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Autoregulation of thyroid-specific gene transcription by thyroglobulin

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ABSTRACT **Thyroglobulin (TG), the primary synthetic product of the thyroid, is the macromolecular precursor of thyroid hormones. TG synthesis, iodination, storage in follicles, and degradation control thyroid hormone formation and secretion into the circulation. Thyrotropin (TSH), via its receptor (TSHR), increases thyroid hormone levels by up-regulating expression of the sodium iodide symporter (NIS), thyroid peroxidase (TPO), and TG genes. TSH does this by modulating the expression and activity of several thyroid-specific transcription factors, thyroid transcription factor (TTF)-1, TTF-2, and Pax-8, which coordinately regulate NIS, TPO, TG, and the TSHR. Major histocompatibility complex class I gene expression, which also is regulated by TTF-1 and Pax-8 in the thyroid, is decreased simultaneously. This helps maintain self-tolerance in the face of TSH-increased gene products necessary for thyroid hormone formation. In this report we show that follicular TG counter-regulates TSH-increased, thyroid-specific gene transcription by suppressing expression of the TTF-1, TTF-2, and Pax-8 genes. This decreases expression of the TG, TPO, NIS, and TSHR genes, but increases class I expression. TG acts transcriptionally, targeting, for example, a sequence within 1.15 kb of the 5′ flanking region of TTF-1. TG does not affect ubiquitous transcription factors regulating TG, TPO, NIS, and/or TSHR gene expression. The inhibitory effect of TG on gene expression is not duplicated by thyroid hormones or iodide and may be mediated by a TG-binding protein on the apical membrane. We hypothesize that TG-initiated, transcriptional regulation of thyroid-restricted genes is a normal, feedback, compensatory mechanism that limits follicular function and contributes to follicular heterogeneity.**

The primary function of the thyroid is the formation, storage, and secretion of thyroid hormones (1, 2). Thyroid hormone formation involves a coordinated series of steps controlled by thyrotropin (TSH) but requiring insulin/insulin-like growth factor-1. This includes thyroglobulin (TG) synthesis and vectorial transport to the lumen of the thyroid follicles making up the gland, where TG is stored (1, 2). This involves concentrative iodide uptake by the sodium iodide symporter (NIS), as well as iodination of TG and coupling of TG iodotyrosine residues by the thyroid peroxidase (TPO) (1, 2). TG stored in the follicular lumen is degraded as needed, and thyroid hormones are secreted into the bloodstream. Despite the same supply of TSH from the blood, the function of thyroid follicles within a gland is not synchronized. Heterogeneity of size and function exists. Quiescent follicles with flattened cells and large accumulations of TG exist near follicles having growing, highly functional cells and little TG (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data).

In cultured thyroid cells, expression of the TG, TPO, NIS, and TSH receptor (TSHR) genes is regulated by thyroid-restricted transcription factors. Thyroid transcription factor (TTF)-1 is essential for maximal expression of all the genes (3–16). TTF-1 functions together with Pax-8, a paired domain protein that binds to a sequence overlapping some TTF-1 sites, and with TTF-2, a factor that binds to an insulin-responsive element (12–14). TSH, which increases TG synthesis, paradoxically decreases TTF-1 mRNA and protein levels, as well as TTF-1 complex formation with the TG promoter (10, 11, 17). TSH, however, simultaneously increases Pax-8 binding to the TTF-1/Pax-8 cis element of the TG promoter (10). This explains the ability of TSH to decrease TSHR, but increase TG (or TPO) gene expression, i.e., TSHR is regulated only by TTF-1, but TG and TPO are regulated by TTF-1 and Pax-8 (3–10). TTF-1 increases, whereas Pax-8 decreases, major histocompatibility complex (MHC) class I gene expression in the thyroid cell (18). This partially explains the ability of TSH to simultaneously decrease class I expression. The decrease in MHC class I would prevent the TSH-increased gene products, TPO, NIS, and TG, from becoming autoantigens and would prevent their initiating an autoimmune response (18).

In the course of studies trying to show that TSH-regulated TTF-1 was relevant to the function or growth of individual follicles *in vivo* (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data), we confirmed the importance of TTF-1 as a direct positive regulator of TG synthesis and of TSH as a suppressor of TTF-1 RNA levels. The data suggested, however, that follicular TG also regulated both TTF-1 mRNA levels and TSH-increased TG synthesis *in vivo* and that follicular TG was a feedback suppressor of both. We supported this by showing that exogenous, follicular TG decreased TTF-1 mRNA levels additively with TSH and decreased TSH-increased TG mRNA levels in rat FRTL-5 thyroid cells. We also provided *in vivo* data suggesting that TG accumulation within a follicle might play a role in follicular heterogeneity (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data).

