Pai, J.-K., Siegel, M. I., Egan, R. W., & Billah, M. M. (1988) *Biochem. Biophys. Res. Commun. 150*, 355-364.
Parkos, C. A., Dinauer, M. C., Walker, L. E., Rodger, A. A., Jesaitis, A. J., & Orkin, S. H. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3319-3323.
Parks, G. D., Duke, G. M., & Palmenberg, A. C. (1986) *J. Virol. 60*, 376-384.
Pember, S. O., Barnes, K. C., Brandt, S. J., & Kinkade, J. M., Jr. (1983a) *Blood 61*, 1105-1115.
Pember, S. O., Shapira, R., & Kinkade, J. M., Jr. (1983b) *Arch. Biochem. Biophys. 221*, 391-403.
Rider, L. G., & Niedel, J. E. (1987) *J. Biol. Chem. 262*, 5603-5608.
Rossi, F., Grzeskowiak, M., Della Bianca, V., Calzetti, F., & Gandini, G. (1990) *Biochem. Biophys. Res. Commun. 168*, 320-327.
Rotrosen, D., & Gallin, J. I. (1987) *Annu. Rev. Immunol. 5*, 127-150.
Rotrosen, D. & Leto, T. L. (1990) *J. Biol. Chem. 265*, 19910-19915.
Rotrosen, D., Kleinberg, M. E., Nunoi, H., Leto, T., Gallin, J. I., & Malech, H. L. (1990) *J. Biol. Chem. 265*, 8745-8750.
Royer-Pokora, B., Kunkel, L. M., Monaco, A. P., Goff, S. C., Newburger, P. E., Baehner, R. L., Cole, F. S., Curnutte, J. T., & Orkin, S. H. (1986) *Nature 322*, 32-38.
Sambrook. J., Fritsch, E. F., & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Seifert, R., & Schultz, G. (1987) *Biochem. Biophys. Res. Commun. 146*, 1296-1302.
Smith, R. M., & Curnutte, J. T. (1991) *Blood 77*, 673-683.
Strum, S. L., Hendricks, C. L., & McPhail, L. C. (1990) *FASEB J. 4*, A2181.
Studier, W., Rosenberg, A. H., Dunn, J. J., & Dubendorff, J. W. (1990) *Methods Ezymol. 185*, 60-89.
Tamura, M., Tamura, T., Burnham, D. N., Uhlinger, D. J., & Lambeth, J. D. (1989) *Arch. Biochem. Biophys. 275*, 23-32.
Tyagi, S. R., Tamura, M., Burnham, D. N., & Lambeth, J. D. (1988) *J. Biol. Chem. 263*, 13191-13198.
Tyagi, S. R., Burnham, D. N., & Lambeth, J. D. (1989) *J. Biol. Chem. 264*, 12977-12982.
Uhlinger, D. J., Burnham, D. N., & Lambeth, J. D. (1991) *J. Biol. Chem. 266*, 20990-20997.
Van Gelder, B. F., & Slater, E. C. (1962) *Biochim. Biophys. Acta 58*, 593-595.
Volpp, B. D., Nauseef, W. M., Donelson, J. E., Moser, D. R., & Clark, R. A. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 7195-7199.
White, J. G., & Estensen, R. D. (1974) *Am. J. Pathol. 75*, 45-52.

Transcriptional Stimulation by Thyroid Hormone of a Cytosolic Thyroid Hormone Binding Protein Which Is Homologous to a Subunit of Pyruvate Kinase M_1

Kiyoto Ashizawa, Takaaki Fukuda,‡ and Sheue-yann Cheng*

Laboratory of Molecular Biology, Division of Cancer Biology, Diagnosis and Centers, National Cancer Institute, National Institutes of Health, Building 37, Room 4B09, Bethesda, Maryland 20892

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ABSTRACT: We have recently shown that the monomer of rat pituitary pyruvate kinase subtype M_1 (p58-M_1) is a cytosolic binding protein for 3,3',5-triiodo-L-thyronine (T_3). To understand the role p58-M_1 plays in thyroid hormone action, we examined the regulation of p58-M_1 by T_3 in GH_3 cells. Expression of p58-M_1 was evaluated by metabolically labeling GH_3 cells cultured in regular medium, thyroid hormone depleted medium (T_d medium), or T_d medium supplemented with T_3 (T_d + T_3 medium) followed by immunoprecipitation. T_3 stimulates the expression of p58-M_1 by 2-fold. Analysis by pulse–chase experiments indicates that the increased expression is not due to the increase of stability of p58-M_1. Northern analysis of mRNA prepared from cells cultured in regular, T_d, or T_d + T_3 medium demonstrates that T_3 increases the accumulation of cytoplasmic mRNA by 2-fold. Nuclei from cells cultured in the three conditions were prepared, and the rates of synthesis of nascent nuclear RNA were compared by an in vitro transcription assay. Addition of T_3 stimulates the rate of transcription by 2-fold. The parallel and identical magnitude in the increase of transcription rate and the accumulation of mRNA indicates that T_3 stimulates the synthesis of p58-M_1 by increasing the transcriptional activity of its gene.

