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Thyroid or glucocorticoid hormone induces pre-growth-hormone mRNA and its probable nuclear precursor in rat pituitary cells

(rat pituitary tumor cells/plasmid pBR322-GH1/RNA gel blot hybridization/RNA dot hybridization)

PAUL R. DOBNER*†, ERNEST S. KAWASAKI*‡, LI-YUAN YU*§, AND F. CARTER BANCROFT*¶‖

*Memorial Sloan–Kettering Cancer Center and ¶Department of Genetics and Molecular Biology, Sloan–Kettering Division, Graduate School of Medical Sciences, Cornell University, New York, New York 10021

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ABSTRACT **Thyroid or glucocorticoid hormone increases the synthesis of growth hormone (GH) by clonal lines of rat pituitary tumor cells. To investigate whether these increases arise from increased accumulation of GH-specific RNA sequences in the cytoplasm and nuclei of these cells, we adapted two existing procedures so that a ^{32}P-labeled hybrid plasmid containing a cDNA sequence could be used to quantitate relative concentrations of the corresponding mRNA. One method (RNA gel blot hybridization) used electrophoresis of RNA, transfer to nitrocellulose paper, and hybridization to ^{32}P-labeled plasmid. The other (RNA dot hybridization) used covalent attachment of RNA to activated cellulose paper squares and hybridization to ^{32}P-labeled plasmid. As probe, we used a hybrid plasmid (pBR322-GH1) which we show by restriction analysis to contain a DNA sequence coding for rat GH. The results were comparable from both techniques and showed that incubation of GH_3 cells with a thyroid hormone (triiodothyronine), a glucocorticoid hormone (dexamethasone), or both hormones caused an increase of cytoplasmic pre-GH mRNA sequences of about 4-, 22-, and 13-fold, respectively. Results obtained with the RNA gel blot hybridization method showed that hormonal stimulation leads to the induction of a single 1.0-kilobase species of pre-GH mRNA in the cytoplasm and of 2.7- and 1.0-kilobase species of GH-specific RNA in the nucleus.**

GH-cells are related lines of rat pituitary tumor cells that produce the protein hormones growth hormone (GH) and prolactin (1, 2). Glucocorticoids increase GH production by GH-cells (3). The use of hypothyroid serum in the growth medium later led to the demonstration that thyroid hormones also increase GH production by these cells (4). Since then, GH-cells have been used extensively to study the mechanisms of action of glucocorticoid and thyroid hormones (5–7).

Recent experiments using *in vitro* mRNA translation systems have shown that increases in GH synthesis in response to these hormones are accompanied by increases in translatable cytoplasmic pre-GH (pGH) mRNA (8–11). However, the intracellular events leading to increased GH synthesis by GH-cells upon exposure to a glucocorticoid or a thyroid hormone remain unclear. For example, it is not known whether the hormone-induced increases in translatable cytoplasmic pGH mRNA arise from an increase in cytoplasmic pGH mRNA sequences or from some cellular alteration in the structure of pGH mRNA that increases its translational efficiency. Furthermore, the recent demonstration of a probable nuclear precursor of pGH mRNA (12) raises the possibility that hormone action on GH-cells could be exerted at the level of processing of this precursor into mature cytoplasmic pGH mRNA.

To investigate these questions, we have adapted the gel blot hybridization technique of Thomas (13) and the dot hybridization procedure of Kafatos *et al.* (14) so that a ^{32}P-labeled GH cDNA plasmid [pBR322-GH1 (15)] can be used as probe to detect and quantitate GH-specific RNA sequences. We report here that exposure of GH-cells to glucocorticoid and thyroid hormones, alone or in combination, leads to an increased cytoplasmic accumulation of mature [1.0 kilobase (kb)] pGH mRNA sequences, and an increased nuclear accumulation of a 2.7-kb probable precursor of cytoplasmic pGH mRNA.