The present studies extend those observations and, in part, uncover the underlying mechanism. We show that TG is a feedback suppressor of TTF-1, Pax-8, or TTF-2 gene expression and that regulation of these thyroid-specific transcription factors appears to be specific. This results in decreased TSHR,

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Abbreviations: TG, thyroglobulin; TTF, thyroid transcription factor; TSHR, thyrotropin receptor; TPO, thyroid peroxidase; NIS, sodium iodide symporter; MHC, major histocompatibility; GAPDH, glyceraldehyde phosphate dehydrogenase; CAT, chloramphenicol acetyltransferase; CRE, cAMP response element; CREB, CRE-binding protein.

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NIS, and TPO, as well as TG gene expression, but increased expression of the MHC class I gene. We propose that the accumulation of TG within the follicular lumen can decrease follicular function by initiating this novel, feedback pathway resulting in suppression of thyroid-specific transcription. We speculate that in some goiters the increase in MHC class I may induce autoantibody formation, for example, the growth antibodies associated with recurrent nodules or unusually sized goiters.

MATERIALS AND METHODS

Cell Culture. The F1 subclone of FRTL-5 rat thyroid cells (Interthyr Research Foundation, Baltimore, MD) was grown in 6H medium: Coon's modified F-12, 5% calf serum, and a mixture of six hormones (6H) including bovine TSH, insulin, cortisol, transferrin, glycyl-L-histidyl-L-lysine acetate, and somatostatin (10, 11, 18). Fresh medium was added every 2 or 3 days; cells were passaged every 7–10 days. Cells grown to 50% confluency in 6H medium were used directly or maintained another 5 days in TSH-free (5H) medium before use.

RNA Isolation and Northern Analyses. Cells were washed with 6H or 5H medium without serum before TG or other agents were added in serum-free medium. RNA was prepared by using a Total RNA Isolation Kit (5 Prime → 3 Prime) with minor modifications of the manufacturer's protocol: a 10-cm dish of cells was used and volumes were doubled. Northern analyses, radiolabeling, hybridization, and washing are described (19). Quantitation was performed by using a BAS-1500 Bioimaging Analyzer (Fuji). The probes for TG, TPO, MHC class I, TSHR, TTF-1, and Pax-8 are described (10, 18, 19–21). The NIS and TTF-2 probes were prepared by reverse transcription–PCR by using FRTL-5 cell poly(A)$^+$ RNA. The NIS primers, AAGTTCCTGTGGATGTGCG and TCACACCGTACATGGAGAGC, amplified a 529-bp fragment (365–893 bp); the TTF-2 primers, TATCTTCACCGCAGAGGTGC and TCTGGTGCCAAGAGATCAGG, amplified a 207-bp fragment (189–395 bp) (14, 22). The glyceraldehyde phosphate dehydrogenase (GAPDH) probe used was cut from pTR1-GAPDH-Rat template (Ambion, Austin, TX) and subcloned into a pBluescript SK(+) vector (Stratagene).

Transient Expression Analysis. FRTL-5 cells in 5H medium were returned to 6H medium for 24 hr, and 5 μg of each reporter gene chimera was transfected by using a DEAE procedure (23, 24). Cells were returned to 6H medium for 24 hr, at which time they were washed with 6H medium without serum. Fresh medium without serum, but containing the agents to be tested, then was added. Chloramphenicol acetyltransferase (CAT) or luciferase activity was measured after 48 hr (10, 11, 18, 24). The coefficient of variation of transfection efficiency in 24 different experiments was 8.7%. The preparation and properties of the TSHR-CAT, class I-CAT, and TG-CAT chimeras have been detailed (10, 11, 18, 24). TPO-luciferase constructs were obtained from TPO-CAT constructs (7) by placing the upstream sequence into the luciferase reporter plasmid, pSV0AL-AΔ5, which was also used to measure transfection efficiency (18, 24). Rat TTF-1(T/EBP)-luciferase constructs were prepared by PCR amplification of various lengths of the TTF-1 gene upstream sequences by using a rat genomic clone as template (6, 7).

Nuclear Extracts and Electrophoretic Mobility-Shift Assays. Nuclear extracts were prepared, oligonucleotides were radiolabeled, and electrophoretic mobility-shift assays were performed as described (10, 11, 18, 24). Protein concentration was determined by Bradford's method (Bio-Rad); recrystallized BSA was the standard.