A cytosolic binding protein for 3,3',5-triiodo-L-thyronine (T_3)[1] was described as early as 1958 by Tata (Tata, 1958). In the following 3 decades, cytosolic T_3 binding protein was reported to be present in various tissues of many species and in many cultured cell lines including rat pituitary GH_1 cells and human epidermoid carcinoma A431 cells (Cheng, 1991). Using A431 cells, this cytosolic T_3 binding protein (p58-M_2) was isolated and purified (Kitagawa et al., 1987a,b). Antibodies against p58-M_2 were prepared and used to isolate the cDNA encoding p58-M_2. Analysis of nucleotide sequence indicated that p58-M_2 is a subunit of pyruvate kinase subtype

* To whom correspondence should be addressed.
‡ Present address: The First Department of Internal Medicine, Nagasaki University School of Medicine, 7-1 Sakamoto-machi, Nagasaki 852, Japan.

[1] Abbreviations: T_3, 3,3',5-triiodo-L-thyronine; p58-M_1, cytosolic thyroid hormone binding protein; PKM_1, pyruvate kinase M_1; GH, growth hormone; PBS, phosphate-buffered saline; CHAPS, 3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate; SDS, sodium dodecyl sulfate; SDS–PAGE, SDS–polyacrylamide gel electrophoresis.

M_2 (PKM_2) (Kato et al., 1989).

PK (ATP:pyruvate O^2-phosphotransferase, EC 2.7.1.40) is an important glycolytic enzyme which catalyzes the conversion of phosphoenolpyruvate to pyruvate with the generation of ATP. There are four pyruvate kinase isoenzymes, L, R, M_1, and M_2, in mammalian systems. They are expressed in a tissue-specific manner and developmentally regulated. The L form is present mostly in liver, the R form exclusively in erythrocytes, and M_1 mostly in muscle; M_2 is found in many tissues such as kidney, intestine, lung, fibroblasts, testis, adipose tissue, and stomach (Imamura & Tanaka, 1982). Each of the isoenzymes consists of four identical or nearly identical subunits with the molecular mass of each subunit ranging from 57 to 60 kDa (Imamura & Tanaka, 1982). However, the L and R isozymes differ from the M_1 and M_2 forms in their electrophoretic, kinetic, and immunological properties. cDNAs and genomic clones for rat L, R, M_1, and M_2 have been isolated and sequenced (Noguchi et al., 1986, 1987). The L- and R-type and the M_1- and M_2-type isozymes are under the control of different genes. The L and R types of rat pyruvate kinase are produced from a single gene by use of different promoters, whereas the M_1 and M_2 types are produced from another single gene by alternative RNA splicing.

Despite the ubiquitous distribution of cytosolic T_3 binding protein, its cellular function is not clearly understood. Its functions have been proposed to act as an intracellular regulator/buffer or as a transporter for thyroid hormones. It was thought that via cytosolic T_3 binding protein, thyroid hormone could be made readily available to mitochondria (Defer et al., 1975; Hashizume et al., 1986), deiodinase (Francon et al., 1985), and nuclei (Defer et al., 1975; Visser et al., 1976; Hashizume et al., 1989). Recently, cytosolic T_3 binding protein was also postulated to be involved in mediating T_3-induced metabolic effects (Kato et al., 1989). To evaluate these possibilities, we chose to use GH_3 cells for our studies. GH_3 cells are a clonal strain of growth hormone producing cells which have long been used as a model system to study thyroid hormone action. Using human p58-M_2 cDNA as a probe, we screened the cDNA library prepared from GH_3 cells to isolate the gene encoding cytosolic T_3 binding protein. Sequence analysis of the isolated cDNA indicated that in GH_3 cells, the cytosolic T_3 binding protein is a monomer of PK subtype M_1 (Parkison et al., 1989). To further characterize its T_3 binding activity, p58-M_1 was overexpressed in *Escherichia coli* and purified to homogeneity. Evaluation of its thyroid hormone binding activity indicated that p58-M_1 binds T_3 with affinity and analogue specificity similar to p58-M_2 (Kato et al., 1989; Ashizawa et al., 1991; Parkison et al., 1991).

