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Transfection of β-casein chimeric gene and hormonal induction of its expression in primary murine mammary epithelial cells

(insulin/glucocorticoid/prolactin/extracellular matrix/milk protein gene)

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ABSTRACT **To study the regulatory sequence elements responsible for casein gene expression, we constructed a chimeric gene containing 5.3 kilobases (kb) of the 5′-flanking sequence and 1.6 kb of the 3′-flanking sequence of the mouse β-casein gene fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. The chimeric gene was transfected by the calcium phosphate-precipitation procedure into primary mouse mammary epithelial cells prepared from pregnant mice. The transfection procedure had negligible effect on expression of the endogenous β-casein gene. Expression of the β-casein–CAT chimeric gene required the synergistic actions of insulin, hydrocortisone, and prolactin. Expression of the chimeric gene also depended on the appropriate substratum because the degree of hormonal induction of the chimeric gene was much higher in cells cultured on a reconstituted basement membrane (Matrigel) than in cells cultured on either type I collagen gel or plastic. On the other hand, the expression of a simian virus 40–CAT chimeric gene in which the CAT gene was driven by the early promoter of the virus was not influenced by the hormonal milieu and occurred at the highest level in cells cultured on plastic. Additional transfection experiments with a series of β-casein–CAT constructs suggested the existence of regulatory elements responsible for hormonal induction and negative regulatory elements.**

Caseins are major milk proteins that are produced in large amounts in mammary glands of lactating animals. The tissue- and stage-specific expression of casein genes is regulated by a variety of factors including peptide and steroid hormones, cell–substratum, and cell–cell interactions (1–4). Thus, casein genes provide an attractive model to study the molecular mechanism of developmentally and hormonally regulated gene expression.

The expression of casein genes is stimulated by the synergistic actions of insulin, glucocorticoid, and prolactin in cultured mammary tissue through enhancement of both transcription of the genes and stability of the transcripts (5). To elucidate sequence elements responsible for regulation of casein gene expression, genomic clones of several caseins from various species have been isolated and characterized. There are several common sequence elements in the 5′-flanking region of casein genes (6–8). It has been shown that the 5′-flanking sequence of rat β-casein gene containing these common sequence elements allows hormonal induction of stably integrated casein chimeric gene in a mammary epithelial cell line, HC11 (9). On the other hand, a study using transgenic animals has shown that the same 5′-flanking sequence allows the tissue-specific expression of a chimeric gene, but that full hormonal induction requires additional sequence elements residing in an intragenic and/or 3′-flanking region of the rat β-casein gene (10).

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To analyze the regulatory sequence elements responsible for regulation of casein genes, it is highly desirable to establish a transient expression system in which regulation of transfected casein gene expression can be studied. Cell lines that express a specific set of genes in response to external stimuli are useful in this regard. At present, there are several mammary epithelial cell lines derived from normal mammary gland (11, 12). However, only small percentages of their cell population can express casein genes in response to hormonal stimuli, and their hormonal response is often lost by subcloning (13), thereby limiting analyses of the regulatory elements of casein genes. On the other hand, it has been shown that >90% of primary mouse mammary epithelial cells (PMME) derived from pregnant mice can express β-casein gene at a high level on a reconstituted basement membrane in response to hormonal stimuli (2). Therefore, PMME may be a preferable alternative for transfection of DNA to study regulatory elements of casein genes.

We report here the demonstration of successful transient expression of the casein chimeric gene in PMME. We constructed chimeric genes that contained both 5′- and 3′-flanking sequences of mouse β-casein gene fused to the structural sequence of the bacterial chloramphenicol acetyltransferase (CAT) gene. PMME were transfected with the chimeric gene on plastic dishes by using calcium phosphate-precipitation protocol to achieve high-transfection efficiency. The cells were then transferred and cultured on Matrigel, a reconstituted basement membrane, to promote functional differentiation. The data showed that transient expression of β-casein–CAT chimeric gene in PMME was regulated by hormones and substratum in a manner analogous to that of the endogenous gene.

