

# Expression of thyroid hormone receptor $\alpha$ in 3T3-L1 adipocytes; triiodothyronine increases the expression of lipogenic enzyme and triglyceride accumulation

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## Abstract

Thyroid hormone receptors (TR) are members of the nuclear receptor superfamily. There are at least two TR isoforms, TR $\alpha$  and TR $\beta$ , which act as mediators of thyroid hormone in tissues. However, the relative expression of each TR isoform in target tissues is still elusive. Herein, we have developed an RT-PCR and restriction enzyme digestion method to determine the expression of TR $\alpha$ 1 and TR $\beta$ 1. We analyzed the expression of TR isoforms in 3T3-L1 preadipocytes induced to differentiate by an adipogenic cocktail in the presence or absence of 100 nM triiodothyronine (T<sub>3</sub>). The TR $\alpha$ 1 isoform was predominantly expressed in 3T3-L1 adipocytes, and its expression was increased at the stage of development concomitant with the emergence of lipid droplets. Little, if any, TR $\beta$ 1 mRNA was detected in adipocytes. Administration of T<sub>3</sub> to the differentiating 3T3-L1 cells enhanced the accumulation of triglyceride. The expression profile of TR $\alpha$ 1 in

T<sub>3</sub>-treated adipocytes was similar to that in non-treated cells. The transcripts of adipogenic factors, CCAAT/enhancer binding protein  $\beta$  (C/EBP $\beta$ ) and peroxisome proliferator activated receptor  $\gamma$  (PPAR $\gamma$ ), were not altered by T<sub>3</sub>. Lipid binding protein, aP2, that is downstream of these transcription factors was also unaffected by T<sub>3</sub>. In contrast, the lipogenic enzyme, glyceraldehyde-3-phosphate dehydrogenase mRNA was significantly increased in the presence of T<sub>3</sub>. Therefore, T<sub>3</sub> appears to be a hormone capable of modulating the expression of lipogenic enzyme and augments the accumulation of lipid droplets. We conclude that the TR $\alpha$  isoform might play an important role in the generation and maintenance of the mature adipocyte phenotype, regulating the expression of lipogenic enzymes.

*Journal of Endocrinology* (2004) **182**, 295–302

## Introduction

Thyroid hormone, 3,5,3'-triiodothyronine (T<sub>3</sub>), exerts profound effects via nuclear thyroid hormone receptors (TRs) on energy metabolism, homeostasis, development and differentiation in all vertebrates (Evans 1988, Lazar 1993). There are at least two thyroid hormone receptor (TR) genes, designated as TR $\alpha$  and TR $\beta$ , each of which generates two or more mRNAs and polypeptide products through alternate splicing or alternative promoter (Sap *et al.* 1986, Weinberger *et al.* 1986, Benbrook & Pfahl 1987, Wrutniak *et al.* 1995). To date, three functional TRs have been described: TR $\alpha$ 1, TR $\beta$ 1 and TR $\beta$ 2, which can bind T<sub>3</sub> and regulate transcription by binding to specific thyroid hormone response elements within promoters of their target genes (Lavin *et al.* 1988, Harvey & Williams 2002). In addition, a variant molecule, TR $\alpha$ 2, does not bind T<sub>3</sub> and may act to inhibit T<sub>3</sub> action due, in

part, to missing a critical portion of the amino terminal ligand-binding region (Koenig *et al.* 1989, Lazar *et al.* 1989). Despite a high degree of structural homologies, minor divergence in the amino-terminal sequences of TR isoforms may modulate an isoform-dependent function.

The extensive and tissue-specific action of thyroid hormone has been well documented, but little is known about the relative contribution of each TR isoform to T<sub>3</sub> action in target tissues. Initial attempts to determine the role of each of the TR proteins indicated the wide distribution of TR $\alpha$ 1, TR $\alpha$ 2, and TR $\beta$ 1 mRNAs among target tissues, whereas TR $\beta$ 2 mRNA is limited to the anterior pituitary or to a certain region of the central nervous system (Thompson *et al.* 1987, Hodin *et al.* 1989, Wood *et al.* 1991). Schwartz *et al.* (1992) compared the concentration of TR $\alpha$  and TR $\beta$  mRNA to nuclear T<sub>3</sub> binding capacity in rat tissues by using specific antibodies against each TR type. Marked variation in the ratio of the

expression of TR isoforms among tissues raises the question of redundancy and specificity of TR isoforms in target tissues.

