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Modulation of thyroglobulin messenger RNA level by thyrotropin in cultured thyroid cells

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ABSTRACT

To examine the influence of thyrotropin (TSH) on the thyroglobulin (Tgb) mRNA content, the latter was evaluated in the cytoplasm of hog thyroid cells cultured in the absence (control cells) or presence of TSH. The Tgb mRNA levels were determined by, (i) kinetics of hybridization to sheep Tgb cDNA, (ii) capacity of coding for peptides immunologically related to Tgb in reticulocyte lysate. In cells cultured for 4 days in the absence of TSH, the content of Tgb mRNA sequences decreased to 30 % of its initial value and the messenger activity to 15 %. Conversely, TSH maintained the initial Tgb mRNA level in cells cultured in its presence, and TSH concentrations 50 µU/ml or 5 mU/ml gave indentical results. At each period tested poly (A) content was the same in TSH-treated and control cells. When TSH was added to media after 4 or 8 days culture without TSH, the Tgb mRNA level was partially restored. These results suggest that TSH exerts a positive control on Tgb gene expression through modulation of Tgb mRNA content of thyroid cells.

INTRODUCTION

The mechanisms of hormonal regulation of gene expression have been extensively investigated. In the case of steroid hormones, it is generally accepted that steroid-receptor complexes interact with chromatin to control gene expression (1, 2). Steroid hormones also affect the translation (3) and stability (4, 5) of specific mRNA molecules. It has recently been demonstrated that polypeptide or protein hormones are able to induce or modulate the accumulation of specific mRNA species (6-11) e.g. casein mRNA by prolactin (6, 7), α_2U globulin mRNA by growth hormone in association with other hormones (8), albumin mRNA by growth hormone (9) and insulin (10), and phosphoenolpyruvate carboxy kinase mRNA by glucagon (11). The initial step in polypeptide hormone action is the interaction with specific receptors on the plasma membrane of target cells and the subsequent modulation of intracellular concentration of messengers (e.g. cAMP, cGMP, Ca^{++} etc...). Their mode of action on gene expression at transcriptional and translational

levels is still unknown. Such action might involve mediation by intracellular regulatory molecules. A possible participation of the internalized hormone molecules (12) cannot be ruled out. The present study constitutes an additionnal example of protein hormone control on specific mRNA accumulation. Evidence for stimulatory action of the glycoprotein hormone thyrotropin (TSH) on thyroid protein and RNA synthesis has been reported in several laboratories. Hyperstimulation by TSH, in vitro (13, 14) or in vivo (15), resulted in a general increase in protein synthesis first, through stimulation of translation (13, 14, 16) and later through increased RNA synthesis (14, 17). Scheinman et al (18) recently found that in rat thyroids incubated with TSH the relative amount of ornithine decarboxylase was specifically increased. In the normal thyroid, synthesis of thyroglobulin (Tgb) represents 40-60 % of the total protein synthesis (15, 19). Nevertheless, in hyperstimulation experiments, neither Tgb synthesis (15) nor Tgb mRNA content (20) were found specifically increased. Conversely, in the thyroid of hypophysectomized Xenopus (19), or rat (15, 21) as well as in rat thyroid explants (22, 23), incorporation of labeled aminoacids into Tgb was preferentially diminished. Also, in the course of Xenopus metamorphosis, under stimulation by endogenous TSH, relative Tgb synthesis increases from 10 to 60 % (19). These results suggested that there is a specific regulation by TSH of the overall Tgb gene expression which does not appear in hyperstimulation experiments; but information is lacking on the level at which regulation operates. Accordingly, in this study we investigated the influence of TSH deprivation, or chronic stimulation on the Tgb mRNA cellular content. We have previously characterized and purified ovine Tgb mRNA (24, 25). The 33 S mRNA has been used as a template for synthesis of a specific Tgb complementary DNA probe (Tgb cDNA), (26). In the present study, the Tgb mRNA was quantitatively evaluated in the cytoplasmic RNA extracted from isolated porcine thyroid cells cultured without (control cells) or with TSH (27), by its capacity to code for Tgb peptide chains in reticulocyte lysate and to hybridize to the Tgb cDNA. In primary culture TSH-treated cells reassociate into follicle-like structures, are able to concentrate and organify iodide and can respond to acute TSH stimulation by adenylate cyclase activation (27, 28). Control cells reorganize into epithelial structures in which cell polarity is inverted (27). Such cells lose their capacity to concentrate and organify iodide and have a decreased cAMP response to TSH stimulation (27, 28). In TSH deprived cells the relative Tgb mRNA level was found to decrease to 20 % of its initial value after 15 days in culture, whereas the Tgb mRNA level was preserved in

cells treated with TSH immediately after their isolation. When TSH was added to control cells several days after isolation the Tgb mRNA decrease was arrested and initial Tgb mRNA level was partially restored.

