Characterization of Double Transgenic Mice Harboring Both Goat β-casein/hGH and Goat β-casein/hG-CSF Hybrid Genes

Keon Bong Oh[1] and Chul-Sang Lee[2†]

[1]*Animal Biotechnology Division, National Institute of Animal Science, RDA, Suwon 441-706, Korea*
[2]*Dept. of Biology, College of Natural Science, Kunsan National University, Gunsan 573-701, Korea*

ABSTRACT : In an attempt to simultaneously produce two human proteins, hGH and hG-CSF, in the milk of transgenic mice, we constructed goat β-casein-directed hGH and hG-CSF expression cassettes individually and generated transgenic mice by co-injecting them into mouse zygotes. Out of 33 transgenic mice, 29 were identified as double transgenic harboring both transgenes on their genome. All analyzed double transgenic females secreted both hGH and hG-CSF in their milks. Concentrations ranged from 2.1 to 12.4 ㎎/㎖ for hGH and from 0.04 to 0.13 ㎎/㎖ for hG-CSF. hG-CSF level was much lower than hGH level but very similar to that of single hG-CSF mice, which were introduced with hG-CSF cassette alone. In order to address the causes of concentration difference between hGH and hG-CSF in milk, we examined mRNA level of hGH and hG-CSF in the mammary glands of double transgenic mice and tissue specificity of hG-CSF mRNA expression in both double and single transgenic mice. Likewise protein levels in milk, hGH mRNA level was much higher than hG-CSF mRNA, and hG-CSF mRNA expression was definitely specific to the mammary glands of both double and single transgenic mice. These results demonstrated that two transgenes have distinct transcriptional potentials without interaction each other in double transgenic mice although two transgenes co-integrated into same genomic sites and their expressions were directed by the same goat β-casein promoter. Therefore goat β-casein promoter is very useful for the multiple production of human proteins in the milk of transgenic animals.

Key words : Goat β-casein promoter, hGH, hG-CSF, Double transgenic, Milk.

INTRODUCTION

Mammary gland is an exocrine organ that produces various proteins, which genes are expressed by independent regulatory mechanisms (Wilmut et al., 1990; Clark, 1998; Houdebine, 2000). Since the concept of mammary gland as a bioreactor was established by Gordon et al. (1987), many studies have accomplished targeted gene expressions to the mammary glands of transgenic animals using mammary gland-specific promoters. Thus, various human proteins destined for clinical use have been produced in biologically active form in the milk of transgenic animals (Goldman et al., 2004; Hunter et al., 2005; Demain & Vaishnav, 2009). In spite of the versatile productivity of mammary glands, however, most studies pursued to produce single human protein from the milk of one transgenic animal. As a modified approach to improve the productivity of bioreactor system, we here attempted a synchronous production of two individual human proteins from the milk of one transgenic animal by simple co-injection of two expression cassettes for human proteins, hGH and hG-CSF.

Previous studies reported that co-injection of two transgenes into pronuclei of fertilized eggs has generally resulted in co-integration at single site on genome (McKnight et al., 1995; Clark et al., 1997). This strategy, originally developed by Clark et al. (1992), have attempted to rescue poorly expressing transgene by another co-integrated transgene expressing highly in the same transgenic mice

† Corresponding author: Chul-Sang Lee, Ph.D., Dept. of Biology, College of Natural Science, Kunsan National University, 68 Miryong-dong, Gunsan 573-701, Korea. Tel: +82-63-469-4588, Fax: +82-63-463-1560, E-mail: chulsangc@kunsan.ac.kr