The regulation of p58-M_1 or PKM_1 by T_3 has never been reported. As a first step to understand whether p58-M_1 plays a role in thyroid hormone action, the present studies evaluated the regulation of p58-M_1 by T_3 in GH_3 cells. We found that T_3 controls the expression of p58-M_1 by stimulation of its gene.

Materials and Methods

[^{35}S]Methionine (1134 Ci/mmol) and nick translation kits were purchased from DuPont New England Nuclear. [α-^{32}P]UTP (>3000 Ci/mmol) and [α-^{32}P]CTP (>3000 Ci/mmol) were obtained from Amersham. Ham's F-10, horse serum, fetal bovine serum, and penicillin–streptomycin were purchased from Gibco. Anion-exchange resin AG-1X-8 (chloride form 200–400 mesh) was from Bio-Rad.

GH_3 Cell Culture. GH_3 cells were cultured as described previously (Kitagawa et al., 1987a,b). Experimental dishes were plated at a density of (1–4) $\times$ 10^3/cm^2 in Ham's F-10 medium supplemented with 12.5% horse serum and 2.5% fetal bovine serum (regular medium). Cells were further cultured in either regular medium, thyroid hormone depleted medium (T_d), or T_d medium supplemented with 50 nM T_3 (T_d + T_3) for the duration as described in the figure legends. In all experiments, media were changed every 48 h.

Thyroid hormone depleted serum was prepared similarly as described in Samuels et al. (1979). In brief, 40 mL of fetal bovine serum was shaken with 2 g of washed AG1-X8 (Bio-Rad, Richmond, CA) for 5 h at 23 °C. The mixture was spun at 1000*g* for 10 min. The serum in the supernatant was removed and shaken with 2 g of AG1-X8 for 22 h. The same process was repeated once more. The T_3 and T_4 contents of the serum were determined by radioimmunoassay. In the thyroid hormone depleted sera, T_3 and T_4 levels were 7.6 ng/dL (0.12 nM) and 0.3 μg/dL (4 nM), respectively. The T_3 and T_4 levels in serum before resin treatment were 173 ng/dL (2.7 nM) and 14.3 μg/dL (184 nM), respectively.

Determination of p58-M_1. The amount of p58-M_1 was measured by immunoprecipitation. GH_3 cells were labeled with [^{35}S]methionine in methionine-free medium at 37 °C for various lengths of time as described in the legends. Extraction of cellular proteins by 3 mM CHAPS and immunoprecipitation were carried out as described previously (Hasumura et al., 1986). J11, a monoclonal antibody which recognizes rat p58-M_1 (Obata et al., 1989), was used in all the experiments. After electrophoresis and autoradiography, the p58-M_1 bands were quantified by densitometry.

RNA Blot Hybridization. GH_3 cells were plated in culture superdishes (600 cm^2) with a density of 1 $\times$ 10^3 cells/cm^2 and cultured in regular medium for 2 days. Cells were further cultured in regular medium, T_d medium, and T_d + T_3 medium for an additional 2 days. Poly(A$^+$) RNAs were prepared as described (Cheng et al., 1987). Using the [^{32}P]-labeled nick-translated cDNA (2.3 kb) insert isolated from pGH35 plasmid (Parkison et al., 1989) as a probe, RNA blot hybridization was carried out as described by Cheng (Cheng et al., 1987).

In Vitro Nuclear Transcription Studies. The runoff transcription assays were performed according to Linial et al. (1985).

(A) Isolation of Nuclei. GH_3 cells were plated in culture dishes (24 $\times$ 24 cm, 600 cm^2) with a density of 1 $\times$ 10^3 cells/cm^2 and cultured in regular medium for 2 days. Cells were further cultured in regular medium, T_d medium, or T_d + T_3 medium for an additional 2 days. GH_3 cells were washed with PBS (2×), and 10 mL of lysis buffer (10 mM Tris, pH 7.4, 3 mM $CaCl_2$, and 2 mM $MgCl_2$) was added. Cells were pelleted (500*g*, 10 min) after being scraped with a rubber policeman. Cell pellets were resuspended in 10 mL of lysis containing 1% NP40. Cells were homogenized in a Dounce homogenizer (loose pestle; 15–17 strokes). The nuclei were collected by centrifugation (700*g*) for 10 min at 4 °C. The excess buffer was drained by inverting the tube for 5 min at 22 °C. Freezing buffer (420 μL) (50 mM Tris, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, and 0.1 M EDTA) was added, and the samples were frozen and stored at −70 °C.