Adipose tissue that consists of white adipose tissue (WAT) and brown adipose tissue (BAT) plays a vital role in lipid metabolism and energy balance. Thyroid hormone modulates the development and metabolism of adipose tissue in both physiological and pathological conditions. Previous studies showed that the numbers of adipocytes were increased or decreased in hyper- or hypothyroid animals (Picon & Levacher 1979). Recently, it has been reported that thyroid hormone is an adipogenic factor in the Ob17 adipocyte cell line where T<sub>3</sub> regulated adipocyte proliferation, as well as induced preadipocyte differentiation (Darimont *et al.* 1993, Gharbi-Chihi *et al.* 1993). On the other hand, some studies supported the view that thyroid hormone increased basal oxygen consumption and lipolysis (Oppenheimer *et al.* 1991, Viguier *et al.* 2002). The contradictory data might be obtained by using different cell types or distinct experimental conditions, which indicate the intricate effects of thyroid hormone on adipose tissue. It has been shown that TRs are expressed in WAT and BAT (Reyne *et al.* 1996), but the precise isoform-dependent function in adipose tissue has not been clarified.

In the present study, we have developed an RT-PCR and restriction enzyme digestion method to define the relative roles of the TR $\alpha$ 1 and TR $\beta$ 1 isoforms in 3T3-L1 adipocytes. Our observations suggest that TR $\alpha$ 1 plays a role in adipocytes through regulating the expression of lipogenic enzymes.

## Materials and Methods

### Cell culture and differentiation

The mouse 3T3-L1 preadipocytes were obtained from the American Type Culture Collection (Manassas, VA, USA) and were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (Gibco), 100 U/ml penicillin and 100 µg/ml streptomycin in 24-well plates or 100 mm<sup>2</sup> plates. Two days after cell confluence (designated as day 0), differentiation was initiated with 1 µg/ml insulin, 1 µM dexamethasone (DEX), and 0.5 mM isobutylmethylxanthine (IBMX) in DMEM containing 10% fetal bovine serum (FBS) in the presence or absence of 100 nM T<sub>3</sub>. After incubation for 2 days (day 2), the culture media were replaced with DMEM supplemented with 10% FBS and 1 µg/ml insulin, and the cells were then fed every two days with DMEM containing 10% FBS. 3T3-L1 cells were fully differentiated by day 8. For T<sub>3</sub>-treated cells, T<sub>3</sub> was added to the media over the full course of the differentiation.

In the above mentioned experiments, FBS was depleted from T<sub>3</sub> by anion exchange resin (Teboul *et al.* 1991).

### Oil red O staining

3T3-L1 cells were grown on 24-well plates and induced to differentiate as described above. After incubation for 8 days (day 8), plates were washed three times with phosphate-buffered saline (PBS), fixed with 7% formaldehyde for 5 min at room temperature, then replaced with fresh fixing solution and incubated for at least one hour. After fixation, cells were stained with a filtered oil red O solution (0.5 g oil red O (Sigma) in 100 ml isopropanol) for 15 min at room temperature. Cells were then washed twice with distilled water and visualized under the microscope.

After the microscopic examination, we quantified the amount of triglyceride in each well. Isopropanol (300 µl/well) was added to the staining plates and the plates were incubated for 10 min at room temperature, then the optical density (OD) of the solution at 500 nm was measured.

### To distinguish between TR $\alpha$ and TR $\beta$ isoforms using RT-PCR and the restriction enzyme digestion method

We designed one common primer set for the amplification of the ligand-binding domain (LBD) of the TR $\alpha$  and TR $\beta$  isoforms. The primer sequences are: TRs P1 5'-GAAGACCAGATCATCCTCCT-3' and P2 5'-AGG AAGCGGCTGGC-3'. PCR amplification of both TR $\alpha$  and TR $\beta$  isoforms produces a 426 bp product, but after digestion with the TR $\alpha$ -specific restriction enzyme, Pst I, the TR $\alpha$  transcript is converted into two fragments of 222 bp and 204 bp; meanwhile, the TR $\beta$  transcript is left intact as a 426 bp fragment.