(McKnight et al., 1995; Barash et al., 1996; Clark et al., 1997; Yull et al., 1997; Langley et al., 1998). Additionally, co-injection strategy has been applied for the production of multimeric protein assembled by more than two subunits (Prunkard et al., 1996) and for the production of mature proteins modified by a processing enzyme (Drews et al., I995; John et al., 1999). One of the important prerequisites for co-expression system from single mammary gland is that each transgene expression must be directed by a strong and mammary gland-specific promoter without an interference on their expressional potential each other. In this study, we used identical 5.5 kb goat β-casein promoter to direct hGH and hG-CSF expression individually to the mammary glands of transgenic mice because goat β-casein promoter has been known to be an efficient and highly specific promoter to the mammary glands of transgenic animals (Echelard, 1996; Lee, 2006). The human grown hormone (hGH) is one of the hormones required for normal body size and an essential therapeutic drug for the treatment of GH deficiency (Reddy et al., 1991). The human granulocyte colony-stimulating factor (hG-CSF), which is a member of the CSF family of hormone-like glycoproteins, stimulates granulocyte colony formation and affects the proliferation, differentiation and activation of hematopoietic cells of the neutrophilic granulocyte lineage (Mire-Sluis, 1999). The hG-CSF has been widely used for treating neutropenia caused by chemotherapy for cancer treatment (Gabrilove, 1992; Bae et al., 1998). Goat β-casein/hGH hybrid gene has been usually expressed at high level in a mammary gland-specific manner (Lee, 2006; Lee et al., 2006). However hG-CSF expressional potential has not been fully addressed and some studies reported relatively low level expression of hG-CSF in the mammary gland of transgenic animals although its expression was directed by a strong goat β-casein promoter (Lu et al., 1999; Ko et al., 2000). Therefore the generation of double transgenic mice by co-injection of both goat β-casein/hGH and goat β-casein/hG-CSF hybrid genes would be interesting to explore their expressional pattern in the same transgenic mice. We examined goat β-casein-directed hG-CSF expression in the mammary glands of double and single transgenic mice at the mRNA and also analyzed its tissue specificity in the various tissues of transgenic mice.

MATERIALS AND METHODS

1. Construction of Transgenes and Generation of Transgenic Mice

A hGH expression vector (pGbc5.5hGH) driven by 5.5 kb goat β-casein promoter was previously described (Lee, 2006). The same goat β-casein promoter was used for the construction of hG-CSF expression cassette. Briefly, 1.9 kb Cla I/Kpn I insert of hG-CSF gene containing its genomic coding region and bGH (bovine growth hormone) terminal sequence was isolated from a vector pGbc-hGCSF

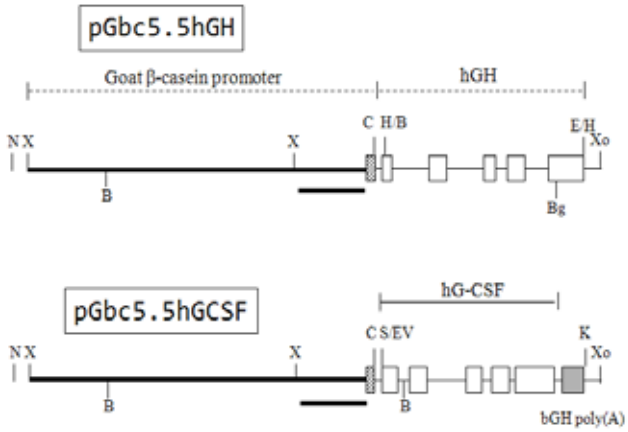

Fig. l. Generation of transgenic mice. (A) Schematic representation of pGbc5.5hGH and pGbc5.5hGCSF vectors. Construction of pGbc5.5hGH was previously described in detail (Lee, 2006). For the construction of pGbc5.5hGCSF, hG-CSF genomic DNA containing bGH terminator isolated from pGbc-hGCSF (Ko et al., 2000) was put into the pGbc5.5hGH with replacement of hGH gene. The 5.5 kb promoter of goat β-casein gene is represented as a bold line. The hatched box indicates first exon (+27) of goat β-casein gene. Open boxes indicate exons of hGH and hG-CSF gene. Introns are represented as thin lines. bGH poly(A) signal region is indicated. Restriction enzymes are abbreviated as B, BamH I; Bg, Bgl I; C, Cla I; EV, EcoR V; H, Hind III; K, Kpn I; X, Xba I; Xo, Xho I. The probe for Southern blot analysis was indicated as a solid bar. The diagram is not shown to scale.