(B) Runoff Transcription Assay and Preparation of Labeled RNA. On the day of experiment, the nuclei were thawed on ice and suspended in 60 μL of 5× transcription buffer (25 mM Tris, pH 8.0, 12.5 mM $MgCl_2$, 750 mM KCl, 1.25 mM GTP, 1.25 mM ATP, and 1.25 mM CTP). Sixty microliters of [^{32}P]UTP (>3000 Ci/mmol) was added to the nuclei. After incubation for 30 min at 30 °C, 25 μL of RQI DNase (Promega) and 5 μL of 100 mM $CaCl_2$ were added and incubated for an additional 10 min at 30 °C. After addition

of 38 μL of 10× SET buffer (10% SDS, 250 mM EDTA, and 2 M Tris, pH 7.4) and 11 μL of proteinase K (10 mg/mL), the reaction mixture was heated for 2 min at 65 °C followed by incubation at 42 °C for 90 min. The mixture was treated with phenol/chloroform, and the RNA was precipitated by the addition of NH_4OAc and isopropyl alcohol. The products were passed through a Sephadex G-50 spin column to remove the smaller RNA species. The RNAs in the eluate were fragmented into 100–200 base-pair pieces by treatment with $^1/_9$th volume of 2 M NaOH on ice for 10 min. The digested RNAs were immediately neutralized with $^1/_3$rd volume of 1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7, and precipitated with 2.5 volumes of ethanol for 2 h at −70 °C. After centrifugation (110000*g*) for 10 min at 4 °C, the pellet was resuspended in 100 μL of 10 mM Tris, pH 7.4, and 0.1 mM EDTA. One microliter was counted in a β-counter to determine the radioactivity.

(*C*) *DNA Excess Hybridization.* Five micrograms of pGH35 which contains the cDNA for p58-M_1, Bluescript SK(+) (used as a control), and the mouse β-actin pUC 18 which contains the cDNA for β-actin (Alonso et al., 1986) were denatured by heating at 65 °C in 0.3 M NaOH for 1 h. After being heated, the solution was neutralized with an equal volume of 2 M NH_4OAc (pH 7.0) and kept on ice. The denatured DNA was transferred to a nitrocellulose paper and baked in a vaccum oven at 80 °C for 2 h. The nitrocellulose filter was prehybridized overnight with hybridization buffer (10 mM TES, pH 7.4, 0.2% SDS, 10 mM EDTA, 300 mM NaCl, 1× Denhardt's solution, and 250 μg/mL *Escherichia coli* RNA). After hybridization at 42 °C for 72 h, the blots were washed with four changes of 2× SSC and 0.2% SDS at 65 °C for 1 h and then washed with two changes of 0.1× SSC and 0.1% SDS for 1 h at 65 °C. The blots were autoradiographed at −70 °C.

Results

Stimulation of p58-M_1 Expression by T_3 in GH_3 Cells. The rat pituitary tumor GH_3 cells used in these studies were cultured in a medium containing 12.5% horse serum and 2.5% calf serum. These sera contain significant amounts of thyroid hormones. In order to study the effects of T_3 on p58-M_1 expression, we compared the amounts of p58-M_1 synthesized in cells grown in regular medium, medium containing serum which was depleted of thyroid hormone (T_d medium), or T_d + T_3 medium. Cells were metabolically labeled with [^{35}S]-methionine and immunoprecipitated by J11, a monoclonal antibody which recognizes p58-M_1 (Obata et al., 1989). Figure 1A shows the autoradiogram of p58-M_1 immunoprecipitated from 20–400 μg of cellular extracts. Evaluation of the intensity of the protein bands indicates that in this range of cellular extracts, the amount of p58-M_1 immunoprecipitated is quantitative. The linear relationship in this range of cellular extracts allowed us to use immunoprecipitation to compare the effect of T_3 on the expression of p58-M_1. Figure 1B shows the results of immunoprecipitation from equal amounts of cellular extracts (30 μg) prepared from cells grown in regular, T_d, or T_d + T_3 medium. Quantification of the radioactive bands by densitometry indicated that after culturing of cells in T_d medium for 2 days, p58-M_1 was reduced by 2-fold (lane 3, Figure 1B). Addition of T_3 to T_d medium increased the level of p58-M_1 by 2-fold (lane 5, Figure 1B), back to the level seen in cells grown in regular medium (lane 1, Figure 1B), indicating the effect is reversible and specific for T_3.

