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MODULATION BY THYROTROPIN OF THYROGLOBULIN SYNTHESIS IN CULTURED THYROID CELLS : CORRELATIONS WITH POLYSOME PROFILE AND CYTOPLASMIC THYROGLOBULIN mRNA CONTENT

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SUMMARY. Porcine thyroid cells were cultured with or without thyrotropin for 4 days. In the absence of thyrotropin the amount of ^{35}S-méthionine incorporated into proteins was reduced 3-4 fold. In this case, relative thyroglobulin synthesis was reduced 3 fold ; this is related to the 3 fold decrease in the relative thyroglobulin mRNA content (previously described). In the presence of hormone a peak of heavy thyroglobulin synthesizing polysomes was observed which contained 60 % of the thyroglobulin mRNA sequences. In absence of the hormone most of the thyroglobulin mRNA sequences were shifted to lighter polysomes, indicating that thyroglobulin mRNA molecules were underloaded with ribosomes. Therefore in the absence of thyrotropin the decreased thyroglobulin synthesis observed was the result of a lower thyroglobulin mRNA content and a lower efficiency of translation.

It has been well established that thyrotropin (TSH) produces a general stimulation of protein synthesis in the thyroid gland (Ref. in (1)). However, a specific effect of TSH on thyroglobulin (Tgb) gene expression has not been elucidated until recently, although in some experiments of TSH deprivation, the incorporation of labeled aminoacids into Tgb was found to be preferentially decreased (2-6).

In previous studies (7) of cultured porcine thyroid cells it was shown that the presence of TSH in the culture medium maintained initial levels of cytoplasmic Tgb mRNA. In contrast, when cells were cultured in absence of the hormone the Tgb mRNA level decreased to 35 % of its initial level after 4 days in culture. In order to evaluate the consequence of this modulation of Tgb mRNA level on Tgb synthesis, we studied the incorporation of [^{35}S]-methionine into proteins and into Tgb peptides after 4 days in culture. The Tgb synthesizing polysomes were identified on sucrose gradients by immunoprecipitation of labeled Tgb growing chains and by hybridization of RNA contained in polysome fractions to Tgb [^{3}H]-complementary DNA (8).

Abbreviations : Thyrotropin, TSH ; Thyroglobulin, Tgb ; Ethyleneglycol-bis-(β aminoethyl ether) N, N'-tetraacetic acid, EGTA ; High salt buffer, HSB ; Sodium dodecylsulfate, SDS.

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Our results suggest that TSH specifically regulates Tgb synthesis mainly through the control of the Tgb mRNA content and allows the translation of the Tgb mRNA sequences on large sized polysomes ; this implies that the protein synthesizing machinery is more efficient in TSH-treated cells.

MATERIALS AND METHODS

Cell culture. Porcine thyroid cells were isolated by a discontinuous trypsin EGTA-treatment (9). Cells were cultured for 4 days as unstirred suspension (7) in the absence (control cells) or presence of 0.05 mU or 5 mU/ml TSH (TSH-treated cells).

Isotopic labeling of thyroid cells. Determination of the total protein and thyroglobulin specific radioactivity. After 4 days in culture about 30 x 10^6 cells in 10 ml initial medium were incubated with 1.2 mCi [^{35}S]-methionine (560 Ci/mmole, N.E.N.) for 55 minutes. Cycloheximide (Sigma) was added to obtain 50 µg/ml final concentration and incubation continued for 5 min. Cells were isolated by centrifugation, twice washed by PBS (7) containing 50 µg/ml cycloheximide, homogenized (30 x10^6 cells/4 ml unless otherwise indicated) in high salt buffer (HSB) (10) containing 2 % Triton X-100 in previously described conditions (7) and centrifuged for 5 min at 27,000 x g. The total radioactivity was counted on duplicate 10 µl aliquots in 8 ml scintillation mixture. The total protein radioactivity was determined on duplicate 10 µl aliquots by hot TCA precipitation. The Tgb specific radioactivity was obtained by successive addition of 1 µl of rabbit serum anti-porcine Tgb (1h30, room T°) and 12 µl of donkey serum antirabbit globulins (Wellcome) (1h30, room T°) on duplicate 50 µl aliquots of 27,000 x g supernatant. The centrifugation of the immunoprecipitates through 1M sucrose cushion and counting of the radioactivity were performed as described elsewhere (10). The non specific radioactivity (precipitated in the presence of normal rabbit serum) was subtracted from all values. The relative Tgb synthesis was calculated as the percentage of immunoprecipitated radioactivity compared with TCA precipitated radioactivity.

