Increased level of prolactin gene sequences in bromodeoxyuridine treated GH cells

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ABSTRACT

The 5-bromodeoxyuridine-resistant (BrdUrd^r) derivative (F₁BGH₁2C₁) of prolactin nonproducing (PRL⁻) rat pituitary tumor cell-subclone GH₁2C₁, synthesize prolactin (PRL) in the presence of the drug. Analysis of nuclear RNA isolated from BrdUrd treated F₁BGH₁2C₁ cells demonstrated several high molecular weight RNA_{PRL} sequences, similar to those observed in the nuclear RNA fraction of PRL producing (PRL⁺) GH₁2C₁ cells. No such RNA_{PRL} sequences could be detected in nuclear RNA fraction of untreated F₁BGH₁2C₁ cells. PRL sequences in the genome of GH₁2C₁ (PRL⁺), GH₁2C₁ (PRL⁻) and F₁BGH₁2C₁ (PRL⁻, BrdUrd^r) GH cells could be identified by blot analysis in 4.8-5.2kb fragment of restriction endonuclease, Hind III digested DNA. Both PRL⁺ and PRL⁻ cells seem to have approximately the same level of PRL gene sequences in total cell DNA. However Hind III digested DNA of BrdUrd treated F₁BGH₁2C₁ cells revealed the presence of significantly higher levels of PRL gene sequences, in comparison, to that observed in total DNA of untreated cells. The increased level of PRL gene sequences was dependent on the period of drug treatment and a parallel increase in the cytoplasmic RNA_{PRL} sequences was also observed.

Several GH cell strains (rat pituitary tumor cells in culture) synthesize different amounts of prolactin (PRL) and growth hormone (GH) (1). Such hormone productions by GH cells are differentiated functions. Though these cells are transformed ones, they retain several properties of hormone synthesis and secretion which are characteristic of a normal pituitary gland. GH cells also respond to physiological modulators such as thyroliberin (TRH) and hydrocortisone (HC) in a manner similar to normal pituitary gland (2). The process of cellular differentiation and development in higher organisms is usually associated with selective gene activation and/or gene suppression. Overproduction of a specific gene product is achieved either via regulation of transcriptional and/or translational rates (e.g. production of ovalbumin in hen oviduct in response to estrogen (3,4), globin synthesis during erythropoiesis (5,6), fibroin synthesis in silk gland (7,8) or by increasing the number of gene copies, i.e. by gene amplification (e.g. rRNA gene amplifica-

tion during oogenesis in xenopus (9), and during macronuclear formation in Tetrahymena (10,11); amplification of chorion protein genes during oogenesis in *D. Melanogaster*, (12), amplification of enzymes of UMP synthesis, in N-pharmacetyl-L-aspartate resistant hamster cells (13) and overproduction of dihydrofolate reductase by gene amplification in methotrexate resistant mouse or hamster cells (14)).

The thymidine analogue, 5-bromodeoxyuridine (BrdUrd) has been found to influence several differentiated functions in various eukaryotic cells (15). The exact mechanism of such actions of BrdUrd on cellular differentiated function is not completely understood. Strom *et al* (16) proposed that BrdUrd irreversibly blocked chondrogenic differentiation by inhibiting the process of amplification of specific sequences necessary for the expression of cartilage phenotype. The PRL⁺ (prolactin producing) GH₃ cells synthesize and secrete in the medium large amounts of PRL. Whereas the PRL⁻ (prolactin non-producing) GH₁2C₁ and its BrdUrd^r derivative, F₁BGH₁2C₁ synthesize PRL only in the presence of the drug (17). We have reported previously that BrdUrd incorporates into cell DNA replacing thymidine, and that induction of PRL synthesis by the drug seems to be dependent on DNA synthesis and such base substitutions (18). Induction of PRL synthesis in these cells could be correlated to the increased level of nuclear and cytoplasmic RNA_{PRL} sequences (19). Results presented in this report demonstrate that BrdUrd-induction of PRL synthesis in F₁BGH₁2C₁ cells was accompanied by increased levels of PRL gene sequences in these cells.

MATERIALS AND METHODS

GH cells are clonal strains of rat pituitary tumor cells in culture (2). Isolation, growth conditions and properties of the three GH subclones used in this investigation have been described previously (1).