Figure 2 shows the kinetics of reduction of p58-M_1 when T_3 was withdrawn from the medium. The depletion of p58-M_1 was slow. After 2 days in T_d medium, p58-M_1 dropped by 2-fold. A 5–10% further decrease was observed on days 3 and 4. These results could imply that the overall effect of T_3 is

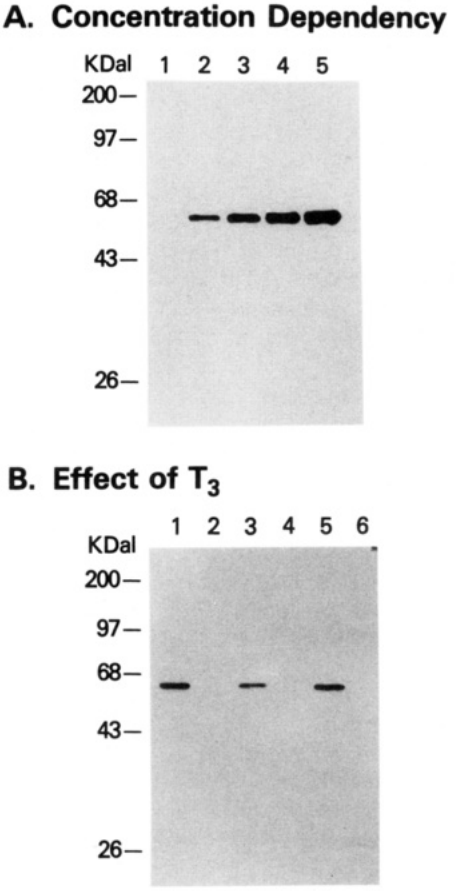

FIGURE 1: Autoradiogram of the immunoprecipitates of p58-M_1. (A) Concentration dependency: GH_3 cells were plated at a density of 2 × 10^5 cells/100-mm dish and cultured for 2 days. Cells were extracted with 1 mL of 3 mM CHAPS. Increasing amounts of cellular extracts were immunoprecipitated with 10 μg of affinity-purified J11 as described in Obata et al. (1989). The immunoprecipitates were analyzed by SDS–PAGE and autoradiographed. The protein concentrations are 20, 50, 100, and 400 μg for lanes 2, 3, 4, and 5, respectively. Lane 1 is the result of immunoprecipitation using 20 μg of cellular extracts and 10 μL of preimmune serum. (B) Effect of T_3 on the expression of p58-M_1: GH_3 cells were plated at a density of 2 × 10^5 cells/100-mm dish. Cells were cultured in regular medium (lanes 1 and 2), T_d (lanes 3 and 4), and T_d + 50 nM T_3 (lanes 5 and 6) medium as described under Materials and Methods. Cellular extracts (30 μg of protein) were immunoprecipitated by 10 μg of affinity-purified J11 (lanes 1, 3, and 5) and preimmune serum (lanes 2, 4, and 6).

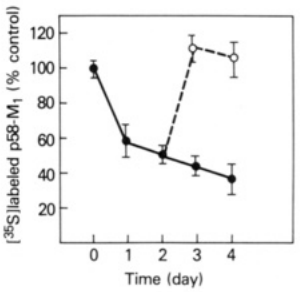

FIGURE 2: Time-dependent regulation of p58-M_1 by T_3. GH_3 cells were plated at a density of 2 × 10^5 cells/100-mm dish. Cells were first cultured in regular medium for 2 days followed by 5 days in T_d. To examine the T_3 effect, cells were added to T_3 (50 nM) after deinduction in T_d medium for 2 days (○). At each day, cells were labeled with [^{35}S]methionine and extracted with 3 mM CHAPS. Cellular extracts (30 μg) were immunoprecipitated by the affinity-purified antibody J11 (10 μg). After electrophoresis and autoradiography, the p58-M_1 bands were quantified by densitometry. The data are a mean of two experiments. The bars represent the variation.

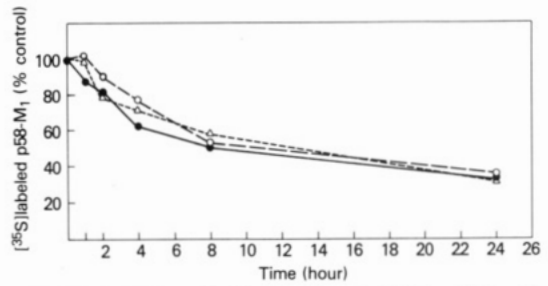

FIGURE 3: Effect of T_3 on the degradation of p58-M_1. GH_3 cells were plated at a density of 2×10^5 cells/100-mm dish. After cells were cultured as described under Materials and Methods, they were pulsed with [^{35}S]methionine (1 mCi/dish) in methionine-free medium for 15 min at 37 °C followed by a chase for 1, 2, 4, 8, and 24 h. Cellular extracts (25 μg) were immunoprecipitated with 10 μg of monoclonal antibody J11. After electrophoresis and autoradiography, the intensity of p58-M_1 bands was quantified by densitometry. Regular medium (●); T_d medium (○); $T_d + T_3$ medium (△). The data represent the mean of two experiments with variation less than 10%.

greater than 2-fold. Figure 2 also shows that addition of T_3 to cells after deprivation of cells of T_3 for 2 days stimulated the synthesis of p58-M_1 by 2-fold within 24 h. The level was maintained in the 2 days examined. As a control, similar experiments were done to see whether the withdrawal of T_3 had any effect on the amounts of β-actin, but no significant effect was found (data not shown).