### RNA isolation and RT-PCR

Total RNA was extracted from 3T3-L1 cells harvested at the indicated time points during differentiation using an RNeasy kit (Qiagen) and quantified by spectrophotometry. First-strand cDNA was synthesized by MuLV reverse transcriptase with oligo (dT) primer (Roche). The cDNA was used as a template for PCR to amplify the specific product. The sequences of each set of primers are listed below: peroxisome proliferator activated receptor  $\gamma$  (PPAR $\gamma$ ) P1, 5'-ATGTCTCATAATGCCATC-3' and P2, 5'-CTAGTACAAGTCCTTGTA-3'; CCAAT/enhancer binding protein  $\beta$  (C/EBP $\beta$ ) P1, 5'-ACGAC TTCCTCTCCGAC-3' and P2, 5'-GCAGCTGCTT GAACAAG-3'; aP2 P1, 5'-GAACCTGGAAGCTT GTC-3' and P2, 5'-ACTCTTGTGGAAGTCACG-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) P1, 5'-CCAGAACATCATCCCTG-3' and P2, 5'-TTA CTCTTGGAAAGGCC-3'; elongation factor- $\alpha$  P1, 5'-CCATGAAGCTTTGAGTGAAGCTCT-3' and P2, 5'-TAGCCTTCTGAGCTTTCTGGGCAG-3'. Each set of primers was designed to include at least one intron in

order to allow the discrimination of contaminating genomic DNA from cDNA. In control experiments, RT-PCR was performed under the same experimental conditions but lacking reverse transcriptase. RT-PCR products were separated by 1% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate pH 8.0, 2 mM EDTA) and stained with ethidium bromide. Identities of the PCR products were confirmed by using an ABI 310 sequencer (Applied Biosystems, Foster City, CA, USA).

#### Northern blot analysis

Total RNA was isolated from 3T3-L1 cells at the indicated time points during the differentiation process using the RNeasy kit (Qiagen). Fifteen micrograms total RNA were size fractionated in 1% denaturing agarose-formaldehyde gel, then transferred to a Hybond-N+ nylon membrane (Amersham Pharmacia Biotech) and cross-linked with UV light (Stratalinker, Stratagene, La Jolla, CA, USA).

Hybridizations were performed in ExpressHyb<sup>TM</sup> hybridization solution (Clontech, Palo Alto, CA, USA) with cDNAs labeled with [<sup>32</sup>P]dCTP using a random hexamer labeling kit (Amersham Pharmacia Biotech). The results were visualized using a Phosphor Imager (Fuji BAS 1500). Blots were stripped and reprobed with cDNA for elongation factor- $\alpha$  that was considered as an internal control.

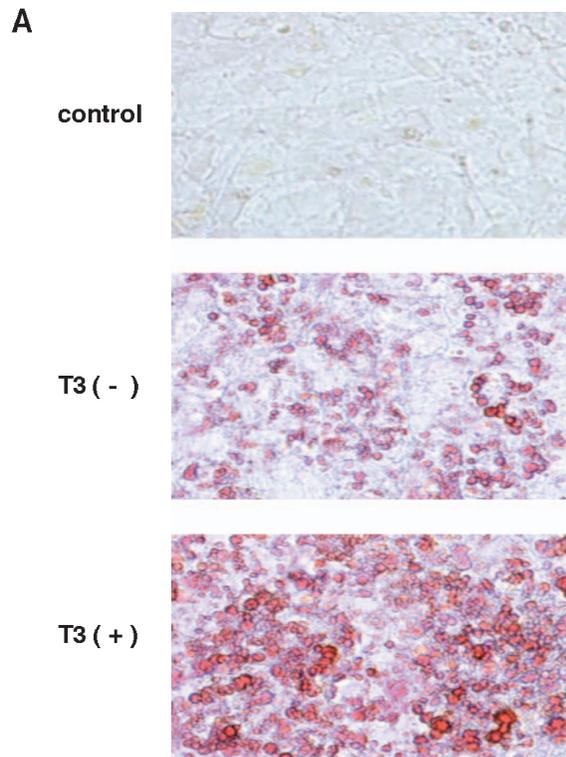
#### Statistical Analysis

Comparison of the accumulation of triglyceride from 3T3-L1 cells grown in the absence or presence of T<sub>3</sub> was performed using the unpaired Student's *t* test. A *P* < 0.05 was considered as statistical significance. The data represent as mean  $\pm$  S.D.