Analysis of Nuclear RNA for the Detection of RNA_{PRL} Sequences: Nuclei, and cytoplasm, and RNA from these fractions were isolated according to the methods described previously (20). Nuclear RNA was denatured and analyzed by agarose (1.1%) slab gel electrophoresis according to the method of Lehrach *et al* (21). The RNA fragments were then transferred and covalently coupled to freshly prepared activated diazobenzyloxymethyl (DBM) paper according to the method of Wahl *et al* (22). Nuclear RNA_{PRL} sequences were then localized by hybridization with [³²P]-labelled pDNA_{PRL} probe, prepared by nick translation (23) of cloned cDNA_{PRL}, and by subsequent autoradiography. The *E. coli* K 12 strain HB101 carrying the plasmid pBR322-PRL-1 was kindly supplied to us by Dr. Richard

Maurer of University of Iowa. The growth of bacteria and isolation of plasmid DNA was carried out in our laboratory with facilities approved by Harvard University Biohazards Safety Committee for Pl/EK1 containment recombinant DNA experiments.

Analysis of Cellular DNA for the Detection of PRL and GH Sequences by Blot

Technique: High molecular weight DNA from different GH cell strains were isolated according to the method of Gross-Bellard *et al* (24). DNA concentrations were determined by measurement of absorbance at 260nm (A_{260} of 1 = 50 μ g/ml) or by Spectrofluorometric method of Hinegardner (25). Restriction endonuclease (Hind III, Bethesda Research Laboratory, Bethesda, MD) digestion of the genomic DNA was carried out under the conditions specified by the manufacturer of the enzyme. Completeness of Hind III digestion was tested by incubating a small aliquot of the genomic DNA (1 μ g) in a reaction mixture containing 1 μ g of Lambda DNA, followed by quick analysis by agarose gel electrophoresis and visualization under UV light of the ethidium bromide stained DNA fragments. The Hind III digested DNA was then resolved by electrophoresis on a 1 percent agarose slab gel and was blot-transferred (26) to activated DBM paper. Subsequent baking, prehybridization and hybridization of DNA fragments to [32 P]-labelled PRL or GH probes were carried out according to the method described by Wahl *et al* (22). [32 P]-labelled GH probe was prepared by nick translation of plasmid DNA isolated from the recombinant *E. coli* strain carrying the plasmid pBR322-GH₁ (27). This recombinant clone was provided to us by Dr. F.C. Bancroft of Memorial Sloan-Kettering Cancer Research Center, N.Y. After the specified period of hybridization (for details see Legends to Figures) the paper was washed and autoradiographed by exposing to Kodak XAR5 film at -80° with an intensifying screen (Cronex, Lightning Plus, Dupont).

Detection of Cytoplasmic RNA_{PRL} Sequences by DBM-Dot Hybridization: Cytoplasmic RNA from BrdUrd treated and untreated F₁BGH₁2C₁ cells were isolated according to the methods described previously (20). Small circles (7mm in diameter) were punched out of a freshly prepared activated DBM-paper and washed twice with ice cold water followed by two washes with ice cold buffer (25mM sodium phosphate, pH6.0). The washed dots were air dried and appropriate amounts of cytoplasmic RNA in 5-50 μ l was then applied to the DBM dots (5 μ l at a time). The dots were then kept at 4° for 2 hrs. for the completion of RNA coupling with the diazotized paper, followed by baking for 2 hrs. at 70°. Each dot was then soaked in 0.2ml of prehybridization solution (28) in a 5ml snap cap polystyrene tube and incubated for 3 hrs. at 50°. The pre-

hybridization solution was then removed and the dots were resuspended in hybridization solution (prehybridization solution plus the [32 P]-labelled pDNA_{PRL}) and incubated for the specified period of time at 50°. After hybridization the dots were rinsed with 0.5ml of rinsing solution (28) until the radioactivity in the rinse solution was less than 50cpm. The dots were then autoradiographed. Subsequent to autoradiography the dots were counted in 5ml of Omniflor (New England Nuclear, Boston, Mass.) in a Packard Liquid Scintillation Counter.

