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Inverse control of prolactin and growth hormone gene expression: effect of thyroliberin on transcription and RNA stabilization

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The hypothalamic tripeptide thyroliberin (TRH) regulates prolactin (PRL) and growth hormone (GH) synthesis inversely by modulating the levels of their specific mRNA. Changes in mRNA levels could involve both transcriptional and post-transcriptional events. To examine further these possibilities, we have investigated the effect of TRH on the biosynthesis and degradation of PRL and GH RNA in a rat pituitary tumor cell line. Newly synthesized PRL and GH RNA sequences were quantified in nuclear and cytoplasmic fractions by hybridization of ^{3}H-labelled RNA to immobilized plasmid DNA containing either PRL or GH cDNA sequences. Steady-state levels of specific RNA were estimated by RNA blot hybridization. The results indicate that TRH increases in a rapid but transient manner the transcription of the PRL gene, and suggest that it does not alter the processing and the transport to the cytoplasm. In contrast, after a lag-time, TRH seems to induce a long-lasting inhibition on GH, as well as on overall gene transcription. Furthermore, we observed an effect of TRH on mRNA stability. TRH significantly increases the half-life of PRL mRNA. Our results also support the hypothesis that TRH decreases the half-life of GH mRNA. Such post-transcriptional action of TRH amplifies and prolongs the regulations exerted at the transcriptional level.

Key words: growth hormone/prolactin/RNA stabilization/thyroliberin/transcription

Introduction

The prolactin (PRL) and growth hormone (GH) genes are thought to originate from a common ancestral gene. This hypothesis is based on comparisons of the respective sequences of the two proteins, of their structural genes and, recently, of their genomic sequences (Niall *et al.*, 1971; Cooke *et al.*, 1980, 1981; Cooke and Baxter, 1982; Barta *et al.*, 1981). These two genes are normally expressed in separate cells of the pituitary gland (Tixier-Vidal *et al.*, 1982). However, several strains of rat pituitary cells, such as the GH3 and their subclones, are able to synthesize and release both hormones (Tashjian *et al.*, 1970; see review in Tashjian, 1979; Gourdji *et al.*, 1982). Extensive studies using these cell lines have shown that the production of GH and PRL is modulated by a variety of hormones: thyroliberin (TRH – thyrotropin releasing hormone), estrogens, glucocorticoids, thyroid hormones, epidermal growth factor, dopamine, etc. Most of these hormones have been shown to mediate their action through a primary effect on the levels of the mRNA coding for PRL and GH (see review in Tashjian, 1979; Gourdji *et al.*, 1982; and Dobner *et al.*, 1981; Evans *et al.*, 1982; Wegnez *et al.*, 1982; Spindler *et al.*, 1982; Maurer, 1982a, 1982b; Murdoch *et al.*, 1982). As far as the hypothalamic tripeptide TRH (pGlu-His-Pro-NH2) is concerned, it is well documented that it increases PRL synthesis and inhibits GH synthesis by modulating the level of their specific mRNAs (Dannies and Tashjian, 1976; Evans and Rosenfeld, 1976; Evans *et al.*, 1978; Morin *et al.*, 1981). Recent work, using cloned cDNA probes, has shown that the increase in rat PRL (rPRL) mRNA concentration is preceded or accompanied by a parallel increase in the concentration of its nuclear precursors (Potter *et al.*, 1981; Biswas *et al.*, 1982). In contrast, the regulation of rat GH (rGH) gene expression by TRH is far less well documented.

We have used the GH3/B6 system to analyse the effect of TRH on the *'in vivo'* biosynthesis and degradation rates of PRL and GH mRNA. Newly synthesized [^{3}H]RNA extracted from control cells or from cells exposed to TRH for increasing times were analyzed for labelled rPRL and rGH RNA sequences by hybridization to filters containing the respective cDNA probes. Additional information was obtained by using the RNA blotting procedure. To measure the effect of TRH on the half-life of the mRNA, pulse-chase experiments were carried out in the presence or absence of TRH. The results indicate that TRH regulates PRL and GH synthesis by two types of mechanisms, the first one acting at the transcriptional level, the second one modifying the half-lives of the cytoplasmic mRNAs.

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Results

Incorporation of [^{3}H]uridine into newly synthesized RNA

Non-specific RNA biosynthesis was analyzed by labelling exponentially growing GH3/B6 cells with [^{3}H]uridine for 30 or 60 min. After thorough washing, the cells were disrupted and the nuclear and the cytoplasmic RNA fractions were isolated. Using the fractionation procedure described, nuclear RNA accounted for ~20% of total cellular RNA. Not surprisingly, the nuclear RNA fraction was labelled to a higher specific activity than that of the cytoplasm. After 30 min of [^{3}H]uridine incorporation, the specific activity of purified nuclear RNA was 33-fold higher than that of cytoplasmic RNA. This ratio decreased to 24 when cells were labelled for 60 min, indicating a time-dependent accumulation of newly synthesized RNA into the cytoplasm (not illustrated).