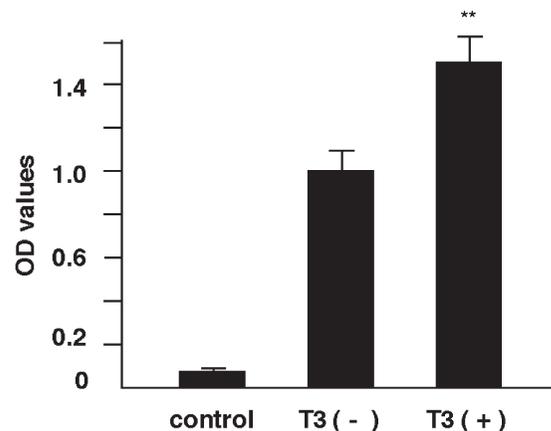
## Results

### T<sub>3</sub> increases the accumulation of lipid droplets in 3T3-L1 adipocytes

To assess the role of thyroid hormone receptors in adipocytes, we first tried to examine the effect of thyroid hormone on the differentiating 3T3-L1 cells. Two days after cell confluence (day 0), differentiation was induced by adipogenic stimuli in the absence or presence of 100 nM T<sub>3</sub>. Cytoplasmic triglyceride droplets were visible by day 3. The oil red O staining of these cells on day 8 is shown in Fig. 1A. In the presence of an adipogenic cocktail, 3T3-L1 cells revealed a morphological appearance of mature adipocytes, converting from a fibroblast-like to a spherical shape and containing large lipid-laden droplets in the cytoplasm (Fig. 1A). Compared with non-treated cells, T<sub>3</sub>-treated adipocytes exhibited more

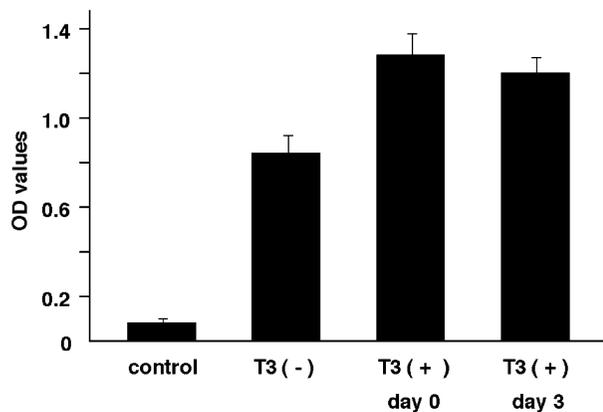


## B



**Figure 1** Effect of T<sub>3</sub> on triglyceride accumulation. (A) Microscopic analysis of 3T3-L1 cells stained with oil red O, 8 days after inducing differentiation with DEX, IBMX and insulin, in the absence (-) or presence (+) of 100 nM T<sub>3</sub> (T<sub>3</sub>). (B) Quantification of the amount of triglyceride. The data represent means  $\pm$  S.D. of four independent experiments. \*\**P* < 0.001 statistical difference between T<sub>3</sub> (-) and T<sub>3</sub> (+). Control, cells were cultured in the media without adipogenic mixture and T<sub>3</sub>.

extensive deposits of lipid droplets (Fig. 1A), but the time course of differentiation was unchanged. We then quantified the amount of triglyceride and found that



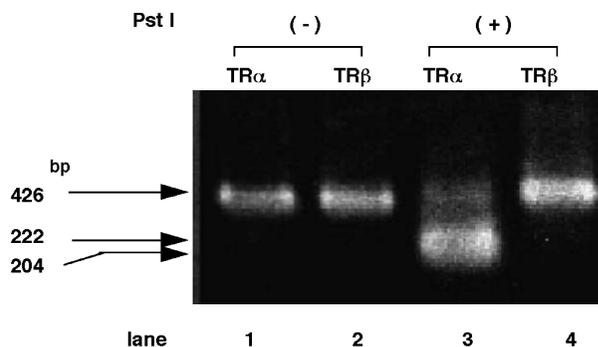
**Figure 2**  $T_3$  exerts its effect at the late stage of differentiation. Quantitative analysis of triglyceride accumulation. 3T3-L1 preadipocytes were treated with differentiation-permissive medium (DEX, IBMX and insulin) and cultured for 8 days. The data are derived from two independent experiments and represent the means  $\pm$  S.D. Control, without adipogenic mixture and  $T_3$ ;  $T_3$  (-), cells were cultured in the differentiating media in the absence of 100 nM  $T_3$ ;  $T_3$  (+) day 0, 100 nM  $T_3$  were added to the differentiating media on day 0;  $T_3$  (+) day 3, 100 nM  $T_3$  were added to the differentiating media on day 3.

$T_3$  increased droplet accumulation by about 1.5-fold (Fig. 1B), indicating that thyroid hormone enhances lipid accumulation in mature adipocytes.

In general, the process of adipocyte differentiation can be divided into three stages: early, intermediate and late (Ntambi & Young-Cheul 2000). To ascertain at which stage  $T_3$  exerts its effect on triglyceride accumulation, 100 nM  $T_3$  were added to the media containing an adipogenic cocktail at various time points during differentiation, and the accumulation of lipid droplets in cytosol was monitored on day 8. Consistent with the results above, a similar increment of lipid accumulation was observed in adipocytes treated with  $T_3$  on the third day (day 3) (Fig. 2), suggesting that the addition of  $T_3$  to the media at the late stage of differentiation is sufficient for increasing lipid accumulation.