MATERIALS AND METHODS

Cell culture -Porcine thyroid cells were isolated by a discontinuous trypsin-EGTA treatment (50). Cells were cultured as unstirred suspension in untreated polystyrene dishes in Eagle's medium containing 10 % new born calf serum or 10 % horse serum for periods up to 15 days. Media were changed every four days. Cells were eventually attached in tissue culture treated Falcon flasks. Cells were incubated in the presence (TSH-treated cells) or absence (control cells) of thyrotropin (0.05 to 5 mU/ml) at 37° C in a 5 % CO_2, 95 % air in a water saturated atmosphere.

Cytoplasmic RNA extraction - Porcine thyroid cells were washed twice with serum-free culture medium and once with 0.15 M sodium chloride, 0.01 M sodium phosphate pH 7.4. They were homogenized in 0.25 M sucrose, 0.2 M KCl, 0.01 M $MgCl_2$, 0.001 M EDTA, 0.05 M Tris-Cl pH 7.6, 0.001 M DTT, containing 2 % Triton X-100,HSB (24),(10^7 cells/5ml) by 30 strokes of a tight fitting pestle in a Dounce homogenizer. After 5 min centrifugation at 27 000 x g, the supernatant was precipitated by two volumes of ethanol (1h, - 20° C). The precipitate was dissolved in 4 M guanidinium-chloride pH 7.0, 0.01 M EDTA and the RNA was precipitated by 0.5 vol of ethanol using the procedure of Cox (29). After three extractions the RNA precipitate was washed twice with 3 M sodium acetate, pH 6.0, dissolved in sterile bidistilled water, supplemented with 0.1 M sodium acetate pH 7.0 and precipitated with ethanol. Ethanol precipitates were dissolved in bidistilled water and clarified by centrifugation for 10 min at 27 000 x g before incubation in reticulocyte lysate or hybridization to ovine Tgb [^{3}H] cDNA.

Polysome isolation and polysomal RNA extraction - Ovine or porcine thyroids were homogenized in high salt buffer containing 2 % Triton X-100 (HSB) and polysomes were precipitated from the 27 000 x g supernatant by magnesium treatment (24). RNA was extracted from the polysome pellets by guanidinium chloride (26, 29).

Ovine Tgb cDNA synthesis and RNA/DNA hybridization procedures - Details of the ovine Tgb mRNA purification, synthesis of its DNA copy (Tgb cDNA) and hybridization procedures will be published elsewhere (26). Briefly, ovine Tgb mRNA

was isolated from polysomal RNA by two passages through oligo (dT)-cellulose and purified on the basis of its size by centrifugation on a sucrose gradient. The purified 33 S mRNA was used as a template for synthesis of [^{3}H]dCTP labeled complementary DNA using avian myeloblastosis virus reverse transcriptase (30, 31). The Tgb cDNA was purified by filtration through G-50 Sephadex and by ethanol precipitation.

RNA excess hybridization kinetic experiments were performed at 70°C in siliconized glass capillaries, in 5 µl of 0.3 M NaCl, 1 mM EDTA, 50 mM Tris-Cl buffer pH 8.3 at 37°C. After incubation, each assay was diluted and divided into two portions for determination of total and S_1 nuclease resistant radioactivities (32). The data are expressed as the percentage of hybridization versus the log equivalent Rot (moles of ribonucleotides per liter multiplied by the incubation time in seconds). The concentration of moles of ribonucleotides per liter is calculated assuming that $1A_{260}$ represents 40 µg RNA and that one mole of nucleotides = 330 g. The Rot $\frac{1}{2}$ is the Rot at which half maximum hybridization of the cDNA occurs. Usually 10-30 µmoles of porcine cytoplasmic RNA were reacted with 80 pmoles (about 1000 cpm) of ovine Tgb cDNA in a 5 µl assay in order to perform hybridization in conditions of RNA excess.

Heat denaturation of RNA/DNA hybrids - Ovine polysomal RNA and porcine total cytoplasmic RNA were hybridized to ovine Tgb cDNA for 16 h at 65° C to reach Rot values (respectively 5 and 3 x 10^2 Moles x l^{-1} x s) leading to maximal hybridization of the cDNA. Temperature was then increased stepwise to 100°C and after 10 min at each desired temperature duplicate assays were tested for total and S_1 nuclease resistant radioactivities.

RNA and DNA quantification - Cells, washed with phosphate buffer saline, were suspended in water at 0°C and precipitated by perchloric acid, (0.2 N final) (33). The precipitate was dissolved in 0.3 N KOH and the RNA was hydrolyzed for 1 h at 37°C. After perchloric acid precipitation (0.5 N final) the RNA concentration was measured by reading the optical density at 260 nm on the supernatant (33). DNA was quantified on the residual perchloric acid precipitate or directly on the cells by the method of Mc Intire and Sproull (34).

