0273-2289/96/5603—0211$08.00

Accelerated Article

An Efficient Expression of Human Growth Hormone (hGH) in the Milk of Transgenic Mice Using Rat β-Casein/hGH Fusion Genes

CHUL-SANG LEE,[1] KYUNGJIN KIM,[2]
DAE-YEUL YU,[1] AND KYUNG-KWANG LEE*,[1]

[1]*Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yusong, Taejon 305-600, Korea; and* [2]*Department of Molecular Biology and Research Center for Cell Differentiation, Seoul National University, Seoul, Korea*

Received August 18, 1995; Accepted November 14, 1995

ABSTRACT

In order to produce human growth hormone (hGH) in the milk of transgenic mice, two expression vectors for hGH differing in their 3′ flanking sequences were constructed by placing the genomic sequences of hGH gene under the control of the rat β-casein gene promotor. The 3′ flanking sequences of the expression constructs were derived from either the hGH gene (pBCN1GH) or the rat β-casein gene (pBCN2GH). Transgenic lines bearing pBCN1GH expressed hGH more efficiently than those bearing pBCN2GH in the milk (19–5500 μg/mL vs 0.7–2 μg/mL). In particular, one of the BCN1GH lines expressed hGH as much as 5500 ± 620 μg/mL. Northern blot analysis showed that the transgene expression was specifically confined to the mammary gland and developmentally regulated like the endogenous mouse β-casein gene in the mammary gland. However, a low level of nonmammary expression was also detected with more sensitive assay

*Author to whom all correspondence and reprint requests should be addressed.

methods. In conclusion, the rat β-casein/hGH fusion gene could direct an efficient production of hGH in a highly tissue-and stage-specific manner in the transgenic mice and the 3′ flanking sequences of hGH gene had an important role for the efficient expression.

Index Entries: Milk; hGH; rat β-casein; transgenic mice.

INTRODUCTION

There is considerable interest in the production of recombinant proteins from the milk of transgenic animals, as an alternative to large-scale mammalian cell culture system. A number of milk protein genes have been isolated and shown to function efficiently in the mammary gland of transgenic mice (*1*). So far, several heterologous proteins have been expressed in the milk of many transgenic animals using regulatory sequences of various milk protein genes (*2–6*). In particular, several groups have reported high level expression of hybrid genes in the milk of transgenic mice (*5–10*). Naturally, this approach is now in the process of industrial application for a large scale production of pharmaceutically active proteins from transgenic livestock (*11*).

The rat β-casein gene encodes the principal murine casein and is mainly expressed during mammary gland development. At midlactation, the rat β-casein mRNA constitutes about 20% of poly(A) mRNA (*12*). In spite of its abundance in the milk, rat β-casein was inefficiently expressed in transgenic mice harboring the entire rat β-casein gene along with 3.5 kb of 5′ and 3.0 kb of 3′ flanking sequences. In these mice, the average expression level of the transgene only ranged between 1 and 0.01% of that of the endogenous mouse gene, although its expression was restricted to the mammary gland (*13*). In addition, the rat β-casein-based expression vectors, in which the rat β-casein promoter was linked to chloramphenicol acetyltransferase gene (*14*) or to bovine follicle stimulating hormone cDNA (*15*), showed poor performance in transgenic mice. Thus, it has been suggested that the low expression level of the rat β-casein gene in transgenic mice might be owing to the lack of potent *cis*-acting regulatory sequences in the expression construct (*13*).

The expression efficiency of hybrid genes does not absolutely depend on the milk protein gene promoter. It is well known that genomic sequences generally show a better performance than their cDNAs when used in an expression construct because introns have some regulatory functions on their expression (*16,17*). The selection of a target gene and the architecture of the fusion gene could then be crucial for the efficient expression of the construct. hGH gene has been widely used for a targeted expression in transgenic mice, and several milk protein gene promoters were shown to direct high level expression of hGH to the mammary gland of transgenic mice (*9,10*).

