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Thyrotropin controls transcription of the thyroglobulin gene

(*in vitro* transcription/cDNA clones/propylthiouracil/triiodothyronine/hypophysectomy)

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ABSTRACT **The availability of rat thyroglobulin cDNA clones was exploited to study the regulation of thyroglobulin gene transcription by thyrotropin (TSH). Groups of rats were subjected to treatments leading to reduction or increase in the rat serum TSH (rTSH) levels. Thyroid gland nuclei were isolated, incubated *in vitro* in the presence of ^{32}P-labeled uridine triphosphate, and thyroglobulin transcripts were quantitated by hybridization to immobilized rat thyroglobulin cDNA clones. Transcription of the thyroglobulin gene was found to be very active in thyroid nuclei from control animals. It represented about 10% of total RNA polymerase II activity. Chronic hyperstimulation of the thyroid glands with endogenous rTSH was achieved in rats treated with the goitrogen propylthiouracil. No significant increase of thyroglobulin gene transcription could be measured in thyroid nuclei from these animals. On the contrary, a dramatic decrease in thyroglobulin gene transcription was observed in those animals in which endogenous rTSH levels had been suppressed by hypophysectomy or by the administration of triiodothyronine. Injection of exogenous bovine TSH in such animals readily restored transcriptional activity of the gene. Our results identify transcription as an important regulatory step involved in TSH action. They suggest that normal TSH levels induce close to maximal expression of the thyroglobulin gene but that continuous presence of TSH is required in order to maintain the gene in an activated state.**

The pituitary hormone thyrotropin (thyroid-stimulating hormone, TSH) is the main agent regulating the activity of the thyroid gland (1). Stimulation of thyroid follicular cells by TSH results in the rapid activation of almost every aspect of their metabolism, including the synthesis and secretion of thyroid hormones. The latter is achieved by the complete lysosomal hydrolysis of a precursor iodoprotein, thyroglobulin, synthesized in large amounts by the gland (2, 3). Most, if not all, of these effects of TSH are mediated by cyclic AMP-dependent mechanisms (1).

TSH stimulates overall protein synthesis by the thyroid gland (4). Indirect evidence suggests that the mechanisms involved may be both translational and transcriptional (5). Attempts to demonstrate a specific effect of TSH on the expression of the thyroglobulin gene have yielded conflicting results, depending on the system and the methodology used. Whereas early studies suggested that the continuous stimulation of the gland by TSH was required to sustain thyroglobulin synthesis *in vivo* (6) and in tissue explants (7), no effect on thyroglobulin production could be demonstrated in primary cell culture (8). More recently, measurements of mRNA levels by cDNA·RNA hybridization have shown an effect of TSH on thyroglobulin mRNA accumulation *in vivo* (9) and in primary cell culture (10). However, the precise level at which the effect took place could not be identified by a methodology involving only quantitation of steady-state mRNA levels.

The availability of cloned rat thyroglobulin cDNA fragments (11, 12) provided an opportunity to directly explore the transcription of the thyroglobulin gene. In the present study, experiments were performed with nuclei isolated from thyroid glands of rats subjected to various manipulations leading to the suppression or stimulation of rat serum TSH (rTSH) levels. The relative proportion of thyroglobulin transcripts was measured by specific hybridization to immobilize cDNA clones. The results indicate that the thyroglobulin gene is already maximally expressed in thyroids from rats exposed to physiological concentrations of TSH. However, the continuous presence of TSH is required to maintain the gene in an activated state, as decreasing rTSH levels by hypophysectomy or by triiodothyronine (T3) administration dramatically decreases its transcription.

MATERIALS AND METHODS

Reagents. Bovine TSH (bTSH; Thytropar) was obtained from Armour (Kankakee, IL); aurin tricarboxylic acid (ammonium salt) from Merck; [^{32}P]UTP, 400 Ci/mmol (1 Ci = 37 GBq) and Na^{125}I from Amersham; α-amanitin from Boehringer Mannheim; T3 (triiodo-L-thyronine, sodium salt) from Sigma; and 4(6)-propyl-2-thiouracil (PrSUra) from Koch-Light Laboratories (Bucks, England).