#### *The expression of TR $\alpha$ 1 in 3T3-L1 adipocytes*

Because the structure of the LBDs of TR $\alpha$ 1 and TR $\beta$ 1 have a high degree of similarity, a common primer set can be used to amplify both receptor isoforms. Although the PCR products from both transcripts are indistinguishable in length, the TR $\alpha$ 1 but not the TR $\beta$ 1 transcript has a unique Pst I restriction enzyme site in the middle region of the PCR products, and produces two fragments after restriction enzyme digestion (Fig. 3). Here we amplified the same array of the LBDs of TR $\alpha$ 1 and TR $\beta$ 1 from plasmid templates containing each cDNA in a single PCR reaction as a 426 bp product (Fig. 3, lane 1 and lane 2), using a common primer set. After digestion of the



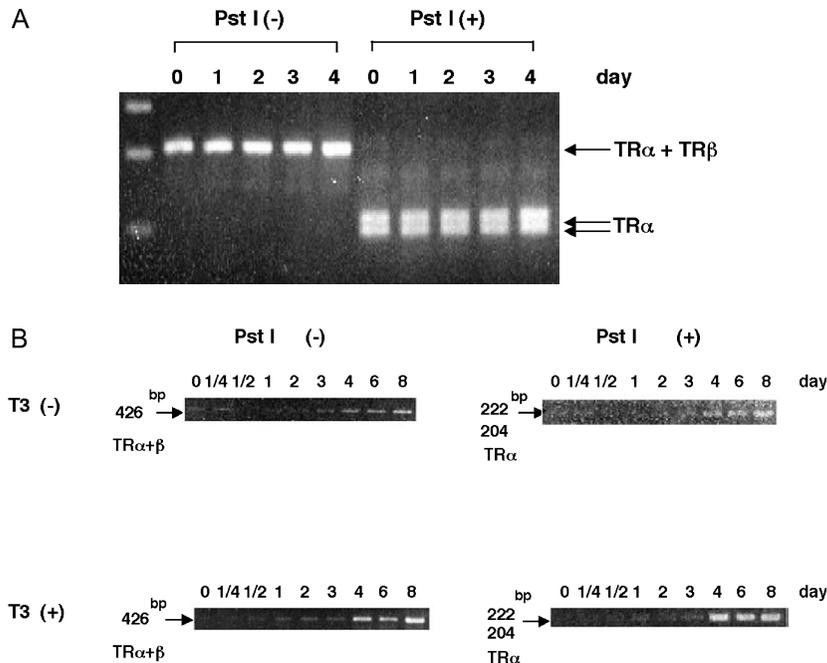
**Figure 3** Determination of the expression of TR $\alpha$  and TR $\beta$  using RT-PCR and the restriction enzyme digestion method. Amplification of the same array of the ligand-binding domains of TR $\alpha$  and TR $\beta$  from each cDNA template (lane 1, TR $\alpha$ ; lane 2, TR $\beta$ ), and digestion of the PCR products with Pst I restriction enzyme (lane 3, TR $\alpha$ ; lane 4, TR $\beta$ ).

PCR product with Pst I enzyme, the TR $\alpha$ 1 transcript produced fragments of 222 bp and 204 bp (Fig. 3, lane 3) while the 426 bp product of the TR $\beta$ 1 transcript remained intact (Fig. 3, lane 4). Thus, in these experiments, a reliable simplified method for the comparison of the expression of TR $\alpha$ 1 with TR $\beta$ 1 isoform has been established.

To determine the expression of TR $\alpha$ 1 and TR $\beta$ 1 in 3T3-L1 adipocytes, PCR amplifications were performed on cells submitted to synchronization experiments. Figure 4A illustrates the RT-PCR results obtained at different days of differentiation (day 0 to 4). It appears that TR was already present in preadipocytes, and the expression level of TR mRNA was increased on day 3 and day 4 after inducing differentiation. Pst I digestion converted almost all of the PCR products into 222 bp and 204 bp fragments, indicating that TR $\alpha$ 1 is predominantly expressed in 3T3-L1 preadipocytes and throughout differentiation. Strikingly, TR $\beta$ 1 mRNA was hardly detectable at any stage, even when PCR cycles were extended up to 35 cycles to increase the sensitivity (Fig. 4A). These results indicate the importance of TR $\alpha$ 1 isoform as a master regulator of thyroid hormone signaling in adipocytes. As shown in Fig. 4, TR $\alpha$ 1 was expressed at all stages examined and its expression level was increased at the stage concomitant with emergence of lipid droplets on day 3 and day 4, which is in close agreement with the results shown in Fig. 2. The expression pattern of the TR $\alpha$ 1 isoform was not altered by  $T_3$  throughout the entire period of differentiation (Fig. 4B). In control experiments, elongation factor- $\alpha$  mRNA levels were constant during the differentiation period.