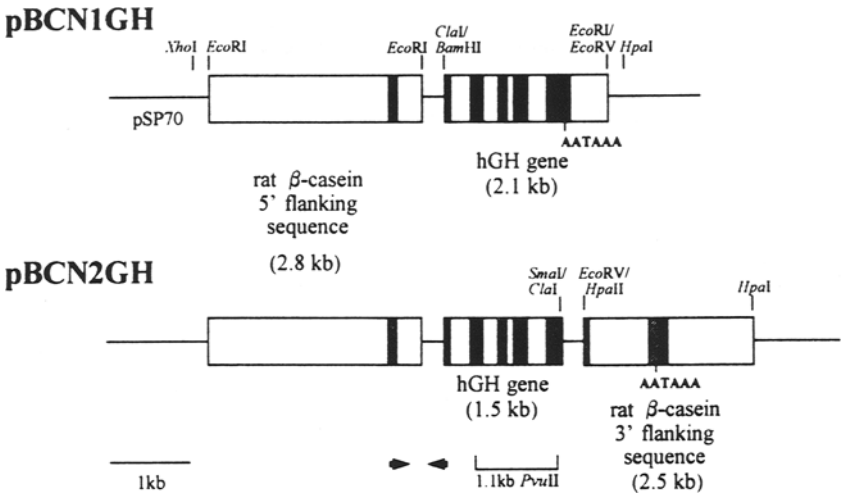

Fig. 1. Schematic representation of the rat β-casein/hGH fusion genes. The flanking sequences and introns are blanked, the five exons of hGH gene are filled in, the exons of rat β-casein gene are shaded, and plasmid regions are shown as horizontal lines. The position of polyadenylation signal (AATAAA) is indicated. The indicated 1.1-kb *Pvu*II fragment was used as a labeled probe for Southern and Northern blot analyses. The arrowheads represent a primer set used for RT-PCR.

In the present study, we examined whether the rat β-casein promoter could direct an efficient production of hGH in the milk of transgenic mice when it was combined with 3′ flanking sequences from different genetic sources.

MATERIALS AND METHODS

Construction of the Rat β-Casein/hGH Fusion Genes

The two rat β-casein-based expression vectors, pBCN1 and pBCN2, were constructed as follows. In the pBCN1 construction, a 2.8-kb *Eco*RI fragment (−2300/+490) of rat β-casein gene consisting of 2.3 kb of 5′ flanking sequences, the first exon and a portion of the first intron (*14*) was subcloned into the *Eco*RI site of pSP70 vector (Promega, Madison, WI). In the pBCN2, a 2.5-kb *Hpa*II/*Hpa*I fragment containing the last intron and exon, and 1.2 kb of 3′ flanking sequences of rat β-casein gene was subcloned into *Eco*RV/*Hpa*I site of the pBCN1. A 2.1-kb *Bam*HI/*Eco*RI fragment of hGH gene including its entire coding and 0.5 kb 3′ flanking sequences was isolated from phGH (*18*) and then inserted into *Cla*I/*Eco*RV site of pBCN1 vector to form pBCN1GH. Another hGH expression vector, pBCN2GH, was constructed by ligating a 1.5-kb *Bam*HI/*Sma*I fragment of hGH gene, which is free of 3′ untranslated/flanking sequences, to *Cla*I site of pBCN2 vector (Fig. 1). The β-casein/hGH hybrid genes were released

from the two hGH expression vectors by *XhoI*/*HpaI* digestion, purified through Elutip (Schleicher & Schuell, Germany) and diluted to a final concentration of 4 ng/μL in 10 m*M* Tris (pH 7.4)/0.1 m*M* EDTA solution for the pronuclear injection.

Generation and Screening of Transgenic Mice

Transgenic mice were generated using a standard procedure (*19*). Purified DNA solution was microinjected into the pronuclei of fertilized mouse eggs obtained from F1 hybrid (C57BL/6 × DBA) females. The injected eggs were transferred to pseudopregnant ICR female mice. Identification of transgenes and determination of their copy numbers in transgenic mice were achieved by Southern blot analysis (*20*) using radiolabeled 1.1 kb *PvuII* fragment of hGH gene as a probe. Identified transgenic mice were mated to the F1 hybrid mice to produce the first generation (G1) of offspring. All subsequent experiments were carried out using G1 transgenic mice.