EXPERIMENTAL PROCEDURES

Preparation of Induction Medium. Fetal calf serum was treated with Dowex 1-X10 resin to remove thyroid hormones as described by Samuels *et al.* (16), except that proportions were 100 mg (wet weight) of resin per ml of serum. Resin was removed by centrifugation (300 × *g*, 10 min) followed by filtration through a 0.8-μm filter. To remove glucocorticoids, the serum was then extracted with activated charcoal [20 mg (wet weight) per ml] for 40 min at 37°C and 30 min at 55°C. Following removal of charcoal by centrifugation (300 × *g*, 10 min), the serum was sterilized and any remaining charcoal was removed by successive filtrations through 0.8- or 0.45-μm and 0.22-μm filters. The efficiency of hormone removal by this protocol was examined by incubating fetal calf serum overnight at 37°C with either 20 nM 1-[3′-^{125}I]triiodothyronine (^{125}I-T3) or 80 nM [^{3}H]hydrocortisone and then treating it with resin plus charcoal. Based on recovery of added radioactivity after treatment, this protocol led to removal of 98.2% and 97.6% of endogenous thyroid and glucocorticoid hormones, respectively. As assayed by radioimmunoassay (kindly performed by Martin Surks), the concentration of T3 in the treated serum was undetectable (<5 ng/100 ml). Serum substitute was prepared essentially as described by Bauer *et al.* (17), except that all hormones and methocellulose and lecithin were omitted. Induction medium was then prepared by combining the following to the indicated final concentrations: Joklik's modified Eagle's medium containing penicillin plus streptomycin (5), 89%; serum substitute, 10%; fetal calf serum extracted with resin plus charcoal, 1%; Hepes, 15 mM. We have found that GH_3 cells (the GH-cell line used in these studies) incubated in suspension culture in this medium will remain ≥95% viable and grow exponentially for at least 17 days (unpublished data).

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Abbreviations: GH, growth hormone (somatotropin); pGH mRNA, pre-GH mRNA; T3, triiodothyronine; kb, kilobase(s).

† Present address: Department of Microbiology and Molecular Genetics, University of Massachusetts Medical Center, Worcester, MA 01605.
‡ Present address: Cetus Corporation, Berkeley, CA 94710.
§ Present address: Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
‖ To whom reprint requests should be addressed.

Measurement of GH Synthesis and Translatable pGH mRNA. Relative GH synthesis (GH synthesis ÷ total protein synthesis) was measured as described (5), except that radioactivity in GH in cytoplasmic lysates was measured by indirect immunoprecipitation with anti-GH antiserum plus fixed *Staphylococcus aureus* bacteria as described (18). To prepare total cytoplasmic RNA, cells were lysed at 4°C with 0.5% Nonidet P-40 in isotonic high pH buffer (12) and the nuclei were pelleted by centrifugation (1100 × *g*, 5 min). The resulting cytoplasmic lysates were made 0.5% in $NaDodSO_4$ and 5 mM in EDTA, and RNA was isolated by phenol/chloroform extraction. After isolation of poly(A)$^+$RNA by oligo(dT)-cellulose chromatography (18), translatable pGH mRNA was measured by translation in nuclease-treated rabbit reticulocyte lysates at three concentrations of poly(A)$^+$RNA from each sample in a range (0–30 μg/ml) that was observed to yield a linear relationship between mRNA concentration and protein synthesis (18), followed by indirect immunoprecipitation exactly as described (18).

Preparation of Plasmid DNA and Labeling by Nick Translation. Plasmid pBR322-GH1 (15) was propagated in an approved EK1 host–vector system under P1 conditions. Supercoiled plasmid DNA was prepared essentially as described (19). For hybridization, plasmid DNA was labeled with [^{32}P]dCTP plus [^{32}P]dGTP (>400 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels; Amersham) by nick translation as described (20) to a specific activity of 2–4 × 10^8 cpm/μg.

