# Regulated Expression of the Prolactin Gene in Rat Pituitary Tumor Cells

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ABSTRACT Prolactin (PRL) gene expression in three strains of GH cells (rat pituitary tumor cells) has been quantitated by measurement of: (*a*) intracellular and extracellular PRL, (*b*) cytoplasmic translatable PRL-specific mRNA ($mRNA_{PRL}$), and (*c*) molecular hybridization of cytoplasmic poly(A) RNA to $cDNA_{PRL}$ (DNA complementary to $mRNA_{PRL}$). Three GH cell lines utilized in this investigation were a PRL-producing ($PRL^+$) strain, $GH_4C_1$, a PRL nonproducing 5-bromodeoxyuridine resistant ($PRL^-$ $BrdUrd^r$) strain, $F_1BGH_12C_1$, and a new strain, 928-9b, derived by fusion of $PRL^+$ cells with a nuclear monolayer of the $PRL^-$, $BrdUrd^r$ GH cell strain. PRL production is a characteristic of 928-9b cells, but the level of PRL production (2–4 μg/mg protein/24 h) is much lower than that of the $PRL^+$ strain, $GH_4C_1$ (15–25 μg/mg protein/24 h). Levels of cytoplasmic translatable $mRNA_{PRL}$ and cytoplasmic PRL-RNA sequences quantitated with a $cDNA_{PRL}$ probe were also much lower in 928-9b as compared to the $PRL^+$ parent. PRL-RNA sequences could not be detected in the $PRL^-$ strain. Thyrotropin-releasing hormone (TRH) stimulates PRL synthesis about threefold and inhibits growth hormone (GH) synthesis 72% in the $PRL^+$ strain. TRH has no effect on the synthesis of either PRL or GH in the 928-9b strain, although TRH receptors could be detected in these cells. Stimulation of PRL synthesis in the $PRL^+$ strain by TRH could be correlated with increases in levels of cytoplasmic translatable $mRNA_{PRL}$ and increases in cytoplasmic PRL-RNA sequences. These results demonstrate that the graded expression of the PRL gene at the basal level, and in response to TRH, is caused by the regulated production of specific mRNA, i.e., $mRNA_{PRL}$ in these three GH cell strains.

Different clonal strains of rat pituitary tumor cells (GH cells) in culture produce different amounts of two protein hormones, prolactin (PRL) and growth hormone (GH). Certain properties of the GH strains used in this investigation are described in Table I. The GH strains differ from each other not only in their basal level production of PRL but also in their response to the physiological modulator, thyrotropin-releasing hormone (TRH) and to the drug, 5-bromodeoxyuridine (BrdUrd) (1, 2). The $GH_4C_1$ and $GH_3$ subclones respond to TRH with a stimulation of PRL synthesis and an inhibition of GH synthesis (for review, see reference 13.). However, $GH_12C_1$ and the $BrdUrd^r$ (BrdUrd resistant) derivative of $GH_12C_1$, the $F_1BGH_12C_1$ substrain, do not produce any detectable PRL and do not respond to TRH. We have previously reported that PRL synthesis can be induced in these two PRL-nonproducing ($PRL^-$) strains by treatment with the drug BrdUrd (1). These results suggest that, in these cells, PRL synthesis is under the influence of a rigid cellular control mechanism/s which does not permit PRL gene expression, but which is affected by incorporation of the drug into the DNA, subsequently permitting the PRL gene to be expressed.

The varied basal level expression of the PRL gene in these GH strains and the varied responses of the cells to modulators such as TRH and BrdUrd, constitutes a system in which a specific eukaryotic gene can be regulated to different degrees under steady-state conditions and can be further modulated in response to physiological and pharmacological agents (6, 8, 9, 15).

In this investigation, a comparative study of the properties of the $PRL^+$ and $PRL^-$ parent GH cells and of the substrain derived from these two strains, has been made. Several characteristics of these three GH strains such as (*a*) level of PRL and GH production, (*b*) TRH response, and (*c*) presence of TRH receptors and BrdUrd induction were examined (Table I "?"). A probe into the different steps, involved in varied PRL production by the two parent strains and by the progeny at



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basal levels and in response to TRH, has been undertaken to clarify the molecular mechanism/s involved in PRL gene regulation in these cells.

## MATERIALS AND METHODS

### Cells and Growth Conditions

GH Cells are clonal strains of rat pituitary tumor cells (18). Properties of different GH cell strains used in this investigation are described in Table I. Cells were generally grown in monolayer culture with complete F10 medium (Ham's F10 medium supplemented with 15% horse serum and 2.5% fetal calf serum) in a humidified atmosphere of 5% $CO_2$ and 95% air. For preparative purposes, cells were grown in suspension culture using complete Eagle's medium (Eagle's medium with Earle's salts, supplemented with 15% horse serum and 2.5% fetal calf serum). Production of PRL and GH by different cell strains was measured after harvesting cells grown to the same density in fresh medium for identical periods of time.

