Volume 225, number 1,2, 238-242 FEB 05382 December 1987

Growth hormone gene expression in myoepithelial cells directed by various eucaryotic transcriptional regulatory sequences

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Received 26 October 1987

Mammary gland myoepithelial cells were isolated from cattle and cell lines were established. Cells were plated onto tissue culture dishes with or without collagen. Cells were transfected with bovine growth hormone rDNA containing one of the following eucaryotic transcriptional regulatory sequences: human cytomegalovirus immediate early promoter, simian virus 40 early promoter, mouse metallothionein I promoter and the Rous sarcoma virus long terminal repeat. These sequences were evaluated for their ability to direct recombinant bovine growth hormone DNA expression in myoepithelial cells. The most effective transcriptional regulatory sequences were the cytomegalovirus immediate early and simian virus 40 early promoters.

Growth hormone; Gene expression; (Myoepithelial cell, Cattle)

1. INTRODUCTION

The use of recombinant DNA technology has resulted in the cloning of a variety of growth hormone genes and cDNAs including those derived from human, rat, bovine, and porcine tissue [1–4]. Growth hormone genes have been expressed in both procaryotic and eucaryotic cells. DNAs complementary to human, bovine and porcine mRNA have been expressed in *Escherichia coli* [1,3]. Rat, human and bovine growth hormones have been expressed in cultured mammalian cells [5–7].

Pasleau et al. [8] demonstrated bovine growth hormone gene expression in rat GH3 cells, directed by the Rous sarcoma virus long terminal repeat and the cytomegalovirus immediate early promoter. No attempts have been made to direct recombinant bovine growth hormone DNA expression in specific mammary gland cell types using these eucaryotic viral promoters.

For the possible use of recombinant-derived peptides, such as growth hormone in animal husbandry and medicine, we evaluated various eucaryotic transcriptional regulatory sequences for their ability to direct recombinant bovine growth hormone DNA expression in cultured bovine mammary gland, myoepithelial cells.

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2. MATERIALS AND METHODS

Myoepithelial cells were originally isolated from mammary tissue of two lactating Holstein cows by collagenase dispersion of tissue and density gradient centrifugation [9]. Cell lines were established from primary cultures. Culture medium (maintenance medium) consisted of Dulbecco's modified Eagles medium containing 5% bovine serum collected from the cows at slaughter, 2.38 mg/ml