Analysis of hGH in Milk, Blood, and Tissue Extracts

Milk, blood, and tissue extracts were prepared from 11-d lactating mice. Mothers were separated from their pups and, 3 h later, anesthetized and injected intraperitoneally with 10 IU of oxytocin (Sigma, St. Louis, MO) to induce enhanced milk secretion. Milking was carried out by gentle suction with hand-controlled vacuum pump (Nalge, Rochester, NY). After milking and blood sampling, various organs were removed and homogenized in phosphate buffered saline. By a brief centrifugation, whey, serum, and tissue extracts were obtained and stored at −20°C until analysis. Radioimmunoassay (RIA) was performed to determine hGH concentrations in the samples using a commercially available RIA kit (Incstar, Stillwater, MN) which quantifies hGH ranging from 0.001 to 0.03 μg/mL. Whey samples were diluted at least to 1/100 in the standard buffer of the kit to limit nonspecific binding. Thus the detection limit for the wheys was 0.1 μg/mL. Total protein contents in tissue extracts were determined using protein assay reagents (Bio-Rad, Hercules, CA) according to the manufacturer's instruction. For electrophoretic analysis, the whey samples were diluted to 1/10 in 10 m*M* Tris (pH 8.0)/10 m*M* $CaCl_2$. SDS-PAGE analysis was performed on a 12% acrylamide gel (*21*) and the protein bands were identified by staining with Coomassie blue.

RNA Isolation and Analysis

Total RNA was isolated from various organs of 11-d lactating mice using one step acid/guanidinium isothiocyanate method followed by phenol/chloroform extraction (*22*). Aliquots (20 μg) of total RNAs were fractionated on duplicate sets of 1% agarose/formaldehyde gel. One set of the gels was stained with ethidium bromide to check the integrity and content of RNA. RNAs in the other set were transferred to a nylon mem-

brane for Northern blot analysis. For analyzing the developmental regulation of hGH transgene, mammary gland RNA samples (5 μg) of virgin, pregnant, and lactating mice were hybridized with either hGH or β-casein probe. Reverse transcriptase-mediated polymerase chain reaction (RT-PCR) was carried out as follows. The first cDNA strand was synthesized from 5 μg of total RNA using 50 pmol oligo dT primer and 1 U of AMV reverse transcriptase (Promega) in 20 μL of reaction volume. An aliquot (2 μL) of the reaction mixture was used for the second-strand DNA synthesis in a 100-μL PCR reaction. After denaturation for 3 min at 94°C, 30 cycles of PCR amplification (94°C for 30 s, 55°C for 30 s, and 72°C for 30 s) were performed with a 5′ primer (5′-ATCCTCTGAGCTTCATCTTC-3′) identical to the first exon sequence of rat β-casein gene and a 3′ primer (5′-GCCATTGCAGCTAGGTGAGC-3′) complementary to the first exon sequence of hGH gene. PCR with these primers should yield a 120-bp product from cDNA of the transgene-specific transcript and a 555-bp product from the transgene constructs. Therefore, our RT-PCR strategy allows one to distinguish cDNA-specific amplification (120-bp product) of the transgene from the contaminating genomic DNA amplification (555-bp product).

RESULTS

Generation of Transgenic Mice

We observed approx 15% efficiency in obtaining transgenic mice in terms of the percentage of mice screened (data not shown). Six and three transgenic founder mice were obtained from the microinjection of pBCN1GH and pBCN2GH constructs, respectively. These founder mice were mated to F1 hybrid mice to produce the first generation (G1). Two of BCNIGH mice with low copy numbers of transgene did not transmit their transgene to their progeny. One of BCN1GH female founders was sterile. Finally, three transgenic lines were established from each vector. These transgenic lines harboured 2–30 copies of transgene (Table 1), as judged by Southern blot analysis and stably transmitted their transgene to their progeny in a Mendelian fashion (data not shown).

hGH Expression in the Milk and Blood of Transgenic Mice

Several G1 transgenic females from each line were milked on d 11 of lactation. hGH concentration in the milk was determined by RIA (Table 1). More than 19 μg/mL of hGH was detected in the milk of BCN1GH transgenic lines (11, 25, and 28), while less than 2 μg/mL hGH were present in the milk of BCN2GH lines (1, 7, and 9). The highest level of hGH was 5500 ± 620 μg/mL produced in line 11. On an SDS-PAGE a unique band was detected only in the milk of the line 11 (Fig. 2). The molecular weight of this band was 21.5 kDa, as expected from that of hGH of human