MATERIALS AND METHODS

Construction of CAT Plasmids. For all construction, pBS(+) (Stratagene) was used as a vector. A 552-base-pair (bp) *Nsi* I–*Hph* I fragment of mouse β-casein gene was cloned into the *Sph* I site of pBS(+) by using *Sph* I linkers (14). This fragment corresponds to the nucleotide position of −545 to +7 of the β-casein gene (8).† The resulting plasmid was designated as pCsn(−545,+7). A small fragment corresponding to nucleotides (nt) −545 to −381 of the cloned DNA was removed by *Hin*dIII digestion, and a 4.9-kilobase (kb) *Hin*dIII fragment corresponding to nt −5300 to −381 of the β-casein gene was inserted. The resulting two clones were designated as pCsn(−5300,+7) and pCsn(−381,−5300/

Abbreviations: CAT, chloramphenicol acetyltransferase; EGF, epidermal growth factor; PMME, primary mouse mammary epithelial cells; nt, nucleotide(s).

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†The sequences of the mouse β-casein gene from position −4857 to position +8194 have been deposited in the GenBank data base (accession nos. X13484, X15991, and X15992).

−380,+7), respectively. pCsn(−5300,+7) has 5.3 kb of continuous genomic sequence from the β-casein gene, whereas pCsn(−381,−5300/−380,+7) has the 4.9 kb of 5′-upstream sequence in inverted orientation. Another clone was constructed with a 636-bp *Nsi* I fragment corresponding to nt −545 to +91 of the β-casein gene in the same way as pCsn(−5300,+7). This clone was designated as pCsn(−5300,+91), which contained exon 1 and 48 bp of intron 1.

A fragment containing the CAT gene (Pharmacia) was inserted into the *Bam*HI site of the above four clones in the same orientation as the β-casein gene fragments. A 1.6-kb *Eco*RI fragment corresponding to nt +6605 to +8194 of the β-casein gene was prepared from a genomic clone in pBS(+) vector, which contained the last 190 bp of exon 9, including a signal for polyadenylylation and 1.4 kb of 3′-flanking sequence. This fragment was inserted into the *Kpn* I site of the above four clones containing the CAT gene in the same orientation as the 5′ sequences and CAT gene. The resulting four clones were designated as pCsn(−545,+7)CAT/3′Csn, pCsn(−5300,+7)CAT/3′Csn, pCsn(−381,−5300/−380, +7)CAT/3′Csn, and pCsn(−5300,+91)CAT/3′Csn (Fig. 1).

Preparation, Culture, and Transfection of PMME. PMME were isolated by enzymatic dissociation and differential centrifugation, according to Lee *et al.* (15) with some modifications. Mammary glands from female mice (C3H/HeN) at their second half of pregnancy were minced with a razor blade. The tissue pieces were dissociated in Dulbecco's modified Eagle's medium containing 0.3% collagenase (type III, Worthington), 1% dispase (grade II, Boehringer Mannheim), and 10% fetal bovine serum with rotation at 100 rpm for 1 hr at 37°C. Incubation was continued in the fresh dissociation mixture for an additional 30 min. Epithelial cells were collected by centrifugation at 600 rpm for 2 min (RT6000 centrifuge with H-1000B rotor, Sorvall). The cells were washed three times with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, penicillin at 50 μg/ml, streptomycin at 50 μg/ml, and neomycin at 100 μg/ml (basal medium).

Cell number was estimated by counting nuclei after brief homogenization of an aliquot of isolated cells (16). Approximately 1.5–2 × 10^6 cells were plated and cultured on a 100-mm plastic culture dish in 10 ml of basal medium supplemented with porcine insulin at 5 μg/ml and mouse epidermal growth factor (EGF) at 10 ng/ml at 37°C in 95% air/5% CO_2. Previous study showed that preincubation with insulin and EGF augmented the induction of β-casein gene expression by insulin, glucocorticoid, and prolactin in cultured mammary tissue (17). After 2-day incubation, medium was changed 4 hr before transfection. Cells were transfected