#### *The effect of T $_3$ on the expression of adipogenic factors*

Adipocyte differentiation is a complex process modulated by a cascade of transcription factors including the PPAR $\gamma$



**Figure 4** RT-PCR analysis of the expression of TRs in differentiating 3T3-L1 adipocyte. (A) After 35 cycles of amplification, the PCR products were treated with (+) or without (-) Pst I, and analyzed by agarose gel electrophoresis. (B) RNA was prepared from 3T3-L1 cells cultured in the absence (-) or presence (+) of 100 nM T<sub>3</sub>. Expression of TRs during the differentiation were analyzed by RT-PCR with 30 cycles of amplification.

and C/EBPs families (Brun *et al.* 1996, Schwarz *et al.* 1997, Rosen *et al.* 2000). These results led us to investigate whether T<sub>3</sub> regulates the expression of these transcription factors.

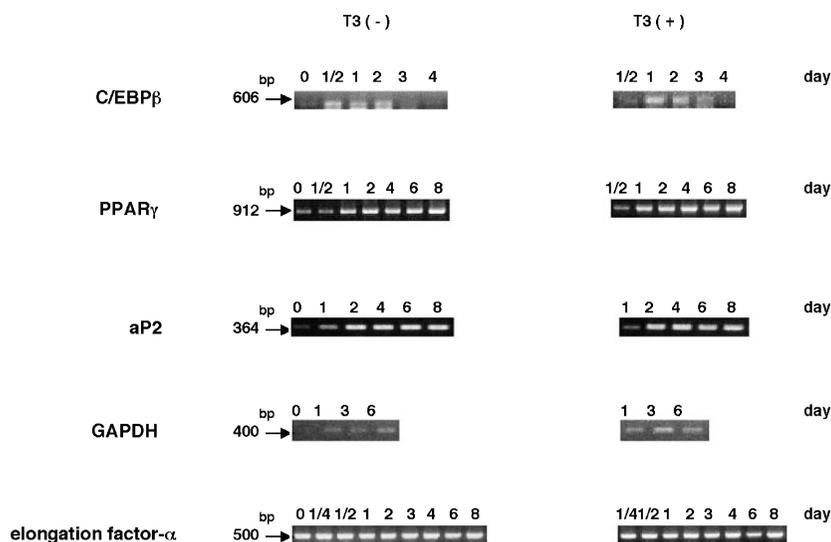
To gain insight into the molecular mechanism of thyroid hormone action in 3T3-L1 cells, RT-PCR was performed with RNA samples isolated at various time points of the differentiation process. Figure 5 illustrates the expression profiles of adipogenic factors during differentiation in the absence or presence of 100 nM T<sub>3</sub>. The expression of C/EBP $\beta$  was increased very early and transiently under standard conditions as reported by Ntambi & Young-Cheul (2000). The expression level of C/EBP $\beta$  was not affected by the administration of T<sub>3</sub>. The transcript for PPAR $\gamma$ , whose induction may be regulated by C/EBP $\beta$ , began to be increased as early as day 1 and rose progressively during the differentiation process. As shown in Fig. 5, no significant difference in the expression pattern of the PPAR $\gamma$  transcript was detected between the absence and presence of T<sub>3</sub>. The adipocyte-selective fatty acid binding protein, aP2, that is known to be downstream of these adipogenic transcription factors, was increased over the basal level from day 2 to day 8. The expression of aP2 was also unaffected by T<sub>3</sub>. However, T<sub>3</sub> increased the expression of the lipogenic enzyme GAPDH involved in lipid metabolism at the terminal phase of differentiation. In control experiments, elongation factor- $\alpha$  mRNA levels were expressed constantly.

As shown in Fig. 6, similar results were obtained in Northern blot analysis. We could not detect any considerable alteration by T<sub>3</sub> in the expression of the adipogenic factors C/EBP $\beta$  and PPAR $\gamma$ , nor in their downstream factor, aP2. However, the peak levels and also the basal expression levels of the GAPDH transcript were augmented by the addition of T<sub>3</sub>. Quantitative determination using Phospho Imager revealed that T<sub>3</sub> increased the expression of GAPDH by 2-fold and 1.7-fold on days 3 and 4 respectively (Fig. 7). Elongation factor- $\alpha$  mRNA expression was not altered by T<sub>3</sub> (data not shown).