(Ko et al., 2000) and put into pGbc5.5hGH in replacement with 2.1 kb Cla I/Hind II insert of hGH. This hG-CSF expression vector was designated as pGbc5.5hGCSF (Fig. 1). Both hGH and hG-CSF expression cassettes were excised with Not I and Xho I and purified by pass through elutip-D column. For microinjection, the concentration of purified DNAs was adjusted to 4 ng/㎖ in Tris-Cl/EDTA buffer (pH 8.0). Double transgenic mice were generated by co-injection of a mixture of two DNAs in an equimolar ratio. Single transgenic mice harboring only pGbc5.5hGCSF hybrid gene was also generated by microinjection. Transgenic founders were screened by PCR analysis using fresh tissue of pups collected from ear punching (Chen & Evans, 1993). PCRs were performed using primer sets specific to hGH and hG-CSF transgenes. The primer sets for detecting hGH and hG-CSF transgene were 5'-ATTCCGACAC GCTCCAACAG-3' and 3'-CATCGTCGAGTGCCGCTCTG-5', and 5'-GGATCTCCCCCGAGTTGGGT-3' and 3'-GCGTT CTACGCCACCTTGCC-5', respectively. The primer sets generated 720-bp products for hGH transgene and 390-bp products for hG-CSF transgenes (Fig. 2A), For Southern hybridization, genomic DNA samples were obtained from tail biopsy using standard phenol-chloroform extraction methods after tissue digestion in DNA extraction buffer [10 mM Tris-pH 8.0, 0.1M EDTA, 0.5% SDS, and protease K (400 ㎍/㎖)] at 55℃. 20 ㎍ of DNA was digested with BamH I and Bgl I, resolved on a 0.8 % agarose gel, blotted onto a nylon membrane, and then hybridized with a ^{32}P-labeled probe specific for goat β-casein promoter. The probe showed 6.2 kb hybridization signal for hGH transgenes and 4.9 kb signal for hG-CSF transgenes.

2. Sample Collection and Analysis

Milk was collected from 10-day lactating mice as described previously (Oh et al., 1999). Concentration of hGH and hG-CSF in milk was determined using radioimmunoassay (RIA) kit specific for human growth hormone (Daichii, Japan) and ELISA kit specific for hG-CSF (R&D systems, USA), respectively. After milking, mammary glands and other organs including liver, spleen, pancreas, kidney, lung, heart, salivary gland, and brain were homogenized in Trizol reagents (InVitrogen, USA) for total RNA isolation (Chomczynski & Sacchi, l987). Total RNA samples were resolved on 1% agarose/formaldehyde gel and transferred to nylon membrane (Boehringer Mannheim, Germany). Northern hybridization was carried out with ^{32}p-labeled probes specific to hGH, hG-CSF, and endogenous whey acidic protein (WAP) mRNA. Hybridization blots were exposed to X-ray film and their signal intensity was quantified using BASS 2000 phosphorimager (Fuji, Japan) and Tina phosphorimage analyzing software (Raytest, Germany).

RESULTS

1. Generation of Transgenic Mice

We screened 33 transgenic mice from 128 mice produced from co-injection of a DNA mixture of hGH and hG-CSF hybrid genes by PCR analysis (Table 1, Fig. 2A). Most of them (29 mice) were double transgenic mice containing both hGH and hG-CSF genes and the others were single transgenic mice, one for hGH and three for hG-CSF. This finding indicates that most co-injected embryos could develop double transgenic mice. This result also demonstrates that co-injection of DNA mixture could be an applicable method to generate multiple transgenic animals containing more than one foreign DNA. We also generated single hG-CSF transgenic mice by injecting goat β-casein/hG-CSF gene alone (Table 1) to compare hG-CSF expression pattern in single and double transgenic mice. Several double and single transgenic mice were randomly selected and applied to Southern analysis to confirm and characterize integration of the transgenes on their genome (Fig. 2B). Most double transgenic samples clearly showed both hGH and hG-CSF signals of expected size and other two samples (#7 and #9) showed either hGH or hG-CSF signal. One double transgenic sample showing very weak hybridization signal was confirmed by a prolonged exposure (data not shown). Band intensity of two transgene-specific