Statistical Significance. All experiments were repeated at least three times using different batches of cells. Values are the mean ± SD of these experiments. Significance between experimental values was determined by two-way ANOVA and is significant if P values were <0.05.

Materials. Bovine TG was from the Sigma; bovine and human follicular 19S TG also were prepared by salt extraction and agarose chromatography as described (25–28); human thyroids were obtained from operative or postmortem samples under approved protocols.

RESULTS

Exogenous bovine 19S follicular TG, added to the medium of FRTL-5 cells, decreases TPO, NIS, TSHR, and TG mRNA levels as a function of TG concentration (Fig. 1). In contrast to TPO, NIS, TSHR, and TG mRNAs, exogenous follicular TG increased MHC class I mRNA levels and had no effect on GAPDH, which thus is used as a reference to normalize data (Fig. 1). The TG concentrations tested are in the range of those measured in the colloid of individual follicles by fine-needle aspiration biopsy, 0.1–3 mg/ml in normal human thyroids and up to 14 mg/ml in colloid nodules (29). Increased class I establishes that TG is not simply a general suppressor of all genes. Unlike NIS, TPO, TG, and TSHR, class I is a ubiquitous gene. TG had no effect on class I RNA levels in Buffalo rat liver cells (BRL), human or rat fibroblasts, 3T3, or HeLa cells

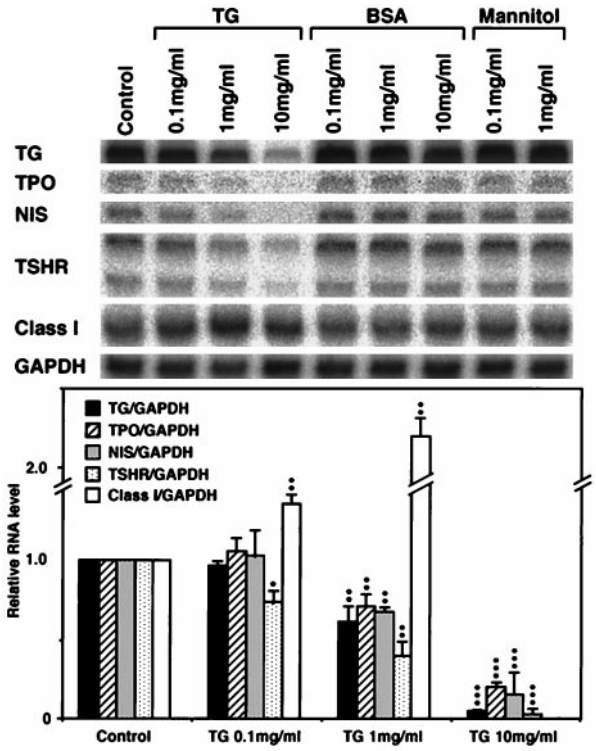

FIG. 1. Ability of exogenous follicular TG to modulate TG, TPO, TSHR, NIS, GAPDH, or MHC class I mRNA levels when added to the medium of rat FRTL-5 thyroid cells. FRTL-5 cells in 6H medium were washed with serum-free medium and the incubation continued in serum-free medium containing TG, crystalline BSA, or mannitol. After 48 hr, Northern analyses were performed by using 20 μg total RNA. Blots were sequentially hybridized with probes for TG, TSHR, TPO, NIS, MHC class I, and GAPDH. The ratio of the binding of each probe to GAPDH was calculated, because GAPDH was not changed by TG. Cells exposed to neither BSA, mannitol, or TG were the control; the ratio of binding in these cells was set at 1 for each probe. Experimental values were compared with their corresponding controls; data are the mean ± SD of three different experiments, each performed in duplicate. One, two, or three stars represent a significant TG-induced decrease or increase at $P < 0.05$, <0.01, or <0.001, respectively. Representative blots are from one experiment.

as measured by Northern blot analysis (data not shown), i.e., the TG effect appeared to be thyroid-specific.

The effects of TG on TPO, NIS, TSHR, TG, and class I mRNA levels were not duplicated by albumin or mannitol (Fig. 1 *Upper*). The effect of mannitol was tested to ensure that the TG action was not an osmotic pressure effect. The osmotic pressure of 1 mg/ml mannitol is 5.5 mOsm; that of 10 mg/ml TG is 0.14 mOsm. The effects also were not duplicated by human or bovine IgG and IgM (data not shown). Neither triiodothyronine (T3), tetraiodothyronine (T4), or iodide, which can be derived from the TG, duplicated the TG effects. This is illustrated for TG and TSHR RNA levels in Fig. 2. There was also no effect of T3, T4, or iodide on thyroid transcription factor-1 (TTF-1) RNA levels; the importance of this observation will be clear below.

