c-Fos* gene by TRs was exerted through transcriptional interference with the AP-1 binding site and the serum response element (SRE) of this promoter (Perez *et al*., 1994). In accordance with these data it has been shown the hypothyroidism was associated with a marked increase of c-fos mRNA in rat cardiomyocytes. This effect could be reversed by T3 replacement (Green *et al*., 1991).

However, other authors showed that T3 treatment triggered a rapid, transient, and strong stimulation of c-Fos and FosB mRNA expression in Schwann cells (but not in NIH-3T3 and CHO cell lines). This was accompanied by an increase of c-Fos protein amount. Functionally active TRs were indispensable for this to occur (Mercier *et al*., 2001).

SUGGESTED REGULATION OF TRANSCRIPTION OF THE GENES ENCODING PROTEINS INVOLVED IN PROLIFERATION CONTROL BY T3 *VIA* YET UNKNOWN MECHANISMS

TGF-β

Transforming growth factor-β was initially identified and named on the basis of its ability to

stimulate fibroblast growth in soft agar, but it is now the best-studied growth inhibitory protein. The TGF-β superfamily of versatile cytokines consists of nearly 30 members. TGF-β binds to a cell-surface receptor that activates Smad signal transduction pathways. Smads assemble multisubunit complexes that regulate transcription (Shi, 2001; Miyazawa *et al*., 2002). The members of the TGF-β family exert many different effects depending on the type and state of the cell (Bachman & Park, 2005). For example, TGF-β can stimulate the proliferation of mesenchymal cells, but it can also act as a growth-inhibitory factor for epithelial, lymphatic, hematopoetic, and endothelial cells.

Treatment with T3 of hepatic HepG2 cells, stably expressing TRs, caused inhibition of their proliferation by arresting them at the transition point between G_1 and S phases. In addition to other alterations, a stimulation of TGF-β on mRNA level was observed. The activity of its promoter was enhanced by T3 up to 8-fold, but this effect was dependent on the presence of the active TRs. It has been also shown that TGF-β neutralizing antibodies, and not control antibodies, could reverse anti-proliferative effect of T3 in HepG2 cells proving that this was TGF-β-mediated mechanism (Yen *et al*., 2006).

Cip/Kip family

As mentioned before, Cip/Kip family members inhibit cdk2 and are required for the assembly of cyclin D-cdk4, -cdk6 complexes, and progression of the cell cycle. The family is well conserved phylogenetically, suggesting its biological importance (Sherr, 2000; Vidal & Koff, 2000).

It has been shown that the incubation of N2a-β neuroblastoma cells with T3 resulted in a strong and long-lasting increase of $p27^{Kip1}$ mRNA amount suggesting a role for T3 in transcriptional regulation of its gene. However, no TRE was found in $p27^{Kip1}$ promoter. Interestingly, the $p27^{Kip1}$ protein half-life was lengthened in T3-treated N2a-β cells leading to its accumulation. This, in turn, increased the level of $p27^{Kip1}$-cdk2 complexes and led to a marked inhibition of the kinase activity of the cyclin E-cdk2 complexes. As a result, RB protein was hypophosphorylated and this caused cell cycle arrest (Perez-Juste & Aranda, 1999). In hepatoma cells, the $p21^{Cip1}$ protein or mRNA levels were up-regulated 5-fold or 7-fold, respectively, following T3 treatment (Yen *et al*., 2006). In contrast, depletion of T3 induced $p21^{Cip1}$ expression in astrocytoma cells. This could be reversed by exogenous T3 (Toms *et al*., 1998).

Transient neonatal hypothyroidism resulted in prolonged postnatal Sertoli cell mitogenesis and doubled adult Sertoli cell numbers. $p27^{Kip1}$ immunostaining was reduced in Sertoli cells from hypothyroid mice, while hyperthyroidism increased $p27^{Kip1}$ immunostaining relative to controls from euthyroid animals, suggesting that T3 effects on Sertoli cells proliferation may be mediated by this protein (Holsberger *et al*., 2003). This result was confirmed by other authors who showed that T3 treatment induced an accumulation of $p27^{Kip1}$ and $p21^{Cip1}$ in these cells (Buzzard *et al*., 2003). A similar observation was made in HepG2 cells where T3 treatment resulted in $p21^{Cip1}$ mRNA and protein accumulation (Yen *et al*., 2006) and in N2a-β cells where T3 treatment resulted in a strong and sustained increase of the levels of the $p27^{Kip1}$(Garcia-Silva *et al*., 2002). This caused a marked inhibition of cyclin-cdk2 complexes, hypophosphorylation of RB and inhibition of the cell cycle progression. On the other hand, hyperthyroidism caused the decrease of $p27^{Kip1}$ level in rat liver after partial hepatectomy (Alisi *et al*., 2005). The precise molecular mechanism of tissue specific $p27^{Kip1}$ and $p21^{Cip1}$ regulation by T3 is not known.