RESULTS

Nuclear RNA_{PRL} Sequences in BrdUrd Treated F₁BGH₁2C₁ Cells: Previous results reported from this laboratory demonstrated that RNA_{PRL} sequences could not be detected either in nuclear or cytoplasmic RNA isolated from PRL⁻, F₁BGH₁2C₁ cells (19). However, quantitation by RNA excess solution hybridization to cDNA_{PRL}, RNA_{PRL} sequences could be detected in BrdUrd treated cells. Several species of RNA_{PRL} sequences of different molecular weight was detected in the nuclear RNA fraction, isolated from PRL⁺, GH₃ cells (19). To determine whether BrdUrd treatment of PRL⁻, F₁BGH₁2C₁ cells led to the synthesis of detectable levels of such high molecular weight nuclear RNA_{PRL} sequences (presumably the precursors of mature cytoplasmic mRNA_{PRL}) total nuclear RNA was isolated from BrdUrd treated cells and was characterized by blot analysis. Several species of RNA_{PRL} sequences of sizes ranging from 25-30, 18-20 and 11-13S could be detected in the total nuclear RNA fraction of BrdUrd treated cells (Fig. 1, Lane A). No nuclear RNA_{PRL} sequences of any size could be detected in untreated F₁BGH₁2C₁ cells (Fig. 1, Lane B). Solution hybridization analysis (19) of nuclear RNA isolated from BrdUrd treated cells showed presence of RNA_{PRL} sequences (19). In addition, blot analysis results presented in this report (Fig. 1, Lane A) revealed that, species of nuclear RNA_{PRL} sequences similar to those observed in control and TRH treated, PRL⁺, GH₃ cells (19) and to those observed by Maurer *et al* (29) in the nuclei of normal pituitary gland, could be detected in the nuclear RNA of BrdUrd treated cells. Thus, previously observed (17) induction of PRL synthesis in PRL⁻, F₁BGH₁2C₁ cells, by BrdUrd could be correlated to the appearance of nuclear precursor RNA_{PRL} sequences.

Levels of PRL Gene Sequences in Different GH Cell Strains: PRL gene sequences in the total cellular DNA of PRL⁺ (GH₃), PRL⁻ (GH₁2C₁) and in DNA of control and BrdUrd treated F₁BGH₁2C₁ cells was identified by blot technique described by Southern (26). Identification of specific DNA sequences

Figure 1. Analysis of Total Nuclear RNA from BrdUrd-Treated $F_1BGH_{12}C_1$ Cells: Nuclei and nuclear RNA from BrdUrd-treated (300µg/ml, 7d) $F_1BGH_{12}C_1$ cells were isolated as described previously (20). The RNA was denatured, electrophoresed (21), transferred to activated paper (21) and hybridized (72 hrs.) with [^{32}P]pDNA_{PRL} (2×10^6 cpm of 6×10^7 cpm/µg DNA in a 5ml hybridization mixture) and autoradiographed (2 wks.) as described in "Materials and Methods" section. Lane A shows nuclear RNA (150µg) from BrdUrd treated cells. Same amounts of nuclear (150µg) RNA isolated from untreated cells when analyzed did not show presence of RNA_{PRL} sequence of any size (Lane B). Numbers with arrows show the position of the marker RNA molecules.

in the total genomic DNA by blot technique generates signals which are proportional to the concentration of a specific sequence in the restriction endonuclease-digested total DNA (30,31). Thus, comparative analysis of the intensities of the autoradiographic bands generated under identical conditions by hybridization with the same [^{32}P]-labelled probe gives a relative estimate of the level of specific sequences in certain amounts of total DNA.

To have an estimate of the relative levels of PRL gene sequences in different GH subclones such a comparative analysis of the total DNA, isolated from these cells was conducted. Results presented in Fig. 2 demonstrate that PRL sequences could be detected in the 4.8-5.2kb fragment of restriction endonuclease, Hind III-digested total DNA of GH cell strains. This radioactive fragment of Hind III treated DNA was subsequently referred to as 5kb fragment for convenience of citation. Though the intensities of the 5kb band in Fig. 2, Lane B, (untreated $F_1BGH_{12}C_1$) and in Lane E ($GH_{12}C_1$) seem to be somewhat lower than that is observed in Lane D, (GH_3) in this experiment, stronger signals comparable to that observed with GH_3 cell DNA (Lane D) was observed in both of these cell lines (untreated $F_1BGH_{12}C_1$ and $GH_{12}C_1$) in other experiments. These