Sucrose gradients. 27,000 x g supernatants were analyzed on 12 ml 15-50 % (w/v) sucrose gradients as described (8). Indirect immunoprecipitation was performed on 100 µl aliquots on each fraction. For hybridization to Tgb [^{3}H]-cDNA, gradient fractions were pooled and ethanol precipitated. RNA was extracted by the guanidium-chloride method (11) then precipitated by 3M Na Acetate pH 6.

Hybridization to Tgb [^{3}H]-cDNA. Hybridization experiments were performed for 17h at 65°C and the percentage of [^{3}H]cDNA hybridized was determined as previously described (8). Two concentrations of each RNA sample were tested and the Tgb mRNA amount was determined by reference to the linear portion of a calibration curve relating the percent of hybridized [^{3}H]-cDNA to the amount of pure Tgb mRNA .

DNA evaluation. DNA was measured on homogenates or 27,000 x g pellets by the method of Mc Intire and Sproull (12).

Sodium dodecylsulfate polyacrylamide gel electrophoresis. Electrophoresis was carried out with a discontinuous buffer system (13) in slab gels (14). Acrylamide gel concentrations were 3 % for the stacking gel and 5 % for the separating gel. Samples were reduced according to Giraud et al. (15). After fixation and staining with Coomassie blue (16), slab gels were dried down under vacuum on Whatman 3MM and autoradiographied for 3 or 12 days on Kodirex film (Kodak).

RESULTS AND DISCUSSION

Protein and thyroglobulin labeling in cultured thyroid cells.

Porcine thyroid cells were cultured for 4 days in the presence or absence of TSH ; [^{35}S]-methionine was added 1 hour before washing and homogenizing the cells. Total and hot TCA precipitable radioactivities were measured (Table I). The amount of radioactivity incorporated into proteins was found to be 3-4 times (0.05 mU/ml TSH) and 5 times (5 mU/ml TSH) higher in TSH treated cells than in control cells. The difference in the radioactivity incorporated into proteins between control and 0.05 mU/ml TSH-treated cells could be due to a difference of the intracellular labeled aminoacid specific activity and/or to an intracellular pool of labeled aminoacid which is more limiting in control cells than in TSH-treated cells. However, the preceding possibilities may be discarded for two reasons : 1) after 60 min incubation in the presence of labeled aminoacid with a 0.2 mM extracellular methionine concentration, the isotopic equilibrium between extra- and intracellular methionine was probably attained (17) ; 2) in the absence of TSH, the free intracellular pool of labeled aminoacid, estimated as the difference between total and TCA precipitated radioactivities, was found to be similar if not higher than that observed in the presence of 0.05 mU/ml TSH (Table I). Therefore, these results suggest that TSH chronically stimulates the aminoacid incorporation into proteins from similar intracellular pools found in these steady state culture conditions. No differences were observed when a fresh medium change was made before incubation with labeled aminoacid.

This stimulating effect of TSH on incorporation of radioactive aminoacid into thyroid protein was also observed by Pavlovic-Hournac and Delbauffe

Table I. Total and protein incorporated [^{35}S]-methionine radioactivities in cultured hog thyroid cells.

	Total Radioactivity[a] Counts min^{-1}/µg DNA	TCA-precipitable Radioactivity[b] Counts min^{-1}/µg DNA	TCA-soluble Radioactivity (a - b)
Control cells	40730	9563	31167
Treated cells			
0.05 mU/ml TSH	55940	33456	22484
5 mU/ml TSH	89817	49855	39962

Total intracellular radioactivity was measured on duplicate 10 µl aliquots of the 27,000 x g supernatant. Protein incorporated radioactivity was determined by hot TCA precipitation (See Methods). Each value is the average of 3 experiments.