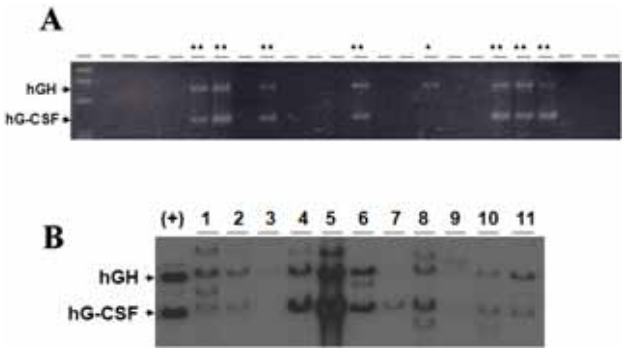

Fig. 2. Detection of hGH and hG-CSF transgenes in transgenic mice by PCR (A) and Southern blot analysis (B). (A) An example of PCR screening data. PCR was performed using two specific primer sets for hGH and hG-CSF, which produced 720 bp- and 390 bp-products, respectively. In this gel image, seven double(**) and 1 hGH single(*) transgenic samples were identified among 23 biopsied samples. The most left-lane was loaded with size markers. (B) Genomic DNA samples isolated from tail biopsies were digested with BamH I and Bgl I and transferred to nylon membrane after gel electrophoresis. The membrane was probed with ^{32}p-labeled 0.8 kb fragment of goat β-casein promoter (Fig. 1). These sequences were included in both hGH and hG-CSF transgenes, but could discriminated them by the size of hybridization signals. Enzyme digestion of genomic DNA samples with BamH I and Bgl I yielded hGH transgene of 6.2 kb and hG-CSF transgene of 4.9 kb. #7 and #9 showing one hybridization band were identified as single hG-CSF and hGH transgenic, respectively, by PCR analysis. The others were double transgenic. #3 showed both transgene signals by a prolonged exposure. (+) indicates co-injected DNA mixture corresponding to approximately 5 copies of each gene.

signals was very similar within samples but highly variable between samples, which means that two hybrid genes could be integrated together into the same genomic site (s) with a variable copy number. In addition, there were several other signals of unexpected size. They could be due to the incomplete enzyme-digestion of DNA samples and/or rearrangement of the transgenes.

2. Secretion of hGH and hG-CSF into the Milk of Transgenic Mice

We selected 5 female double transgenic mice showing clear integration signals (Fig. 2B) and bred them for milk collection. They all successfully bred their offsprings and secreted both hGH and hG-CSF into their milks (Table 2). The hGH level in the milk of 10-day lactating mice was very high regardless of copy numbers of hGH transgene, ranging from 2.1 ㎎/㎖ to 12.4 ㎎/㎖, which were comparable to that of single hGH mice previously reported (Lee, 2006). In contrast, hG-CSF concentrations in the same milk samples ranged between 0.04 and 0.13 ㎎/㎖, which were very low in comparison with hGH level, and it was not significantly different from that of single hG-CSF transgenic mice, which ranged from 0.05 to 0.16 ㎎/㎖.