Table 1
Concentration of hGH in the Milk and Blood of Transgenic Mice

Transgenic line	Copy number[a]	hGH[b], μg/mL		
			Blood	
		Milk	Lactating	Cyclic
BCN1GH				
11	20	5500 ± 620	0.556	0.02
25	10	19 ± 4	0.005	< 0.001
28	20	30	0.027	0.002
BCN2GH				
1	2	0.7	0.002	< 0.001
7	30	2.0 ± 0.4	0.002	< 0.001
9	30	0.7	0.003	< 0.001
Nontransgenic control		< 0.1[c]	< 0.001	< 0.001

[a]The transgene copy numbers were approximately estimated from Southern blot analysis.
[b]The values are mean (± SEM) of hGH concentrations determined in more than two mice from each line by RIA kit which detection limit is 0.001 μg/mL. SEM was represented when three mice were analyzed.
[c]The detection limit for milk was 0.1 μg/mL because of its dilution to 1/100 to decrease nonspecific binding.

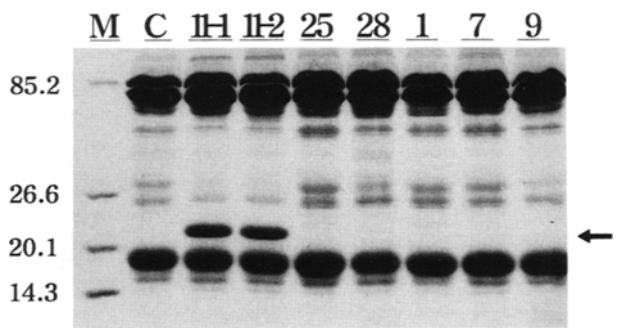

Fig. 2. Secretion of hGH into the milk of transgenic mice. Wheys, equivalent to 1 μL of milk, from a pool of two nontransgenic control (C), two individuals of line 11 (11-1, 11-2), and one from each of lines 25, 28, 1, 7, and 9 were applied to SDS-PAGE alongside protein size markers (M) and stained with Coomassie blue. Numbers on the left side are molecular weight (kDa) of the markers. The arrow indicates the position of hGH.

origin. However, the specific hGH band was not detected in the whey samples of the other transgenic lines because their milk hGH levels were below the detection limit of the SDS-PAGE.

hGH concentration in blood was also measured in cyclic and lactating mice (Table 1). In all lactating transgenic lines, variable amounts of hGH

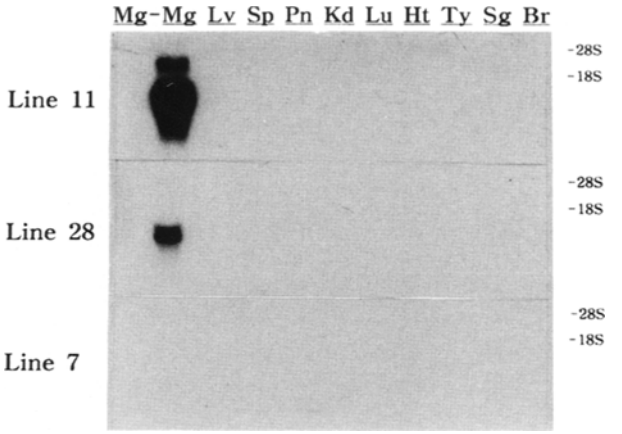

Fig. 3. Tissue-specific hGH expression by Northern blot analysis. Total RNAs (20 μg) from various organs of lines 11, 28, and 7 were applied to a nylon filter and hybridized with the ^{32}P-labeled 1.1-kb *Pvu*II fragment of hGH gene shown in Fig. 1. The analyzed organs are mammary gland (Mg), liver (Lv), spleen (Sp), pancreas (Pn), kidney (Kd), lung (Lu), heart (Ht), thymus (Ty), salivary gland (Sg), and brain (Br) of transgenic mice and mammary gland of a nontransgenic mouse (Mg-).

were detected in the blood and their levels were higher than those of cyclic mice. For instance, in line 11, blood hGH level of virgin mouse was 0.02 μg/mL; this value increased to 0.556 μg/mL by d 11 of lactation.