Quantitation of GH-specific RNA Sequences. *RNA gel blot hybridization.* Total cytoplasmic RNA was isolated as described above. Nuclear pellets (see above) were washed once with isotonic high pH buffer, and nuclear RNA was isolated essentially as described by Penman (21). Cytoplasmic or nuclear RNA was totally denatured by heating (60°C, 5 min) in electrophoresis buffer (5 mM sodium acetate/1 mM EDTA/20 mM morpholinopropanesulfonic acid, pH 7.0) containing 6% formaldehyde and 50% formamide (22). Electrophoresis in a 1.1% agarose slab gel containing 6% formaldehyde was then performed as described (22). After electrophoresis, RNA was transferred to nitrocellulose paper (13), prehybridized as described (23) except that the glycine was omitted, and hybridized as described (23) with 1–2 × 10^7 cpm of ^{32}P-labeled pBR322-GH1 DNA. After hybridization, the paper was washed four times for 5-min each at room temperature in 250 ml of 0.30 M NaCl/0.03 M sodium citrate/0.1% $NaDodSO_4$ and then for 15–30 min at 50°C in 250-ml portions of 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$ until the radioactivity in the final wash was close to background (usually three or four washes). The nitrocellulose paper was then exposed to x-ray film at −70°C with a Dupont Cronex Lightning-Plus intensifying screen. When cytoplasmic RNA was analyzed, the intensities of the resulting pGH mRNA bands were quantitated by scanning in a Quick Scan Jr. TLC densitometer with a peak area integrator (Helena Laboratories, Beaumont, TX). Preliminary analyses were performed to determine the amounts of each RNA preparation to apply to a gel and the autoradiographic exposure time to be used so that, for all samples on a given gel, the pGH mRNA band intensity was proportional to the amount of RNA analyzed.

RNA dot hybridization. Aminophenylthioether-paper, a modification of the aminobenzylmethoxymethyl-paper described by Alwine *et al.* (24), was prepared according to a protocol developed by Brian Seed (see footnote 51 in ref. 24). This paper was then cut into 1.0-cm^2 squares and activated to the diazophenylthioether form as described (24). Poly(A)$^+$RNA was isolated from total GH_3 cell cytoplasmic RNA as described above. Yeast RNA was added to each RNA sample to be analyzed to bring the total amount of RNA to 5 μg. Samples (10–20 μl) of RNA in 0.2 M sodium acetate (pH 4.0) were spotted in duplicate onto diazophenylthioether-paper squares. The papers were dried (1 hr, room temperature) and then prehybridized as described (23). Duplicate papers for each point plus a control paper containing only yeast RNA were then hybridized as described (23) in 200 μl in a siliconized glass vial containing 2 × 10^6 cpm of ^{32}P-labeled pBR322-GH1 DNA per ml. After hybridization, the papers were washed as described above, dried, and assayed in a toluene-based scintillant. For each point, radioactivity in the control paper has been subtracted from the average value in the experimental papers. Control experiments with [^{3}H]uridine-labeled total GH_3 cell cytoplasmic RNA showed that 60–70% of the applied RNA remained on the paper through the procedures described above.

RESULTS

Restriction Analysis of Hybrid Plasmid pBR322-GH1. This plasmid will specifically hybridize pGH mRNA (15). Cleavage of pBR322-GH1 DNA with various restriction enzymes showed that the insert has the same restriction map as a portion of the rat GH structural gene sequence (Fig. 1). The insert contains the 3′ end of pGH mRNA, is about 700 base pairs long [including poly(dA-dT) tails (15)], and extends in the 5′ direction to within about 160 nucleotides of the pGH mRNA initiator methionine codon.

Hormonal Induction of GH Synthesis and Translatable pGH mRNA in GH_3 Cells. GH_3 cells growing exponentially in suspension culture as described (5) were pelleted, washed twice with induction medium, resuspended in induction medium, and grown for 3 days. This culture was then split into four cultures and grown for 3 days either in the absence of added hormones ("control") or in the presence of 50 nM T3, 80 nM dexamethasone, or both hormones. The stimulation of relative GH synthesis in these intact GH_3 cells was 3-, 14-, and 9-fold, respectively (Table 1). Translation of poly(A)$^+$RNA isolated from the same cultures showed that *in vitro* synthesis of preGH directed by pGH mRNA closely paralleled relative GH synthesis, in agreement with previous observations (8–10).