The decrease in TG RNA induced by adding 10 mg/ml exogenous 19S follicular TG to the medium of the FRTL-5 cells (Fig. 1) was accompanied by a 77 ± 7% decrease in immunoreactive TG secreted into the medium of methionine-labeled cells (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data). Analysis of TPO and TSHR protein in detergent extracts of the methionine-radiolabeled cells, using specific antibodies and similar procedures (19, 20), revealed decreases of 64 ± 10% and 59 ± 14%, respectively. Flow cytometry analysis (30) of the TG-treated cells indicated a 270 ± 42% increase in MHC class I antigen presentation. These results are consistent with the RNA changes, i.e., there are concomitant changes in protein.

To determine whether the TG effect was transcriptional, promoter constructs were transiently transfected into FRTL-5 cells and cells were placed in medium in the presence or absence of TG. Exogenous follicular TG decreased the promoter activity of TSHR-CAT, TG-CAT, and TPO-luciferase chimeras, but increased MHC class I–CAT chimera activity (Fig. 3). There was no effect on the control CAT or luciferase vectors (Fig. 3). The TG effect was not duplicated by albumin (Fig. 3) or mannitol (data not shown). The likelihood that NIS mRNA levels are also transcriptionally regulated will be evident from studies of the underlying mechanism below.

Salt-extracted/agarose-purified human 19S follicular TG obtained from extremely large iodide-deficient goiters associated with growth antibodies (31) or from colloid adenomas was more effective, at the same protein concentration, than salt-extracted/agarose-purified bovine or normal human 19S follicular TG or TG from Sigma (Table 1). This is exemplified by TPO-luciferase activity (Table 1), but was also true in assays measuring RNA levels (data not shown). By comparison with normal human TG, the goiter and adenoma TG preparations are enriched in TG molecules with a low iodide and low sialic acid content. These exhibit the highest affinity for the TG-binding protein on FRTL-5 cell, human, and bovine thyroid membranes (2, 25–28).

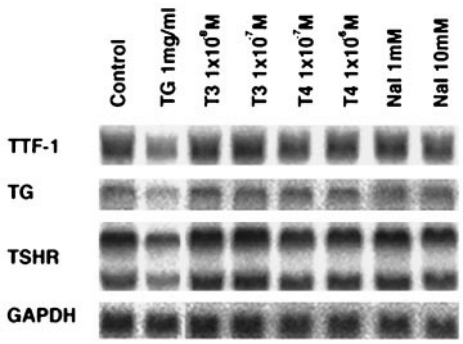

FIG. 2. Ability of exogenous TG, T3, T4, or iodide to modulate TG, TSHR, TTF-1, or GAPDH RNA levels when added to the medium of rat FRTL-5 thyroid cells. The experiment was performed exactly as in Fig. 1. The autoradiograph is representative of three separate experiments with similar results.

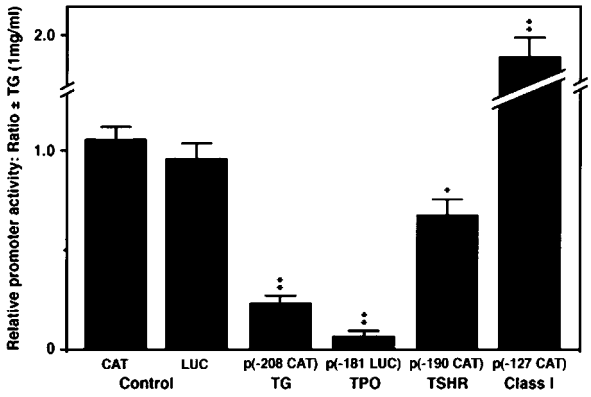

FIG. 3. Ability of exogenous follicular TG to modulate TG, TPO, TSHR, or MHC class I promoter activity in rat FRTL-5 thyroid cells, as assessed by transient expression analysis. FRTL-5 cells in 6H medium were transfected with 5 μg of the noted chimera DNAs or their control vectors. After 24 hr, cells were washed and exposed to 10 mg/ml TG or BSA as in Fig. 1. After 48 hr, promoter activity was measured and normalized for transfection efficiency. Values are expressed relative to values measured in the presence of TG vs. BSA; a ratio of 1 indicates no TG effect. Data are the mean ± SD of three different experiments. One or two asterisks represent a significant TG-induced decrease or increase at $P < 0.05$ or $P < 0.01$, respectively.