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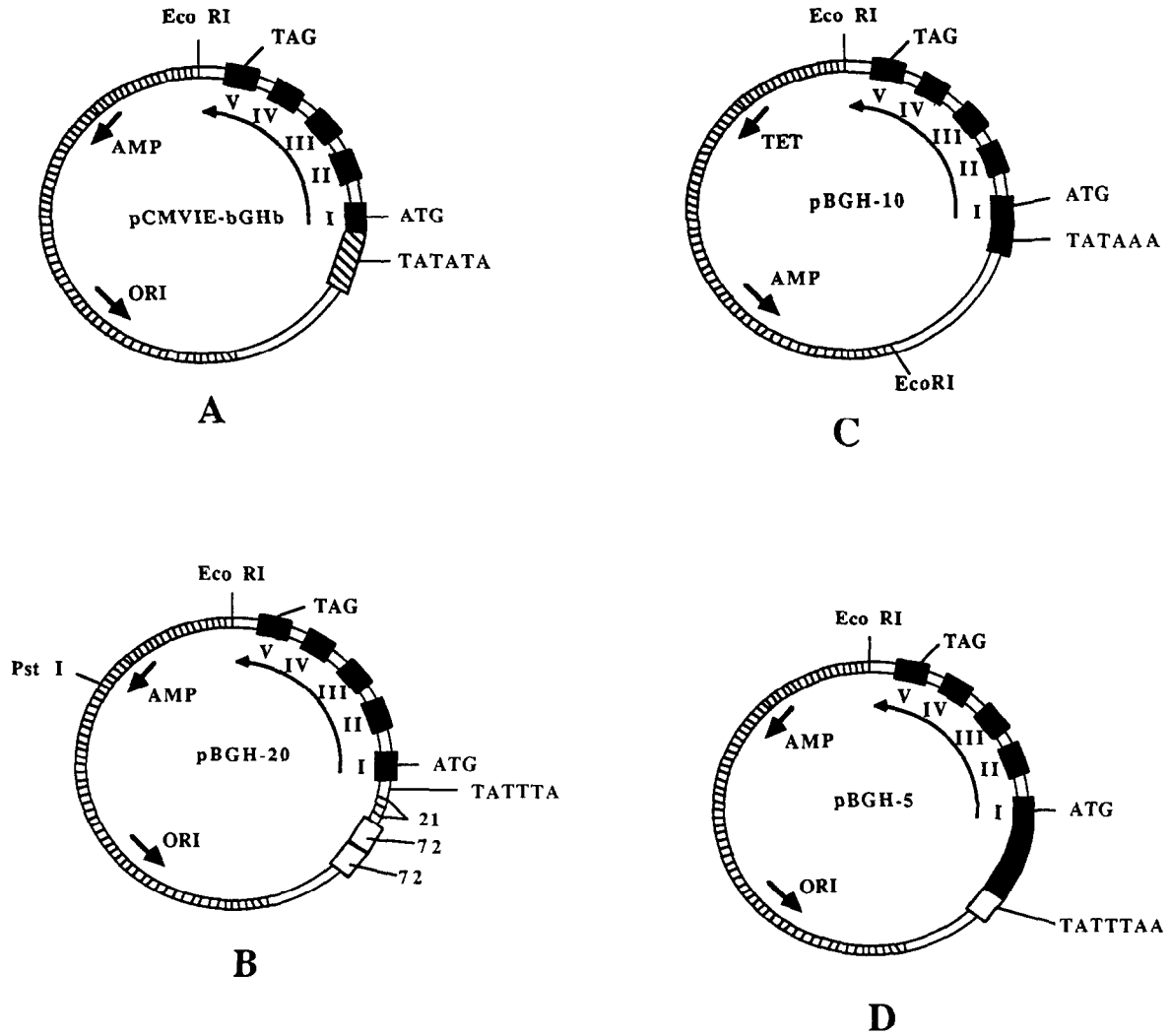

Fig.1. Constructs of plasmid DNAs: (A) pCMVIE-bGHb, (B) pBGH-20, (C) pBGH-10 and (D) pBGH-5. All plasmids contain a *Bam*HI-*Eco*RI fragment from the bGH gene [13,14]. Black boxes represent bGH exons and hatched lines represent pBR322 sequences containing the ampicillin-resistance gene (AMP) and the origin of replication (ORI). The 'TATA box' for each promoter is indicated. Details of pCMVIE-bGHb and pBGH-5 constructions have been published [7,8]. The SV40 early promoter was ligated to the bGH gene as follows. pSVO (from Peter Lomedico, Hoffmann La Roche) was cleaved with *Hin*dIII and *Eco*RI and a 415 base pair fragment containing the SV40 early promoter was isolated. pBGH-2 DNA was cleaved with *Sal*I and *Bam*HI. All restriction endonucleases used in these cleavages generate 5'-protruding termini. The ends were made flush by addition of deoxynucleotides using DNA polymerase I. The resulting blunt end molecules were ligated and transfected into *E. coli* RRI cells. A plasmid containing the SV40 early sequence attached to the bGH gene was isolated (pBGH-20). The mouse metallothionein I promoter was ligated to the bGH gene as follows: pMK DNA (from Palmiter, R.) was cleaved with *Bgl*II and *Eco*RI and a 750 bp fragment containing the promoter isolated. Similarly, pBGH-2 was cleaved with *Eco*RI and *Bam*HI and a 2.2 linear fragment isolated. pBR322 was linearized with *Eco*RI. Following a three-fragment ligation, *E. coli* RRI cells were transfected using standard procedures. A plasmid containing the metallothionein I promoter ligated to the bGH gene was isolated (pBGH-10).