Poly(A) content determination - Cytoplasmic RNA (100 to 500 ng) or poly (A) (Collaborative Research, 50 to 500 pg) was reacted with 15000 cpm [^{3}H] poly (U) (New England Nuclear 6.29 Ci/mmol) in 0.3 M NaCl, 0.03 M sodium citrate buffer pH 6.8 (35), in the presence of 20 µg of E. Coli tRNA (Boehringer Mannheim) in 50 µl assays, processed for hybrid formation and for

determination of pancreatic RNase resistant radioactivity (35).

Cell-free protein synthesis - Cytoplasmic or polysomal thyroid RNA_s (0.4 to 1.2 µg/50 µl assay) were incubated with nuclease-treated reticulocyte lysate (36) in the presence of 3H-labeled leucine (100 Ci/mmole - Amersham), 5 µCi/assay (25). Total radioactivity in peptide linkage was determined on an aliquot by hot TCA precipitation, Tgb radioactivity was immunoprecipitated under previously described conditions (25) by antiovine- or antiporcine Tgb antibodies.

RESULTS

DNA and RNA content in TSH-treated and control cells - DNA content was measured in samples of a known number of freshly isolated porcine thyroid cells and was found to be 7.5 pg per cell (at least 8 determinations were made in each of 4 isolation experiments). The RNA to DNA ratio was measured on day 0 and after 4 and 8 days of culture. This ratio varied from 0.9 to 1.1 in freshly isolated cells and was maintained at this level in TSH-treated cells. The ratio decreased in control cells to reach approximately 80-85 % of its initial value at day 4 and 60-70 % at day 8. In the studies reported below the cytoplasmic RNA was extracted by guanidinium-Cl and then washed with 3 M sodium acetate. In both TSH and control cells the RNA yield represented 60-65 % of total cell RNA.

Poly(A) content of the cytoplasmic RNA in cultured thyroid cells - Poly(A) content was measured in the cytoplasmic RNA of TSH-treated and control cells by hybridization with $[^3H]$poly(U) and used as an estimation of the mRNA concentration in cytoplasmic RNA. The poly(A) was found similar in both cell types (Fig. 1), for up to 15 days in culture. The relative poly(A) content increased as a function of time in culture but the increment was similar in TSH-treated and control cells. At present there is no explanation for the increase in Poly(A) content.

We next investigated whether or not the steady state level of Tgb mRNA was dependent on the presence of TSH. The relative content of Tgb mRNA sequences in total cytoplasmic RNA of TSH-treated and control porcine thyroid cells was estimated by hybridization to ovine Tgb cDNA. The concentrations of functional cytoplasmic Tgb mRNA were evaluated by its template activity in a nuclease treated reticulocyte lysate.

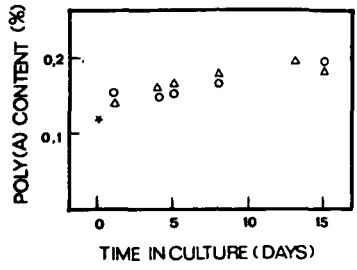

Fig. 1 - Poly(A) content of cytoplasmic RNA as a function of culture time.

Cytoplasmic RNA from freshly isolated cells (*) or from cells cultured for the indicated times in the absence (o - o) or in the presence of TSH (Δ - Δ), 50 μU or 5 mU/ml, were hybridized to [^{3}H]poly(U) as detailed in Methods.

Hybridization of ovine thyroglobulin cDNA to ovine and porcine thyroid RNA - The thyroglobulin [^{3}H]cDNA probe has been previously characterized (26). The Rot $\frac{1}{2}$ of the kinetics of hybridization of the cDNA to an excess of the Tgb mRNA template was 4.8×10^{-3} M.l^{-1}.s (Fig. 2).

Ovine and porcine thyroid polysomal RNA, both extracted by guanidinium chloride from Mg^{++}-precipitated polysomes, were annealed to ovine Tgb cDNA. In both species hybridization occured with a single transition until completion for 75 % of the cDNA hybridized. The reaction proceeded 5 times slower

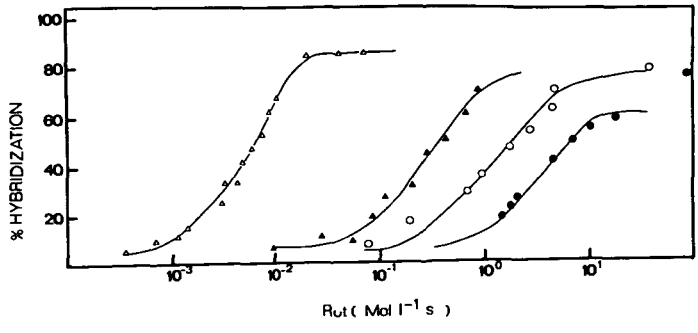

Fig. 2 - Hybridization kinetic curves of ovine thyroid RNA and porcine thyroid RNA to Tgb [^{3}H]-cDNA.