The amounts of ^{3}H-labelled rPRL and rGH-RNA from the nuclear and cytoplasmic fractions were measured by hybridization to the rPRL and rGH cDNA probes immobilized on nitrocellulose filters. The hybridizable radioactivity was then referred to the input radioactivity (p.p.m.) to determine the relative distribution of newly synthesized RNA between the cytoplasmic and nuclear fractions (Table I). This quantitative analysis shows that after labelling the cells for 30 min, a 9-fold enrichment in ^{3}H-labelled rPRL RNA is observed in the cytoplasmic fraction as compared with the nuclear frac-

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Table I. Distribution of newly synthesized rPRL and rGH RNA in the cytoplasmic and nuclear fractions isolated from cells grown in control conditions

	Duration of [^{3}H]uridine incorporation	Fractions		Whole cell[b]
		Cytoplasm[a]	Nucleus[a]	
[^{3}H]cRNA hybridized with rPRL filters (in p.p.m.)	30 min	147 ± 37	17 ± 4	
	60 min	210 ± 37	15 ± 1	37.7
[^{3}H]cRNA hybridized with rGH filters (in p.p.m.)	60 min	16 ± 3	9 ± 3	9.8
Ratio of the number of RNA molecules rPRL/rGH	60 min	13	1.7	3.9

[a]Each value is the mean ± standard deviation of six independent experiments (60 min of incorporation) or eight independent experiments (30 min of incorporation.
[b]Data for whole cell are calculated. This was done by combining the experimental values obtained for the cytoplasmic and nuclear fractions taking into account that the absolute nuclear radioactivity was 7.6-fold higher than the absolute cytoplasmic radioactivity (~56 x 10^6 d.p.m. *versus* 7.3 x 10^6 d.p.m./dish).

tion. When the cells were allowed to incorporate for 60 min this ratio increased to 14. The distribution of newly synthesized rGH RNA followed a different pattern. In contrast to what was observed with rPRL RNA, the relative amount of newly synthesized rGH RNA in the cytoplasmic RNA fraction was only 1.7-fold higher than in the nuclear RNA fraction. This indicates a preferential accumulation of newly synthesized rPRL RNA in the cytoplasm of GH3/B6 cells as compared with rGH RNA (Table I).

Since both rPRL and rGH cytoplasmic mRNAs have the same size (1.0 kb), the ratio of the radioactivity incorporated into each species is a measure of the ratio of the number of mRNA molecules. Therefore, data from Table I show that, in our cells under normal conditions, the cytoplasmic rPRL mRNA is 13-fold more abundant than the cytoplasmic rGH mRNA.

Effect of TRH on RNA biosynthesis

To determine whether TRH could specifically modulate the transcription of the rPRL and rGH genes, GH3/B6 cells were exposed to TRH for increasing periods of time. The RNA was labelled by adding [^{3}H]uridine to the culture medium during the last 60 min of the incubation. The labelled nuclear and cytoplasmic RNAs were then extracted and analysed as a function of time.

Effect of TRH on the general RNA biosynthesis. As illustrated in Figure 1C, TRH treatment significantly affects the incorporation of [^{3}H]uridine into all the RNA species, nuclear as well as cytoplasmic. Although the initial effect is a weak and temporary stimulation, the main result turned out to be an inhibition detectable after 10 h. This TRH-induced effect reached its maximal level (50%) within 20–48 h of treatment.

Effect of TRH on rPRL and rGH RNA biosynthesis. Experimental data concerning rPRL and rGH RNA were obtained by means of filter hybridization. Figure 1A shows that TRH, rapidly and drastically, increases the rate of rPRL RNA synthesis. The effect reaches its maximum (250% of control) within 90 min (the first time point considered) at the nuclear level and after 10 h at the cytoplasmic level. Finally, 20 h after its addition, TRH does not seem to have any further effect on rPRL RNA synthesis (Figure 1A). As far as rGH RNA sequences are concerned (Figure 1B), TRH does