Epidermal growth factor (EGF) and its receptor (EGFR)

EGF was one of the first growth stimulating peptides described. It has a characteristic structure with three disulfide bridges, which is essential for its activity (Carpenter & Cohen, 1990; Van Zoelen *et al*., 2000). The receptor for EGF (EGFR or ErbB-1 or HER-1) is a member of the ErbB family of receptor tyrosine kinases. It is a cell-surface protein consisting of an extracellular ligand-binding domain, a single transmembrane region, and an intracellular domain with tyrosine kinase activity. Activation of the receptor occurs when a ligand, such as EGF or transforming growth factor-α binds to the ectodomain of the receptor, resulting in its dimerization, activation of the intracellular kinase domain, autophosphorylation, and activation of downstream signaling molecules (Yarden & Sliwkowski, 2001).

The treatment of mouse submandibular gland cells by T3 resulted in a rapid increase of EGF mRNA and protein. In hypothyroid mice the amount of EGF mRNA in these cells was low and increased after T3 administration in a dose-dependent manner (Fujieda *et al*., 1993). T3 exposure led to a significant increase of EGF-promoted DNA synthesis in renal tubule cells. The level of EGFR mRNA markedly increased in proximal tubule cells upon treatment with this hormone. This was accompanied by increase of EGFR protein on the cell surface (Humes *et al*., 1992).

A significant increase in the total tyrosine kinase activity measured in bovine thyroid cell protein extracts was induced by physiological concentrations of T3. Tyrosine phosphorylation of EGFR was significantly stimulated by this hormone. However, the level of EGFR protein was not changed by T3 treatment as determined by Western blot (Di Fulvio *et al*., 2000).

INTERACTIONS OF THYROID HORMONE RECEPTORS WITH PROTEINS INVOLVED IN CELL CYCLE CONTROL

p53

The tumor suppressor p53 is a short-lived phosphoprotein present in low amounts in the nuclei of normal cells. *p53* expression, protein level and activity are regulated in a cell cycle-dependent manner. It plays a central role in the protection against permanent DNA damage and other consequences of physiological stress (Cadwell & Zambetti, 2001). When the DNA is damaged, p53 induces the expression of $p21^{Cip1}$ and G_1 arrest. p53 either forms homotetramers that act as a transcription factors, or interacts with other proteins leading to the induction of cell cycle arrest or to the induction of apoptosis (Levine, 1997; North & Hainaut, 2000). p53 inactivation (most commonly due to mutation) is considered a key event in human carcinogenesis.

A physical interaction between the DNA binding domain of TRβ1 and p53 was initially detected (Yap *et al*., 1996). Subsequently, it has been shown that DNA binding domain and the carboxy terminus of p53 were involved in this interaction (Barrera-Hernandez *et al*., 1998). TRβ1 increased the binding of p53 to its recognition sites in the promoters of target genes. This resulted in the repression of p53-dependent activation of certain genes such as *bax* and *gadd45*, while transcription of others was not affected (Barrera-Hernandez *et al*., 1998). Other authors show that overexpression of p53 blocks TR-mediated constitutive activation of transcription that is not dependent on the presence of T3, and suggest that this is due to the reversal of p53-mediated repression (Qi *et al*., 1997).

Thyroid hormones may influence the function of p53 by altering its expression, as shown in T47D human breast ductal carcinoma cell line where p53 amount increased in a T3 concentration-dependent manner (Dinda *et al*., 2002), or by affecting its post-transcriptional modification, as shown in T4-treated HEK293 and in HeLa cells that do not express TRs. Such treatment promoted p53 phosphorylation by mitogen-activated protein kinase (MAPK) (Shih *et al*., 2001).

OTHER FUNCTIONS OF T3 IN CELL CYCLE CONTROL

Counteracting Ras-induced proliferation

The *Ras* protooncogenes encode GTP-binding proteins (H-, K-, and N-Ras) acting at the cellular membrane. Ras activate several pathways of signal transduction including the mitogenic Ras/mitogen-activated protein kinase (MAPK) pathway. While Ras has usually been regarded as a transforming oncogene, its activation in normal cells causes growth arrest *via* the induction of p53 and cyclin-dependent kinase inhibitors (Crespo & Leon, 2000).

It has been shown that hypothyroidism was associated with a marked increase in myocardial H-ras mRNA. This effect was reversed by T3 treatment. Administration of T3 to euthyroid rats resulted in inhibition of H-ras mRNA (Green *et al*., 1991).

T3 inhibits Ras-induced proliferation of neuroblastoma cells. This hormone is able to block the response to the oncogenic forms of all three Ras isoforms by interfering with the activity of the Ras/Erk/Rsk pathway and CRE-mediated transcription (T3 inhibits transcriptional activity of b-Zip transcription factors such as CREB and ATF-2 that are direct targets of Rsk2), and both TRα and TRβ can mediate this action. As a result, Ras-dependent induction of *cyclin D1* and of other genes containing CRE element in their promoters is markedly decreased in the presence of T3 (Garcia-Silva & Aranda, 2004).

Induction of secretion of mitogenic factors

This effect of T3 was observed in the central nervous system. Cerebellar astrocytes and C6 glioma cells (immortalized glial cells with properties of both astrocytes and oligodendrocytes) treated with T3 secrete basic fibroblast growth factor, and, to a lesser extent, acidic fibroblast growth factor, tumor necrosis factor-β and TGF-β (Trentin *et al*., 2001). Cerebellar astrocytes also secrete EGF which directly induces neuronal proliferation (Martinez & Gomes, 2002; Martinez & Gomes, 2005).

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