Cytoplasmic RNA from freshly dispersed porcine thyroid cells (● - ●), polysomal RNA isolated from porcine thyroids (o - o) or ovine thyroids (▲ - ▲) by magnesium precipitation (25) and purified ovine Tgb mRNA (Δ - Δ) (26) were hybridized to thyroglobulin [^{3}H]-cDNA (1000 cpm) in kinetic experiments.

for porcine than for ovine polysomal RNA (Fig. 2). The slower rate of hybridization is probably related to the mismatching of the ovine cDNA-porcine RNA hybrids relative to ovine cDNA-RNA hybrids, whose melting temperatures have been found to be respectively 85° C and 93° C (Fig. 3). Therefore the difference in Rot $\frac{1}{2}$ values probably does not reflect an actual difference in Tgb mRNA concentration. In fact, the relative Tgb synthesis in a cell free system programmed by porcine thyroid polysomal RNA was only 1.3 times lower than that programmed by ovine thyroid polysomal RNA (Table I). Total cytoplasmic RNA from freshly isolated porcine thyroid cells hybridized about 5 times slower than porcine polysomal RNA (Fig. 2). The Tgb template activity was 4-5 times lower in total cytoplasmic RNA from porcine cells than in porcine polysomal RNA (Table I). Thus, when two porcine RNA samples are compared, the difference in the Rot $\frac{1}{2}$ value most probably reflects an actual difference in the Tgb mRNA content. Accordingly, this shows that, although the homology of sequences between ovine and porcine Tgb mRNAs was not perfect it was nevertheless sufficient to base the comparison of the Tgb mRNA content in porcine RNA samples on comparison of their kinetics of hybridization to ovine Tgb cDNA.

Tgb mRNA quantitation in the cytoplasm of TSH-treated and control cells as a function of time in culture and TSH concentration - Cells were maintained in culture in suspension for 4 days, in the absence (control cells), or presence of 5 mU/ml TSH (TSH treated cells). Cytoplasmic RNA was extracted from freshly dispersed cells (D_o), from cells cultured for 16 h (D_1), and then from cells collected at 24 h intervals. Hybridization kinetic experiments revealed that the differences in the Rot $\frac{1}{2}$ values of RNA extracted from control and TSH-treated cells increased during the culture period (Fig. 4). For a given culture time the hybridization reaction proceeded more slowly with control cell RNA than with TSH-treated cell RNA, indicating that the Tgb

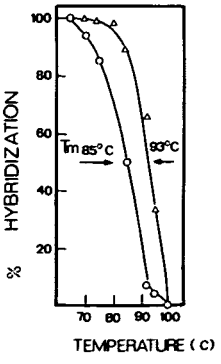

Fig. 3 - Thermal denaturation of RNA-cDNA hybrids

Maximal hybridization of porcine cell RNA (o - o) and ovine thyroid polysomal RNA (Δ - Δ) to ovine Tgb cDNA was achieved at 65° C and denaturation realized by step temperature increases as described in Methods. Percentage of hybridization obtained at 65° C was used as 100 % value.

TABLE I - Total messenger and thyroglobulin messenger activities of polysomal RNA_S from ovine and porcine thyroids and of cytoplasmic RNA from freshly isolated cells.

RNA from	TCA precipitable radioactivity (counts . min^{-1} . µg RNA^{-1})		Relative thyroglobulin synthesis %
	Total	Thyroglobulin	
sheep polysomes	119 584	19 000	15.8
Porcine polysomes	57 300	6 900	12.0
Porcine cells (Do) cytoplasm	45 892	1 478	3.2

Total protein synthesis was determined by TCA precipitated radioactivity on aliquots (5 µl) of two-fold diluted assays. Thyroglobulin messenger activity was determined by radioactivity immunoprecipitated by anti-porcine or anti-ovine thyroglobulin antibodies (duplicate 40 µl aliquots of two-fold diluted assay).

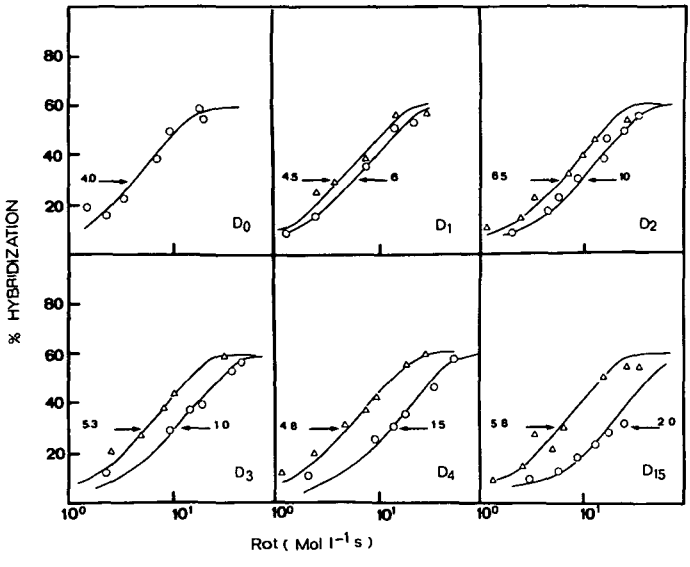

Fig. 4 - Variation of Tgb mRNA content as a function of time in a typical experiment