Use of a Plasmid Containing pGH cDNA to Quantitate Relative Amounts of pGH mRNA Sequences. We have used two techniques in which ^{32}P-labeled pBR322-GH1 DNA is used as

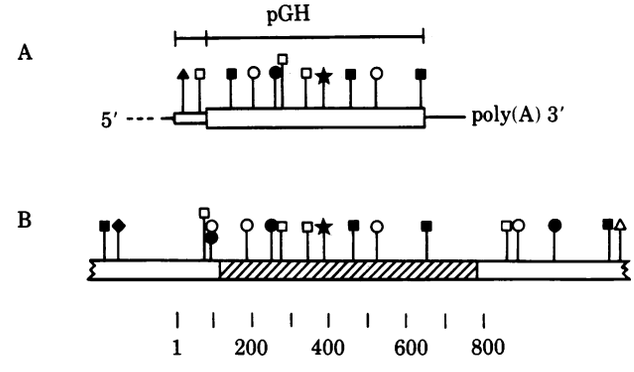

FIG. 1. (*A*) Restriction map of pGH mRNA, as determined by Seeburg *et al.* (25). (*B*) Restriction map of pBR322-GH1. The hybrid plasmid described previously (15) was recloned, thus avoiding the smeared restriction patterns obtained in preliminary experiments. Plasmid DNA was cut with various restriction enzymes singly or in combination. Fragments containing pGH mRNA sequences were identified by the DNA gel blot hybridization technique (24). The probe was [^{32}P]cDNA prepared from partially purified pGH mRNA (about 60% pure by translation assay) (18). The open and hatched regions represent pBR322 and insert [including the poly(dA·dT) tails] DNA, respectively. All restriction fragments containing the hatched region hybridized with the probe. Nucleotide numbers are shown counting from the pGH mRNA initiator methionine codon. ▲, *Pst* I; □, *Hae* III; ■, *Alu* I; ○, *Hha* I; ●, *Hpa* II; ★, *Kpn* I; ◆, *Sal* I; △, *Eco*RI.

probe to investigate whether the hormonal effects described in the preceding section arose from increases in cytoplasmic pGH mRNA sequences.

The first technique involved adapting the RNA gel blot hybridization procedure of Thomas (13), in which RNA is prepared for hybridization by transfer from a gel to nitrocellulose paper, for use in the quantitation of relative amounts of a particular mRNA sequence. Various amounts of total cytoplasmic RNA from control and hormone-stimulated cells were analyzed by this technique, with pBR322-GH1 [^{32}P]DNA as probe. Control cells (Fig. 2, lanes a, b, and c) contained a single 1.0-kb band of GH-specific RNA (i.e., pGH mRNA). An increase in a GH-specific RNA band indistinguishable in size from that observed in control cells was observed in cells grown in the presence of T3 (lanes d, e, and f), dexamethasone (lanes g, h, and i), or T3 plus dexamethasone (lanes j, k, and l).

Relative concentrations of pGH mRNA sequences were quantitated by scanning the autoradiogram shown in Fig. 2. The resultant plots of pGH mRNA detected versus the amount of RNA applied to the gel were linear for RNA from all four cultures over the ranges examined (Fig. 3 *Left*). Hence, the stimulation of cytoplasmic pGH mRNA sequences resulting from incubation of GH_3 cells with T3 or dexamethasone or both could be calculated from the slopes (Table 1).

The second technique was an adaptation of the dot hybridization procedure of Kafatos *et al.* (14) in which cloned DNAs are attached to nitrocellulose filters and probed with radioactive cellular RNA or DNA. In the present technique (RNA dot hybridization), various amounts of poly(A)$^+$RNA from control or hormone-stimulated cells were covalently linked to squares of diazophenylthioether-paper which were then hybridized with pBR322-GH1 [^{32}P]DNA. Plots of radioactivity hybridized versus poly(A)$^+$RNA applied were linear for RNA from all four cultures over the ranges examined (Fig. 3 *Right*), permitting calculation, from the slopes, of the stimulation of cytoplasmic pGH mRNA sequences by incubation of GH_3 cells with T3 or dexamethasone or both (Table 1). The values of relative pGH mRNA sequences measured according to either technique were in good agreement and show that incubation of GH_3 cells with T3, dexamethasone, or T3 plus dexamethasone caused an increase of cytoplasmic pGH mRNA sequences of about 4-, 22-, and 13-fold, respectively.