Figure 2. Blot Analysis of PRL Gene Sequences in Total DNA of PRL⁺ and PRL⁻GH Substrains: DNA from different GH cell strains were isolated according to the method of Gross-Bellard *et al* (23). Hind III digested DNA was electrophoresed in 1 percent agarose slab gel and blot-transferred to activated DBM paper, and probed with [³²P]pDNA_{PRL} (2x10⁶ cpm/5ml of hybridization mixture, specific activity of [³²P]pDNA_{PRL} 7x10⁷/μg DNA) as described in "Materials and Methods". Hybridization was carried out for 72 hrs. and autoradiography was carried out for 7d. 40μg of Hind III digested DNA from untreated F₁BGH₁2C₁ (Lane B), from treated (300μg/ml, 7d) F₁BGH₁2C₁ (Lane C), from GH₃ (Lane D) and GH₁2C₁ (Lane E) cells were loaded on the gel. Lane A shows the resolution pattern of Hind III digested Lambda DNA after staining the ethidium bromide. The numbers indicated by the arrow designate the sizes of the resolved DNA fragments.

results (Fig. 2 and results from other experiments not shown here) suggest that PRL⁻, F₁BGH₁2C₁, and GH₁2C₁ and PRL⁺, subclones have approximately the same level of PRL gene sequences (within an experimental variation of 2-3 fold) in equal amounts (40μg each) of total cellular DNA (Lanes B, D, & E). Similar conclusions can be drawn by densitometric measurement of the intensities of these radioactive bands (data not shown). However, the intensity of the autoradiographic (5kb) band in DNA isolated from BrdUrd treated cells was significantly higher (Lane C). These results demonstrate that the concentration of PRL-gene sequences are higher in drug treated cells suggesting that BrdUrd induced amplification of the PRL gene in these cells.

Results presented in Fig. 3 show the level of PRL gene sequences in different amounts of Hind III digested genomic DNA, isolated from BrdUrd

Figure 3. Levels of PRL Gene Sequences in Different Amounts of Total DNA of BrdUrd Treated F_1 BGH $_1$ 2C $_1$ Cells: F_1 BGH $_1$ 2C $_1$ cells were treated with 300µg/ml, BrdUrd for 7d. Different amounts (µg, indicated by the numerals on top of each Lanes, B) of Hind III digested DNA was then electrophoresed, and PRL-DNA sequences were identified as described in the Legend of Figure 2. 1×10^6 cpm of [32 P]pDNA $_{PRL}$ of specific activity $5 \times 10^7/\mu$ g DNA was used in 5ml hybridization mixture. Hybridization was carried out for 72 hrs. and autoradiography was for 4d. 40µg of Hind III digested DNA from untreated F_1 BGH $_1$ 2C $_1$ cells was loaded on Lane A.

treated F_1 BGH $_1$ 2C $_1$ cells. It is evident from these results that PRL sequences could be detected in the 5kb fragment in as low as 1µg of total cellular DNA (Fig. 3, Lane B, 1). Comparison of the intensity of the autoradiographic band in 5kb DNA fragment demonstrate that level of PRL gene sequences that could be detected in 40µg of untreated F_1 BGH $_1$ 2C $_1$ cells (Fig. 3, Lane A) was approximately the same as that was detected in 1µg of BrdUrd treated

cells (Fig. 3, Lane B, 1). These results suggest that BrdUrd treatment led to approximately 40 fold increase in the level of PRL gene sequence in $F_1BGH_12C_1$ cells over the basal level of untreated cells.

The levels of growth hormone (GH) gene sequences in total DNA isolated from control and BrdUrd treated $F_1BGH_12C_1$ cells was also determined. Treatment of PRL⁻, $F_1BGH_12C_1$ cells with BrdUrd does not effect the GH production in these cells (17). Results presented in Fig. 4 demonstrate that GH sequences could be detected in the 4.3kb fragment of Hind III digested DNA of control (Fig. 4, Lane A) and BrdUrd treated (Fig. 4, Lane B) $F_1BGH_12C_1$ cells. These results demonstrate that BrdUrd treatment of $F_1BGH_12C_1$ cells did not lead to the apparent amplification of GH gene.