Cytoplasmic RNA, from freshly isolated porcine thyroid cells (D_O) and from cells cultured for the indicated time in the absence (o - o) or presence (Δ - Δ) of 5 mU/ml TSH, was hybridized to ovine Tgb $[^3H]$-cDNA in kinetic experiments. D_{15} cells were cultured in the presence of 1 µg/ml hydrocortisone.

mRNA content relative to cytoplasmic RNA was lower in control cells - (Fig.4). The Tgb mRNA content was 2.8 ± 0.4 times lower, at D_4, in control cells (result based on 4 cell isolation experiments). The Rot $\frac{1}{2}$ values were determined for cytoplasmic RNA in cells issued from various cell isolation experiments and cultured for varying time intervals. In Fig. 5 A, the ratios of the Rot $\frac{1}{2}$ values to the mean of the Rot $\frac{1}{2}$ values obtained for 4 D_o cell RNA samples are reported as a function of culture time. The Tgb mRNA content relative to total cytoplasmic RNA was reduced to 50 % of its D_o value within a period ranging from 16 h (D_1) to 40 h (D_2) in culture without TSH. After 4 days this value decreased to 30 % and reached 20 % within 15 days. During the same period Tgb mRNA content did not significantly vary in cells cultured in the presence of TSH (Fig. 5 A). Taking in account the decrease of total RNA content per cell during incubation without TSH, the Tgb mRNA content per cell was diminished to 25 % by 4 days and to 12 % by 8 days.

The variation of the Tgb mRNA level was also examined by translation

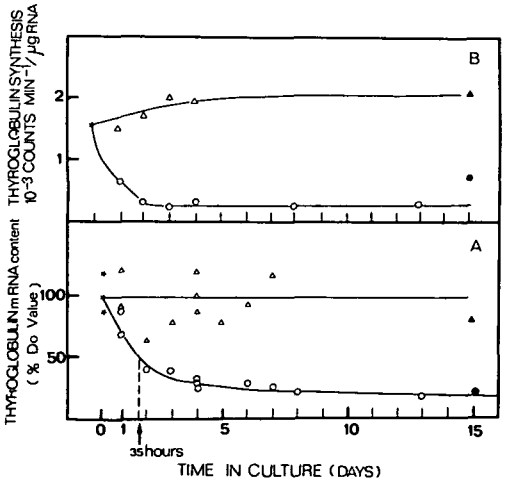

Fig. 5 - Tgb mRNA quantitation in cytoplasm of TSH-treated and control cells as a function of culture time.

Thyroglobulin mRNA content was evaluated in freshly isolated cells (*) or in cells cultured in the absence (o - o) or presence (Δ - Δ) of TSH, according to either the Rot $\frac{1}{2}$ values of hybridization kinetics observed at different days in several cell isolation experiments (part A), or the Tgb specific messenger activity in reticulocyte lysate in a typical experiment (part B). Black symbols refer to RNA from cells cultured for 15 days in presence of hydrocortisone (1 µg/ml). TSH concentration was 5 mU/ml in this experiment.

assays (Fig. 5B). The Tgb messenger activity in reticulocyte lysate of the RNA from control cells maintained for 40 h in culture (D_2) was decreased to 15-20 % of its initial level. After this delay no appreciable decrease occured for up to 15 days; during the same period the Tgb messenger activity in TSH-treated cells was stabilized at its original level or was slightly increased (Fig. 5 B).

Based on the kinetics of molecular hybridization of cytoplasmic RNA to Tgb [^{3}H]cDNA, the Tgb mRNA content in thyroid cells cultured without TSH fell to 30 % of its initial value within 4 days. Based on the Tgb messenger activity in a heterologous cell-free system the mRNA content was reduced to 15 % by 4 days. The translational activity thus declined more rapidly than the number of hybridizable sequences. This suggests that control cell RNA contains a lower proportion of functional Tgb mRNA molecule than TSH- treated cell RNA.

When cells were exposed for 1 to 6 days to 5 mU/ml TSH, or to 50 µU/ml TSH, the latter dose being near physiological levels (10 µU/ml), it was observed that 50 µU/ml was sufficient to maintain Tgb mRNA at its maximal level as evidenced by both hybridization and translation activities (Fig. 6 and Table II).