Hormonal Regulation of Nuclear GH RNA Sequences. To examine the sizes and relative amounts of nuclear GH-specific RNA in control and hormone-induced cells in the experiment described in Table 1, total nuclear RNA from cells in each culture was analyzed by the RNA gel blot hybridization procedure (Fig. 4). Analysis of 10 or 20 μg of nuclear RNA from control

Table 1. Hormonal stimulation of GH synthesis and of cytoplasmic pGH mRNA in GH_3 cells

	Relative GH synthesis, hormone/control	pGH mRNA, hormone/control		
		Translation	RNA gel*	RNA dot†
Control	1	1	1	1
+ T3	3.3	2.2	3.4	4.5
+ Dex	13.9	15.7	22.5	21.3
+ T3 + Dex	9.3	10.1	11.4	15.4

For each experimental condition, cytoplasmic RNA was extracted and relative GH synthesis was measured simultaneously in aliquots of cells from the same suspension culture. In control cells, relative GH synthesis was 0.8%; when assayed by translation, pGH mRNA was 1.8% of total cytoplasmic mRNA. The two hybridization techniques used can yield only ratios (hormone/control) of pGH mRNA sequences. Dex, dexamethasone.

* RNA gel blot hybridization from the data shown in Fig. 3 *Left*.

† RNA dot hybridization from the data shown in Fig. 3 *Right*.

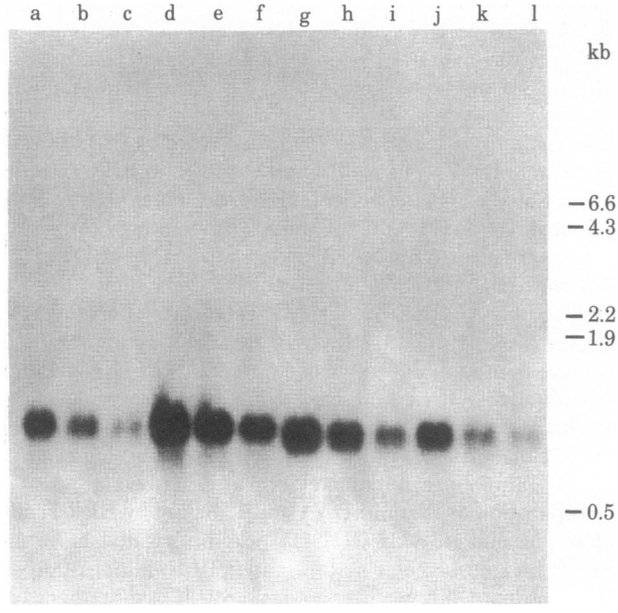

FIG. 2. Analysis of total cytoplasmic RNA from control and hormone-treated GH_3 cells. Cytoplasmic RNA was isolated from cells in the four cultures described in Table 1 and analyzed by RNA gel blot hybridization with pBR322-GH1 [^{32}P]DNA as probe. The size markers were λ DNA cleaved with *Hin*dIII, end-labeled with ^{32}P, and treated identically to the RNA samples prior to electrophoresis. The film was exposed for 4 hr. The lanes contained RNA from control cells (a, 10 μg; b, 5 μg; c, 2.5 μg) or from cells grown in the presence of T3 (d, 10 μg; e, 5 μg; f, 2.5 μg), dexamethasone (g, 1 μg; h, 0.5 μg; i, 0.25 μg), or T3 plus dexamethasone (j, 1 μg; k, 0.5 μg; l, 0.25 μg). Note that the amounts of RNA applied from cells grown under the first two conditions were 10-fold higher than for cells grown under the second two conditions.