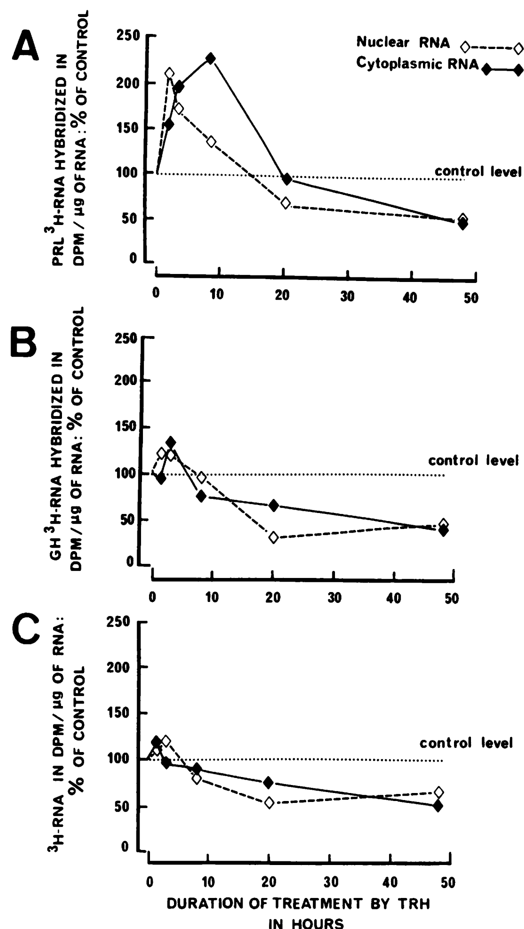


Fig. 1. Time course of TRH effect on newly synthesized RNA. GH3/B6 cells were treated with 100 nM TRH for 90 min to 48 h. During the last 60 min of TRH treatment, [^{3}H]uridine (4.2 µM) was added to the culture medium. Cytoplasmic and nuclear RNA were isolated as described in Materials and methods and the levels of newly synthesized rPRL and rGH RNA were quantitated by hybridization to nitrocellulose filters containing immobilized rPRL or rGH cDNA recombinant plasmids. The specific hybridized d.p.m. were referred to the concentration of non-labelled total RNA in the hybridization reaction. Concerning overall RNA synthesis, the data were obtained by measuring the radioactivity and the concentration of purified nuclear and cytoplasmic RNA (1 OD260 nm = 40 µg of RNA). Each point is the mean of independent determinations corresponding to two culture dishes. The results are expressed in percent of the control level (100% – dashed line) which is the mean of six independent determinations, that is two dishes at 90 min, 20 h and 48 h. **(A)** rPRL RNA, **(B)** rGH RNA, **(C)** total RNA, (◇----- ◇) nuclear RNA, (◆—— ◆) cytoplasmic RNA.

not seem to affect their synthesis in a manner very different from that observed with total RNA.

To investigate early action of TRH, a similar experiment was carried out but the duration of the [^{3}H]uridine incorporation was reduced to 30 min (Figure 2). To avoid interference from the effect of TRH on total RNA synthesis, the levels of newly synthesized rPRL RNA were related to the total ^{3}H d.p.m. input in the hybridization reaction. Under these conditions, a 1.7-fold stimulation of rPRL RNA synthesis was detected at the nuclear level as early as 30 min after TRH treatment. The concentration of newly synthesized cytoplasmic rPRL RNA was simultaneously increased. After maximal stimulation was reached within 5 h of exposure to TRH, the rPRL RNA synthesis decreased following a pattern similar to that shown in Figure 1A.

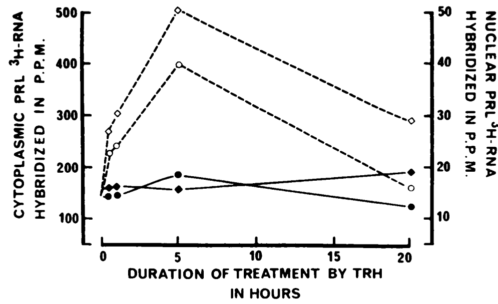


Fig. 2. Time course of TRH effect on newly synthesized rPRL RNA. GH3/B6 cells were treated with 100 nM TRH for various times from 30 min to 20 h. Untreated cultures were carried in parallel. Cells were processed as described in Materials and methods and in the legend to Figure 1 to determine the level of newly synthesized rPRL RNA, except that the duration of [^{3}H]uridine incorporation was reduced to 30 min and its concentration was increased to 5 μM. The specific hybridized d.p.m. were referred to the input d.p.m. in the hybridization reaction. Each point is the mean of two independent determinations each corresponding to one culture dish. (◆——◆) nuclear RNA and (●——●) cytoplasmic RNA from control cells, (◇-----◇) nuclear RNA and (○-----○) cytoplasmic RNA from TRH-treated cells.