### Isolation of 928-9b GH Cell Strain

Isolation of 928-9b involved preparation of a nuclear monolayer of one of the parent cell lines by adopting the method described by Mauck and Green (14). These authors demonstrated that when 3T6 cells in monolayer were subjected to mild detergent treatment, the cytoplasm leaked out but the nuclei remained attached to the plate forming a monolayer of nuclei which was referred to by these authors as a "Ghost monolayer." Such nuclei maintain RNA and DNA synthesis at a level comparable to that of intact cells for several hours (3, 14). A nuclear monolayer was prepared from the $PRL^-$, $F_1BGH_12C_1$ cells by treatment of the cells (grown in 60-mm dishes, in F10 medium, approximately $10^6$/plate) with 0.1% Nonidet-P 40 (Shell Oil Co., Houston, Tex.) for 30–45 s at room temperature. The nuclear monolayer was then washed three times very gently with serum minus minimal essential medium. A typical electron micrograph of such a nuclear monolayer is shown in Fig. 1. Approximately $5 \times 10^6$ of the $PRL^+$, $GH_4C_1$ cells (the other parent) in 2–3 ml of F10 medium was then overlayered on the same plate containing this nuclear monolayer. The plates were then incubated under normal cell growth conditions, to permit the $GH_4C_1$ cells to attach. After this period, medium was removed and polyethylene glycol (PEG) mediated fusion under the conditions described by Davidson and Gerald (7) was conducted. The plates were then washed free of PEG and incubated for 48 h in F10 medium. During this period the plates were examined under a phase-contrast microscope and cells with multiple nuclei were observed. Control plates in duplicate, containing either nuclear monolayer alone or $GH_4C_1$ cells alone were subjected to similar treatments. After this period, 3 ml of selection medium (F10 + 30 μg/ml BrdUrd) was added to both control and experimental plates, and plates were incubated for 72 h. No survivors were noticed in the control plates (less than one in $10^6$ cells). However, 10–15 colonies were located in the experimental plates. The 928-9b strain, whose properties have been studied in this investigation, is one of them.

### Karyotype Analysis

Karyotype analysis of the two parent strains and that of 928-9b was carried out by following the method described by Steve Mento (1979, Ph.D. thesis, Rutgers University). Cells in exponential growth were treated with 1 μg/ml of colcemid and incubated for 3 h. The cells were centrifuged (1,000 *g*, 5 min) and 5 ml of hypotonic buffer was added to the pellet which was then incubated at 37°C for 5 min. The cell pellet obtained after centrifugation of this cell suspension was fixed with 1 ml of fixative (one part acetic acid: three parts methanol). Two to three drops of the mixed suspension were then placed onto a clean glass slide and the slide was thrust through a flame to ignite the fixative. This step was repeated two to three times. The slide was washed in absolute alcohol, dried, and subsequently treated in 1 N HCl at 60°C for 10 min. The slide was stained with Giemsa and destained by sequential treatment with acetone, acetone:xylene (1:1), and xylene. Sets of chromosomes of 100 different cells were counted for each cell strain.

### Assays for PRL and GH Production and Synthesis

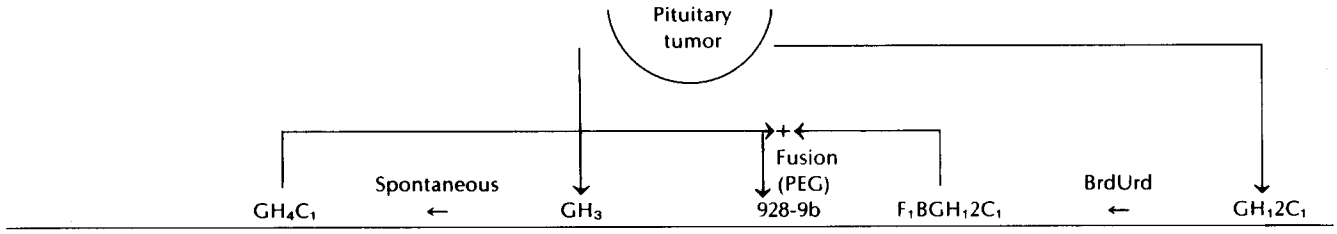
The production of PRL and GH was measured by microcomplement fixation assay of the culture media and sonic extracts of cells (18). PRL synthesis was quantitated by indirect antibody precipitation of PRL in culture media and in cells after pulsing for 6 h with [$^3$H]leucine. Aliquots of cell lysates or media samples were brought to 0.5 ml in 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5, 1% Triton X-100, 0.1% leucine. After centrifugation in an Eppendorf microfuge, prolactin was indirectly immunoprecipitated from the supernatent by the addition of 5 μl of a diluted rabbit anti-PRL antiserum, incubation at 37°C for 1 h and subsequent addition of an appropriate volume of sheep anti-rabbit globulin (Grand Island Biological Co., Grand Island, N. Y.). After overnight incubation at 4°C, immunoprecipitates were collected, washed, and prepared for liquid scintillation counting and $NaDodSO_4$-polyacrylamide gel electrophoresis as described previously (1). Gels were sliced into 2-mm sections, treated with 0.5 ml of 50% Protosol (New England Nuclear, Boston, Mass.) in toluene overnight at 37°C and radioactivity was determined in the samples after the addition of 5 ml of Omnifluor (New England Nuclear). Radioactivity in the PRL and pre-PRL regions of the gel, usually 95% of the antibody-precipitated radioactivity, was used to calculate the quantity of PRL synthesized during the 6-h pulse.