in hypostimulated rats (2). The decrease of total protein synthesis in the absence of TSH cannot be explained only by the 15-20 % decrease of total RNA and the 30-40 % reduction of total messenger activity (tested in reticulocyte lysate) in control cells (7), although the percent of poly(A) was the same for both cell types (7). Therefore, it should be considered that a decrease of translation efficiency occurs in the absence of TSH. [^{35}S]-methionine radioactivity incorporated into Tgb peptides was determined by immunoprecipitation of the 27,000 x g supernatant. The relative Tgb synthesis was 3 times lower in control cells compared to TSH-treated cells (Table II). The particulate fraction was isolated from 27,000 x g supernatant by centrifugation at 100,000 x g. The proportion of radioactive immunoprecipitated polysomes was 3 times higher in TSH-treated cells (24 %) than in control cells (8 %). These results are in good agreement with preceding observations (7) which showed that the relative Tgb mRNA content displayed a 3-fold decrease in control cells. Accordingly, the specific effect of TSH on relative Tgb synthesis appears to be mainly the consequence of the hormone modulation of Tgb mRNA content in thyroid cells. In agreement with our results, others have demonstrated a specific decrease of Tgb synthesis in hypophysectomized (4) and T_4-treated (18) rat thyroids. In the latter paper the proportion of 19S Tgb diminished following T_4-treatment.

The TSH increase from 0.05 to 5 mU/ml does not change the relative Tgb synthesis or the relative Tgb mRNA content (7). The 0.05 mU/ml TSH concentration seems sufficient to maintain the specific properties of cultured thyroid cells (24).

The radioactivity incorporated into Tgb peptides was 12 times (0.05 mU/ml TSH) and 18 times (5 mU/ml TSH) higher in TSH-treated cells than in control cells (Table II). This result is at variance with other observations (19) in cultured thyroid cells showing that the amount of accumulated Tgb evaluated by radioimmunoassay in cells and medium was the same in control and TSH-treated cells. However, it must be kept in mind that the Tgb degradation is likely to occur in TSH-treated cells which are associated into follicles and not in the monolayers of control cells.

The Tgb mRNA content/µg DNA was 3-4 times lower in control cells, whereas the incorporated Tgb radioactivity/µg DNA was 12 times lower in control cells compared to TSH-treated cells. This difference results from a specific effect of TSH on Tgb mRNA content (x3) and a general effect on overall protein synthesis (x4). Since the latter observation suggests that translation might

Table II. Protein and thyroglobulin incorporated [^{35}S]-methionine radioactivities in cultured hog thyroid cells.

	TCA-precipitable Radioactivity Counts $min^{-1}/\mu g$ DNA	Immunoprecipitated Radioactivity Counts $min^{-1}/\mu g$ DNA	Relative Tgb Synthesis %
Control cells	9563	297	3,5
Treated cells			
0.05 mU/ml TSH	33456	3574	11
5 mU/ml TSH	49855	5305	10,8

TCA-insoluble radioactivity and Tgb specific immunoprecipitated radioactivity were measured on duplicate aliquots of the 27,000 x g supernatant. Results are expressed as the mean value of 3 experiments.

be less efficient in cells cultured in the absence of TSH, we analyzed the size distribution of Tgb synthesizing polysomes.

Identification of Tgb synthesizing polysomes and thyroglobulin mRNA distribution.

27,000 x g supernatants of homogenates made in the presence of 2 % Triton X-100 were layered on 15-50 % sucrose gradients. When TSH was present in the culture medium a peak of rapidly sedimenting polysomes was observed (Fig. 1A). This peak was barely perceptible in supernatants of control cells (Fig. 1B). Labeled Tgb peptides were identified in each fraction by immunoprecipitation. Newly synthesized Tgb peptides were associated with the heavy polysome fraction in the supernatant of TSH-treated cells (Fig. 1C). Conversely, only trace amounts of immunoprecipitated heavy polysomes were present in control cells (Fig. 1D). In the supernatant of control cells a small peak of immunoprecipitated polysomes was observed in the area of the lightest polysomes (Fig. 1D). Consequently, the individual fractions of the gradients were pooled in 4 parts and the Tgb mRNA content of each was determined by hybridization to Tgb [^{3}H]-cDNA (Fig. 1A,B). When the culture was performed in the presence of TSH, 61 % of the total Tgb mRNA sequences present in the gradient were found associated with heavy Tgb specific polysomes (part I). In contrast, when TSH was absent during the culture only 7 % of the Tgb mRNA sequences was associated with this fraction and 70 % of the Tgb mRNA was found in light polysomes (III). This shift of the Tgb mRNA sequences towards the lighter fractions of the gradient suggests that either, 1) a ribonuclease was more active (20) in control cells and degraded the heavy Tgb polysomes into small fragments in the cells or during the homogenization, and/or 2) Tgb mRNA