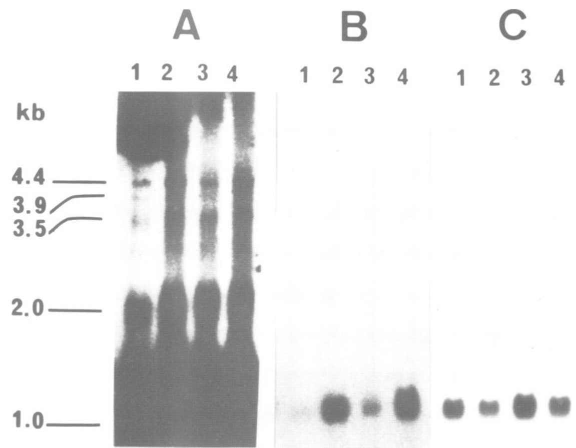


Fig. 3. Effect of TRH on the steady-state level of specific RNA species. Total RNA was extracted as described in Materials and methods from cells grown in serum-supplemented medium **(A)**, or in serum-free medium **(B** and **C)**, and untreated **(lanes 1** and **3)** or treated **(lanes 2** and **4)** with 100 nM TRH for 4 h **(A)** or with 5 nM TRH for 48 h **(B** and **C)**. Glyoxalated RNA **(A: lanes 1** and 2, 20 μg; **lanes 3** and **4**, 40 μg; **B** and **C: lanes 1** and 2, 30 μg; **lanes 3** and **4**, 60 μg) was submitted to gel electrophoresis, transferred to nitrocellulose paper and hybridized with ^{32}P-labelled rPRL cDNA probe **(A** and **B)** or ^{32}P-labelled rGH cDNA probe **(C)**. Ribosomal RNAs (16S, 18S, 23S and 28S) were co-electrophoresed as size markers to compute the length of RNA species (Schaffer and Sederoff, 1981). Radioautography was for 10 and 20 h **(B** and **C)** or for 10 days **(A)** with intensifying screens.

Effect of TRH on the steady-state level of specific mRNA

Short-term effect of TRH on rPRL mRNA. To test whether TRH affects the processing of rPRL mRNA precursors, total cellular RNA from control cells and from cells treated for 4 h with TRH were analyzed using the RNA blotting procedures (Thomas, 1980) after size fractionation on a 1.5% agarose gel.

Figure 3A shows the results obtained using the rPRL cDNA (rPRL 800) as a probe. Several RNA species larger than the mature mRNA are visible; however, all of them seem to be present in both TRH-treated and control cells. Furthermore, TRH does not appear to influence the relative abundance of these RNA species in any given lane, thus suggesting that TRH does not act on the processing of the rPRL mRNA precursor.

The same blot was also hybridized (data not shown) with a 6-kb genomic DNA probe corresponding to the 5′ half of the rPRL gene extending from the 5′-non-coding region to the middle of the third intron (*Hind*IIIA, Cooke and Baxter, 1982). Except for a few additional bands, the pattern of RNA precursors revealed by the genomic probe was similar to the one shown by the cDNA probe. One of these measured 2.9 ± 0.2 kb and the others were of high mol. wt. (>10 kb).

Whatever the probes used, TRH increased by 2-fold both the concentration of mature mRNA species (1.0 kb) and the steady-state level of larger mRNA species.

Long-term effect of TRH on the steady-state level of rPRL and rGH mRNA. To reconcile the transitory effect of TRH on rPRL RNA synthesis and its long lasting effect on rPRL synthesis, we investigated the long-term effect of TRH on the steady-state level of rPRL mRNA. This analysis was simultaneously carried out for rGH mRNA.

For this purpose, the cells were grown in serum-free medium, under culture conditions which enhance the secretory response to TRH (Brunet *et al.*, 1981). The cells were exposed for 48 h to 5 nM TRH and total RNA was extracted and analyzed as above by hybridization with rPRL (Figure 3B) or rGH (Figure 3C) cDNA probes. The TRH treatment resulted in a marked increase in the steady-state level of mature rPRL mRNA. In contrast, in the same experiment, the steady-state level of mature rGH mRNA was slightly decreased.

Effect of TRH on RNA degradation

Changes in the steady-state concentrations of mRNAs could result from alteration of their half-lives. Consequently, we performed experiments to detect a possible effect of TRH on the stability of rPRL and rGH RNA. To this end, GH3/B6 cells were pulse-labelled for 2 h with [^{3}H]uridine. After washing the cells, fresh medium containing unlabelled uridine and with or without TRH was added. The chase period varied from 3 to 72 h.

Effect of TRH on the total RNA degradation. The radioactivity associated with total cellular RNA was determined and the specific activity (d.p.m./μg) was plotted as a function of time using a semi-logarithmic scale. This yielded a straight line, the slope of which corresponds to the turnover rate constant. As illustrated in Figure 4C, TRH seems to decrease the turnover rate of total cellular RNA. Under control conditions, the 50% decrease of ^{3}H-labelled RNA occurred within 76 h of chase. This half-life increases up to 105 h in TRH-treated cells.

Effect of TRH on rPRL and rGH RNA degradation. The semi-logarithmic representation, used for the analysis of rPRL mRNA selected by hybridization, revealed a more complex and biphasic phenomenon (Figure 4A). The radioactivity associated with rPRL mRNA first increased up to 16–20 h and thereafter decreased slowly to the initial level after 72 h of chase. TRH treatment also resulted in this biphasic pattern, but elicited a greater increase in the concentration of ^{3}H-labelled rPRL RNA during the first 20–24 h of treatment. Thereafter, the marked difference between the level of ^{3}H-labelled rPRL RNA in treated as compared with untreated cells remained fairly constant during the experiment. These data were analyzed as a two-pool open system