Table 1

Mean concentrations of BGH (ng/ml) ± SE in medium of myoepithelial cell (cell lines)[a]

Promoter	Substrate[b]	Days post-transfection											
		1	2	3	4	7	10	13	16	19	22	25	28
pCMVIE-	P	−1.6 ± 0.5	1.2 ± 0.4	16.8 ± 0.6	22.9 ± 0.9	59.2 ± 0.8	18.6 ± 1	11.5 ± 0.9	10.1 ± 1	12.2 ± 0.7	5.4 ± 1	3.0 ± 0.5	−8.6 ± 0.3
	C	2.7 ± 0.8	35.0 ± 1	82.0 ± 1	93.0 ± 1	115.0 ± 2	42.0 ± 1	31.0 ± 1	20.0 ± 0.8	21.0 ± 2	14.1 ± 0.8	14.3 ± 1	−5.8 ± 1
pBGH-20	P	−0.1 ± 0.1	−0.9 ± 0.4	2.0 ± 0.8	1.8 ± 0.5	26.0 ± 1	15.0 ± 0.9	122.0 ± 5	28.0 ± 1	16.0 ± 0.8	1.3 ± 0.5	15.0 ± 0.3	−5.6 ± 0.1
	C	2.8 ± 0.2	9.8 ± 1	23.9 ± 1	31.0 ± 2	46.0 ± 1	26.0 ± 2	20.0 ± 0.9	17.0 ± 0.8	11.0 ± 1	9.4 ± 0.7	−5.1 ± 4	−5.1 ± 0.8
pBGH-10[c]	P	0.0 ± 0.5	−0.9 ± 0.7	−0.6 ± 0.3	−0.6 ± 0.1	−1.9 ± 0.8	0.0 ± 0.3	0.1 ± 0.1	−0.8 ± 0.4	−0.6 ± 0.2	−0.5 ± 0.1	0.0 ± 1	−2.0 ± 0.4
	C	1.3 ± 0.8	1.0 ± 0.5	−0.8 ± 1	−1.4 ± 0.4	−0.1 ± 0.6	−0.9 ± 0.2	1.0 ± 0.1	0.0 ± 0.1	−0.8 ± 0.4	−0.1 ± 0.2	−0.9 ± 0.3	−1.1 ± 0.1
pBGH-5	P	−0.4 ± 0.1	0.0 ± 0.5	−0.9 ± 0.2	−0.3 ± 1	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.6	−0.1 ± 0.2	−0.1 ± 0.1	−0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.2
		0.9 ± 0.2	0.4 ± 2	0.3 ± 0.1	−0.1 ± 0.1	0.0 ± 0.4	−0.1 ± 0.1	0.0 ± 0.4	−0.1 ± 0.3	0.0 ± 0.2	−0.1 ± 0.3	−0.1 ± 0.3	0.0 ± 0.3

[a] Data corrected for concentrations of bGH in the maintenance medium as described in section 2. Means were calculated from quadruplicates

[b] P, plastic; C, collagen

[c] Data from cell cultures incubated in the presence of 100 μM $ZnCl_2$. No difference was seen between cells cultured in the presence or absence of $ZnCl_2$

Hepes, 0.35 mg/ml L-glutamine, 0.2 mg/ml streptomycin and 0.2 U/ml penicillin.

A transient eucaryotic expression assay system was employed. The technique used was as described in [7,8,10] with minor modifications. Briefly, approx. 5.0×10^5 cells were plated onto 35 mm plastic tissue culture plates in the absence or presence of bovine epidermal collagen (Vitrogen 100, Collagen Corp., Palo Alto, CA) in maintenance medium, pH 7.4). Following an overnight incubation, cells were transfected with bovine growth hormone genomic (bGH) rDNA (1 μg) and DEAE-dextran (20 μg) in phosphate-buffered saline, containing one of the following eucaryotic promoters shown in fig.1: human cytomegalovirus immediate early promoter (pCMVIE-bGHb), simian virus 40 early promoter (pBGH-20), the mouse metallothionein I promoter (pBGH-10) and the Rous sarcoma virus long terminal repeat (pBGH-5). In these plasmid constructions the bovine growth hormone 5′-flanking sequences were removed and replaced by the various eucaryotic promoters.