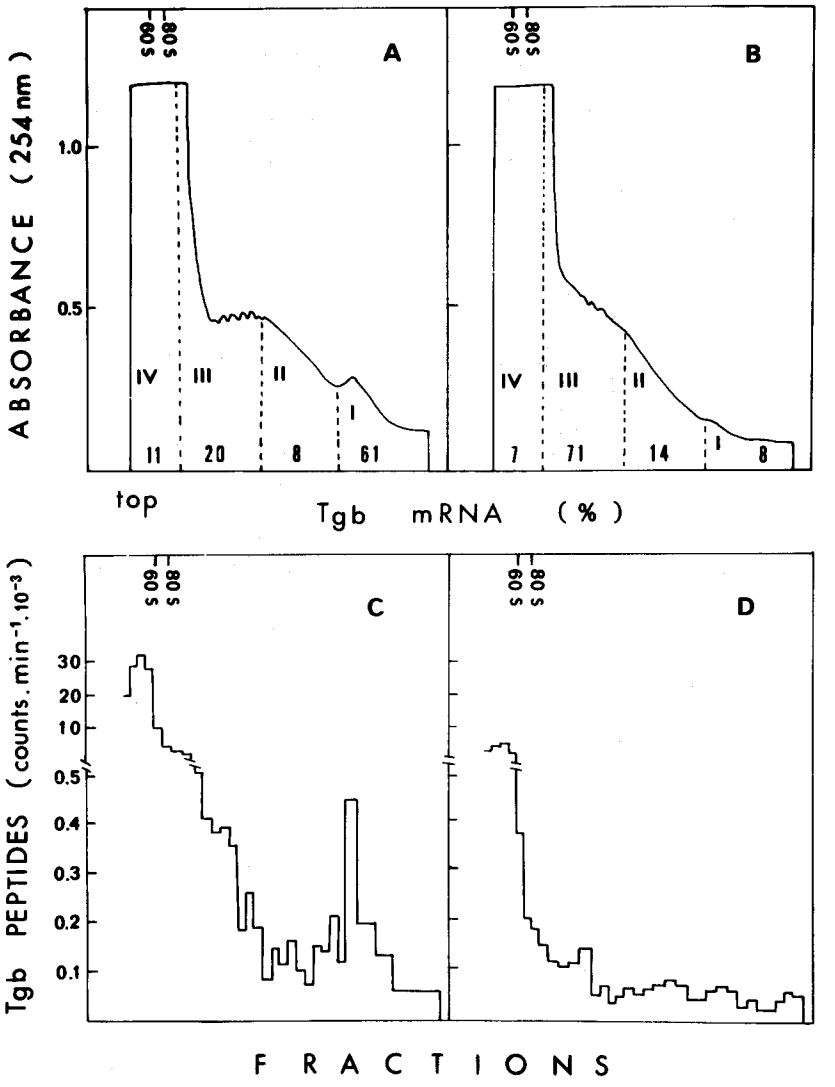

Fig. 1. Polysomal profile, thyroglobulin peptides and thyroglobulin messenger RNA distribution in 27,000 x g supernatants analyzed on sucrose gradient.
27,000 x g supernatants from 5 mU/ml TSH-treated cells (A,C) and control cells (B,D) were run on sucrose gradients containing 50 µg/ml cycloheximide (See Methods and (9)). A,B : $30x10^6$ cells were homogenized in 1 ml HSB and 0,8 ml ($24x10^6$ cells) 27,000 x g supernatant was layered on the gradient ; Tgb mRNA was quantified by hybridization with Tgb [^{3}H]-cDNA. In each pooled fraction of the gradient, Tgb mRNA was expressed as the percentage of the total amount of Tgb mRNA on the gradient. C,D : Tgb peptides in each gradient fraction were measured by immunoprecipitation (27,000 x g supernatant from $7.5x10^6$ cells).