Tissue-Specific Expression of hGH Transgenes

In order to investigate the tissue specificity of transgene expression, total RNAs obtained from various organs of lines 7, 11, and 28 on d 11 of lactation were subject to Northern blot analysis (Fig. 3). hGH mRNA was detected in the mammary gland, but not in other tissues of lines 11 and 28. The hybridization signal from line 11 was much stronger than that of line 28. No hGH mRNA was detected in any examined tissues of line 7 or the mammary gland of nontransgenic control. When the exposure time was extended up to 4 d, a very faint hybridization signal was detected in the mammary gland of line 7 (data not shown). However, there was no change in the tissue specificity of hGH expression in the three lines. Therefore, according to the Northern blot analysis, hGH was exclusively expressed in the mammary gland of the transgenic mice and the amounts of hGH mRNA were shown to correlate well with the amounts of hGH secreted into the milk (Table 1).

Although Northern blot analysis showed that hGH expression was strictly restricted to the mammary gland, a sensitive RIA identified the production of hGH in several nonmammary organs, although their levels

Table 2
hGH Contents in Various Tissues of Transgenic Mice

Transgenic line	hGH contents, ng/mg protein									
	Mg[a]	Lv	Sp	Pn	Kd	Lu	Ht	Ty	Sg	Br
BCN1GH										
11-1	6000	4.0	0.6	1.3	5.4	2.0	1.3	2.0	16.6	1.2
-2	6500	4.2	2.0	1.0	3.8	1.7	3.3	14.4	18.9	5.2
25-1	130	—	—	—	0.3	0.2	—	0.8	1.4	—
-2	90	—	—	—	—	—	—	0.6	0.3	—
28-1	360	—	—	—	—	—	—	3.4	—	—
-2	100	0.2	—	—	2.0	—	—	2.7	0.4	—
BCN2GH										
1-1	2.3	—	—	—	—	—	—	0.4	—	—
-2	4.0	—	—	—	—	—	—	0.4	—	—
7-1	9.4	—	—	—	—	—	—	1.1	—	—
-2	11.0	—	—	—	—	—	—	5.3	0.3	—
9-1	2.3	—	—	—	—	—	—	7.8	0.3	—
-2	1.9	—	—	—	—	—	—	1.2	0.3	—

[a]Tissue extracts were prepared from two lactating mice from each transgenic line. Organs are abbreviated by Mg, mammary gland; Lv, liver; Sp, spleen; Pn, pancreas; Kd, kidney; Lu, lung; Ht, heart; Ty, thymus; Sg, salivary gland; Br, brain.
—: <0.2 ng/mg protein.

were drastically lower than that of the mammary gland in each line (Table 2). The hGH was produced from the thymus of all the transgenic lines and from the salivary gland of some transgenic lines regardless of the transgene constructs. hGH production was also observed in several additional tissues of BCN1GH lines such as kidney and lung from line 25, kidney and liver from line 28, and all tissues examined from line 11. To ensure the tissue specificity of the expression, RT-PCR analysis was performed with lines 7, 11, and 28 (Fig. 4). The expected PCR products of 120 bp were obviously seen in the mammary gland of the three transgenic lines. Faint signals were also seen in several nonmammary organs. The tissue specificity of hGH expression determined by hGH RIA and RT-PCR generally remained consistent in the two assay methods.

hGH Transgene Expression During Mammary Gland Development

It has been known that the rat β-casein gene expression is developmentally regulated (*12,14*). To determine whether the rat β-casein/hGH fusion genes also exhibit developmental regulation, slot blot analysis was performed using total RNAs isolated from the mammary gland of lines 11 and 28 at virgin, d 17 of pregnancy, and d 11 of lactation (Fig. 5). hGH mRNA levels were markedly increased from virgin through pregnancy to