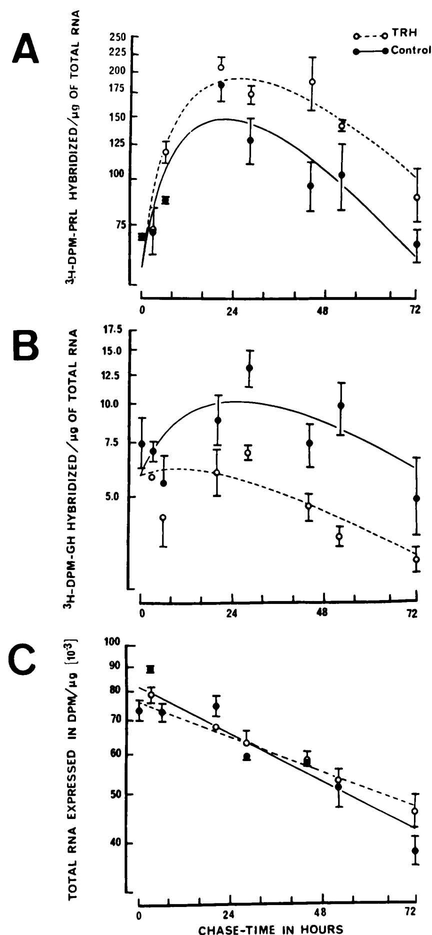
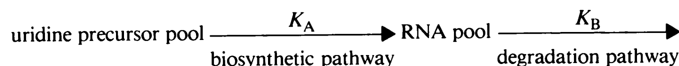


Fig. 4. Time course of TRH effect on RNA degradation. GH3/B6 cells were pulse-labelled with [^{3}H]uridine (4.2 μM) for 2 h. At the end of the labelling period the radioactive medium was replaced by a new culture medium containing non-radioactive uridine supplemented with 100 nM TRH (○-----○) or not (●——●). The cells were subsequently incubated for various durations from 6 to 72 h and total RNA extracted as described in Materials and methods. ^{3}H-labelled rPRL RNA **(A)**, rGH RNA **(B)** and total RNA **(C)** were quantitated as described in the legend to Figure 1 and in Materials and methods. The bars represent the range of duplicate experiments. Statistical significance of TRH *versus* control rate constants of degradation: **Panel A** (PRL RNA): $P<0.001^a$. **Panel B** (GH RNA): $0.05<P<0.1^a$. **Panel C** total RNA: $0.05<P<0.1^a$, $P<0.01^b$. ^{a}P values estimated as described in Materials and methods. ^{b}P values estimated by the student t test applied to the comparison of the regression coefficients calculated by least square regression analysis.

(Shipley and Clark, 1972), where the observed RNA pool depends on a pool of labelled precursors. As shown in the diagram below, the precursor and RNA pools represent spatial compartments undergoing molecular interchange, and K_A and K_B are the turnover rate constants of the precursor and RNA pools, respectively.



By non-linear regression analysis of the data according to methods described and assuming the absence of reflux of RNA pools, the half-life of rPRL RNA was estimated as ~17 h in untreated cells, whereas TRH treatment increases significantly this half-life to ~27 h (Figure 4A), i.e., TRH stabilizes rPRL RNA.

Analysis of rGH RNA extracted from control cells resulted in a similar although less pronounced biphasic pattern (Figure 4B). In TRH-treated cells, however, the initial increase in [^{3}H]uridine incorporation was barely detectable. In addition, the level of ^{3}H-labelled rGH RNA remained constantly lower in this group as compared with control cells, the maximal difference being reached following 24 h of chase. Using the same statistical analysis as described above, the half-life of rGH RNA was estimated to be ~24 h in control cells and ~15 h in TRH-treated cells. This suggests an accelerated rate of degradation of rGH mRNA following exposure to TRH although the significance of the comparison was estimated to be between 5% and 10% only.

Discussion

The control of mRNA level may involve a variety of mechanisms: change in the rate of specific gene transcription, modification of the processing of the mRNA precursors, including capping and poly(A) addition and splicing and, finally, change in the stability or in the rate of transfer of mRNA from the nucleus to the cytoplasm (Darnell, 1982). The differential expression of the genes coding for rPRL or rGH may result from a modulation of any of these mechanisms.

Here we have compared the levels of newly synthetized specific mRNA, i.e., PRL and GH RNA to that of total RNA in both nuclear and cytoplasmic compartments. The technique used, very similar to those applied by others to GH cells and pituitary cells (Murdoch *et al.*, 1982, Evans *et al.*, 1982), lead to nuclear fractions containing substantial amounts of RNA (~20% of total RNA). Phase contrast microscopic observations, together with the high specific activity of RNA of the nuclear fractions, indicated a rather low cytoplasmic contamination or nuclear leakage. These fractions appeared, therefore, suitable for comparative investigations.