### Cell-free Translation

Isolation of polysomes, RNA, and poly(A) RNA by oligo (dT) cellulose chromatography has been described previously (3, 4). Poly(A) RNA, isolated from polysomes, was translated in a cell-free system prepared from a nuclease-

TABLE I

*Certain Properties of Different GH Cell Strains*




| | $GH_4C_1$ | ← | $GH_3$ | 928-9b | $F_1BGH_12C_1$ | ← | $GH_12C_1$ |
|---|---|---|---|---|---|---|---|
| PRL production | +++ | | ++ | ? | − | | − |
| GH production | + | | + | ? | + | | + |
| TRH receptor | + | | + | ? | − | | − |
| TRH response: | | | | | | | |
| PRL induction | Yes | | Yes | ? | No | | No |
| GH inhibition | Yes | | Yes | ? | No | | No |
| BrdUrd response: | | | | | | | |
| PRL induction | No | | No | ? | Yes | | Yes |
| GH induction | No | | No | ? | No | | No |

The GH strains, $GH_3$ and $GH_12C_1$ were obtained as independent cultured lines from the pituitary tumor (13). The $GH_4C_1$ strain that produces the highest amounts of PRL (+++) is a spontaneous derivative of $GH_3$ (++). The $GH_12C_1$ strain and $F_1BGH_12C_1$, a BrdUrd$^r$ (5-bromodeoxyuridine resistant) derivative of $GH_12C_1$ (1) do not produce any detectable PRL (−). Both $F_1BGH_12C_1$ and $GH_12C_1$ produce significant amounts of PRL after treatment with BrdUrd. The GH strain, 928-9b, was isolated by fusion of a nuclear monolayer derived from the $F_1BGH_12C_1$ strain with $GH_4C_1$ cells by the method described in Materials and Methods. "?" Stands for several questions posed in regard to different properties of the 928-9b strain.

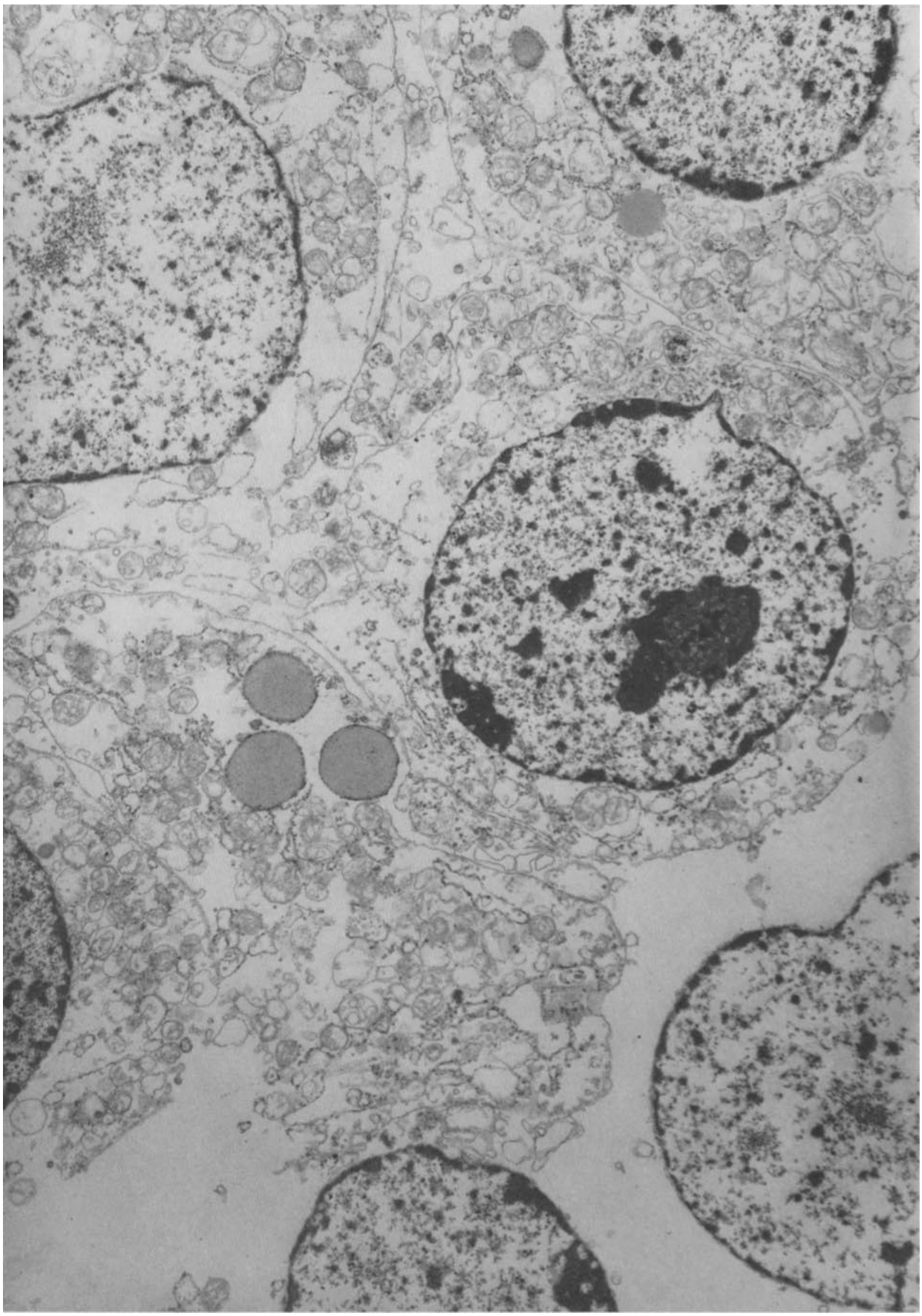


FIGURE 1 Electron micrograph of nuclear monolayer of $F_1BGH_22C_1$ cells. Nuclear monolayers were prepared from the $PRL^-$ $F_1BGH_12C_1$ parent cells after brief detergent treatment as described in Materials and Methods. Electron micrograph prepared by Evelyn Flynn through the courtesy of Dr. George Szabo. × 4,500.

treated reticulocyte lysate (16). In vitro synthesized pre-PRL was measured using mRNA concentrations in the linear response range of the lysate assay. After a 90-min incubation period at 26°C, samples were analyzed for pre-PRL by the indirect immunoprecipitation method described above.