Effect of T_3 on the in Vivo Stability of p58-M_1. To examine whether the in vivo stability of p58-M_1 was affected by T_3, cells which were grown in regular, T_d, and $T_d + T_3$ medium were labeled with [^{35}S]methionine for 15 min followed by a chase with unlabeled methionine for 1, 2, 4, 8, and 24 h. After immunoprecipitation of the cellular extracts with J11, the [^{35}S]methionine-labeled p58-M_1 was quantified. Figure 3 shows the decay curves of p58-M_1 from cells grown under three conditions. Analysis of the data indicated that p58-M_1 from cells cultured in regular, T_d, and $T_d + T_3$ medium had a half-life of 11.55 ± 1.96, 12.6 ± 2.52, and 12.6 ± 2.52 h, respectively. These degradation rates were not significantly different from each other. These results indicated that T_3 had no effects on the in vivo stability of p58-M_1.

T_3 Regulates the Expression of p58-M_1 at the mRNA Level. To understand whether the regulation of p58-M_1 by T_3 occurs at the mRNA level, poly(A$^+$) RNAs were prepared from cells grown in regular, T_d, or $T_d + T_3$ medium. Figure 4A shows that one single species of mRNA with a size of ~2.7 kb was detected. Quantitation of the mRNA bands by densitometry showed that the mRNA of p58-M_1 was reduced by 2-fold in cells grown in T_d medium (lane 2). When T_3 (50 nM) was added to the T_d medium, a 2-fold increase of p58-M_1 mRNA was detected. The blot was also probed for β-actin mRNA as an internal control. Scanning by a densitometer indicated that no effect of T_3 on the level of β-actin mRNA was found (Figure 4B). These results indicate that the effect on p58-M_1 by T_3 was selective. The extent of increase in p58-M_1 at the protein level was accounted for by the increase in its mRNA level in the cytoplasm. These results indicate that T_3 controlled the synthesis of p58-M_1 at the mRNA level.

T_3 Increases the Transcription Rate of the p58-M_1 Gene. The increase of mRNA induced by T_3 could be due to the change of transcriptional rate, the increase in the stability of mRNA, or both. To understand the mechanism by which T_3 regulates the mRNA of p58-M_1, nuclear runoff experiments were carried out. Nuclei from cells grown in three conditions were prepared, and the rates of synthesis of the nascent nuclear p58-M_1 RNA were compared. The rates of synthesis of β-actin RNA in Table I show that T_3 did not have an overall

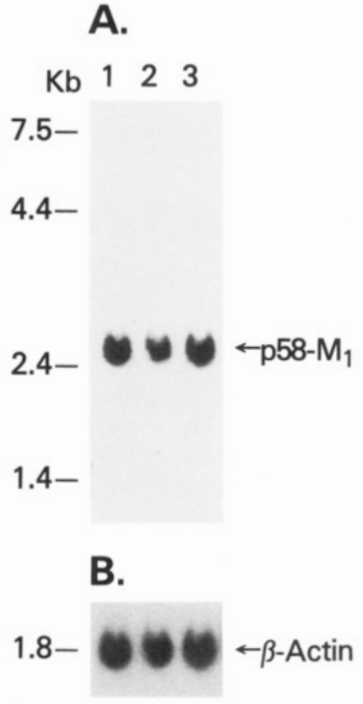

FIGURE 4: Northern analysis of p58-M_1 mRNA. Poly(A$^+$) RNAs were isolated from GH_3 cells under three cultured conditions, and the blot was prepared as described under Materials and Methods. mRNA (20 μg) from cells cultured in regular medium (lane 1), T_d medium (lane 2), or $T_d + T_3$ (50 nM) medium (lane 3) was loaded onto each lane and probed with [^{32}P]-labeled cDNA for p58-M_1 (A). The blot was subsequently heated (100 °C) in 15 mM NaCl and 1.5 mM sodium citrate for 3 min and rehybridized with [^{32}P]-labeled cDNA for β-actin (B).