We show that, under basal conditions, the concentration of rPRL mRNA and rGH mRNA depends both on the transcriptional activity of their respective genes and on the transfer rate of the RNA from the nucleus to the cytoplasm. Such conclusions were drawn as follows. The radioactivity incorporated within 1 h into each specific RNA species was assumed to correlate directly with the level of transcription and processing of each gene since no significant degradation of the mRNAs was observed within this short period of time (Figure 4A,B). Hence, data reported in Table I show that GH3/B6 cells synthesize in 1 h ~4-fold more rPRL than rGH RNA. If transcription was the only mechanism of regulation, a similar ratio would be expected between the number of rPRL and rGH RNA molecules in both the nuclear and cytoplasmic fractions. In fact, the cytoplasmic to nuclear ratios were found to be 13 and 1.7, respectively (Table I). This, therefore, indicates that, in our cells – which in basal conditions produce 10 times more rPRL than rGH – the rPRL mRNA is processed and/or transported more rapidly and efficiently than rGH mRNA.

Our kinetic analysis shows that TRH has a biphasic effect on total RNA biosynthesis, consisting of an initial, transient and discrete augmentation followed by a long lasting and significant inhibition (Figure 1C). The observed alteration of [^{3}H]uridine incorporation into total RNA could originate in TRH-induced modifications of the specific activity of RNA precursors as suggested by Martin and co-workers (Martin and Tashjian, 1978; Martin *et al.*, 1978). Although this hypothesis cannot be ruled out, previous reports from Morin *et al.* (1981) showed that TRH does not decrease the incor-

poration of [^{3}H]uridine into poly(A)$^+$ RNA in contrast to its effect on the total RNA. This finding adds significance to the observed delayed and long lasting inhibition of rGH mRNA biosynthesis.

Our results indicate a strong, specific and acute stimulation of transcription of the PRL gene (Figures 1A, 2), which is consistent with the increased steady state level of the rPRL mRNA precursors (Figure 3A). The stimulation of rPRL gene transcription, however, is very short lived and disappears after 20 h of treatment with TRH. Potter *et al.* (1981) have drawn similar conclusions from blotting procedures.

No effect of the hormone on RNA transport could be detected by comparing the TRH-induced changes on the concentration of newly synthesized RNA at the nuclear and cytoplasmic level (Figures 1 and 2).

Analysis by gel electrophoresis and Northern blotting yielded some additional information on the processing of the PRL transcripts. Several bands corresponding to nuclear RNA precursors could be detected. The size difference between some of these precursors was smaller than the shortest of the introns (intron A 1.5 kb, Cooke and Baxter, 1982). Similar findings were reported by others (Maurer *et al.*, 1980; Hoffman *et al.*, 1981; Potter *et al.*, 1981). These observations are consistent with the hypothesis that multiple events may be required to excise a single intervening sequence. Concerning TRH regulation, identical patterns of precursors were found in control and TRH-treated cells, indicating that TRH does not appear to affect either the transport or the processing of the PRL mRNA.

Comparison of the precursor patterns obtained with cDNA and genomic DNA probes, yielded corresponding results. However, the main difference, relating to the 2.9 ± 0.2 kb band, was revealed only by the genomic probe. Considering its size and its hybridization characteristics, we postulate that it could correspond to intron C. Similar results were obtained by some of us (Cathala and Martial, unpublished data) and can be explained either by a peculiar stability of this intron, or because it is the last to be spliced out.

After 48 h of exposure to TRH, the steady-state level of rPRL RNA is increased although the stimulation of rPRL gene transcription is no longer detectable. These observations suggest a longer half-life for rPRL mRNA under TRH treatment; this was confirmed by the studies of RNA degradation.

The pulse-chase experiments measuring rPRL and rGH degradation were interpreted as a two-pool open system, which takes into account an intracellular pool of uridine, the existence of which is highly probable (Martin *et al.*, 1978). However, the semi-logarithmic plot of the specific activity of total RNA as a function of time yielded a straight line (Figure 4C) which raises doubts about the existence of the uridine pool. Indeed optimization procedures applied to the data: (i) revealed that an additional uridine pool is compatible with the experimental results, (ii) indicated that the constant rate of the uridine flow is very high in the case of total RNA synthesis. The latter observation, together with the absence of experimental data from 0 to 3 h of the chase, makes the determination of the kinetic parameters very speculative with a two-pool open model. Consequently, the degradation of total RNA was analyzed as a single pool model.

The results are noteworthy in so far as they show that TRH may have different effects on two specific mRNA species. On the one hand, it stabilizes the rPRL mRNA. The half-life of 27 h measured for rPRL mRNA would indicate that it is sufficient to account for persistence of the acute effect of TRH on rPRL gene transcription in TRH-treated cells (Berlin and Schimke, 1965). In the case of rGH RNA the low statistical significance of the difference between TRH and control allows only speculation. However, it is of interest that TRH seems to act in an opposite but symmetrical manner. The stabilization of total RNA by TRH treatment could possibly be related to the simultaneous increase of the amount of protein per cell (Gourdji, 1980).