## Preparation of $cDNA_{PRL}$ and Molecular Hybridization Assays

$cDNA_{PRL}$ was prepared from the RNA of a PRL-producing GH cell strain and isolated after hybridization to RNA from $PRL^-$, GH cells, as described previously (4). $cDNA_{PRL}$ preparations were routinely characterized by alkaline sucrose gradient analysis and polyacrylamide gel electrophoresis. Highly enriched (>75%) $mRNA_{PRL}$ was prepared and characterized by following the method described previously (4). These $cDNA_{PRL}$ preparations back hybridized to pure $mRNA_{PRL}$ with an $eR_ot_{1/2}$ of 0.008. When hybridized to total RNA from $PRL^+$ cells to an $eR_ot$ of 50, a single transition was observed. Under the same conditions, there was no significant hybridization of $cDNA_{PRL}$ to RNA from the $PRL^-$ substrain used for $cDNA_{PRL}$ preparation (4).

RNA excess hybridizations were carried out in plastic conical tubes using 1,000–3,000 cpm of [$^3$H]$cDNA_{PRL}$ and 0.1–10 μg of RNA in a total volume of 25 μl hybridization buffer (0.1 M HEPES, pH 7.0, 0.6 M NaCl, 0.005 M EDTA). Samples were overlayered with mineral oil, heated to 100°C for 1 min, and incubated at 68°C for various times. The reactions were terminated by freezing in a dry ice-methanol bath. The degree of hybridization was assessed after $S_1$ nuclease (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) treatment (37°C for 30 min) and subsequent trichloroacetic acid precipitation of $S_1$-resistant hybrids. When hybridization was observed, >95% of the $cDNA_{PRL}$ was involved in hybrid formation.

PRL-RNA sequences were determined from the $eR_ot_{1/2}$ values of the hybridization curves generated by plotting percent hybridization as a function of $eR_ot$. $eR_ot$ = RNA concentration (moles of nucleotide per liter) × time (seconds) corrected to standard reaction conditions.

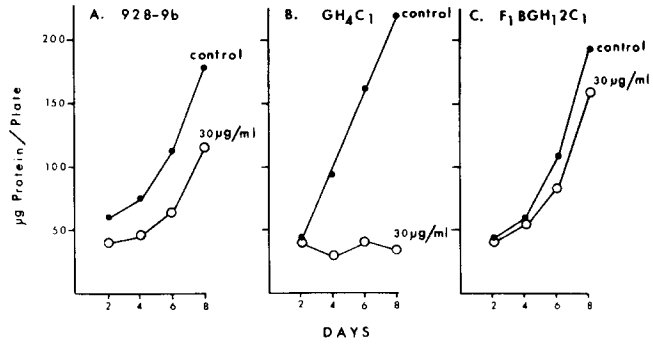



FIGURE 2 Growth characteristics of GH cells. The two parent strains, $GH_4C_1$ and $F_1BGH_12C_1$, and the 928-9b derivative were grown in complete medium (F10, 2 ml) in 35-mm dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) in the absence (●) and in the presence (○) of 30 μg/ml BrdUrd. Plates were withdrawn after every 2 d of growth, and washed free of medium. Cells were scraped off the plate with 2 ml of 0.1 NaOH and protein in an aliquot was determined by the method of Lowry et al. (12). Each point represents an average of protein determinations in duplicate plates.

## RESULTS

The new GH cell strain, 928-9b, was isolated after fusion of a nuclear monolayer of one GH strain to intact cells of another GH strain. The nuclear monolayer, prepared by detergent treatment of $F_1BGH_12C_1$ cells (described in Materials and Methods) retained elements of cytoskeleton structure (Fig. 1). No surviving cells (less than one in $10^6$) could be detected when the nuclear monolayers or $GH_4C_1$ cells alone were subjected to identical fusion and selection in the presence of BrdUrd (30 μg/ml). Several properties of 928-9b described below are distinctly different from either of the parent strains from which it is derived. Nuclear monolayers were preferred for the fusion so that inability to survive could be exploited for selection purposes. After selection, 928-9b cells were compared to the parent GH strains with respect to growth pattern and chromosome number, and then used in studies of regulated PRL gene expression. All parameters of PRL gene expression in these three strains were quantitated under identical growth conditions.

## Growth Pattern and Karyotype Analysis

The growth of $GH_4C_1$, 928-9b, and $F_1BGH_12C_1$ cells in the absence and presence of the drug, BrdUrd (30 μg/ml) has been studied. Results presented in Fig. 2 show that the growth pattern of 928-9b is comparable to that of both parent strains in the absence of the drug. The 928-9b cells, as well as the $BrdUrd^r$ parent, $F_1BGH_22C_1$, can grow in the presence of BrdUrd (30 μg/ml). The growth pattern of the 928-9b strain was studied after culturing cells in the absence of the drug for several generations, and then reexposing cells to the drug. The fact that the growth of these cells was similar whether or not the drug was present demonstrates that the drug resistance property of these cells is a stable phenotype and that the cells are not dependent on BrdUrd for growth.

Karyotype analysis demonstrates that 928-9b cells contain an average of 70 chromosomes/cell, which is significantly higher than that of the $PRL^+$ and $PRL^-$ parent strains (63 and 57, respectively, Table II). The number of chromosomes and the characteristic phenotype described below have been found to be constant over the last 6–12 mo of continuous culture, suggesting that these features of the new cell line, 928-9b, are stable ones.