Duration of Drug Treatment, Level of PRL Gene Sequences and Appearance of Cytoplasmic RNA_{PRL} Sequences: We have previously demonstrated that induction of PRL synthesis by BrdUrd in $F_1BGH_12C_1$ cells was dependent on the drug con-

Figure 4. Blot Analysis of Growth Hormone (GH) Gene Sequences in BrdUrd Treated and Untreated Cells: DNA samples (35 μ g each) isolated from untreated $F_1BGH_12C_1$ (Lane A) and from BrdUrd treated (300 μ g/ml, 7d) $F_1BGH_12C_1$, (Lane B) were digested with Hind III, electrophoresed and blot-transferred to DBM paper. The GH gene sequences were detected by hybridization (72 hrs.) with [³²P]pDNA_{GH} probe (1.5 $\times$ 10⁶ cpm of 5 $\times$ 10⁷ cpm/ μ g DNA, was used per 5ml hybridization mixture). Number indicated by the arrow designates the size of the resolved DNA fragment as extrapolated from the sizes of the Hind III digested Lambda DNA, resolved under similar conditions.

centration and duration of the drug treatment (18). Induction of PRL synthesis could also be correlated with the increased synthesis of nuclear and cytoplasmic mRNA_{PRL} sequences from undetectable to the detectable amounts (19). To vary the possible relationship between duration of drug treatment and the extent of PRL gene amplification, F₁BGH₁2C₁ cells were treated with BrdUrd for 24, 48, 72, and 120 hrs. Levels of PRL-gene sequences in each of these DNA preparations were determined by Southern blot analysis of the equal amounts (40µg) of Hind III treated DNA (26). Results presented in Fig. 5A demonstrate that the intensity of the autoradiographic band, i.e. the 5kb fragment of Hind III digested DNA, increased in a manner which could be correlated with the duration of drug treatment of the cells. Though the total DNA applied on each lane of the gel was the same (40µg), the observed signal generated by hybridization with [³²P]-labelled probe, under identical conditions, was different. This was least in the DNA preparation obtained from untreated cells (Fig. 5A, Lane 0) and increased in DNA preparations which were obtained from cells with longer periods of drug treatment (24hr.-120hr.). This was presumably due to the increasing concentration of PRL gene sequences in DNA preparations of cells treated with drugs for increasing periods of time. These results demonstrated that the level of PRL gene sequences in genomic DNA increased in parallel with the increasing duration of drug treatment (Fig. 5A).

To have an estimate of the gene product during different periods of drug treatment, cytoplasmic RNA_{PRL} sequences were measured. Total cytoplasmic RNA was isolated from cells treated with drug for the indicated periods. The level of RNA_{PRL} sequences were then determined by dot hybridization with [³²P]pDNA_{PRL} (Fig. 5B). DBM dots containing the same amounts of (100µg) *E. coli* tRNA (Fig. 5B, I control) or yeast RNA (not shown) did not hybridize with [³²P]-pDNA_{PRL} demonstrating that nonspecific binding of [³²P]-labelled probe was insignificant under the described hybridization conditions. This was further substantiated by the results that DBM dots containing 100µg of cytoplasmic RNA from PRL⁻, untreated cells, did not show any significant amounts of [³²P]-pDNA_{PRL} associated with it (Fig. 5B, II, 0 and Table 1). These results demonstrate and confirm our previous observation that PRL⁻, F₁BGH₁2C₁ cells do not contain any detectable (by RNA excess/cDNA_{PRL} solution hybridization) cytoplasmic RNA_{PRL} sequences (18,19). However, when the same amounts of cytoplasmic RNA (100µg) isolated from the BrdUrd treated cells (Fig. 5B, 24 hr.-120hr.) was analyzed by DBM dot hybridization, the [³²P]pDNA_{PRL} hybridizable sequences could be detected and these amounts of RNA_{PRL} sequences in cytoplasm increased in parallel with the duration of drug treatment (Fig. 5B).