Handling of Animals. Intact and hypophysectomized albino male rats of the IUPS-OFA strain, weighing 180–240 g, were purchased from Iffa Credo (St. Germain sur l'Arbresle, France). Housed in groups of five per cage, they had access to laboratory Purina rat chow and to tap water ad lib; hypophysectomized rats were given 0.9% NaCl in the drinking water.

The normal concentration of rTSH in serum was reduced by either hypophysectomy or suppression of pituitary rTSH secretion by T3 treatment of intact rats. Supraphysiological serum rTSH levels were achieved by either PrSUra-mediated stimulation of endogenous rTSH secretion in intact rats or by the administration of bTSH.

T3-treated rats received the hormone in a single daily intraperitoneal dose of 5 μg/100 g of body weight or an equivalent amount given in the drinking water. PrSUra was given as a 0.05% solution in the drinking water. bTSH was administered intraperitoneally as a single dose or five consecutive doses at 12-hr intervals, each adjusted to deliver 0.2 unit/100 g of body weight.

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Abbreviations: TSH, thyrotropin; rTSH, rat TSH; bTSH, bovine TSH; PrSUra, propylthiouracil; T3, triiodothyronine; T4, thyroxine; prTg, plasmids containing rat thyroglobulin cDNA fragment; kb, kilobase(s).

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The *in vivo* manipulation was terminated at various intervals after different treatments: 15 days after hypophysectomy; 3 and 7 days after the initiation of T3 treatments; at the termination of 7 and 12 days of PrSUra treatment; and 2 to 3 hr after the last injections of bTSH.

Blood was obtained between times 0900 and 1130 by cardiac puncture under ether anesthesia, following which the animals were killed by decapitation. The thyroid glands were immediately removed and dissected free of connective tissue.

Measurement of Hormones in Serum. T3 and thyroxine (T4) were measured by using commercial reagents (Clinical Assays, Gammacoat). rTSH was assayed with the reagents obtained from the National Institutes of Arthritis, Metabolism and Digestive Diseases.

***In Vivo* Transcription and Isolation of Nuclear RNA.** The procedure was essentially that of McKnight and Palmiter (13). Pools of thyroid tissue from 10 rats, containing 0.2–0.4 g, were homogenized in 10 ml of 10 mM Tris·HCl, pH 8.0/2.5 mM Mg acetate/0.25% Triton X-100/0.3 M sucrose/0.5 mM dithiothreithol/0.5 mM phenylmethylsulfonyl fluoride. The nuclei were prepared as described (13) and stored at −70°C in 150 μl of 50 mM Tris·HCl, pH 8.0/5 mM $MgCl_2$/0.1 mM EDTA/1 mM $MnCl_2$/28% glycerol. Transcription was performed in a final volume of 250 μl containing 100 μl of nuclei and 35 mM Tris·HCl (pH 8.0), 100 mM $(NH_4)_2SO_4$, 5 mM Mg acetate, 1 mM ATP, 0.5 mM GTP and CTP, 250 μCi of [α-^{32}P]UTP (2.5 μM), 1 mM $MnCl_2$, 5 mM spermidine, 6 mM aurintricarboxylic acid, 5 mM dithiothreitol, 2 mM creatine phosphate, creatine phosphokinase at 30 μg/ml, nucleotide diphosphokinase at 6 μg/ml, and 28% glycerol. Incubation was for 60 min at 26°C. We have tested that during this period of time, the incorporation of UTP was linear provided that aurintricarboxylic acid was included in the reaction mixture (14). α-Ammanitin at 1 μg/ml inhibited total incorporation of UMP into RNA by 48 ± 7.8% (mean ± 1 SD; n = 8). No significant modification of this percentage was observed in the various experimental conditions explored in the present study. We conclude that RNA polymerase II is responsible for about 50% of the transcriptional activity in our system. Nuclear RNA was extracted as described (13).