## PRL and GH Production at the Basal State and in Response to TRH

As previously demonstrated (1) for parent strains, 928-9b cells do not store PRL intracellularly (data not shown). Furthermore, there is very little degradation of PRL in the culture media of GH cells under these growth conditions (5). There-

TABLE II

*Karyotype Analysis of GH Cell Strains*

| Strains | No. chromosomes | | | | | | | | Average No. chromosomes |
|---|---|---|---|---|---|---|---|---|---|
| | 50–54 | 55–59 | 60–64 | 65–69 | 70–74 | 75–79 | 80–84 | 85–89 | |
| | *% cells counted* | | | | | | | | |
| $F_1BGH_12C_1$ (100) | 38 | 32 | 17 | 13 | — | — | — | — | 57 |
| $GH_4C_1$ (100) | 3 | 22 | 36 | 32 | 7 | — | — | — | 63 |
| 928-9b (100) | — | — | 14 | 35 | 26 | 16 | 8 | 1 | 70* |

The karyotypes of the three strains were examined as described in Materials and Methods. The number in parentheses designates the number of sets of chromosomes counted for each cell type.

* The significance of difference between the three average numbers of chromosomes was calculated by $\chi^2$ analysis with two degrees of freedom. The $P$ value has been found to be <0.001.

fore, the measurement of PRL in the medium reflects PRL production, i.e., the net PRL synthesized and secreted by the cells within a specific period of time. PRL synthesis in these cells, on the other hand, is measured as anti-PRL antibody precipitable radioactivity after pulse labeling the cells with [$^3$H]leucine.

The results presentted in Fig. 3 show the production on PRL by $GH_4C_1$, $F_1BGH_12C_1$, and 928-9b cells at the basal state and after treatment with TRH. 928-9b cells synthesize and secrete into the medium an amount of PRL (2–4 μg/mg protein/24 h) which is about 5- to 10-fold lower than the PRL$^+$, $GH_4C_1$ cells (15–25 μg/mg protein/24 h) and substantially higher than the PRL$^-$, $F_1BGH_12C_1$ cells in which PRL could not be detected in the medium either by complement fixation assay (sensitivity level of 0.005 μg/ml) (Fig. 3*A*) or by precipitation with anti-PRL antibody from the total radioactive proteins of cells labeled with [$^3$H]leucine for 6 h (data not shown). These results demonstrate that PRL synthesis in three GH strains follows the order $GH_4C_1$ > 928-9b > $F_1BGH_12C_1$, thus reflecting a gradation in the basal synthesis of a cell-specific protein.

GH is another cell-specific protein that is synthesized by all three GH cell strains. GH production by 928-9b (0.4–0.6 μg/mg protein/24 h) is not substantially different from GH production observed in either of the two parent strains (0.7–0.8 μg/mg protein/24 h) (Fig. 3*B*).

TRH stimulation of PRL synthesis in $GH_4C_1$ cells is evident from the results presented in Fig. 3*A* (●). Maximal stimulation of PRL synthesis in $GH_4C_1$ cells was seen at TRH concentrations ranging between 5 and 10 ng/ml. When examined under identical growth conditions, the PRL$^-$, $F_1BGH_12C_1$ cells (Fig. 3*A*, ▲), do not respond to TRH at concentrations ranging from 0.1–100 ng/ml. Although 928-9b produced substantial amounts of PRL, its synthesis could not be further stimulated over the basal level by treatment with TRH at concentrations ranging from 0.1–100 ng/ml (Fig. 3*A*, ○). Treatment of $F_1BGH_12C_1$ cells and 928-9b cells with 10 ng/ml TRH for as long as 8 d did not stimulate PRL synthesis in either strain. TRH at these concentrations has no effect on the growth rate of any of these cells.

Results presented in Fig. 3*B* show GH production by the three GH strains. Treatment of the $GH_4C_1$ parent with TRH inhibited GH production to the extent of 72% (●). However, no effect of TRH could be observed in either 928-9b (○) or in the $F_1BGH_12C_1$ (▲) parent cells. These results show that, among the three strains, only the PRL$^+$ parent, $GH_4C_1$, responds to TRH with regard to stimulation of PRL synthesis and inhibition of GH synthesis.

## *TRH-specific Receptor*

Results presented in Table III show $^3$H-TRH binding to $GH_4C_1$, $F_1BGH_12C_1$, and the 928-9b cells. It is evident from these results that the PRL$^+$, $GH_4C_1$ strain and 928-9b, specifically bind $^3$H-TRH whereas the PRL$^-$, $F_1BGH_12C_1$ cells do not demonstrate any specific $^3$H-TRH binding. These results indicate that $GH_4C_1$ and 928-9b cells contain specific TRH receptors and, as previously shown (1), the $F_1BGH_12C_1$ cells do not contain specific receptors.

## *Translatable $mRNA_{PRL}$ Levels*

To examine the possibility that amounts of PRL synthesized by the three GH cell strains is a reflection of corresponding amounts of cytoplasmic translatable $mRNA_{PRL}$, the amount of $mRNA_{PRL}$ in the cytoplasm of the three strains has been quantitated. It is evident from the results presented in Table IV that the $GH_4C_1$ (PRL$^+$) strain contains the highest amounts of translatable $mRNA_{PRL}$ when compared to 928-9b and $F_1BGH_12C_1$ cells. Although the amount of translatable