In conclusion, the data presented here demonstrate that TRH regulates gene expression by acting both at the transcriptional level and at the level of RNA stability. Although the molecular mechanisms by which such regulation occurs are presently unknown, some features can be discussed. The delayed inhibition of rPRL gene transcription could be related to the down-regulation of TRH receptors (Hinkle and Tashjian, 1975; Gourdji *et al.*, 1982) or to a possible counteracting effect of TRH metabolites (Bauer *et al.*, 1978; Prasad *et al.*, 1980). However, previous reports (Morin *et al.*, 1981; Biswas *et al.*, 1982), showed a persistent augmentation of rPRL mRNA biosynthesis after a treatment with TRH of a similar duration. The significance of this observed variability remains unclear. The stimulation of rPRL gene transcription was already clearly evident at 30 min when the first time point was examined. This very quick response implies an equally quick transfer of the hormonal message(s) from the cell surface to the nucleus. On the one hand, it is commonly proposed that peptide hormones act *via* an intracellular messenger. In this respect Ca^{2+} ions have been shown to regulate specifically the level of rPRL mRNA, without altering that of rGH mRNA (White *et al.*, 1981). Since TRH was found to be able to mobilize intracellular calcium (Tan and Tashjian, 1981; Ronning *et al.*, 1982), this ion may well participate in the control by TRH of the expression of the rPRL gene. On the other hand, some of us have found specific binding sites for TRH in the nuclei of GH3/B6 cells using biochemical and autoradiographical approaches (Bournaud *et al.*, 1977; Laverrière *et al.*, 1981). Of interest is the rapid transfer of TRH to the nucleus where it is detectable within 5 min and reaches its maximum by 30 min (Laverrière *et al.*, 1981). The comparison of these kinetics to those of the TRH-induced stimulation of the rPRL gene transcription may suggest a possible causal relationship between these two phenomena.

Materials and methods

Cell culture

GH3/B6 cells, a subclone of the GH3 tumor-derived rat pituitary cell line, were selected for their high TRH binding capacity. They are routinely grown in monolayers, as previously described, in Ham's F10 medium supplemented with horse serum and fetal calf serum plus antibiotics. Under these conditions, they secrete from 3 to 10 times more PRL than GH (Gourdji, 1980). The culture medium was renewed 20 h before experiments to limit the alterations of the cell metabolism elicited by addition of fresh medium. In some experiments, cells were grown for 4 days in a serum-free, hormonally-defined medium (Brunet *et al.*, 1981) before exposure to 5 nM TRH for 48 h.

Cell labelling and TRH treatment

For studying the biosynthesis of RNA, GH3/B6 cells were plated (4 x 10^6 cells/40 ml/150 mm diameter Falcon Culture dish) and grown for 6 days before the experiment. 20 h after the last addition of fresh medium, TRH (100 nM final) was added and the cultures incubated for various times from 30 min to 48 h. 30 or 60 min before termination of the incubation, 30 ml of medium was discarded and [^{3}H]uridine (30 Ci/mmol, CEA, Saclay) was added to the remaining 10 ml to a final concentration of 150 or 125 μCi/ml, respectively. At the end of the incubation the radioactive medium was removed, the monolayer was washed twice with room temperature Ham's F10 and

once with phosphate buffered saline. Cytoplasmic and nuclear RNA were then extracted as described below.

For the determination of the mRNA half-lives, GH3/B6 cells were cultured for 6 days ($1-1.5 \times 10^6$ cells plated per 100 mm diameter Falcon culture dish) and submitted to pulse-chase experiments. 20 h after the last medium renewal, 6 ml of culture medium were removed and 125 μCi/ml of [^{3}H]uridine was added to the remaining 4 ml medium. Following a 2 h incubation at 37°C, the cell monolayer was washed twice with room temperature Ham's F10 medium, and post-incubated for 30 min at 37°C, to eliminate trapped extracellular [^{3}H]uridine. The culture medium was then discarded and fresh medium containing cold uridine, and supplemented or not with 100 nM TRH, was added. TRH treated and untreated cultures were carried in parallel for increasing chase time, from 3 to 72 h. Total cellular RNA were extracted at the end of the incubation.

Isolation of the nuclear and cytoplasmic RNA fractions

The cytoplasmic RNA fraction was isolated as described by Morin *et al.* (1981) using 0.5% Nonidet P-40 (NP-40) detergent. Simultaneously the nuclear pellet resulting from the 10 000 *g* centrifugation of the cell lysate was processed as follows for the isolation of nuclear RNA. Nuclei isolated from each dish were resuspended in 3 ml of buffer containing 0.14 M NaCl, 10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$ and 0.1% NP-40. An equal volume of 4 M LiCl, 8 M urea was added and the viscous solution was homogenized by using a polytron at a moderate speed. The homogenate was stored overnight at 4°C and precipitated RNA were pelleted down by centrifugation at 20 000 *g* at 4°C for 30 min. The resulting pellet was resuspended in 2 M LiCl, 4 M urea and collected by centrifugation as above. This second pellet, free from most contaminating DNA, was resuspended in TES (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.1% SDS) extracted with phenol/chloroform and ethanol-precipitated.