Preparation of ^{125}I-Labeled Rat Thyroglobulin mRNA. Thyroglobulin mRNA was isolated from rats treated for 20 days with PrSUra (15) and labeled with ^{125}I as described by Scherberg and Refetoff (16).

Hybridization of Nuclear RNA to Immobilized Plasmid DNA. Plasmids containing a fragment of rat thyroglobulin cDNA (prTg) used in this study have been described (11). Plasmid DNA (1 mg/ml) was denatured by heating for 15 min at 80°C in 0.5 M NaOH/1 M NaCl. Aliquots (3 μl) of this solution were spotted onto 6-mm-diameter nitrocellulose filter discs (Schleicher & Schuell BA 85). After drying in air, the filters were neutralized by two successive transfers onto pads of 3 MM Whatmann paper moistened with 1 M Tris·HCl (pH 8.0). By the same technique they were equilibrated with 3 M NaCl/0.3 M Na citrate, dried, and baked for 2 hr at 80°C. Before use, the discs were washed for 10 min at room temperature by immersion in 10 mM Tris·HCl, pH 7.5/300 mM NaCl/2 mM EDTA/0.1% sodium dodecyl sulfate. Prehybridization (incubation overnight at 45°C) and hybridizations (48 hr at 45°C; final volume, 80 μl) were performed in 50 mM Hepes, pH 7.0/210 mM NaCl/0.4% sodium dodecyl sulphate/2.0 mM EDTA/0.2% Ficoll/0.2% polyvinyl pyrrolidone/50% formamide/poly(adenylic acid) at 10 μg/ml. Each hybridization vial contained one prTg cDNA disc and one pBR322 disc as control. ^{32}P-labeled RNA (0.5–3.0 × 10^6 cpm) was included in the mixture, together with 2000 cpm of ^{125}I-labeled rat thyroglobulin mRNA to allow for the monitoring of the efficiency of hybridization. The filters were washed, treated with RNase as described (13), and then assayed separately for ^{125}I and for ^{32}P (100 min).

The specificity of the hybridization was documented by the fact that inclusion of rat thyroglobulin mRNA in the hybridization reaction resulted in the parallel displacement of both the ^{125}I-labeled tracer RNA and of the ^{32}P-labeled RNA synthesized *in vitro*.

RESULTS

The Thyroglobulin Gene is Actively Transcribed under Physiological Conditions. In preliminary experiments, attempts to measure transcription of the thyroglobulin gene in rat thyroid explants were unsuccessful. This was mainly due to a limited amount of tissue and to the relatively low specific activity of the tritiated RNA precursor used. Since the transcriptional activity of isolated nuclei has been shown to reflect transcription in the intact tissue at the time of the animal death (13), this method was used to study thyroglobulin gene transcription and its control by TSH.

The radioactive RNA bound to filters containing prTg cDNA (Tables 1–4) is likely to faithfully represent thyroglobulin transcripts, as it was specifically displaced by purified thyroglobulin mRNA and depended on the activity of RNA polymerase II (see *Materials and Methods*). The background radioactivity bound to filters containing wild-type plasmid was extremely low. Nuclei isolated from intact and untreat-

Table 1. Effect of chronic hyperstimulation by endogenous TSH on transcription of thyroglobulin (Tg) mRNA in isolated nuclei