## Discussion

As lipid metabolism is closely associated with a number of health problems, regulation of adipocytes represents an area of emerging interest. So far, TR has been implicated as a major factor in the regulation of the development and function of adipose tissue (Flores-Delgado *et al.* 1987, Chawla & Lazar 1993, Dace *et al.* 1999, Yen 2001). In this paper, we demonstrate that triiodothyronine increases the accumulation of lipid droplets and the expression of lipogenic enzyme in 3T3-L1 adipocytes. Moreover, we show that the TR $\alpha$  isoform is predominantly expressed in adipocytes, suggesting that TR $\alpha$  seems to be involved in a complex process of lipid accumulation in adipocytes.

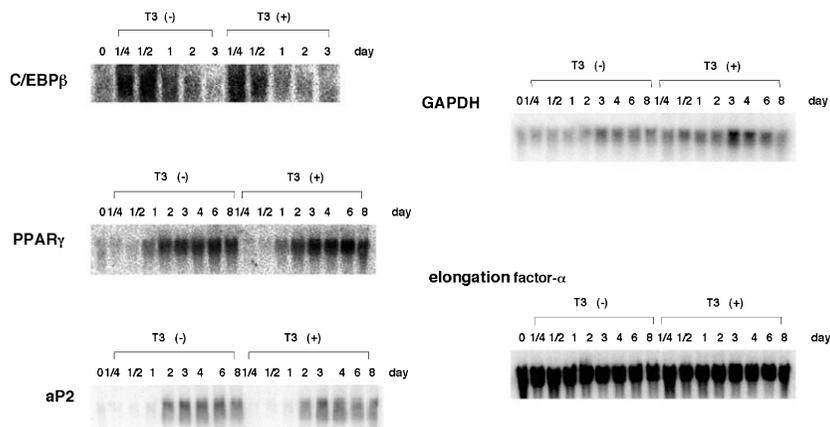
The mouse 3T3-L1 preadipocyte is a well-established model for studying adipogenesis *in vitro* (Green & Meuth



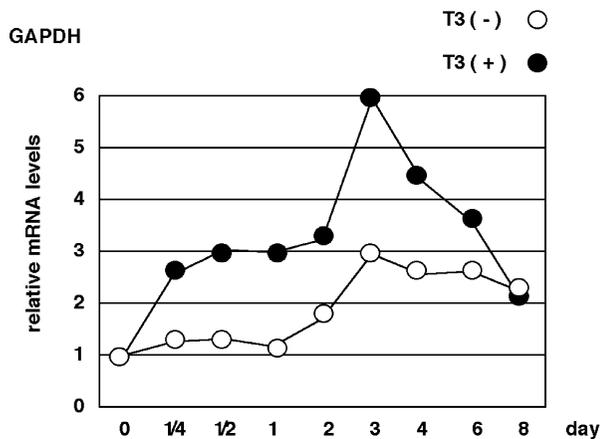
**Figure 5** RT-PCR analysis of the mRNA of adipogenic factors in the absence (-) or presence (+) of T<sub>3</sub> (T3). Total RNA was prepared from 3T3-L1 cells at different time points of the differentiation process in culture under conditions lacking T<sub>3</sub> (left side) and with thyroid hormone (right side). The transcripts were estimated by RT-PCR analysis. The transcripts of elongation factor- $\alpha$  are shown as an internal control.

1974). When 100 nM T<sub>3</sub> were added to the 3T3-L1 cells in the presence of adipogenic mixture, the numbers of triglyceride droplets were significantly augmented at the terminal phase. We tried to explain which TR isoform mediates the action of thyroid hormone in 3T3-L1 adipocytes. There are several reports regarding the determination of the expression of TR isoforms using specific antibodies or specific probes (Strait *et al.* 1990, Schwartz *et al.* 1992). In the present study, we have employed a simple and reliable method to determine the relative amount of TR $\alpha$ 1 and TR $\beta$ 1 transcripts in the cells. It has

been shown that both TR $\alpha$  and TR $\beta$  are expressed in Ob17 preadipocytes, although there is a predominance of TR $\alpha$  (Teboul *et al.* 1991, Dace *et al.* 1999). In these experiments, we have come to the same conclusion that the TR $\alpha$  subtype is the predominant TR transcript in 3T3-L1 adipocytes. Because of its extremely low expression, the role of the TR $\beta$  isoform is less clear; however, TR $\beta$  might maintain a basal responsiveness to thyroid hormone in adipocytes (Dace *et al.* 1999). Furthermore, we have shown that TR $\alpha$  expression is increased at the time of conversion from the intermediate to the late stage



**Figure 6** Northern blot analysis of mRNA of adipogenic factors in the absence (-) or presence (+) of T<sub>3</sub> (T3). The mRNA samples from Fig. 5 were analyzed by Northern blot. Elongation factor- $\alpha$  levels are shown to demonstrate that equivalent amounts of RNA were loaded in each lane.