The TG, TPO, TSHR, NIS, and MHC class I genes require TTF-1 for maximal expression (3–11, 15, 16, 18). TG and TPO genes also are positively regulated by TTF-2 (12, 13). TG and TPO genes are positively regulated (7, 12), but MHC class I genes are negatively regulated, by Pax-8 (18). Exogenous follicular TG added to the medium of the FRTL-5 cells decreased TTF-1, TTF-2, and Pax-8 mRNA levels when tested over the same concentration range used in Figs. 1 and 2 (Fig. 4). The effect was not duplicated by albumin or mannitol (Fig. 4), IgG or IgM (data not shown), nor by T3, T4, or iodide (see, for example, TTF-1 in Fig. 2). Exogenous TG did not, in contrast, decrease the mRNA levels of three ubiquitous transcription factors that regulate the expression of the TSHR, TG, TPO, or MHC class I genes (11, 32–34): Sox-4, the single strand binding protein-1 (SSBP-1), and TSHR suppressor element-binding protein-1 (TSEP-1) (Fig. 4). TSEP-1 and

Table 1. Relative ability of different TG preparations to decrease TPO gene expression in FRTL-5 thyroid cells

TG source and characteristics	Relative inhibition activity
Bovine TG	
Sigma	1
Salt-extracted/agarose-purified 19S	***6.8 ± 3***
Human TG–salt-extracted/agarose-purified 19S	
Normal thyroid	***8.5 ± 2***
Iodide deficiency goiter	***15 ± 3****
Colloid adenoma	***18 ± 3****

Bold and italicized values represent significantly improved inhibition relative to the action of Sigma TG ($P < 0.01$); asterisks indicate values that are significantly higher than TG from normal human thyroids ($P < 0.05$). FRTL-5 cells in 6H medium containing 5% calf serum were transfected with 5 μg of the TPO-luciferase chimera, pTPO-1372-LUC, by using a DEAE procedure. Cells were washed with 6H medium without serum, and the different types of TG were added; luciferase activity was measured 48 h later. The activity of 1 mg/ml of each of the TG preparations was compared; the activity of 1 mg/ml Sigma TG was the arbitrary standard having 1 unit of activity. The same data were obtained with two other TPO-luciferase chimeras (pTRO-6300-LUC; pTRO-181-LUC) and were not duplicated by albumin.

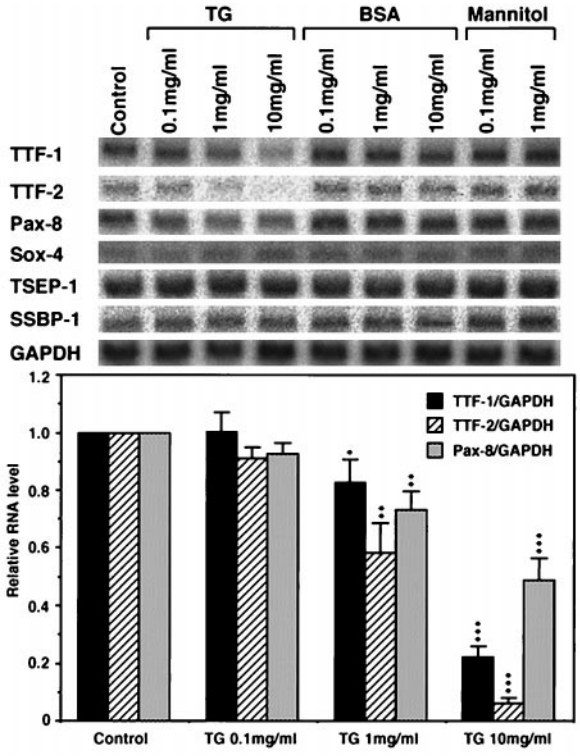

FIG. 4. Ability of exogenous follicular TG to decrease TTF-1, TTF-2, or Pax-8 mRNA levels, by comparison with Sox-4, TSEP-1, SSBP-1, or GAPDH mRNA levels, when added to the medium of rat FRTL-5 thyroid cells. FRTL-5 cells in 6H medium were washed and exposed to TG, BSA, or mannitol as in Fig. 1. After 48 hr, RNA was isolated and Northern analyses were performed as in Fig. 1 except that blots were sequentially hybridized with probes for TTF-1, TTF-2, Pax-8, Sox-4, TSEP-1, SSBP-I, and GAPDH. After quantitative analysis, the ratio of each to GAPDH was calculated. TG values are expressed relative to the respective control values in cells with no TG, BSA, or mannitol, which are set as 1. Data are the mean ± SD from three experiments. One, two, or three asterisks represent a significant TG-induced decrease at $P < 0.05$, <0.01, or <0.001, respectively. A representative blot from one experiment is presented.