Isolation of total cellular RNA

Total RNA was extracted by a modification of the guanidium thiocyanate method (G.Cathala, personal communication). Briefly, the culture medium was discarded and 2 ml of guanidium thiocyanate 50 mM Tris-HCl pH 7.5, 10 mM EDTA and 100 μl of β-mercaptoethanol was directly added to each 100 mm diameter dish. This treatment immediately disrupted the cells and prevented RNA degradation. This viscous solution was transferred to 15 ml polypropylene tubes and LiCl was added to a 3.5 M final concentration. The mixture was homogeneized using a polytron at a moderate speed and allowed to precipitate overnight at 4°C. The precipitate was collected by 15 000 *g* centrifugation for 30 min, dissolved in TES and extracted 2 to 3 times with phenol-chloroform. The aqueous phase was ethanol-precipitated.

Analysis of ^{3}H-labelled RNA

RNA precipitates were dissolved in water and an aliquot of each sample was used to determine the RNA concentration and the associated radioactivity. Another aliquot was used to measure the ^{3}H-labelled rPRL and rGH RNA concentration by filter hybridization. For that purpose, we used recombinant pBR322 DNA containing either the 823-bp rPRL cDNA sequences (Cooke *et al.*, 1980) or the 800-bp rGH cDNA sequences (Seeburg *et al.*, 1977); the pBR322 wild-type DNA was used as a control. 300 μg of each plasmid were attached to 47 mm nitrocellulose filter disc (Schleicher and Schull, BA 85), using the filtration procedure described by Gillespie and Spiegelman (1965). The large filter disc was subsequently cut into small square filters (25 mm^2) which were baked at 80°C under vacuum for 2 h. Following a $4-6$ h hybridization step in the presence of *Escherichia coli* tRNA (750 μg/ml), Ficoll 400 (0.02%) and polyvinyl pirrolidone (0.02%), hybridization was performed in a final volume of 100 μl as described by McKnight and Palmiter (1979) except that PIPES buffer was replaced by HEPES. Three filters containing, respectively, immobilized pBR322 wild-type DNA, rPRL recombinant plasmid and rGH recombinant plasmid were added to each tube. In addition to the ^{3}H-labelled RNA being assayed, [^{32}P]cRNA for rPRL was included in each reaction as internal standard. After hybridization the filters were washed and digested with RNase A and T1 RNase essentially as described by Maurer (1981) except the temperature which was 45°C instead of 68°C. The filters were dried and the radioactivity was measured after addition of HP-B scintillation fluid (Beckman). Under the conditions used, a linear relationship was obtained between the amount of labelled RNA and the count hybridized indicating that the plasmid was present in excess and there was no competition with endogenous non-labelled specific RNA.

Analysis of non-labelled cellular RNA

$20-60$ μg of total cellular RNA treated by glyoxal (McMaster and Carmichael, 1977) were submitted to 1.5% agarose gel electrophoresis in Tris/acetate/NaCl/EDTA buffer. RNA was transferred on the nitrocellulose paper, which was prehybridized, hybridized with ^{32}P-labelled probe and washed as described by Thomas (1980). Autoradiography was carried out with Kodak X-ray films with or without intensifying screens at -80°C for various times.

Preparation of ^{32}P-labelled cDNA probe and ^{32}P-labelled standard cRNA

Non-labelled rGH RNAs were characterized using the [^{32}P]rGH cDNA plasmid (Seeburg *et al.*, 1977). Non-labelled rPRL RNAs were characterised using two different ^{32}P-labelled probes: one corresponding to the mRNA complete coding sequence (823-bp cDNA), the other to a 6 kb long DNA sequence complementary to the 5′ part of the rPRL gene (Cooke and Baxter, 1982). These two probes were labelled by nick-translation (Rigby *et al.*, 1977) to a specific activity of $2.5-5.0 \times 10^8$ d.p.m./μg.

[^{32}P]cRNA used as internal standard in filter hybridization was synthetized *in vitro* using 10 μg rPRL cDNA recombinant plasmid as template for *E. coli* RNA polymerase (kindly provided by Dr.Flamee). The 100% hybridization efficiency was determined assuming that out of the 1000 ^{32}P d.p.m. input of rPRL cRNA, 440 d.p.m. hybridized with rPRL filter, 280 d.p.m. with rGH and 280 d.p.m. with pBR322 filter.

Analysis of degradation experiments

Curve fitting of the experimental data was carried out by non-linear regression analysis where all parameters were estimated simultaneously by minimizing the sum of the square of residuals. Optimization procedures were conducted on Digital computer PDP 11.34 using the interactive system COSMOS (Hamrouni *et al.*, 1979). Alternatively, two iterative methods were applied, one direct and the other gradient based up to the point at which both methods failed. Standard deviation(s) of parameters was estimated through the evaluation of the square approximate co-variance matrix (Beck and Arnold, 1977). The T test was applied to check the significance of differences between degradation pathways.

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