cells (lanes a and b) yielded a faint band corresponding in size to mature 1.0-kb pGH mRNA but no detectable high molecular weight GH-specific RNA [however, a longer exposure of lane b revealed a very faint band of 2.7-kb GH-specific RNA (data not shown)]. Analysis of 10 or 20 μg of total nuclear RNA from hormone-induced cells showed that incubation of GH_3 cells with T3 led to an increased nuclear accumulation of both 1.0-kb pGH mRNA and the 2.7-kb GH-specific RNA (lanes d and e), that incubation with dexamethasone led to a much larger accumulation of both of these GH-specific RNA bands (lanes g and h), and that incubation with T3 plus dexamethasone led to an accumulation of both GH-specific RNA bands which was in-

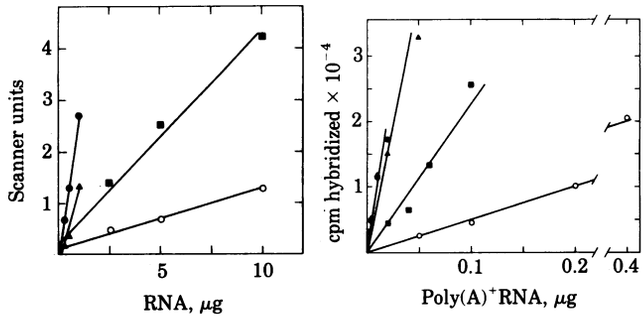

FIG. 3. Quantitation of relative cytoplasmic pGH mRNA sequences. (*Left*) By RNA gel blot hybridization. The autoradiogram shown in Fig. 2 was scanned, and the area under the pGH mRNA band in each lane was determined. ○, control; ■, plus T3; ●, plus dexamethasone; ▲, plus T3 plus dexamethasone. (*Right*) By RNA dot hybridization. Poly(A)$^+$RNA was isolated from cells from the four cultures described in Table 1, and relative pGH mRNA sequences were determined. Symbols as in *Left*.

termediate in amount to that seen with T3 alone and dexamethasone alone (lanes j and k). It should be noted that mature pGH mRNA and the 2.7-kb RNA sequence were the only GH-specific RNA bands detected in the nuclei of either control or hormone-stimulated GH_3 cells. Furthermore, in agreement with previous results (12), the 2.7-kb GH-specific RNA was present only in the nucleus; none was detected in the cytoplasm of control or hormone-stimulated GH_3 cells (Figs. 2 and 4).

DISCUSSION

We have presented here two techniques for using a ^{32}P-labeled hybrid cDNA plasmid to quantitate relative levels of a specific mRNA sequence during hormonal induction. The observation for each technique that, under the proper conditions, the signal obtained is proportional to the RNA input (Fig. 3) supports the validity of that technique. Furthermore, the good agreement obtained when the results of a hormonal induction experiment are analyzed by both techniques (Table 1) shows that either technique can be used for following relative levels of a specific mRNA sequence during hormonal induction, differentiation, etc.

The RNA gel blot hybridization technique can be used to obtain information about both the sizes of particular RNA sequences in control and induced cells and the relative number of such sequences. The RNA dot hybridization technique is more convenient to perform, but it yields no information about the size(s) of the RNA sequence under investigation. It should be noted that, as used here, neither of these techniques can be used to measure the absolute concentration of an mRNA sequence. However, this could probably be achieved by including