Reversion by TSH of the Tgb mRNA decrease in control cells - Control cells cultured for 4 or 8 days were incubated with 5 mU/ml TSH for respectively 4 or 5 additional days. The RNA from cells treated by TSH at day 4 hybridized

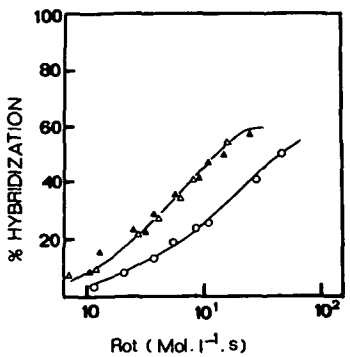

Fig. 6 - Effect of the amount of TSH, added at day 0, on the Tgb mRNA content after 4 days in culture.

Cytoplasmic RNA was extracted from cells cultured for 4 days in the absence of TSH (o - o), or in the presence of TSH 50 µU/ml (Δ - Δ) or 5 mU/ml (▲ - ▲), and hybridized to Tgb [^{3}H]-cDNA.

TABLE II - Total messenger and thyroglobulin messenger activities of cytoplasmic RNA_S from control and TSH-treated cells in culture for 4 days. Values are determined as in Table I.

Cytoplasmic RNA from	TCA precipitable radioactivity (counts. min^{-1}. µg RNA^{-1})		Relative thyroglobulin synthesis %
	Total	Thyroglobulin	
Control cells	33 000	518	1.6
TSH-treated cells			
0.05 mU/ml	46 645	2 496	5.4
5 mU/ml	46 500	2 417	5.2

to Tgb cDNA about 1.5 times faster than the RNA of control cells at day 4 (Fig. 7 A). In contrast, the RNA from corresponding cells without TSH treatment (D_8 control cells) hybridized 1.3 fold slower than the RNA of D_4 control cells. After 4 days of TSH treatment, the cell RNA contained 2.8 times more Tgb mRNA than the corresponding D_8 control cells. When TSH was added to control cells at day 8, Tgb mRNA content at day 13 was doubled relative to that in control cells at D_{13}. Tgb messenger activity was increased 3 fold when TSH was added at day 4 (Fig. 7B). Tgb messenger activity increased to a lower extent when TSH was added only at day 8. Thus the treatment by TSH not only stopped the Tgb mRNA decrease occuring in control cells but also induced an increase in its steady-state level above that level observed before TSH

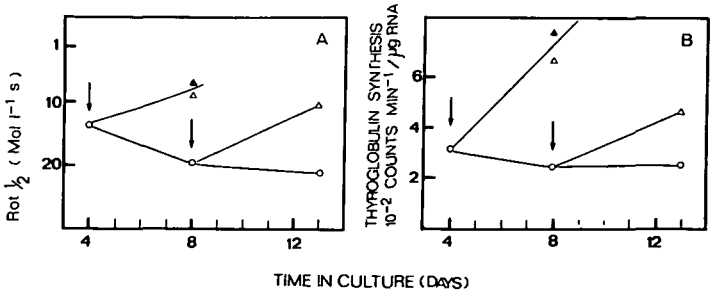

Fig. 7 - Influence of TSH added at different times to culture medium on the restoration of Tgb mRNA content.

At times indicated by the arrows, culture medium was renewed and cells were further incubated without TSH (o), with 5 mU/ml (Δ), or with 50 mU/ml (▲) TSH.

addition. These results imply that in thyroid cells maintained in culture without TSH for 4-8 days, TSH responsiveness is preserved.

DISCUSSION

In the present study, we evaluated the influence of TSH in the culture medium on the relative cytoplasmic Tgb mRNA content in cultured thyroid cells.

Both translation and hybridization assays showed that the cytoplasmic Tgb mRNA content in thyroid cells decreased during culture without TSH. After 4 days, 70 % of the messenger sequences disappeared in control cells. The Tgb mRNA decrease occured much faster than the decline in whole cell RNA. The lower RNA/DNA ratio observed in control cells might be related to the decrease of alkaline ribonuclease inhibitor activity per cell as reported in the thyroid of triiodothyronine-treated or hypophysectomized rats (37). The relative Tgb mRNA decrease seems to be a specific process, since during the same time interval the relative poly(A) content was similar in both control and TSH-treated cells.

A 50 % diminution of the Tgb mRNA sequences was observed within about 35 hours in culture without TSH. Previous experiments using thyroid slices incubated with actinomycin D to block mRNA synthesis suggested that the half life of the Tgb messenger was longer than 20 h (38). The kinetics of the decay that we observed might well be induced by a block of synthesis, without acceleration of degradation processes. The decrease of Tgb mRNA slackened during the following days, suggesting that either hormonal factors in the calf (or horse) serum partly mask the lack of TSH, or the degradation and synthetic processes slow down (39). In the same way, insulin or growth hormone deprivation was able to induce only a 50 % or 70 % decrease, respectively, in albumin mRNA in rat liver (9, 10). Such modulation cannot be compared to larger variations observed in differentiation events in the course of embryologic development (40) or under the influence of hormones (41).