Sox-4 are suppressors where tested, SSBP-1 is an enhancer. TG had no effect on TSEP-1, SSBP-1, or Sox-4 RNA levels in BRL cells or rat fibroblasts (data not shown).

Consistent with the decrease in TTF-1, TTF-2, and Pax-8 mRNA levels, TTF-1, TTF-2, and Pax-8 protein in nuclear extracts from TG-, but not albumin-treated FRTL-5 cells, exhibited a decreased ability to form protein/DNA complexes with their specific binding sites on the TG promoter (Fig. 5). The identification of these complexes as TTF-1, TTF-2, or Pax-8 complexes has been established previously and was based on site-directed mutagenesis, oligonucleotide competition, and recombinant protein binding (10, 32–34). Extracts from TG- or albumin-treated cells did not exhibit differences in complex formation when using oligonucleotides with the sequence of the SSBP-1 or TSEP-1 sites on the TSHR (data not shown). SSBP-1 and TSEP-1, as noted earlier, are ubiquitous transcription factors that also regulate the activity of the TSHR, TG, TPO, or MHC class I genes (11, 32–34).

These results are consistent with the conclusion that TG-decreased TTF-1, Pax-8, and TTF-2 gene expression caused a decrease in TG, TPO, TSHR, and NIS gene expression, as well as the increase in class I expression. Decreased TTF-1, Pax-8, and TTF-2 would decrease maximal TG and TPO gene expression (3–8, 12–14) and decreased TTF-1 would decrease maximal TSHR and NIS gene expression (9–11, 15, 16). Decreased TTF-1 and Pax-8 would allow the cAMP response element (CRE)-binding protein (CREB) to bind to the CRE

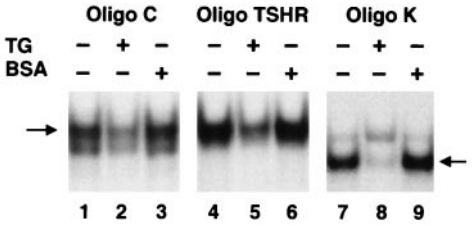

FIG. 5. Ability of nuclear extracts from TG- or albumin-treated FRTL-5 cells to bind to oligonucleotides with the sequence of the TG TTF-1/Pax-8 site (Oligo C), the TSHR TTF-1 site (Oligo TSHR), or the TG TTF-2 site (Oligo K). FRTL-5 cells grown in 6H medium were washed and exposed to 10 mg/ml 19S follicular TG or BSA as in Fig. 1. After 48 hr, extracts were prepared and incubated with the oligonucleotides noted: the oligo C site of TG, which binds TTF-1 and Pax-8 (5, 10), lanes 1–3; the TTF-1 site of the TSHR that binds only TTF-1 (9–11), lanes 4–6; and the oligo K site of TG, which binds TTF-2 (13), lanes 7–9. Complexes were evaluated by electrophoretic mobility-shift assays. The left arrow denotes the TTF-1 complex; the right arrow denotes the TTF-2 complex. The lower complex in lanes 1–3 is the Pax-8 complex, which is not present in lanes 4–6 (10). The upper complex, which appears in lane 8, represents the increased binding of Sox-4, which can interact with the TTF-2 site (34).

that lies between the TTF-1 and TTF-1/Pax-8 sites on the class I 5′ flanking region, −127 to −80 bp (18, 35). CREB is a positive regulator and thereby increases class I gene expression (18, 35).

In FRTL-5 cells, basal expression of a TSHR-CAT chimera, pTRCAT 5′-220 TTF-1/NS, which has a mutation in the TTF-1 site (10, 32), is decreased 50% in transient transfections by comparison with its wild-type control; residual activity results from the action of SSBP-1 (11). TG had no effect on pTRCAT 5′-220 TTF-1/NS activity (data not shown), i.e., mutation of the TTF-1 site appears to eliminate the TG action. Similarly, TG suppression of the activity of a −880-bp TG-CAT chimera was lost (data not shown) when deletions removed the most 5′ TTF-1 and TTF-2 sites, because they are required for TTF-1, Pax-8, and TTF-2 activity (3–5, 8, 12–14).