	RNA synthesized in isolated nuclei						
	[^{32}P]RNA input/ hybridization,	[^{32}P]RNA hybridized to filters, cpm		Tg gene transcription,‡	Hormones in serum†		
Rat treatments*	cpm × 10^{-6}	pBR322	prTg	ppm	rTSH, ng/ml	T4, μg/dl	T3, ng/dl
Untreated control	0.48	7	68	2467	454 ± 249	3.9 ± 0.5	60 ± 11
	1.35	13	186	2564			
PrSUra 7 days	2.40	16	324	2772	1020 ± 224	1.6 ± 0.6	<50
	1.62	14	254	2964			
PrSUra 12 days	1.00	13	180	3204	1485 ± 325	<1	<50
	1.97	16	271	2757			

Nuclei were prepared from two pools of 10 thyroid glands for each group of animals and used in *in vitro* transcription experiments in the presence of [^{32}P]UTP. The ^{32}P-labeled transcripts were hybridized to filters containing immobilized DNA from pBR322 plasmid or from recombinant prTg plasmids. Results from individual transcription reactions are presented.

*PrSUra was given in the drinking water for the period of time indicated.

†Serum levels of hormones were determined on blood samples obtained from individual rats and expressed as the mean ± SD.

‡The rate of Tg gene transcription in ppm was calculated as the difference between the cpm measured on prTg and pBR322 filters. The difference was divided by the input radioactivity, corrected for the efficiency of hybridization as determined by the internal ^{125}I-labeled mRNA standard (from 25% to 50%) and multiplied by the factor 5.4, accounting for the size ratio of the mRNA to the cDNA inserts.

Table 2. Effect of hypophysectomy on transcription of the thyroglobulin (Tg) gene

Rat treatment*	RNA synthesized in isolated nuclei: [^{32}P]RNA input/ hybridization, cpm × 10^{-6}	[^{32}P]RNA hybridized to filters, cpm: pBR322	[^{32}P]RNA hybridized to filters, cpm: prTg	Tg gene transcription,† ppm	Hormones in serum†: rTSH, ng/ml	T4, μg/dl	T3, ng/dl
Intact control	2.0	11	112	1771	220 ± 160	3.9 ± 0.5	59 ± 12
	3.3	9	202	1512			
Hypophysectomy	1.9	7	15	(128)	<100	1.2 ± 0.2	<50
(15 days)	1.5	2	9	(168)			
Hypophysectomy	2.9	18	60	494	—	4.5 ± 0.7	91 ± 32
(15 days) + TSH (2 days)	3.2	40	145	1144			

*Animals hypophysectomized 15 days were handled as described under *Materials and Methods*. bTSH was administered intraperitoneally in five doses over a 2-day period.
†Results are expressed exactly as in Table 1. As the results obtained from the hypophysectomized groups of rats are close to the limit of detection, they appear in parentheses.

ed control rats transcribed the thyroglobulin gene efficiently. Although comparison to the transcription of other genes requires some caution (see *Discussion*), the observed level of about 2500 ppm is similar to that obtained for the ovalbumin gene in chicken oviducts subjected to a maximal stimulation by estrogens (13). The ratio between the estimated size of the gene, about 150 kilobases (kb) (17, 18) and that of its mature RNA, 8 kb (19, 20), is about 20. As ≈50% of the total transcription in our system can be inhibited by low doses of α-amanitin, it could be computed that about 10% of RNA polymerase II activity was devoted to transcription of the thyroglobulin gene in nuclei from thyroid glands exposed to physiological concentrations of TSH.

Hyperstimulation by TSH Does Not Preferentially Increase Thyroglobulin Gene Transcription. In order to investigate the effect of chronic hyperstimulation by supraphysiological concentrations of TSH, rats were treated for 7 or 12 days with PrSUra, an inhibitor of thyroid peroxidase (21). As a consequence, the serum levels of T3 and thyroxine (T4) decreased dramatically, serum rTSH concentration increased (Table 1), and thyroid gland weight doubled. Although it is known that accumulation of total RNA is stimulated by such treatment (9, 15), no significant effect was observed on the specific transcription of the thyroglobulin gene.