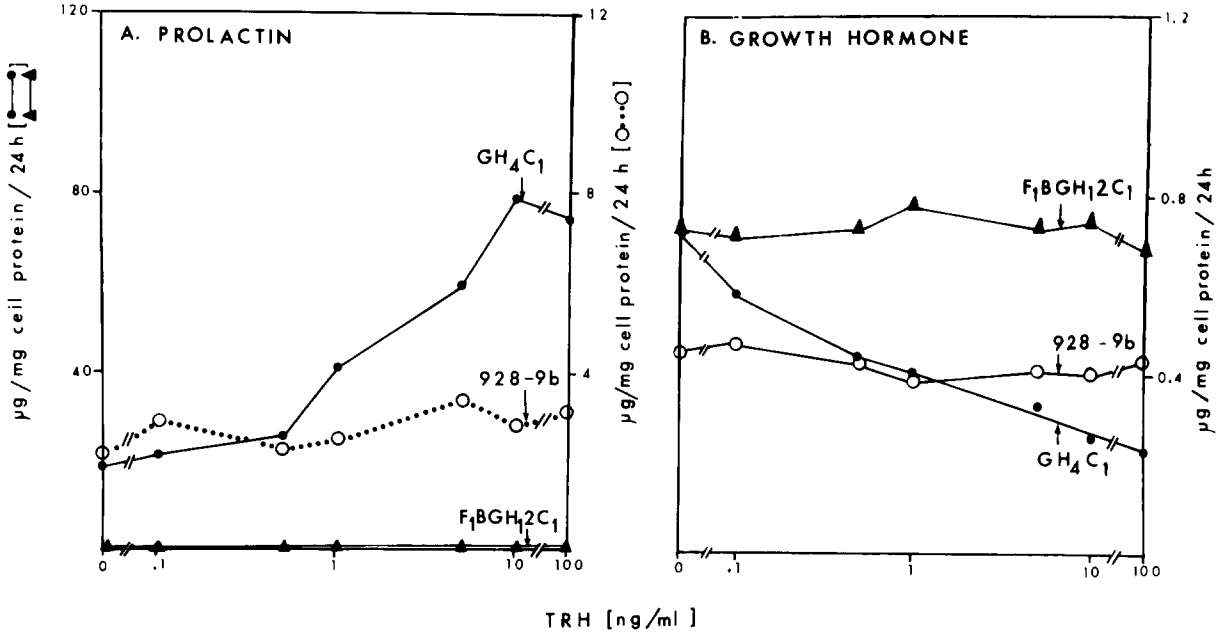



FIGURE 3 Effect of TRH on PRL and GH synthesis in GH cells. GH cells were grown in F10 medium (4 ml) in 60-mm Falcon Labware tissue culture dishes, in the presence of indicated concentrations of TRH. Medium was withdrawn from the plates after 4 d of growth under these conditions, and PRL (*A*) and GH (*B*) in the medium were determined by microcomplement fixation assay (18). The cells in the dishes were washed free of medium and protein in the cell suspension was determined as described in the legend to Fig. 2. Each point represents an average of determinations of hormone concentrations in medium withdrawn from duplicate plates. (*a*) PRL content in $GH_4C_1$ (●), in 928-9b (○), and $F_1BGH_12C_1$ (▲); (*B*) $GH_4C_1$ (●), 928-9b (○), and $F_1BGH_12C_1$ (▲).

$mRNA_{PRL}$ in the cytoplasm of 928-9b cells could not be exactly determined because of inaccuracy in quantitating low levels of anti-PRL antibody precipitable radioactivity, the translatable $mRNA_{PRL}$ assayed on several occasions was always higher than 0.1% and lower than 1% of the total. No anti-PRL antibody precipitable radioactivity was ever detected in the $PRL^-$, $F_1BGH_12C_1$ strain. These findings, which suggest that the 928-9b cells synthesize a constant low level of $mRNA_{PRL}$ and that $PRL^-$ cells do not contain any $mRNA_{PRL}$, were further substantiated by RNA/$cDNA_{PRL}$ molecular hybridization studies described in the following section.

After TRH treatment, the amount of translatable $mRNA_{PRL}$ in the cytoplasm of $PRL^+$, $GH_4C_1$ cells was increased substantially (about threefold). The levels of translatable cytoplasmic $mRNA_{PRL}$ in 928-9b cells after TRH treatment was similar to that observed in control cells. However, the possible stimulation of translatable $mRNA_{PRL}$ levels by TRH in 928-9b cells could not be ruled out from these results because of the insensitivity of this assay.

The quantitation of cytoplasmic translatable $mRNA_{PRL}$ determines the amounts of processed and functional PRL-specific mRNA. However, this does not take into consideration the nonfunctional cytoplasmic $mRNA_{PRL}$ sequences (if there are any). Furthermore, the low level of $mRNA_{PRL}$ in 928-9b could not be accurately determined.

In consideration of these limitations of the translational assay, the amounts of PRL produced by these cells were correlated with their content of PRL-specific RNA sequences by molecular hybridization assays using $cDNA_{PRL}$, prepared as described previously (4). This $cDNA_{PRL}$ preparation has been found to be at least 75–80% enriched for $cDNA_{PRL}$ sequences, shows a single transition with an $eR_ot_{1/2}$ of 0.008 when hybridized to highly enriched $mRNA_{PRL}$ (95% of the translatable $mRNA_{PRL}$ preparation encoded for PRL) and displays no hybrid formation with mRNA preparations from the $PRL^-$ strain, $GH_12C_1$.

The cytoplasmic poly(A)-containing RNA from the $F_1BGH_12C_1$, ($PRL^-$) strain, did not react with $cDNA_{PRL}$ even when hybridized to an $eR_ot$ value of 10 (Table IV). This is consistent with the results reported in Fig. 3 and Table IV, which show that $F_1BGH_12C_1$ cells do not produce detectable amounts of PRL (<0.005 μg/ml) and do not contain any detectable translatable $mRNA_{PRL}$. In contrast, cytoplasmic poly(A) RNA from control and TRH-treated $GH_4C_1$ ($PRL^+$) cells, hybridized rapidly to $cDNA_{PRL}$ with pseudo-first-order kinetics (Fig. 4). TRH treatment resulted in an approximately 2.5 fold increase in the percent of PRL-RNA sequences in these RNA preparations (Table IV). This result parallels the observations in the case of translatable $mRNA_{PRL}$ from control and TRH-treated cells.