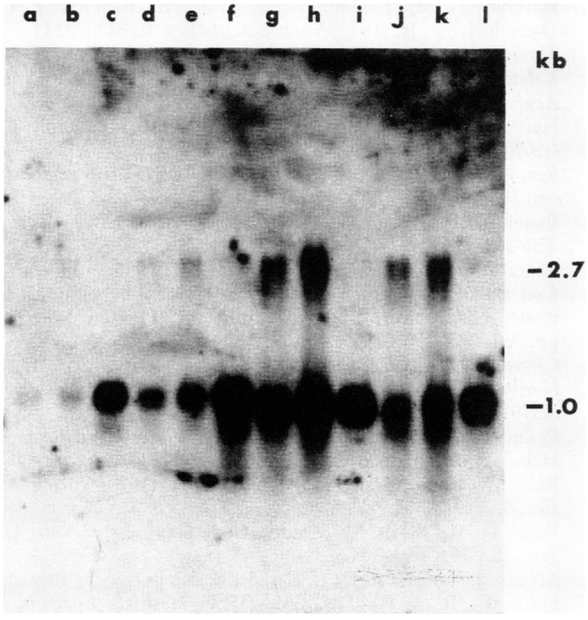

FIG. 4. Analysis of total nuclear RNA from control and hormone-treated GH_3 cells. Nuclear RNA was isolated from the four cultures described in Table 1 and analyzed by RNA gel blot hybridization as in Fig. 2. The indicated sizes of the RNA bands that hybridized to the pBR322-GH1 [^{32}P]DNA probe were calculated from the migration of ^{32}P-labeled *Hin*dIII-cleaved λ DNA analyzed on the same gel. The film was exposed for 4 days. The lanes contained nuclear RNA from control cells (a, 10 μg; b, 20 μg) or from cells grown in the presence of T3 (d, 10 μg; e, 20 μg), dexamethasone (g, 10 μg; h, 20 μg), or T3 plus dexamethasone (j, 10 μg; k, 20 μg). For comparison, total cytoplasmic RNA from control cells (c, 10 μg), or from cells grown in the presence of T3 (f, 10 μg), dexamethasone (i, 1 μg), or T3 plus dexamethasone (l, 1 μg) was analyzed.

in each measurement a control sample containing a known amount of the mRNA under investigation.

It should be noted that in previous similar investigations of hormonal regulation of GH synthesis in GH-cells (6, 7, 9, 10, 26), T3 alone stimulated GH synthesis; glucocorticoids alone yielded a moderate (2- to 3-fold) increase in GH synthesis detectable only after 48 hr of incubation, either in medium containing 10% hypothyroid, hyposteroid serum or in serum-free medium (26); and both hormones together yielded a large "synergistic" stimulation of GH synthesis (6, 7, 9, 10, 26). In the present experiments, in which GH_3 cells were incubated with T3 or dexamethasone or both for 72 hr, we also observed that T3 alone stimulates GH synthesis. However, with dexamethasone alone our results were quantitatively different from those reported previously (26): we reproducibly observed a large stimulation of GH synthesis by dexamethasone. Furthermore, in a number of experiments of the type reported here, we detected no consistent difference, at 72 hr, between the stimulation by dexamethasone alone and by T3 plus dexamethasone. Although the presence of both hormones usually yielded a somewhat greater stimulation than did dexamethasone alone, we occasionally observed, as in the experiment analyzed in detail here, that the stimulation of GH synthesis by T3 plus dexamethasone actually was lower than that obtained with dexamethasone alone.

These differences from previous observations, particularly the large induction by glucocorticoid alone, probably arise from one or more differences in the induction conditions used. The present investigations were performed with cells grown in suspension culture, whereas all previous studies used monolayer culture. However, the large dexamethasone stimulation does not appear to be related to the low calcium concentration in the suspension culture medium used because this stimulation is not reduced by addition of $CaCl_2$ (final concentration, 0.4 mM) to the induction medium (unpublished data). This effect is more likely due to our use of a medium containing a low (1%) concentration of hypothyroid, hyposteroid serum plus a serum substitute. For example, either lowered concentration of a serum component or increased concentration of a component of the serum substitute could result in the loss of the previously observed T3 requirement for a large stimulation of GH synthesis by glucocorticoids.

The induction conditions that we used enabled us to investigate the independent effects as well as the combined effects of T3 and dexamethasone on GH-specific RNA sequences in GH_3 cells. Using the RNA gel blot hybridization technique, we have shown that incubation of GH_3 cells with T3, dexamethasone, or T3 plus dexamethasone causes an increase in the cytoplasmic concentration of a single band of pGH mRNA (Fig. 2). This result, obtained with a pure probe, confirms an earlier suggestion, based upon measurements using hybridization with cDNA to partially purified pGH mRNA, that either T3 or dexamethasone increases pGH mRNA sequences in GH-cells (27). The size of the pGH mRNA sequences detected in the present investigations in control or hormone-treated cells, 1.0 kb (Fig. 2), agrees well with previous measurements of the size of this mRNA (8, 15, 25). Furthermore, this result shows that hormonal stimulation does not lead to the induction of any new species of cytoplasmic pGH mRNA of a size detectably different from that present in control cells.