**Figure 7** Quantitative analysis of the expression of GAPDH in the absence (-) or presence (+) of T<sub>3</sub> (T<sub>3</sub>). The abundance of GAPDH transcripts was estimated by quantitative Imager analysis and normalized with elongation factor- $\alpha$  mRNA levels, in the absence of T<sub>3</sub> (open circles) and in the presence of T<sub>3</sub> (closed circles). The relative mRNA level of GAPDH at day 0 was estimated as 1.

of differentiation, which is coincident with the accumulation of lipid droplets, suggesting a prominent role of TR $\alpha$  isoform in the generation and maintenance of the adipocyte phenotype. Compared with previous reports showing that T<sub>3</sub> is an adipogenic factor necessary at an early stage of differentiation in Ob17 cells, we found that T<sub>3</sub> alone could not promote the conversion from preadipocyte to mature adipocyte (data not shown). These results suggest that T<sub>3</sub>, by itself, is not an adipogenic hormone in 3T3-L1 cells, and that the role of T<sub>3</sub> in adipocytes might differ between cell types.

To understand the role of TRs in 3T3-L1 adipocytes, mRNA expression of the adipogenic factors was monitored. It is known that up-regulation of lipogenic enzymes is required for lipid storage (Dugail *et al.* 1992, Briquet-Laugier *et al.* 1994, Ratledge 2001). GAPDH is one of the lipogenic enzymes involved in glycolysis, which is a major metabolic pathway for providing lipogenic substrates (Barroso *et al.* 1999). Our results showed that administration of T<sub>3</sub> to the differentiating 3T3-L1 cells augmented the accumulation of lipid droplets and significantly increased transcripts of GAPDH and glycerol-3-phosphate dehydrogenase (data not shown). Similar regulation of malic enzyme in adipocytes was also documented in previous studies (Flores-Delgado *et al.* 1987, Lorenzo *et al.* 1988). Collectively, we speculate that T<sub>3</sub> enhances lipid accumulation in 3T3-L1 adipocytes by inducing the expression of these lipogenic enzymes. In the present experiments, we could not elucidate the molecular mechanism whereby T<sub>3</sub> could regulate the transcript of GAPDH. There is an hypothesis based on the fact that the promoter region of malic enzyme has response elements for TR and is regulated by thyroid hormone (Petty *et al.*

1990, Desvergne *et al.* 1991), and a similar mechanism might be involved in the regulation of GAPDH by T<sub>3</sub> (Barroso *et al.* 1999).

By contrast, T<sub>3</sub> does not affect the expression of an early transcription factor, C/EBP $\beta$ , which belongs to a family of transcription factors containing a highly conserved basic/leucine zipper (bZIP) domain and has been implicated in transcriptional control of cell growth and differentiation (Porse *et al.* 2001). This observation might be in conflict with earlier investigations showing that C/EBP $\beta$  and C/EBP $\alpha$  are regulated by thyroid hormone in the rat liver (Menendez-Hurtado *et al.* 2000). It is possible that hormonal control of C/EBP genes is regulated in a tissue- or cell-specific manner. Furthermore, thyroid hormone does not alter the expression of PPAR $\gamma$ , which is considered to be a potent adipogenic inducer, or of aP2, which is a downstream factor under the control of PPAR $\gamma$  (Gurnell *et al.* 2000, Tamori *et al.* 2002). These results are consistent with the finding that T<sub>3</sub> itself is not an adipogenic inducer.

The findings reported here, as well as recent studies suggest that TR $\alpha$  plays a pivotal role in mediating the action of thyroid hormone in adipocytes. It is possible that T<sub>3</sub> increases lipid accumulation through regulating the expression of lipogenic enzymes. However, the function of TRs in adipose tissue is not entirely clear, and the preadipocyte line does not precisely reflect the *in vivo* process. Further investigations will be required to fully elucidate the role of TR $\alpha$  in adipocytes.

## Funding

This work was supported in part by a grant from the Grand-in-Aid for Scientific Research of the Japan Society for the Promotion of Science, Japan. Conflict of interest: none.

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Received in final form 20 May 2004  
Accepted 21 May 2004