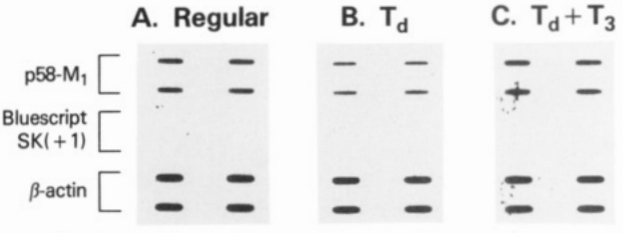

FIGURE 5: Autoradiogram from nuclear runoff assays. Five micrograms of each of the different plasmid DNAs was immobilized onto nitrocellulose filters and hybridized with 5 mL of [^{32}P]-labeled RNA (5.4×10^6 cpm/mL) prepared from cells grown in regular (A), T_d (B), and $T_d + T_3$ (C) medium. Quadruplicates blots were used for each condition.

Table I: Effect of T_3 on the Transcriptional Rate of p58-M_1[a]

culture medium	total ^{32}P synthesized [(cpm × 10^{-6})/10^7 nuclei]	band intensity (arbitrary units)[b]		ratio[c]
		p58-M_1	β-actin	
regular	3.19 ± 0.5	451 ± 36	942 ± 126	2.1
T_d	2.92 ± 0.13	226 ± 28	1022 ± 130	1
$T_d + T_3$	2.60 ± 0.84	431 ± 30	1012 ± 63	2.0

[a] Nuclei were isolated from GH_3 cells cultured in regular, T_d, or $T_d + T_3$ medium. The runoff transcription assays were carried out as described under Materials and Methods. The values were mean ± standard deviation of three experiments. [b] Intensity was determined by densitometry. Each experiment was performed in quadruplicate. The contribution of nonspecific hybridization due to the vector was less than 5% and was corrected. [c] Ratio calculated after normalization using β-actin as an internal control.

effect on the synthesis of nuclear RNAs. However, as shown in Figure 5, T_3 stimulated the transcription of the p58-M_1 gene by 2-fold. Addition of T_3 to the T_d medium reversed the

reduction of mRNA level which was caused by depletion of T_3 in the medium. The magnitude of the increase in the transcriptional rate was the same as the increase in the accumulation of the steady-state mRNA in the cytoplasm (Figure 5 and Table I).

Discussion

The present study demonstrates that T_3 stimulates the synthesis of p58-M_1 in GH_3 cells. The stimulatory effect is not due to protein stabilization since the half-life of p58-M_1 is not affected by T_3 treatment. The increase in the synthesis of p58-M_1 is the result of an increase in the transcriptional rate of the p58-M_1 gene. This effect is selective as T_3 has no significant effect on total RNA synthesis in GH_3 cells and the transcription of a control gene (β-actin) is unaffected by T_3 (Table I). Previously, it was also found that T_3 does not affect total RNA synthesis in livers of euthyroid, thyrodectomized, and T_3-treated rats (Dozin et al., 1986). The parallel and identical magnitude in the transcription activation is sufficient to account fully for the accumulation of p58-M_1 in cytoplasm. These results demonstrate that p58-M_1 synthesis is regulated by T_3 primarily at the level of transcription. This mode of regulation has also been found for growth hormone in GC cells (Yaffe & Samuels, 1984), phosphoenolpyruvate carboxyl kinase (Loose et al., 1985), cytochrome *c* (Scarpulla et al., 1986), and the uncoupling protein in rat liver (Bianco et al., 1988).

The extent of activation in the transcriptional rate of the target genes by T_3 studied so far ranged from 1.4-fold for the α-subunit of Na^+,K^+-ATPase in rat liver (Gick et al., 1988) to a ~10-fold increase for growth hormone in GC cells (Yaffe & Samuels, 1984). The present study demonstrates that T_3 stimulates the rate of transcription of p58-M_1 by 2-fold which is similar to that found for ornithine aminotransferase (Mueckler et al, 1984). The T_3-induced stimulating effect on the expression of p58-M_1 has important implications. p58-M_1 is a multifunctional protein. It associates to form the tetrameric PKM_1. PKM_1 is a key enzyme for the generation of ATP in the glycolytic pathway. The reduction in the expression of this enzyme responding to the decrease of T_3 is entirely consistent with the lowering of metabolic rate observed in hypothyroidism. p58-M_1 also functions as a T_3 binding protein in the cytosol. It could act as a carrier to transport T_3 to the nucleus, as a regulator to control the availability of T_3 to the nuclear receptor, and/or as a mediator for other metabolic effects which are yet to be identified. We are currently evaluating these possibilities. Preliminary results indicated that p58-M_1 could function as a regulator for the cytoplasmic T_3 level (unpublished results). The reduced gene expression in responding to the lowering of the hormonal level demonstrated in the present studies is consistent with its function as an intracellular regulator.

Synthesis and hormonal regulation of GH in GH-producing cell lines (e.g., GH_1, GC, and GH_3) have been well characterized. Synthesis of GH is stimulated by T_3 (Samuels et al., 1988). The present studies demonstrate that the synthesis of p58-M_1 is stimulated by T_3. This correlation suggests that GH_3 cells should be a suitable cell line to evaluate the involvement of p58-M_1 in T_3 function.