After incubation with the respective plasmids, cells were washed three times with Dulbecco's medium without serum. Cells treated with pCMVIE-bGHb, pBGH-5 and pBGH-20 were then incubated in maintenance medium. Cells treated with the mouse metallothionein I promoter bGH DNA were divided into 2 treatment groups. One group of cultures was incubated with medium containing 5% bovine serum and 100 μM $ZnCl_2$, the other being incubated with Dulbecco's medium containing 5% bovine serum without $ZnCl_2$. $ZnCl_2$ was used to activate the metallothionein I promoter. Medium was collected every 24 h for 3 days and then every 3rd day for 28 days. Bovine growth hormone in medium was determined by radioimmunoassay [11]. The concentration of bovine growth hormone was determined in fresh maintenance medium. The growth hormone concentrations measured from cells post-transfection were all adjusted. This was done by subtracting the bGH concentration contained in the maintenance medium from them.

3. RESULTS

pCMVIE-bGHb acted as a potent regulatory sequence in directing bGH gene expression in myoepithelial cell lines (table 1). Cells showed maximum gene expression on day 7 post-transfection. At this time, the peak concentration of bGH was 59.2 ng/ml in medium from cells grown on plastic and 115 ng/ml in medium of cells grown on collagen.

Concentrations of bGH in medium from cells cultured on collagen were almost 2-times greater than those found in medium from cells cultured on plastic. Concentrations of bGH remained elevated in cells grown on both substrates up to 25 days post-transfection (table 1).

pBGH-20 was also effective in directing bGH gene expression in myoepithelial cells (table 1). Concentrations of bGH fluctuated somewhat from days 7 to 16 (17–122 ng/ml), but peaks in bGH were reached during this time interval. Concentrations of bGH in medium from cells cultured on plastic were nearly 3-times greater than those found in medium from cells grown on collagen. Growth hormone concentrations remained elevated for up to 25 days post-transfection for cells grown on plastic (table 1).

pBGH-10 and pBGH-5 were ineffective in directing gene expression in mammary myoepithelial cell lines (table 1).

4. DISCUSSION

Pasleau et al. [8] have shown that the cytomegalovirus immediate early gene regulatory region was 3–4-fold more efficient than the Rous sarcoma retroviral long terminal repeat in directing expression of the bovine growth hormone gene in rat GH3 cells. These promoters as well as the simian virus 40 early and mouse metallothionein I were tested for their ability to direct a 'reporter' gene (bGH) in bovine mammary epithelial cells.

The cytomegalovirus immediate early promoter and the simian viral 40 early promoter were effective in directing gene expression in mammary myoepithelial cells, while the Rous sarcoma virus long terminal repeat and mouse metallothionein I promoters were not. The concentrations of bGH in medium were highest for cells transfected with the pCMVIE-bGHb DNA when cultured on collagen. The simian viral promoter appeared more effective in expressing bGH when it was introduced into myoepithelial cells cultured on plastic. We have shown that the fine structure of the bovine mam-

mary myoepithelial cell can change depending on the substrate on which it is cultured [12].

Our data support the fact that eucaryotic transcriptional regulatory sequences, used to express recombinant derived genes in higher eucaryotic cells, are cell specific. Furthermore, the matrix on which the cells are grown and the degree of their subcellular differentiation, at the time of plasmid insertion, may be important factors to consider when introducing recombinant derived genetic material into higher eucaryotic cells.

The techniques described here will be useful in studying the physiological and biochemical mechanisms whereby mammotropic agents such as growth hormone influence the development and function of the mammary gland.

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