The effect of exogenous follicular TG on the thyroid-specific or -restricted transcription factors appears to be transcriptional, as is the effect of exogenous TG on TG, TPO, TSHR, and class I gene expression. Thus, TG, but not albumin (Fig. 6) or mannitol (data not shown), decreased the promoter activity of four different constructs spanning 5.18 kb of the

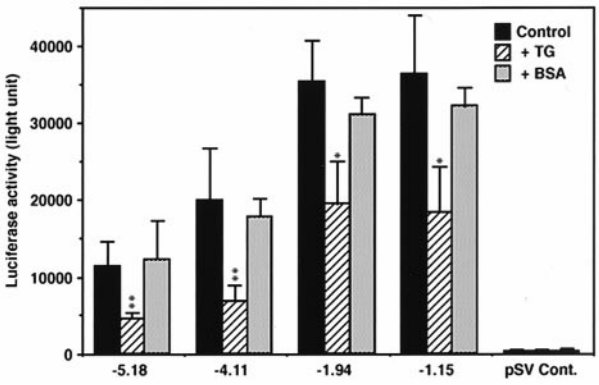

FIG. 6. Ability of exogenous follicular TG to decrease TTF-1 promoter activity in FRTL-5 thyroid cells, as measured by using transient expression analysis. FRTL-5 cells in 6H medium were transfected with 5 μg of TTF-1-luciferase chimeras containing different lengths of 5′ flanking region then exposed to medium with or without 1 mg/ml bovine TG or BSA as described in Fig. 1. Promoter activity was measured 48 hr later and normalized to the pSV control and for transfection efficiency. Data are the mean ± SD of three different experiments. One or two asterisks represent a significant TG-induced decrease at $P < 0.05$ or $P < 0.01$, respectively.

TTF-1 5′ flanking region. In contrast, TG had no effect on the activity of the pSV vector or a construct that contained only −50 bp of 5′ flanking region. These data indicate that the action of TG on the TTF-1 promoter is localized within −1.15 kb of the start of transcription.

The action of exogenous follicular TG on TTF-1-luciferase or TG-CAT activity was additive with that of TSH. This was measured by using cells maintained in 5H medium (no TSH), transfecting them with TTF-1-luciferase or TG-CAT promoter constructs, and challenging them with 1 mg/ml 19S follicular TG, 1×10^{-10} M TSH, or both. The relative activities for the 1.15-kb TTF-1-luciferase construct were 1, 0.6, 0.6, and 0.2, respectively, in the absence of TSH, the presence of TSH, the presence of TG, and the presence of TSH plus TG. These data are consistent with the additive effects of exogenous TG to decrease TTF-1 and TG mRNA levels in FRTL-5 cells (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data).

DISCUSSION

In a separate report (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data), we suggested that TG that accumulated in the lumen of the thyroid follicle *in vivo* was a feedback suppressor of TTF-1 mRNA levels and of TG biosynthesis in thyroid cells comprising that follicle. This conclusion was supported by the observation that, in FRTL-5 thyroid cells, purified, exogenous bovine and rat 19S follicular TG decreased TTF-1 mRNA levels additively with TSH and overcame the effects of TSH to increase TG biosynthesis. In this report we expand these observations and begin to clarify the underlying mechanism.

We show that purified, salt-extracted, bovine and human 19S follicular TG, added to the medium of FRTL-5 cells, suppresses the RNA level of, and protein/DNA complexes formed by, the three critical regulators of maximal TG gene expression: TTF-1, Pax-8, and TTF-2. The effect of TG on these thyroid-specific or -restricted transcription factors is to suppress the expression of the TG, TPO, TSHR, and NIS genes, because each is regulated by one or more of these transcription factors. The effect of TG seems to involve only the thyroid-specific or -restricted transcription factors regulating these genes, because expression of three ubiquitous transcription factors regulating one or more of them was not changed by exogenous TG, when measured at the same time and under identical conditions. The action of the TG on TTF-1 is transcriptional and localized to 1.15 kb of the start of transcription; it is reasonable to anticipate that TG also will regulate Pax-8 and TTF-2 at a transcriptional level. Finally, we show that expression of the MHC class I gene, which is present in all cells but regulated only in the thyroid by TTF-1 and Pax-8 (18, 35), is increased when 19S follicular TG is added to the FRTL-5 cell medium. We suggest that class I expression is increased because the decrease in TTF-1 and Pax would allow CREB, a positive regulator of the gene, to interact with a CRE-containing silencer element having overlapping sites for TTF-1, Pax-8, and CREB.