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Registry No. T_3, 6893-02-3; pyruvate kinase, 9001-59-6.

References

Alonso, S., Minty, A., Bourlet, Y., & Buckingham, M. (1986) *J. Mol. Evol. 23*, 11–22.

Ashizawa, K., McPhie, P., Lin, K., & Cheng, S. (1991) *Biochemistry 30*, 7105–7111.

Bianco, A. C., Sheng, X., & Silva, E. J. (1988) *J. Biol. Chem. 263*, 18168–18175.

Cheng, S. (1991) in *Thyroid Hormone Metabolism: Regulation and Clinical Implications* (Wu, S., Ed.) pp 145–166, Blackwell Scientific Publications, Boston, MA.

Cheng, S., Gong, Q., Parkison, C., Robinson, E., Appella, E., Merlino, G., & Pastan, I. (1987) *J. Biol. Chem. 262*, 11221–11227.

Defer, D., Dastugue, B., Sabatier, M. M., Thomopoulos, P., & Kruh, J. (1975) *Biochem. Biophys. Res. Commun. 67*, 995–1004.

Dozin, B., Magnuson, M. A., & Nikodem, V. M. (1986) *J. Biol. Chem. 261*, 10290–10292.

Francon, J., Osty, J., Chantoux, F., & Lennon, A. M. (1985) *Mol. Cell. Endocrinol. 39*, 197–207.

Gick, G. G., Ismail-Beigi, F., & Edelman, I. S. (1988) *J. Biol. Chem. 263*, 16610–16618.

Hashizume, K., Kobayashi, M., Miyamoto, T., & Yamauchi, K. (1986) *Endocrinology 119*, 1063–1070.

Hashizume, K., Miyamoto, T., Ichikawa, K., Yamauchi, K., Sakurai, A., Ohtsuka, H., Kobayashi, M., Nishii, Y., & Yamada, T. (1989) *J. Biol. Chem. 264*, 4864–4871.

Hasumura, S., Kitagawa, S., Lovelace, E., Willingham, M. C., Pastan, I., & Cheng, S. (1986) *Biochemistry 25*, 7881–7888.

Imamura, K., & Tanaka, T. (1982) *Methods Enzymol. 90*, 150–165.

Kato, H., Fukuda, T., Parkison, C., McPhie, P., & Cheng, S. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 7861–7865.

Kitagawa, S., Obata, T., Hasumura, S., Pastan, I., & Cheng, S. (1987a) *J. Biol. Chem. 262*, 3903–3908.

Kitagawa, S., Obata, T., Willingham, M. C., & Cheng, S. (1987b) *Endocrinology 120*, 2591–2596.

Linial, M., Gunderson, N., & Grondine, M. (1985) *Science 230*, 1126–1132.

Loose, D. S., Cameron, D. K., Short, H. P., & Hanson, R. W. (1985) *Biochemistry 24*, 4509–4512.

Mueckler, M. M., Moran, S., & Pitot, H. C. (1984) *J. Biol. Chem. 259*, 2302–2305.

Noguchi, T., Inone, H., & Tanaka, T. (1986) *J. Biol. Chem. 261*, 13807–13812.

Noguchi, T., Yamada, K., Inone, H., Matsuda, T., & Tanaka, T. (1987) *J. Biol. Chem. 262*, 14366–14371.

Obata, T., Fukuda, T., Willingham, M. C., Liang, C.-M., & Cheng, S. (1989) *Biochemistry 28*, 617–623.

Parkison, C., Kato, H., & Cheng, S. (1989) *Nucleic Acids Res. 17*, 7106.

Parkison, C., Ashizawa, K., McPhie, P., Ling, K., & Cheng, S. (1991) *Biochem. Biophys. Res. Commun. 179*, 668–674.

Samuels, H. H., Stanley, F., & Sananova, J. (1979) *Endrocrinology 105*, 80–85.

Samuels, H. H., Forman, B. M., Horowitz, D., & Ye, Z. (1988) *J. Clin. Invest. 81*, 957–967.

Scarpulla, R. C., Kilar, M. C., & Scarpulla, K. M. (1986) *J. Biol. Chem. 261*, 4660–4662.

Tata, J. R. (1958) *Biochim. Biophys. Acta 28*, 91–94.

Visser, T. J., Bernard, H. F., Docter, R., & Hennemann, G. (1976) *Acta Endrocrinol. 82*, 98–104.

Yaffe, B. M., & Samuels, H. H. (1984) *J. Biol. Chem. 259*, 6284–6291.