molecules were underloaded with ribosomes in control cells as a result of a slackened initiation rate. The first hypothesis can be partly tested by the

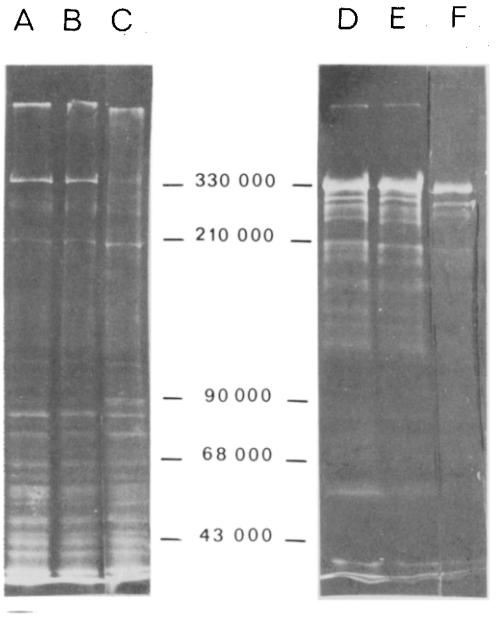

Fig. 2. Autoradiography of [^{35}S] -methionine labeled proteins analyzed by electrophoresis in SDS polyacrylamide gels.
Total proteins (A,B,C) of 10 µl 27,000 x g supernatant and Tgb peptides (D,E,F) isolated by indirect immunoprecipitation from 50 µl 27,000 x g supernatant were electrophoresed (Methods). Radioactivity corresponding to an equal number of cells were used for control cells (C,F), 0.05 mU/ml (B,E) and 5 mU/ml (A,D) TSH-treated cells. A,B,D,E were exposed for 3 days and C,F for 12 days. Positive print of autoradiogram negative.

analysis of immunoreactive Tgb peptides. Indeed, the translation of undegraded Tgb mRNA molecules should result in the synthesis of complete 330,000 Tgb subunits (10).

Analysis of labeled peptides.

27,000 x g supernatants and immunoprecipitated Tgb peptides from 27,000 x g supernatants were analyzed on SDS-polyacrylamide gel electrophoresis (Fig. 2). Supernatants displayed the characteristic band of the 330,000 Tgb subunit (Fig. 2 A,B,C) (10) that was the major component of the immunoprecipitates (Fig. 2 D,E,F). TSH-treated cells (A,B) contained more Tgb subunits than control cells (C). These observations corroborate the quantitative results obtained by immunoprecipitation. Smaller peptides observed in immunoprecipitates could be due to Tgb degradation during the homogenization in presence of 2 % Triton X-100 which broke open lysosomes.

Although some Tgb mRNA molecules may have been degraded, it is likely that most of them were undamaged when being translated in control cells, since 330,000 Tgb peptide was the major component. This observation strongly suggests that in the absence of TSH in the culture medium, intact Tgb mRNA molecules do not

bear their full ribosomal complement as a result of a decreased rate of initiation. In the absence of TSH this could occur through some structural alterations of ribosomes (21) or mRNA molecules (e.g. a defect in cap formation). Moreover, short term incubation of dog thyroid slices (22) with TSH resulted in a higher amount of Tgb specific polysomes and a lower amount of monoribosomes compared to incubation without TSH suggesting that this hormone regulates the number of monoribosomes present on the Tgb mRNA molecules. Therefore, the lower rate of Tgb mRNA translation could result from the effect of TSH on the overall protein synthesizing system, for example through the modulation of the ribosomes'activation state (21). It cannot be excluded that TSH also exerts a specific regulation on the translation of Tgb mRNA, similar to the specific regulation by growth hormone of α_{2u}-globulin mRNA translation (23).

CONCLUSION. In cultured thyroid cells, TSH modulates the relative Tgb synthesis mainly through the control of Tgb mRNA content. Total protein synthesis is diminished in the absence of TSH by modifications of both the RNA content (7) and the translational system. Particularly in the absence of TSH the Tgb mRNA molecules are underloaded with ribosomes which gives evidence in favor of a lower translation efficiency.

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