Figure 5. Duration of Drug Treatment and Levels of PRL Gene Sequences and RNA. Sequences in BrdUrd Treated F₁BGH₁2C₁ Cells: F₁BGH₁2C₁ cells were grown in the presence of 300µg/ml of BrdUrd for the indicated (numerals on the top of each Lane) period (hr.). **Panel A.** DNA from nuclei of untreated and drug treated cells were isolated and 40µg of Hind III digested DNA was electrophoresed. PRL gene sequences in each of the digested DNA preparations were then detected by Southern blot-analysis as described in the Legend of Figure 2. The 5kb fragment in the resolved Hind III digested DNA preparation was identified by comparison of the mobility of this band in relation to that of the Hind III digested Lambda DNA resolved by gel electrophoresis under similar conditions. The density of the autoradiographed 5kb DNA fragment containing PRL gene sequences is shown in Fig. 5A. In Lane 0, DNA from untreated F₁BGH₁2C₁ cells and in other Lanes, DNA from treated cells for the indicated period (24, 48, 72, and 120 hrs.) were loaded for analysis. **Panel B.** Cytoplasmic RNA from treated and untreated F₁BGH₁2C₁ cells were isolated. RNA_{PRL} sequences were then detected by DBM-dot technique. 100µg of cytoplasmic RNA from F₁BGH₁2C₁ cells after treatment for indicated periods (0, 24, 72, and 120 hrs. in Fig. 5 B I and II) were loaded on DBM dot and RNA_{PRL} sequences were detected by hybridization with [³²P]pDNA_{PRL}. In "control" DBM-dot, 100µg of *E. coli* tRNA was applied subsequently subjected to identical hybridization conditions. The dots were autoradiographed for 48 hrs. RNA (100µg) isolated from untreated cells (Dot 0, Fig. 5 B II) when subjected to similar analysis gave results similar to that observed with the control dot.

Table 1. [32 P] DNA_{PRL} Hybridizable RNA_{PRL} Sequences in
Cytoplasmic RNA of BrdUrd Treated and Untreated Cells

DBM Dot	RNA	Treatment Period (hr.)	CPM
1	E.coli tRNA	--	20
2	F ₁ BGH ₁ 2C ₁	0	33
3	F ₁ BGH ₁ 2C ₁	24	72
4	F ₁ BGH ₁ 2C ₁	48	132
5	F ₁ BGH ₁ 2C ₁	72	210
6	F ₁ BGH ₁ 2C ₁	120	430

F₁BGH₁2C₁ cells were grown in suspension culture in absence or presence of 300µg/ml BrdUrd for the indicated period of time. Cells were harvested after the drug treatment period, washed and cytoplasm and nuclei were prepared as described before (20). Nuclei were used for DNA isolation and RNA from cytoplasm was prepared as described in "Materials and Methods." Details of the preparation of DBM dots, coupling of RNA (100µg of each), and hybridization with [32 P] labelled probe are described in "Materials and Methods." After the autoradiographic exposure, the dots were taken in 5ml of Omniflor (New England Nuclear) and counted in a Packard Liquid Scintillation Counter.

These DBM dots, when counted to determine the actual amounts of [32 P]pDNA_{PRL} hybridizable cytoplasmic RNA, provided results which led to the similar conclusion (Table 1). These results demonstrate that the level of cytoplasmic RNA_{PRL} sequences in F₁BGH₁2C₁ after BrdUrd treatment increased concomitantly with the increased levels of PRL gene sequences in the cell DNA.

DISCUSSION

The mechanism of differential levels of PRL gene expression in different subclones of GH cells at the basal state is not completely understood. We have previously reported that the different basal level expression of PRL gene in these cells could be correlated to the corresponding increase, decrease or absence of nuclear and cytoplasmic RNA_{PRL} sequences (19). Although PRL gene is not expressed (or expressed at an undetectable level) in PRL⁻ GH subclones, GH₁2C₁ and F₁BGH₁2C₁, it is clear from the previously reported results (17) that this gene can be expressed only in the presence of BrdUrd. This gene exists at a suppressed state in PRL⁻ cells. Results presented in this report demonstrate that the concentration of PRL gene sequences in PRL⁺ (PRL gene expressing cells) and PRL⁻ (PRL gene nonexpressing cells) are approximately the same, even though

the gene is expressed at a very high level in the former and not expressed in the latter.