Lowering of TSH Results in a Preferential Inhibition of Thyroglobulin Gene Transcription. The effect of lowering the normal serum rTSH was investigated by comparing thyroglobulin gene transcription in thyroid gland nuclei from hypophysectomized rats to that obtained by matched intact control rats. Suppression of the serum rTSH by hypophysectomy was associated with barely detectable levels of thyroglobulin gene transcription (Table 2). Since hypophysectomy reduces not only the TSH level but also causes deficiency in all hormones of pituitary origin, the effect of specific inhibition of rTSH secretion by the administration of T3 was examined (Table 3). Again, reduction of the serum rTSH produced a 65% and 80% decrease in thyroglobulin gene transcription after 3 and 7 days of T3 treatment, respectively.

Readministration of TSH to TSH-Deprived Rats Restores Transcription of the Thyroglobulin Gene. Clear reactivation of thyroglobulin gene transcription was observed after the readministration of bTSH for 2 days to both hypophysectomized (Table 2) and T3-treated (Table 3) rats. However, values fell short of reaching the levels observed in intact and untreated control rats. The effect of TSH was rapid because significant stimulation of thyroglobulin gene transcription could be detected 3 hr after a single bTSH injection (Table 4). Total transcription almost doubled during the same period.

DISCUSSION

In vitro transcription experiments in isolated nuclei present inherent limitations including the low level of *de novo* initiation of transcription (22, 23), little if any processing of the elongated transcripts (24), and, depending on the system, a relatively short period during which incorporation of labeled precursor is linear with time (13, 14). Nevertheless, this system has been shown to reflect faithfully transcriptional activity *in vivo* (13). The exceptionally large size of the thyroglobulin gene, of ≈150 kb (17, 18), requires correction for the size of the gene to make direct quantitative comparisons of thyroglobulin gene transcription to that of other genes in terms of ppm. For example, transcription of the fully in-

Table 3. Effect of suppression of endogenous TSH by T3 on transcription of the thyroglobulin (Tg) gene

Rat treatment*	RNA synthesized in isolated nuclei: [^{32}P]RNA input/ hybridization cpm × 10^{-6}	[^{32}P]RNA hybridized to filters, cpm: pBR322	[^{32}P]RNA hybridized to filters, cpm: prTg	Tg gene transcription,† ppm	rTSH in serum,† ng/ml
Untreated control	1.65	20	248	1565	484 ± 79
	2.48	18	235	963	
T3 (3 days)	1.58	16	83	477	<100
	1.55	17	80	467	
T3 (7 days)	2.24	20	75	287	<100
	1.65	18	59	265	
T3 (7 days)	1.80	19	184	1122	—
+ TSH (2 days)	2.45	18	206	983	

*The rats were treated for 3 or 7 days with one daily injection of T3 (5 μg/100 g of body weight). bTSH was administered intraperitoneally in 5 doses during the last 2 days of T3 treatment.
†Results are expressed as in Table 1.

Table 4. Effect of acute stimulation with exogenous TSH on transcription of the thyroglobulin (Tg) gene in rats chronically treated with T3

Rat treatment*	Total [^{32}P]UTP incorporation,† pmol/μg DNA	[^{32}P]RNA input/ hybridization, cpm × 10^{-6}	[^{32}P]RNA hybridized to filters, cpm		Tg gene transcription,‡ ppm
			pBR322	prTg	
T3 (7 days)	0.11	0.52	13	19	(118)
	0.10	0.54	14	21	(141)
T3 (7 days)	0.17	0.72	14	49	517
+ TSH (3 hr)	0.18	0.68	15	50	542

*Rats received a daily dose of 5 μg of T3 per 100 g of body weight for 7 days given in their drinking water. Thyroid tissue was obtained on the last day of T3 treatment, 3 hr after a single injection of bTSH.
†Total incorporation of [^{32}P]UTP was measured on aliquots of the transcription mixture as the radioactivity precipitable with trichloroacetic acid normalized to the DNA content of the sample.
‡Results are expressed as in Tables 1 and 2.