The specific decrease of the Tgb mRNA relative to other messengers is in keeping with, and might provide a molecular explanation of observations according to which incorporation of labeled aminoacids into Tgb is preferentially decreased relative to other proteins, in TSH deprivation experiments (15, 19, 21). However these results are at variance with previous observations that the total amount of Tgb formed by cultured porcine thyroid cells is not dependent on the presence of TSH in the culture medium (42). It is possible that the overall regulation of Tgb synthesis and Tgb content involves several control steps, not only in the mRNA content, but also on mRNA translation and

on Tgb degradation processes. Analysis of the protein synthesizing machinery in the experimental system of cultured thyroid cells is currently in progress.

The relative thyroglobulin mRNA content was fully maintained in cells treated with 50 µU/ml TSH from the onset of culturing. TSH concentrations, up to 100 times higher, did not further increase the thyroglobulin mRNA level. This is in agreement with previous observations that 50 µU/ml TSH preserve most tissue-specific functions of the thyroid cell, although this concentration does not promote an acute increase in basal cAMP synthesis over that of control cells (43). Higher TSH doses raise cAMP synthesis without further stimulating iodide organification (27). This is also in agreement with the observation that in the thyroids of rats treated by propylthiouracil, which produces hypersecretion of TSH, the relative Tgb mRNA content was not changed (20). In the present study, TSH added to control cells after 4-8 days of culture stopped the Tgb mRNA decay and within 4-5 days raised the relative Tgb mRNA sequence content by 1.5 to 2 fold and the Tgb mRNA activity by 2-3 fold compared to levels before TSH addition. This occurs despite the apparent 70 % loss at day 4 of plasma membrane TSH receptors in control cells (44). Furthermore, control cells at D_4 are no longer able to organify iodide and this property cannot be restored in a simple way by TSH addition (unpublished observation). Nevertheless, control cells still display low activation of adenylate cyclase under acute TSH stimulation (43) and our experiments demonstrate that control cells are still responsive to TSH, regarding a long-term effect of the hormone. The specific decrease of Tgb mRNA content in the absence of TSH and its accumulation in cell cytoplasm under chronic TSH stimulation following deprivation raise questions as to the levels at which regulation operates (i.e. transcription, mRNA processing and transfer, degradation) and the mechanisms of such regulation in the thyroid cell. Particularly , the role of cAMP and cAMP-dependent protein kinases could be examined at the nuclear and cytoplasmic level (13, 14, 45-46, 49). In addition, possible effects of TSH on ionic fluxes by alterations of membrane properties as postulated for other hormones (46, 47, 48) and (or) the hypothetical effects of internalized hormone molecules (12) should be taken in account.