Table 2. Concentrations of hGH and hG-CSF in the milk of transgenic mice

Type	Transgenic mouse	hGH (㎎/㎖)	hG-CSF (㎎/㎖)
Double (hGH+hG-CSF)	4	5.2	0.10
	6	12.4	0.13
	8	10.0	0.12
	10	2.1	0.04
	11	7.4	0.08
Single (hG-CSF)	S1		0.09
	S2		0.16
	S3		0.06
	S4		0.05

Table 1. Generation of double and single transgenic mice

Injection DNAs	No. of mice analyzed	No. of transgenic mice	Transgenic type		
			Double	Single	
				hGH	hG-CSF
hGH+hG-CSF	128	33	29	1	3
hG-CSF	97	19			19

3. Distinct Transcriptional Activity of hGH and hG-CSF Transgenes in Double Transgenic Mice

We had to explain the marked difference of the concentration between hGH and hG-CSF in the milk of double transgenic mice because their expression was controlled by the same strong promoter, goat β-casein promoter. To test whether the concentration difference in milks came from different transcriptional potential of two transgenes, we compared their mRNA level in the mammary glands of double transgenic mice. Total RNA samples were isolated from mammary glands of 5 double transgenic mice (#4, #6, #8, #10, and #11) and applied to Northern blot analysis. Because mRNA size of hGH and hG-CSF transgenes is very similar, we could not discriminate them on a single blot. For this reason, we measured signal intensity of hGH and hG-CSF mRNA on individual blots and compared them by normalization with mouse endogenous WAP mRNA level (Fig. 3). In comparison with endogenous WAP mRNA level, hGH mRNA level was similar to or maximally 2.3-fold higher than that of endogenous WAP mRNA in each transgenic mouse, whereas hG-CSF mRNA level approximately ranged from 3% to 22% of the endogenous WAP mRNA level. These results demonstrated the main cause of expressional difference of hGH and hG-CSF transgenes in milks could be their distinct transcriptional potentials in their mammary glands of double transgenic mice.

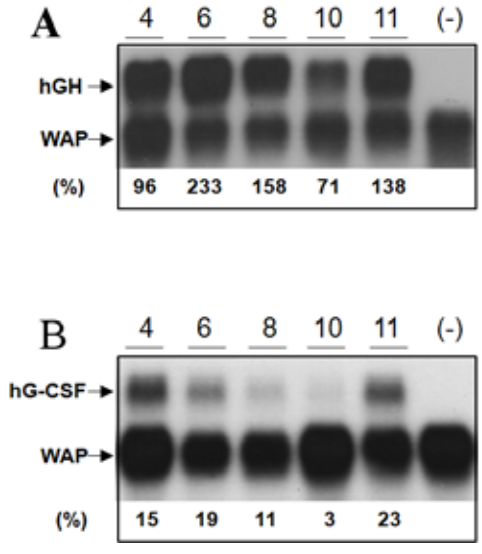

Fig. 3. Relative expression level of hG-CSF or hGH mRNA in the mammary gland of double transgenic mice. Total RNA samples (10 μg) of mammary glands from 5 double transgenic mice (#4, #6, #8, #10, and #11) and non-transgenic control (-) were analyzed by Northern blot analysis. Each blot was hybridized with a mixture of hGH and mWAP cDNA probes (A) or a mixture of hG-CSF and mWAP cDNA probes of same radioactivity (B). Hybridization signals were scanned and quantified. Relative hGH or hG-CSF mRNA level to the endogenous WAP mRNA level were represented as a percentage (%).

4. Tissue Specificity of Goat β-casein/hG-CSF Transgene Expression in Double and Single Transgenic Mice

Since other studies demonstrated that co-integration of two transgenes linked to a milk protein promoter, β-lactoglobulin, resulted in a more relaxed tissue specificity in their expression (Barash et al., 1996; Langley et al., 1998), we investigated whether activity of goat β-casein promo-