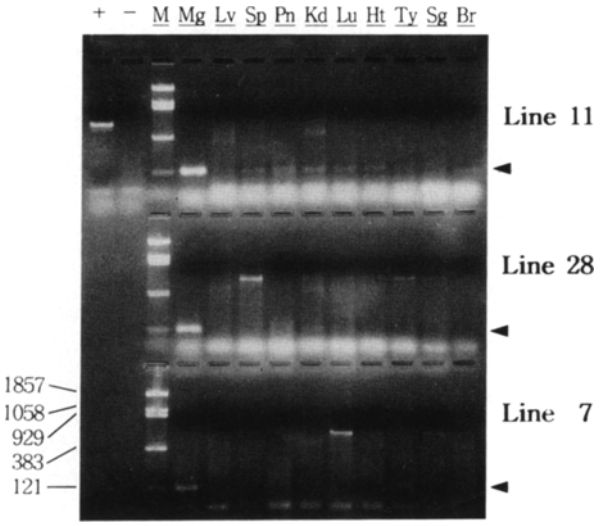

Fig. 4. The tissue specificity of hGH expression determined by RT-PCR. Reverse transcription reaction was performed with total RNA isolated from various organs of lines 11, 28, and 7, and mammary gland of nontransgenic mice on d 11 of lactation. After reverse transcription reaction, PCR amplifications were carried out with a primer set, which specifies the first exon sequence of rat β-casein gene and the first exon sequence of hGH gene, respectively. The RT-PCR products were separated on a 1.8% agarose gel. Lanes: +, PCR amplification from pBCN1GH construct without reverse transcription reaction; −, mammary gland of nontransgenic control mice; M, *Bst*NI digested pBR322 DNA size markers (basepairs); Mg, mammary gland; Lv, liver; Sp, spleen; Pn, pancreas; Kd, kidney; Lu, lung; Ht, heart; Ty, thymus; Sg, salivary gland; Br, brain. The arrowheads indicate the position of PCR products amplified from cDNA of the transgene-specific transcript.

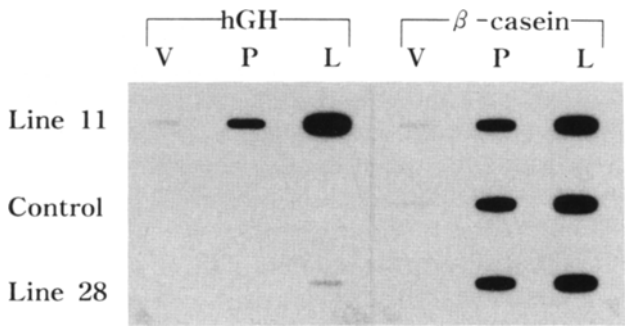

Fig. 5. Developmental regulation of hGH and β-casein expression in the mammary gland of transgenic mice. Total RNAs (5 μg) isolated from the mammary gland of lines 11, 28, and nontransgenic control mice at virgin (V), 17-d pregnant (P), or 11-d lactating (L) stage were blotted on a nylon filter and hybridized with either a 1.1-kb hGH fragment shown in Fig. 1 or a 0.8-kb *Sal*I/*Eco*RI fragment of rat β-casein gene containing exon VII.

midlactation in the two transgenic lines. Moreover, this expression pattern of hGH transgene was similar to that of the endogenous mouse β-casein gene. These results indicate that with the promoter sequences of the rat β-casein gene, hGH expression can be developmentally regulated in the mammary gland just like the endogenous mouse β-casein gene.