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REFERENCES

1. O'Malley, B.W., Tsai, M.J. and Towle, H.C. (1977) Receptors and hormone action, O'Malley and Birnbaumer eds, Acad. Press. N.Y., Vol. I p. 359-379.
2. Palmiter, R.D., Moore, P.B., Mulvihil, E.R. and Emtage, S. (1976) Cell, 8, 557-572.
3. Robins, D.M. and Schimke, R. (1978) J. Biol. Chem. 253, 8925-8934.
4. Cox, R.F. (1977) Biochemistry 16, 3433-3443.
5. Land, H., Hofer, E. and Sekeris, C.E. (1978) Biochem. Biophys. Res. Comm. 83, 607-615.
6. Matusik, R. J. and Rosen, J.M. (1978) J. Biol. Chem. 253, 2343-2347.
7. Devinoy, E., Houdebine, L.M. and Delouis, C. (1978) Biochim. Biophys. Acta, 517, 360-366.
8. Roy, A.K. and Dowbenko, D.J. (1977) Biochemistry 16, 3918-3922.
9. Keller, G.H. and Taylor, J.M. (1979) J. Biol. Chem. 254, 276-278.
10. Peayy, D.E., Taylor, J.M. and Jefferson, L.S. (1978) Proc. Natl. Acad. Sci. USA, 75, 5879-5883.
11. Garcia-Ruiz, J.P., Ingram, R. and Hanson, R.W. (1978) Proc. Natl. Acad. Sci. USA 75, 4189-4193.
12. Goldfine, I.D. (1978) Life Sces 23, 2639-2648.
13. Le cocq, R.E. and Dumont, J.E. (1972) Biochim. Biophys. Acta. 281, 434-441.
14. Sherwin, J.R. and Tong, W. (1976) Biochim. Biophys. Acta, 425, 502-510.
15. Pavlovic-Hournac, M. and Delbauffe, D. (1973) Endocrinology 92, 1273-1276.
16. Le cocq, R.E. and Dumont, J.E. (1973) Biochim. Biophys. Acta, 299, 304-311.
17. Caviedes, R. and Stanbury, J.B. (1074) Endocrinology 95, 447-454.
18. Scheinman, S.J., Burrow, G.N., Theoharides, T.C. and Canellakis, Z.N. (1977) Life Sces 21, 1143-1148.
19. Regard, E. and Mauchamp, J. (1971) J. Ultrastructure Research 37, 664-678.
20. Scherberg, N.H. (1978) Biochem. Biophys. Res. Commun. 85, 1415-1423.
21. Pavlovic-Hournac, M., Rappaport, L. and Nunez, J. (1971) Endocrinology 89, 1477-1484.
22. Pavlovic-Hournac, M., Rappaport, L. and Nunez, J. (1971) Exp. Cell. Res. 68, 332-338.
23. Pavlovic-Hournac, M., Rappaport, L. and Nunez, J. (1971) Exp. Cell. Res. 68, 339-346.
24. Chebath, J., Chabaud, O., Becarevic, A., Cartouzou, G. and Lissitzky, S. (1977) Eur. J. Biochem. 77, 243-252.
25. Chebath, J., Chabaud, O., Berge-Lefranc, J.L., Cartouzou, G. and Lissitzky, S. (1977) Biochem. Biophys. Res. Comm. 79, 267-273.
26. Chebath, J., Chabaud, O. and Jacquet, M. (1979) Submitted for publication.
27. Mauchamp, J., Charrier, B., Takasu, N., Margotat, A., Chambard, M. and Dumas, D. (1979) in Hormones and cell regulation Vol. 3, J. Dumont and J. Nunez Eds., North Holland (1979) p. 51-68.
28. Takasu, N., Charrier, B., Mauchamp, J. and Lissitzky, S. (1978) Eur. J. Biochem. 90, 139-146.
29. Cox, R.A. (1968) Methods enzymol. (Grossmann, K. and Moldave K. eds) vol. XII p. 120-129. Academic Press, N.Y.
30. Baltimore, D. (1970) Nature 226, 1209-1211.
31. Temin, H. and Mizutani, S. (1970) Nature 226, 1211-1213.

32. Milcarek, C., Price, R. and Penman, S. (1974) Cell. 3, 1-10
33. Hallinan, T., Fleck, A. and Munro, H.N. (1963) Biochim. Biophys. Acta 68, 131-133.
34. Mc Intire, F., and Sproull, M.F. (1957) Proc. Soc. Exp. Biol. 95, 458-462.
35. Rosbach, M. and Ford, P.I. (1974) J. Mol. Biol. 85, 87-111.
36. Pelham, H.R. and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247-256.
37. Greif, R.L. and Eich, E.F. (1972) Biochim. Biophys. Acta, 286, 350-359.
38. Seed, R.W. and Goldberg, I.H. (1965) J. Biol. Chem. 240, 764-773.
39. Benecke, B.J., Ben-Zeev, A. and Penman, S. (1978) Cell. 14, 931-939.
40. Harding, J.D. and Rutter, W.J. (1978) J. Biol. Chem. 253, 8736-8740.
41. Baker, H.J. and Shapiro, D.J. (1978) J. Biol. Chem. 253, 4521-4524.
42. Lissitzky, S., Fayet, G., Giraud, A., Verrier, B. and Torresani, J. (1971) Eur. J. Biochem. 24, 88-99.
43. Takasu, N., Charrier, B., Mauchamp, J. and Lissitzky, S. (1977) FEBS Lett. 84, 191-194.
44. Lissitzky, S., Fayet, G., Verrier, B., Hennen, G. and Jacquet, P. (1973) FEBS Lett. 29, 20-24.
45. Pisarev, M.A., Aiello, L.O. and Kleiman De Pisarev, D.L. (1976) Acta Endocrinologica 83, 313-320.
46. Dumont, J.E. (1971) Vitamins and Hormones 29, 287-412.
47. Bisbee, C.A., Machen, J.E. and Bern, H.A. (1979) Proc. Natl. Acad. Sci. USA, 76, 536-540.
48. Chen, J-L.J., Babcock, D.F. and Lardy, H.A. (1978) Proc. Natl. Acad. Sci. USA, 75, 2234-2238.
49. Wagner-Capodano, A.M., Mauchamp, J., Delgrossi, M.H., Stahl, A. and Lissitzky, S. (1978) Biol. Cell. 33, 181-184.
50. Mauchamp, J., Margotat, A., Chambard, M., Charrier, B., Remy, L. and Michel-Bechet M., Cell and Tissu Res., in press.