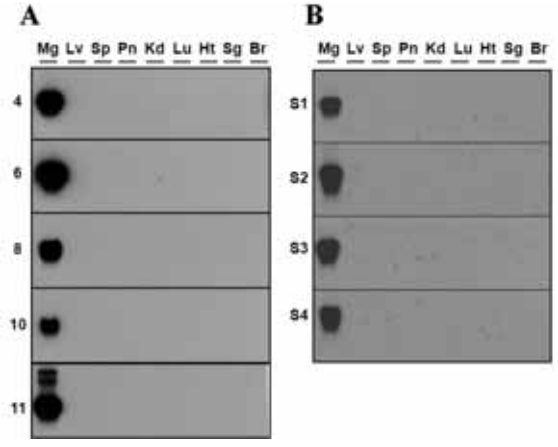

Fig. 4. Tissue-specific expression of hG-CSF transgene in double (A) and single (B) transgenic mice. Total RNA samples (20 μg) isolated from various tissues of lactating mice were subjected to Northern blot analysis using ^{32}P-labeled hG-CSF probe. Analyzed tissues were mammary gland (Mg), liver (Lv), spleen (Sp), pancreas (Pn), kidney (Kd), lung (Lu), heart (Ht), salivary gland (Sg), and brain (Br). Transgenic mouse lines were indicated at the left side of each photograph.

ter, which has been shown to be tightly regulated for mammary gland specific-hGH expression (Lee, 2006), would be impaired in double transgenic mice by examining the tissue specificity of goat β-casein/hG-CSF transgene expression in lactating transgenic mice. We isolated total RNA samples from various organs of 5 double and 4 single transgenic mice and applied to Northern blot analysis (Fig. 4). Transgene-specific hG-CSF mRNA signals were detected only in mammary glands but not in other organs including liver, spleen, pancreas, kidney, lung, heart, salivary gland, and brain of both double and single hG-CSF transgenic mice. These results demonstrated that tissue specificity of goat β-casein/hG-CSF transgene expression is not interfered by co-integrated genes and its integration site on the genome.

DISCUSSION

This study presents a possible strategy to improve commercial value of mammary gland system as a bioreactor by co-injection of two individual genes for the production of multiple target proteins from one transgenic animal. Co-injection method efficiently generated transgenic mice and most of them were double transgenic mice expressing both transgenes (Table 1 and 2). However, microinjection method has innate defect originating from a random integration of transgenes on the genome. Variable chromosomal localization of transgenes can cause variable frequency, level, and tissue specificity of transgene expression in transgenic animals (Palmiter & Brinster, 1986). In a previous study, however, Lee (2006) showed an efficient and consistent expression of goat β-casein/hGH gene in a mammary gland-specific manner in transgenic mice. Those results made it possible for us to test whether co-injected gene together with goat β-casein/hGH gene could achieve a consistent and tissue-specific expression when they integrated into the same locus on the genome. Additionally, we expected a rescue effect on the poorly expressing hG-CSF transgene by highly expressing goat β-casein/hGH gene in the same transgenic mice. Generally, DNA microinjection has resulted in a random integration of multiple copies of the gene in a tandem array (Clark et al., 1997). In this study, we did not examined whether two transgenes were arrayed at a single chromosomal site, but the similar integration signals (Fig. 2) and correlation between hGH and hG-CSF expression (Table 2) might reflect co-localization of two transgenes at the same site on the genome. Although two transgenes co-localized on the genome with similar copy number and also their expression was directed by the same promoter, the expression level of hG-CSF transgene was much lower than hGH level and there was no rescue effect on hG-CSF expression by goat β-casein/hGH expression (Table 2). However, the rescue phenomenon is not universal. Rescue activity has been mainly associated with β-lactoglobulin gene (Yull et al., 1997). There was no clear rescue effect when mouse WAP gene co-integrated with other WAP-based hybrid genes in the same transgenic mice (McKnight et al., 1995). Even β-lactoglobulin gene itself completely abrogated or suppressed the expression of co-integrated β-lactoglobulin/humam serum albumin constructs (Barash et al., 1996). Moreover, the β-lactoglobulin gene distorted tissue specificity of co-integrated transgenes (Barash et al., 1996; Langley et al., 1998).