Similar to the lower PRL production levels and lower translatable $mRNA_{PRL}$ observed when 928-9b cells were compared to the $PRL^+$, $GH_4C_1$ cells, cytoplasmic poly(A) RNA from 928-9b cells contained about fivefold fewer PRL-RNA sequences (Table IV). In agreement with the observation that TRH does not alter PRL production levels and does not cause

TABLE III

*$^3$H-TRH Binding to Parent and Hybrid Cell*

| Strain | $^3$H-TRH bound | |
|---|---|---|
| | Total | Nonspecific |
| | *cpm/ mg protein* | |
| $GH_4C_1$ | 65,558 | 2,297 |
| $F_1BGH_12C_1$ | 2,523 | 3,171 |
| 928-9b | 72,600 | 2,400 |

$^3$H-TRH binding to cells in monolayer was carried out according to the method described by Hinkle and Tashjian (10). Each cell type was grown in four dishes (35 mm) in F10 medium to the semiconfluent state. The plates were rinsed with fresh pre-equilibrated F10 medium on the day of the experiment. 1 ml of fresh medium containing 25 nM $^3$H-TRH (0.5 μCi) was added to all four plates of each cell type. Nonradioactive TRH was then added to two of the four plates to achieve a final concentration of 25 μM (excess) to determine the nonspecific binding. All the plates were then incubated at 37°C (5% $CO_2$ and 95% air) for 1 h. After this period of incubation, the medium was removed by gentle suction and the cells were washed with chilled (4°C) phosphate buffered saline five times. To the washed plates, 1 ml of 0.1 N NaOH was added. An aliquot of the lysed cells was then taken in 10 ml of Aquasol (New England Nuclear) and counted in a liquid scintillation counter. Protein was determined in another aliquot of the same lysed cell suspension by the method of Lowry et al (12).

TABLE IV

*Translatable $mRNA_{PRL}$ Levels and Cytoplasmic $mRNA_{PRL}$ Sequences in the Parents and in the Hybrid GH Cell Strain*

| Strains | Translatable $mRNA_{PRL}$ | | $mRNA_{PRL}$ sequences | |
|---|---|---|---|---|
| | −TRH | +TRH | −TRH | +TRH |
| | *% total translatable RNA* | | *% total cytoplasmic poly(A) RNA* | |
| $GH_4C_1$ | 2 | 6.4 | 0.58 | 1.85 |
| 928-9b | 0.1-1* | 0.1-1* | 0.19 | 0.15 |
| $F_1BGH_12C_1$ | 0 | 0 | 0 | 0 |

Poly(A)-RNA was isolated from polysomes of the various cell strains by following the procedures described previously (4), and translated in a cell-free system prepared from reticulocyte lysates (16). After incubation at 26°C for 90 min, the amount of pre-PRL synthesized was determined by indirect immunoprecipitation as described in Materials and Methods.

* Amounts of radioactive pre-PRL identified in the cell-free system primed with poly(A)-RNA from 928-9b cells were very low, and the absolute amount could not be accurately determined. In repeated experiments, the amount of pre-PRL detected ranged between 0.1 and 1% of the total proteins synthesized. The PRL sequences of cytoplasmic poly(A)-RNA samples were determined by molecular hybridization to $cDNA_{PRL}$ as described in Materials and Methods. $eR_ot_{1/2}$ values for hybridization were extrapolated from the hybridization curve shown in Fig. 4, and PRL-mRNA sequences were quantitated by using a standard curve generated by hybridization of $cDNA_{PRL}$ to $mRNA_{PRL}$ under conditions identical to those described previously (4).

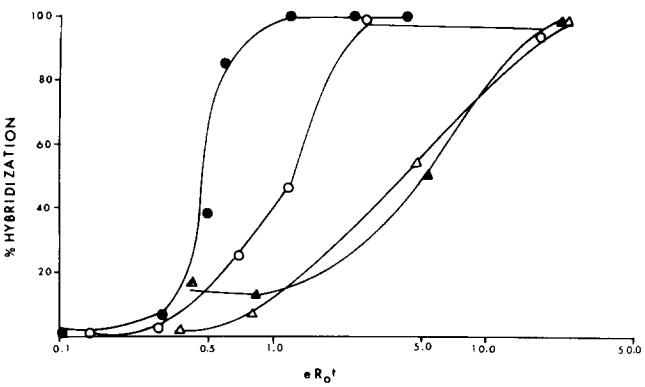



FIGURE 4 Effect of TRH on hybridization of $cDNA_{PRL}$ to cytoplasmic poly(A) RNA in GH cell strains. Molecular hybridization of $cDNA_{PRL}$ to cytoplasmic poly(A) RNA samples was determined as described in Materials and Methods. Percent hybridization was assessed after $S_1$ nuclease treatment and comparison of $S_1$-resistant radioactivity in hybridized samples to radioactivity in $cDNA_{PRL}$ controls that did not receive $S_1$ treatment. $S_1$-resistant radioactivity in $cDNA_{PRL}$ preparations (5% of total) was subtracted from control and sample values before plotting percent hybridization. Each point on the curve represents average of duplicate determinations. Cytoplasmic poly(A) RNA from $GH_4C_1$ (○), $GH_4C_1$ + TRH cells (●), 928-9b cells (▲), and 928-9b + TRH cells (△).

major changes in translatable $mRNA_{PRL}$ levels, TRH treatment of 928-9b cells did not affect the quantity of cytoplasmic poly(A) PRL-RNA sequences (Table IV). The $eR_0t_{1/2}$ values (Fig. 4) from which the percent PRL-RNA sequences were calculated in the case of control and TRH-treated 928-9b cells are not significantly different from each other.