DISCUSSION

In the present study, we examined the efficiency and tissue specificity of hGH expression in transgenic mice from two rat β-casein/hGH gene constructs differing in their 3′ flanking sequences. The BCN1GH lines, containing 3′ untranslated/flanking region (3′-UT/FR) of hGH gene as the 3′ sequences of their transgene, highly efficiently expressed hGH in the milk (19–5500 μg/mL), while the BCN2GH lines, containing 3′-UT/FR of rat β-casein gene, poorly expressed the transgene (0.7–2.0 μg/mL). Interestingly, one of the BCN1GH lines (line 11) showed extraordinarily high hGH levels among others, although such a high level of expression could be in part owing to the effect of insertion position of the transgene. The expression level of BCN2GH lines was as low as that of rat β-casein in the transgenic mice carrying the entire rat β-casein gene along with 3.5 kb of 5′ and 3.0 kb of 3′ flanking sequence. It has been previously suggested that the poor expression of the rat β-casein could be owing to a lack of potent *cis*-elements necessary for the enhancement of the transgene expression in the 5′ and/or 3′ flanking sequences of the transgene (*13*). Therefore, it can be concluded that the efficient expression from BCN1GH construct is a result of the activity of a certain regulatory element(s) provided by the 3′-UT/FR of hGH gene. A detailed analysis is required for the regulatory elements residing in the hGH 3′ UT/FR before the mechanism for the enhanced expression of hGH in our experiments can be understood at molecular level.

According to the Northern blot analysis, the expression of hGH gene in BCN1GH lines was mainly confined to the mammary gland and thus appeared to be properly controlled as one would expect from the known expression pattern of β-casein gene. With a more sensitive assay method such as RIA, the transgene was again shown to be properly regulated according to the β-casein gene expression paradigm. However, with the RIA, a very low level of ectopic expression was also detected from various tissues. Thymus was the only organ other than the mammary gland showing consistent expression of hGH regardless of the gene constructs. This result confirms the previous finding that several casein genes are endogenously expressed in thymus through a different regulatory mechanism from that in the mammary gland (*14,23*). The low level hGH detected in various tissues by RIA could be the result of nonmammary expression of hGH owing to a nonspecific, basal-level activity of our transgene promoter

in many tissue types. This is because the transgene message, as determined by an RT-PCR analysis, is also seen in various tissues. However, the possibility that a portion of blood hGH might be a result of leakage from the mammary gland cannot be ruled out, since some milk proteins of low-mol-wt such as whey acidic protein (WAP) and α-lactalbumin have been shown to be normally present in the blood (*24–26*).

High level production of foreign proteins in the milk of a transgenic animal may lead to their presence in blood as well. The presence of hGH in the blood of cyclic animal, even at low level, can cause serious problems on animal reproductive physiology because of the suppression of endogenous prolactin release by serum hGH (*27*). Female sterility has been described in transgenic mice overexpressing hGH in the milk using WAP (*9*) or bovine casein promoters (*10*), in which hGH was also detected in the blood of cyclic and lactating mice. In our results, one female founder of BCN1GH lines that secreted hGH into blood at the level of 0.03 μg/mL was sterile (data not shown). The sterility may be owing to the serum hGH because the mouse showed the highest blood hGH level among all of the transgenic mice (Table 1) and this level of blood hGH was comparable to that previously reported for sterile mice (*10*).

In two transgenic lines, 11 and 28, temporal regulation of hGH expression was studied (Fig. 5). hGH expression pattern faithfully paralleled the endogenous pattern of mouse β-casein gene expression during mammary gland development. Thus it appears that the rat β-casein promoter sequences included in the BCN1GH construct contain all the sequences necessary for the proper temporal control of a transgene expression in the developing mammary gland.

In summary, the rat β-casein promoter could direct an efficient production of hGH in a highly tissue- and stage-specific manner when it was combined with the 3′ sequences of the hGH gene.

ACKNOWLEDGMENTS

We are grateful to Young II Yeom for a critical reading of the manuscript and Jungsun Park for a skillful microinjection. The rat β-casein gene was kindly provided by J. M. Rosen (Baylor College, Houston, TX). This study was supported by a grant (N80231) from the Korea Ministry of Science and Technology.

REFERENCES

1. Hennighausen, L. (1990), *Protein Expres. Purif.* **1,** 3–8.
2. Gordon, K., Lee, E., Vitale, J. A., Smith, A. E., Westphal, H., and Hennighausen, L. (1987), *Bio/Technology* **5,** 1183–1187.