For each condition of hormonal stimulation, the increase in cytoplasmic pGH mRNA sequences was apparently about 30–40% larger than the increase in GH synthesis and of translatable pGH mRNA (Table 1). Further investigations will be required to determine what significance, if any, there is in this difference. However, it does suggest that hormonal stimulation of GH_3 cells may cause the induction of some pGH mRNA se-

quences that cannot be translated *in vivo* nor *in vitro*, as has been reported recently for a myeloma immunoglobulin light chain mRNA (28).

The detection, by pulse-labeling experiments, of a 2.5- to 3.0-kb nuclear GH-specific RNA sequence in GH_3 cells, which is probably a precursor of mature cytoplasmic pGH mRNA, has been reported (12). In the present experiments, we used the RNA gel blot hybridization technique to examine the effect of hormonal stimulation on accumulation of nuclear GH-specific RNA sequences in GH_3 cells. We found that incubation of GH_3 cells with either T3, dexamethasone, or both hormones leads to increased accumulation of both pGH mRNA and a 2.7-kb GH-specific RNA sequence in our nuclear preparations (Fig. 4). Although we used washed nuclei from detergent-lysed cells for these experiments, we cannot exclude the possibility that some of the "nuclear" pGH mRNA we detect may arise from slight cytoplasmic contamination of our nuclear preparations. However, because it has been shown (12) with pulse-labeling experiments that the nuclei of GH_3 cells contain mature pGH mRNA sequences, we know that at least some of the pGH mRNA sequences detected in the experiment shown in Fig. 4 do not arise from cytoplasmic contamination. It is clear that the 2.7-kb RNA is located only in the nucleus and probably corresponds to the nuclear GH-specific RNA sequence detected previously (12).

Because the amount of the 2.7-kb RNA in control nuclei was too low to measure, we have not been able to quantitate the hormonal induction of this sequence. However, the results obtained (Fig. 4) show that the accumulation of this sequence induced by T3, dexamethasone, or both hormones agrees qualitatively with the accumulation of cytoplasmic pGH mRNA induced by T3 and dexamethasone, either alone or in combination (Table 1). This result has important consequences for understanding the regulation, by T3 and dexamethasone, of GH gene expression in GH_3 cells. It could be envisaged that T3 or dexamethasone or both increase pGH mRNA by increasing the processing of the nuclear 2.7-kb RNA into mature cytoplasmic pGH mRNA. In that case, nuclei of control cells would be expected to contain more of the 2.7-kb RNA than would hormone-induced cells. Our experiments clearly yield the opposite result and thus show that neither hormone stimulates GH gene expression by stimulating processing to cytoplasmic pGH mRNA of 2.7-kb GH-specific RNA that had been stored in the nucleus of control cells. Similar studies have shown that this is also the case for estrogen regulation of ovalbumin gene expression (29).

In both the present experiments and our previous studies (12), no nuclear GH-specific RNA sequences larger than 2.7 kb were detected. By contrast, Maurer *et al.* (30), using similar techniques, recently found 1.0-, 2.3-, 5.6-, and 6.7-kb nuclear GH-specific RNA sequences in a related cell line. The 2.3-kb RNA detected by Maurer *et al.* probably corresponds to the 2.7-kb RNA detected in the present studies. Our failure to detect the 5.6- and 6.7-kb RNA species is not understood but may arise from differences in either the cell line or the techniques used.

In summary, we have shown that growth of GH_3 cells in the presence of T3, dexamethasone, or T3 plus dexamethasone stimulates the cytoplasmic accumulation of pGH mRNA molecules and the nuclear accumulation of a probable precursor of pGH mRNA. These results are clearly consistent with the concept that T3 and dexamethasone both regulate GH gene expression at the level of transcription. However, the results are also consistent with at least one other model in which either or both hormones act by preserving the 2.7-kb GH-specific nuclear RNA species from rapid degradation. A resolution of this question will require pulse-labeling experiments using either whole cells or isolated nuclei and, ultimately, use of a soluble transcription system in which a cloned GH gene is the template.

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