These data clarify one aspect of the underlying mechanism, but open the important question as to how such a large, extracellularly stored molecule could physiologically exert its regulatory action at a transcriptional level. In our separate report (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data), we observed *in vivo* that high levels of TSH induced by propylthiouracil caused the follicular TG to be degraded. However, a rim of TG bound to the apical membrane of the follicle remained, despite the decrease in follicular TG (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data). There is a protein on the apical membrane that binds TG that has been vectorially transported to the follicular lumen (25–28). TG is released from the binding protein as it is reiteratively iodinated and sialylated by, respectively, TPO and a sialotransferase bound to the apical membrane (25–28). In this report, we show that TG preparations from colloid nodules or iodine deficient glands, which are poorly iodinated, poorly sialylated, and have the highest apical membrane-binding activity (25–28), are more effective suppressors than normal follicular TG. We thus raise the possibility that TG bound to the apical membrane and unable to complete its reiterative iodinated/sialylation cycle at that membrane is the important TG moiety initiating the suppression. This hypothesis is being validated by studying the effects of TG preparations with specific changes in carbohydrate moieties and iodide content that affect binding to the TG-binding protein (25–28). The hypothesis is, nevertheless, consistent with observations that higher concentrations of TG inhibit NIS-dependent iodination and iodide coupling *in vitro* and that this inhibition is influenced by the iodination and sialylation of the TG molecule (36). It is also consistent with the observation that poorly iodinated TG in nontoxic, multinodular sporadic goiter inhibits TSH-induced TG utilization from the follicular lumen (37).

Iodide intake is episodic, and TG accumulated in the follicular lumen can act as an iodide trap. We suggest that, as TG accumulates in the follicular lumen, particularly with transient nutritional or chronic endemic iodide deficiency, recently synthesized TG molecules that are low in iodide and not yet sialylated bind to and pile up on the apical membrane. This results in suppression of the thyroid-specific or -restricted transcription factors and, in turn, TSHR, TG, TPO, and NIS. The function of the cells in that follicle becomes quiescent and their growth abates, leaving follicles packed with TG and surrounded by flattened cells. Nearby, follicles with little TG accumulated in their lumens continue to have active, highly functional cells, which are still responsive to TSH. The phenomenon may, therefore, contribute to follicular heterogeneity, as suggested in the *in vivo* observations (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data).

TG bound to the membrane may be the initiating event, but the nature of the actual transcriptional effector is unknown. The TG interaction with its binding protein on the cell surface is not known to activate a signal-transducing pathway. TG has been reported to have a kinase-A-like activity and have phosphorylated tyrosine, serine, and threonine residues (28, 38). However, the TG action is additive with TSH (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data; this report), whose independent suppressive effect on TTF-1 is cAMP- and kinase-A-mediated (10, 11). The kinase-A-like action of TG *per se* seems, therefore, redundant to, and different from, the action of TSH. 19S TG, its 12S subunits, or its fragments can enter the cell by fluid pinocytotic mechanisms (39); internalization via the apical-binding protein is less clear but probable (25–28, 39). A TG fragment may, therefore, be the direct transcriptional suppressor. This may be a phosphorylated fragment, one that is not fully glycosylated and/or one that is poorly iodinated. These possibilities are readily tested in the FRTL-5 cell system, as is the relationship of their action to TSH-induced suppression. However, the most useful data identifying the effector may emerge from studies defining the cis element linked to the TG action on the 5′ flanking region of TTF-1, Pax-8, or TTF-2.

Goiters can be associated with autoimmune thyroid disease, for example, growth autoantibodies causing enormous thyroid enlargement (31, 40). The basis for the development of the autoimmune disease is not understood. The current data provide a potential scenario. Increased class I could result in abnormal presentation of thyroid peptides that can be autoantigens and thereby activate T cells in the immune system. The increased class I might, therefore, initiate an autoimmune response as a result of lost tolerance (41). We currently are

testing whether class I is abnormally expressed *in vivo* in patients with goiters and autoantibodies.

The thyroid gland has a unique structural feature, the thyroid follicle (1, 2). TG is stored, our episodic iodide intake is trapped, and thyroid hormones are synthesized within this storage facility. TG is degraded and thyroid hormones are secreted when needed for our metabolic homeostasis. TSH is one well recognized positive controller of this process (1, 2); we now show that TG is a hitherto unrecognized negative feedback regulator of the process. TG acts as an autocrine/paracrine regulator within a single follicle (K.S., A.M., S.L., E. Miyagi, R.K., L.D.K., and A.K., unpublished data; this report); it contributes to follicular heterogeneity by limiting individual follicle size, cell growth, and cell function. This would allow individual follicles to respond to gradations of TSH, despite their exposure to similar levels of TSH in the blood at any one point in time. It would allow maximal thyroid hormone storage together with the ability to fine-tune the pattern of thyroid hormone release to meet both the short- and long-term needs of physiologic homeostasis.

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