3. Bühler, T. A., Bruyére, T., Went, D. F., Stranzinger, G., and Bürki, K. (1990), *Bio/-Technology* **8,** 140-143.
4. Ebert, K. M., Selgrath, J. P., DiTullio, P., Denman, J., Smith, T. E., Memon, M. A., Schindler, J. E., Monastersky, G. M., Vitale, J. A., and Gordon, K. (1991), *Bio/Technology* **9,** 835-838.
5. Wall, R. J., Pursel, V. G., Shamay, A., McKnight, R. A., Pittius, C. W., and Hennighausen, L. (1991), *Proc. Natl. Acad. Sci. USA* **88,** 1696-1700.
6. Wright, G., Carver, A., Cottom, D., Reeves, D., Scott, A., Simons, P., Wilmut, I., Garner, I., and Colman, A. (1991), *Bio/Technology* **9,** 830-834.
7. Archibald, A. L., McClenaghan, M., Hornsey, V., Simons, J. P., and Clark, A. J. (1990), *Proc. Natl. Acad. Sci. USA* **87,** 5178-5182.
8. Meade, H., Gates, L., Lacy, E., and Lonberg, N. (1990), *Bio/Technology* **8,** 443-446.
9. Devinoy, E., Thepot, D., Stinnakre, M. G., Fontaine, M. L., Grabowski, H., Puissant, C., Pavirani, A., and Houdebine, L. M. (1994), *Transgenic Res.* **3,** 79-89.
10. Ninomiya, T., Hirabayashi, M., Sagara, J., and Yuki, A. (1994), *Mol. Reprod. Dev.* **37,** 276-283.
11. Carver, A. S., Dalrymple, M. A., Wright, G., Cottom, D. S., Reeves, D. B., Gibson, Y. H., Keenan, J. L., Barrass, J. D., Scott, A. R., Colman, A., and Garner, I. (1993), *Bio/Technology* **11,** 1263-1270.
12. Hobbs, A. A., Richards, D. A., Kessler, D. J., and Rosen, J. M. (1982), *J. Biol. Chem.* **257,** 3598-3605.
13. Lee, K. F., DeMayo, F. J., Atiee, S. H., and Rosen, J. M. (1988), *Nucleic Acids Res.* **16,** 1027-1041.
14. Lee, K. F., Atiee, S. H., and Rosen, J. M. (1989), *Mol. Cell. Biol.* **9,** 560-565.
15. Greenberg, N. M., Anderson, J. W., Hsueh, A. J. W., Nishimori, K., Reeves, J. J., deAvila, D. M., Ward, D. N., and Rosen, J. M. (1991), *Proc. Natl. Acad. Sci. USA* **88,** 8327-8331.
16. Brinster, R. L., Allen, J. M., Behringer, R. R., Gelinas, R. E., and Palmiter, R. D. (1988), *Proc. Natl. Acad. Sci. USA* **85,** 836-840.
17. Whitelaw, C. B. A., Archibald, A. L., Harris, S., McClenaghan, M., Simons, J. P., and Clark, A. J. (1991), *Transgenic Res.* **1,** 3-13.
18. Namgung, U., Kim, Y. J., Lee, K. K., Han, M. H., and Kim, J. (1988), *Korean Biochem. J.* **21,** 226-232.
19. Hogan, B., Costantini, F., and Lacy, E. (1986), *Manipulating the Mouse Embryo: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
20. Sambrook, J., Fritsch, E., and Maniatis, T. (1989), *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
21. Laemmli, U. K. (1970), *Nature* **227,** 680-685.
22. Chomczynski, P. and Sacchi, N. (1987), *Anal. Biochem.* **162,** 156-159.
23. Grusby, M. J., Mitchell, S. C., Nabavi, N., and Glimcher, L. H. (1990), *Proc. Natl. Acad. Sci. USA* **87,** 6897-6901.
24. Akers, R. M., McFadden, T. B., Beal, W. E., Guidry, A. J., and Farrell, H. M. (1986), *J. Dairy Res.* **53,** 419-429.
25. McFadden, T. B., Akers, R. M., and Kazmer, G. W. (1987), *J. Dairy Sci.* **70,** 259-264.
26. Grabowski, H., Le Bars, D., Chene, N., Attal, J., Malienou-Ngassa, R., Puissant, C., and Houdebine, L. M. (1991), *J. Dairy Sci.* **74,** 4143-4150.
27. Chandrashekar, V., Bartke, A., and Wagner, T. E. (1992), *Endocrinol.* **130,** 1802-1808.