These results demonstrate that the different amounts of PRL synthesized and different levels of translatable $mRNA_{PRL}$ observed in these three GH strains is a reflection of the different levels of PRL-specific mRNA produced by these cells. Similarly, the stimulation of PRL synthesis by TRH in $PRL^+$, $GH_4C_1$ cells, is also caused by increased production of cytoplasmic PRL-specific mRNA sequences.

## DISCUSSION

Different GH strains express the PRL gene to different degrees, thus providing a system to determine the point/s of control of the regulated expression of a specific gene in eukaryotic cells. The primary objective of this investigation was to determine the level of PRL gene expression in a cell strain derived from fusion of genetic elements from a strain that produces large amounts of PRL and a strain in which PRL production is undetectable. Results presented in this investigation demonstrate that 928-9b, the strain isolated by fusion of $PRL^+$, $GH_4C_1$ cells with a nuclear monolayer of $PRL^-$, $F_1BGH_12C_1$ cells, shares properties with both parents. Like the $GH_4C_1$ parent ($PRL^+$), 928-9b cells (*a*) produce a constant amount of PRL (*b*) do not retain PRL inside the cell, but rather secrete almost all of the newly synthesized PRL into the media, (*c*) contain TRH-specific receptors, and (*d*) cannot be stimulated to produce increased PRL by the drug, BrdUrd. Resembling the $PRL^-$ parent strain, 928-9b cells are (*a*) resistant to the drug, BrdUrd, and (*b*) do not respond to TRH.

In contrast to these similarities, 928-9b cells also display several phenotypes that are distinctly different from either of the parent strains: (*a*) the basal level production of PRL is about 5- to 10-fold lower than the $PRL^+$ parent, but significantly higher than the $PRL^-$ strain, (*b*) the number of chromosomes in these cells (Table II) is significantly higher than that of either parent, (*c*) unlike the $PRL^+$ strain, PRL synthesis cannot be further modulated by TRH, and unlike the $PRL^-$ parent strain, PRL synthesis cannot be stimulated by BrdUrd. These observations suggest that the 928-9b cells inherited genetic elements from both parents and display characteristics which appear to represent a composite of traits from both parents.

This investigation has utilized the $PRL^+$ and the $PRL^-$ parent strains and the newly isolated GH strain, 928-9b, to locate the point/s of control of PRL gene expression at the basal level and after treatment with TRH. These studies revealed that graded PRL production by these cells ($GH_4C_1$ > 928-9b > $F_1BGH_12C_1$) could be correlated with cytoplasmic translatable $mRNA_{PRL}$ and PRL-RNA sequences in cytoplasmic poly(A) RNA.

A similar finding was observed with regard to PRL gene expression in the parent and in 928-9b strains in response to TRH. Increased PRL synthesis in the $PRL^+$, $GH_4C_1$ cells, could be closely correlated with increased $mRNA_{PRL}$ as well as increased PRL-RNA sequences in the cytoplasm. In the $PRL^-$, $F_1BGH_12C_1$ strain, which does not respond to TRH, $mRNA_{PRL}$ and PRL-RNA sequences in cytoplasmic RNA fractions remained undetectable before and after TRH treatment. 928-9b cells, which produce lower levels of PRL than the $PRL^+$ parent strain, do not respond to TRH. In the 928-9b strain, TRH did not alter cytoplasmic poly(A) PRL-RNA sequences from control values.

Taken together, the above observations suggest that control of graded PRL-gene expression in the two parents and in the hybrid GH strain at the basal state and after treatment with TRH is exerted at a point before the appearance of PRL-mRNA sequences in the cytoplasm, most probably at the level of transcription. However, possible regulation at the level of post-transcriptional steps is not completely eliminated. Such transcriptional modulation of cell-specific protein synthesis in response to the hormone estrogen has been demonstrated by other investigators (19, 20).

The peptide hormone, TRH, exerts its effect on cell-specific protein synthesis via an initial interaction with a specific class of plasma membrane receptors. After the initial hormone receptor complex formation, the resulting signal is transmitted by some as yet unknown mechanism/s. The action of the hormone eventually results in altered PRL and GH synthesis. The 928-9b strain, isolated in this investigation, possesses specific TRH receptors, but does not respond to TRH with regard to this hormone's biological effect on PRL and GH synthesis (Fig. 3). The TRH receptor itself may be responsible for this effect, for example by exhibiting altered affinity for TRH. Such anomalies in receptor properties have been described for insulin receptors in various diseases (For review, see reference 11.). However, in this strain, initial hormone receptor complex formation appears to be uncoupled from the sequence of events leading to the effect of TRH on PRL and GH gene expression. This suggests a defect in the step/s subsequent to hormone binding so that even though hormone receptor interaction takes place as in the $GH_4C_1$ parent strain, no transmission of the signal follows. Such defects have been observed in corticotropin stimulation of adrenal steroidogenesis (17). The new GH cell strain, 928-9b, described in this investigation may be an ideal cell system for examining the role of peptide hormone receptors and processes subsequent to hormone-receptor interaction on the modulation of the expression of a specific gene.

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