In addition to the integration site of transgene and its promoter activity, target gene itself and its combination with a specific promoter can influence the expression of transgenes in transgenic animals. Low level expression of hG-CSF in this report could be due to unsuitable association of goat β-casein/hG-CSF hybrid gene and/or due to incompatible system of mammary gland for hG-CSF expression. Strong murine WAP promoter failed to lead an efficient expression of hG-CSF gene, resulting in 0.12-0.25 ㎍/㎖ in the milk of transgenic mice (Lu et al., 1999). In other report, goat β-casein promoter also directed low level expression of hG-CSF in transgenic goat (Ko et al., 2000), although its expression potential could not be confirmatively examined because they produced only one transgenic animal. Therefore it could be postulated that

hG-CSF gene is innately unsuitable for mammary gland expression. In addition to the expression level of transgenes, their tissue specificity could be also affected by the target gene itself and its combination with a promoter. In our previous report (Oh et al., 1999), bovine β-casein/bGH hybrid gene expressed in lung, as well as mammary gland, in transgenic mice. This dual-tissue specificity disappeared when bGH gene was replaced with luciferase gene (unpublished data). Taken these studies, target gene itself and/or its combination with a specific promoter could be an important factor to control the efficiency and tissue specificity of transgene expression in transgenic animals.

Although we could not augment a weak expressional potential of hG-CSF gene in this report, we showed a consistent expression of both hGH and hG-CSF in a highly mammary gland-specific manner without detectable detrimental effects on the animal physiology. Therefore goat β-casein promoter must be a nice promoter for the multiple production of human proteins in the mammary glands of transgenic animals because it can confer position-independent, mammary gland-specific expression of target genes only if the target genes are compatible with mammary gland-based expression system.

REFERENCES

Bae CS, Yang DS, Chang KR, Seong BL, Lee J (1998) Enhanced secretion of human granulocyte colony-stimulating factor directed by a novel hybrid fusion peptide from recombinant *Saccharomyces cerevisiae* at high cell concentration. Biotechnol Bioeng 57:600-609.

Barash I, Ilan N, Kari R, Hurwitz DR, Shani M (1996) Co-integration of beta-lactoglobulin/human serum albumin hybrid genes with the entire beta-lactoglobulin gene or the matrix attachment region element: repression of human serum albumin and beta-lactoglobulin expression in the mammary gland and dual regulation of the transgenes. Mol Reprod Dev 45:42l-430.

Chen S, Evans GA (1993) Use of polymerase chain reaction for screening transgenic mice. In: White BA (ed.). Methods in Molecular Biology, V115, PCR protocols: Current Methods and Applications. Humana Press, Totoma, NJ, pp 74-80.

Chomczynski R, Sacchi N (1987) Single step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Anal Biochem 162:156-159.

Clark AJ (1998) The mammary gland as a bioreactor: expression, processing, and production of recombinant proteins. J Mammary Gland Biol Neoplasia 3:337-350.

Clark AJ, Harold G, Yull FE (1997) Mammalian cDNA and prokaryotic reporter sequences silence adjacent transgenes in transgenic mice. Nucleic Acids Res 25: 1009-1014.

Clark AJ, Cowper A, Wallace R, Wright G, Simons JR (1992) Rescuing transgene expression by co-integration. Biotech 10:1450-1454.

Demain AL, Vaishnav P (2009) Production of recombinant proteins by microbes and higher organisms. Biotechnol Adv 27:297-306.

Drews R, Paleyanda RK, Lee TK, Chang RR, Rehemtulla A, Kaufman RJ, Drohan WN, Lubon H (1995) Proteolytic maturation of protein C upon engineering the mouse mammary gland to express furin. Proc Natl Acad Sci USA 92:10462-10466.

Echelard Y (1996) Recombinant protein production in transgenic animals. Cur Opin Biotech 7:537-540.