No PRL gene products could be detected either in the nuclei or in the cytoplasm of F_1 BGH $_1$ 2C $_1$ cells. This was evident from the previously reported results (19) where sensitive assay such as RNA excess/cDNA_{PRL} solution hybridization was utilized to quantitate the RNA_{PRL} sequences in nuclear and cytoplasmic RNA fractions of treated and untreated F_1 BGH $_1$ 2C $_1$ cells. Similar results i.e. absence of RNA_{PRL} sequences in untreated F_1 BGH $_1$ 2C $_1$ cells (data not shown) and appearance of RNA_{PRL} sequences after drug treatment, in nuclei of F_1 BGH $_1$ 2C $_1$ cells were observed when nuclear RNA from treated cells were analyzed by blot technique (Fig. 1). To elucidate the mechanism by which BrdUrd can overcome such a state of suppression of PRL gene in PRL⁻ strain, the levels of PRL gene sequences in F_1 BGH $_1$ 2C $_1$ before and after drug treatment were examined. The results presented in this report demonstrate that the concentration of PRL gene sequences in BrdUrd treated cells increased by 30-40 fold over that could be detected in the DNA of untreated cells. In contrast, the GH gene, whose expression was unaffected by BrdUrd treatment was not amplified following drug treatment of these cells (Fig. 4). These results also demonstrate that PRL gene product i.e. the cytoplasmic RNA_{PRL} sequences increased in parallel with the increased levels of PRL gene sequences which was dependent on the duration of the drug treatment. PRL gene in F_1 BGH $_1$ 2C $_1$ cells which were maintained in the absence of BrdUrd was not amplified and amplification of the gene was observed only in the presence of the drug. These results suggest that amplification state is unstable in these cells. Thus BrdUrd treatment and withdrawal may serve as a very useful handle in turning the switch, "on" or "off" for the process of gene amplification in this system. Follow up studies of this observation may thus provide evidence towards the understanding of the as yet unresolved mechanism of gene amplification in eukaryotic cells.

Overproduction of gene products during cellular differentiation by increasing number of gene copies, such as, rRNA gene amplification during cellular differentiation in xenopus (9) and in Tetrahymena (10, 11) and amplification of chorion protein genes in *D. Malanogaster* (12) during early development, was observed. A more commonly observed means of overproduction of cell specific protein during differentiation is achieved through variation of transcriptional and/or translational rates without causing any significant change in the concentration of the specific gene (3-8). Gene amplification leading to the excessive synthesis of the end product is also observed in

several instances in eukaryotic systems where cells, through this mechanism, resist antiproliferating drugs and continue to survive. Excessive production of dihydrofolate reductase could be detected in methotrexate resistant cells in the presence of the drug in mouse and in hamster cells for example (14).

Though the primary selection pressure on the GH subclone, $F_1BGH_{12C_1}$ was BrdUrd resistance, the ultimate objective of the selection was to obtain a PRL^+ subclone out of the PRL^- GH cells. Thus the second selection pressure during the process of isolation of this BrdUrd^r, GH substrain, was to induce the PRL gene expression and hormone production. It is clear from our previous results that the growth of the resistant cells was not dependent either on the drug or on the hormone (17). The mechanism or mechanisms by which $F_1BGH_{12C_1}$ cells resist antiproliferating action of BrdUrd is not understood yet. The results presented in this report do not differentiate, whether the increased level of PRL gene sequences is a circumstantial event of amplification of some neighbouring gene/s, which are directly involved in the process of conferring the resistant phenotype to these cells, or whether the increased level of PRL gene sequences is an event of gene amplification, a mechanism occasionally utilized for overproduction of a cell-specific protein during cellular differentiation (9-12). However, results presented in this report elucidate the mechanism of induction of PRL synthesis by BrdUrd in GH cells which apparently is achieved by increasing the concentration of PRL gene sequences, i.e. by gene amplification.

FOOTNOTES

Abbreviations: GH cells, rat pituitary tumor cells in culture; BrdUrd^r, 5 bromodeoxyuridine resistant; PRL^+ , prolactin producing; PRL^- , prolactin nonproducing; TRH, thyroliberin; DBM paper, diazobenzyloxymethyl paper; cDNA_{PRL}, DNA complementary to prolactin mRNA; pDNA_{PRL}, cloned cDNA_{PRL} in plasmid pBR322; kb, kilobases.

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