duced genes of both ovalbumin (13) and thyroglobulin (Table 1) amount to about 2500 ppm. As the size ratio of the mRNAs is 4.3 in favor of thyroglobulin (19, 25), and results are normalized to the full length of the mature mRNA, the efficiency of thyroglobulin gene promoter must be lower by a factor of 4.3 relative to that of ovalbumin. Since little if any processing of transcripts occurs in this kind of experiments, simple interpretation of results expressed in ppm is misleading because it compares incorporation of UTP into mature transcripts to total incorporation, which includes the labeling of introns. Thus, estimation of the fraction of total transcriptional activity devoted to the gene of interest should take into account the actual size of the gene rather than its sole exonic portions. Although comparison between distant systems must be taken as indicative, it can be concluded that despite the higher efficiency of the ovalbumin promoter, transcription of its gene represents only 2% of the total RNA polymerase II activity, while that of the thyroglobulin gene would represent about 10%. Finally, the recombinant plasmids used in the present study are only representative of about 20% of the 3′ end of thyroglobulin mRNA (11). As the transcription time of a 150-kb gene may be estimated to be about 30–45 min (26), there is a significant delay between any alteration of the efficiency of initiation of transcription and its measurement in our system.

Although indirect arguments have been proposed in favor of a TSH effect at the translation level (27, 28), our results clearly identify transcription as one of its important regulatory actions. Lowering the level of circulating rTSH leads to a dramatic decrease in thyroglobulin gene transcription, which could be readily restored by readministration of the hormone (Tables 2, 3, and 4). In contrast, chronic stimulation by supraphysiological levels of rTSH, achieved by PrSUra treatment, induced goiter but failed to produce an important increase in thyroglobulin gene transcription above that observed under exposure to normal physiological concentrations of rTSH (Table 1). The mean concentration of the latter varied among experiments from 220 to 484 ng/ml in keeping with the normal fluctuations of serum rTSH concentration and its diurnal rhythm. These results differ from those obtained with porcine thyroid cells in primary culture (8), which supported the concept that even in serum-free medium the thyroglobulin gene would be constitutively expressed (28). However, the sensitivity of the *in vitro* transcription assay used in the present study does not allow us to exclude the consideration that low levels of thyroglobulin gene transcription would be TSH independent. It is noteworthy that evidence for a positive control of thyroglobulin gene expression by TSH has been obtained recently in other cell culture experiments (10, 29).

Our results suggest that under normal physiological conditions, TSH would induce close to maximal expression of the thyroglobulin gene relative to other genes. This conclusion is in agreement with that drawn from the measurement of steady-state thyroglobulin mRNA levels (9, 15) and from analysis of pulse-labeled thyroid proteins both *in vivo* and *in vitro* (6, 7). Such a pattern of gene regulation may serve to prevent the accumulation of excessive amounts of thyroglobulin, which would result in the dilution of the available iodine and consequently reduce the hormonogenic capability of thyroglobulin. In this context, it would be of interest to determine whether transcription of the thyroglobulin gene is in any way directly regulated by the iodine supply. Evidence of a direct control by iodide has been provided for another parameter of thyroid hormone synthesis, namely the activity of the pentose phosphate pathway (30). It is also possible that this feature of thyroglobulin gene regulation simply reflects the well-known hyperactivity of the rat thyroid gland. Against the latter interpretation are the observations that other parameters of thyroid gland activity can be stimulated by TSH in the normal rat (31, 32).

TSH may be considered as one of the prototype hormones acting via adenylate cyclase activation (1). Although a direct demonstration that the effect observed in the present study is cAMP-mediated should await *in vitro* experiments, it is highly probable that the thyroglobulin gene is one of the few for which a transcriptional control by cAMP has been shown (33–37). When cloned sequences upstream of the gene become available, their study may lead to the identification of the target for cAMP-dependent regulatory mechanisms.

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