Gabrilove JL (1992) Clinical applications of granulocyte colony stimulating factor. Growth Factors 6:187-191.

Goldman IL, Kadulin SG, Razin SV (2004) Transgenic animals in medicine: integration and expression of foreign genes, theoretical and applied aspects. Med Sci Monit 10:RA274-285.

Gordon K, Lee E, Vitale JA, Smith AE, Westphal H, Henninghausen L (1987) Production of human tissue plasminogen activator in transgenic mouse milk. Bio/Technology 5:1183-1187.

Houdebine LM (2005) Transgenic animal bioreactors.

Transgenic Res 9:305-320.

Hunter CV, Tiley LS, Sang HM (2005) Developments in transgenic technology: applications for medicine. Trends Mol Med 11:293-298.

John DCA, Watson R, Kind AJ, Scott AR, Kadler KE, Bulleid NJ (1999) Expression of an engineered form of recombinant procollagen in mouse milk. Nature Biotech 17:385-389.

Ko JH, Lee CS, Kim KH, Pang MG, Koo JS, Fang N, Koo DB, Oh KB, Youn WS, Zheng GD, Park JS, Kim SJ, Han YM, Choi IY, Lim J, Shin ST, Jin SW, Lee KK, Yoo OJ (2000) Production of biologically active human granulocyte colony stimulating factor in the milk of transgenic goat. Transgenic Res 9:215-222.

Langley B, Vilotte J, Stinnakre M, Whitelaw CBA, L'Huillier P (1998) Rescue of an MMTV transgene by co-integration reveals novel locus control properties of the ovine β-lactoglobulin gene that confer locus commitment to heterogeneous tissues. Transgenic Res 7:205-212.

Lee CS (2006) Goat β-casein promoter directs hGH expression at a high level and in a mammary-specific manner in transgenic mice. Korean J Genetics 28:131-137.

Lee CS, Lee DS, Fang N, Oh KB, Shin ST, Lee KK (2006) Integration and expression of goat β-casein/hGH hybrid gene in a transgenic goat. Reprod Dev Biol 30:293-301.

Lu YF, Tian C, Deng JX, Xiao CZ, Ma QJ (1999) Cloning of human G-CSF genomic gene and its expression in transgenic mice mammary gland. Chuan Hsueh Pao 26:281-287.

McKnight RA, Wall RJ, Hennighausen L (1995) Expression of genomic and cDNA transgenes after co-integration in transgenic mice. Transgenic Res 4:39-43.

Mire-Sluis AR (1999) Cytokines: from technology to therapeutics. Trends in Biotech 17:319-325.

Oh KB, Choi YH, Kang YK, Choi WS, Kim MO, Lee KS, Lee KK, Lee CS (1999) A hybrid bovine β-casein/bGH gene directs transgene expression to the lung and mammary gland of transgenic mice. Transgenic Res 8:307-311.

Palmiter RD, Brinster RL (1986) Germ-line transformation of mice. Ann Rev Genet 20:465-499.

Prunkard D, Corttingham I, Garner I, Bruce S, Dalrymple M, Lasser G, Bishop P, Foster D (1996) High-level expression of recombinant human fibrinogen in the milk of transgenic mice. Nature Biotech 14:867-871.

Reddy VB, Vitale JA, Wei C, Montoya-Zavala M, Stice SL, Balise J, Robl JM (1991) Expression of human growth hormone in the milk of transgenic mice. Animal Biotech 2:15-29.

Wilmut I, Archibald AL, Harris S, McClenaghan M, Simons JR, Whitelaw CBA, Clark AJ (1990) Modification of milk composition. J Reprod Fert Suppl 41: l35-l46.

Yull F, Binas B, Harold G, Wallace R, Clark AJ (1997) Transgene rescue in the mammary gland is associated with transcription but does not require translation of BLG transgenes. Transgenic Res 6:11-17.

(received 11 August 2009, received in revised form 18 September 2009, accepted 18 September 2009)