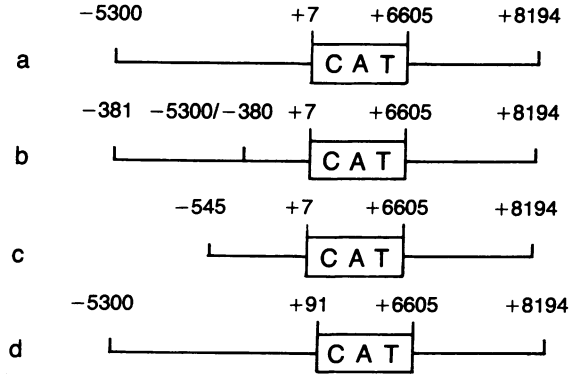

FIG. 1. Structures of the mouse β-casein–CAT chimeric genes. (*a*) pCsn(−5300,+7)CAT/3′Csn. (*b*) pCsn(−381,−5300/−380, +7)CAT/3′Csn. (*c*) pCsn(−545,+7)CAT/3′Csn. (*d*) pCsn(−5300, +91)CAT/3′Csn. The numbers represent the nucleotide positions from the transcription initiation site of the mouse β-casein gene (8).

with 20 μg of DNA per dish using calcium phosphate-precipitation procedure described by Chen and Okayama (18). When different constructs were transfected for comparison, an equimolar amount of construct DNA was used, and the total amount of transfected DNA was adjusted to 20 μg per dish with pBS(+). After transfection, cells were washed twice with phosphate-buffered saline and harvested by brief trypsinization. Cells were washed once with basal medium and transferred onto a 35-mm plastic culture dish coated with Matrigel (0.3 ml per dish, Collaborative Research), a reconstituted basement membrane prepared from Engelbreth–Holms–Swarm tumor (19), and cultured in 3 ml of basal medium containing appropriate hormone supplements. Medium was changed every other day. Unless stated otherwise, PMME were cultured on Matrigel for 4 days.

For hormonal induction of casein gene expression, bovine prolactin at 5 μg/ml, hydrocortisone at 1 μg/ml, and insulin at 5 μg/ml were added to basal medium. When the effects of various combinations of hormones were examined, charcoal-stripped fetal bovine serum (20) was used. In experiments to examine the effect of substratum, 35-mm plastic culture dishes and those coated with Matrigel or type I collagen gel (0.3 ml per dish) (Vitrogen, Collagen Corp.) were used.

CAT Assay. Cells were harvested by treatment with 0.3% collagenase/1% dispase for 30 min at 37°C, washed twice with phosphate-buffered saline, and suspended in 0.25 M Tris·HCl (pH 7.8). Cell extract was prepared by three cycles of freeze-thawing followed by centrifugation at 16,000 × *g* for 20 min. Aliquots (10–20 μl) of the extract were used for measurement of protein amount (21).

CAT assay was carried out at 37°C in 75 μl of reaction mixture, which contained [^{14}C]chloramphenicol (0.1 μCi, 57 mCi/mmol, 1 Ci = 37 GBq) (Amersham) and 4 μl (<1-hr incubation) or 10 μl (1- to 4-hr incubation) of 10 mM acetyl-CoA in 0.25 M Tris·HCl (pH 7.8) (22). The amount of extracts used contained 0.4–4.0 μg of protein. Under these conditions, CAT activity was linear. After TLC, CAT activity was determined by identifying the radioactive spots corresponding to the substrate and acetylated forms by autoradiography and measuring the radioactivity of the spots by liquid scintillation counting. CAT activity was expressed as percent conversion of the substrate to 1- and 3-acetylated forms per hr per μg of protein.

RNA Dot Blot Hybridization. Extraction of total cellular RNA and dot blot hybridization were carried out by using an RNA probe prepared from mouse β-casein cDNA (23), as described (17). This RNA probe had no homology to the constructs used in the transfections. The extent of hybridization was determined by liquid scintillation counting of the dots.

RESULTS

Temporal Expression of Transfected β-Casein–CAT Chimeric Gene in PMME. To establish a transfection system for study of regulatory sequence elements in β-casein gene, we examined the potential use of PMME as recipient cells for DNA transfection. In preliminary experiments, various transfection procedures including electroporation, protoplast fusion, and other methods using calcium phosphate, DEAE-dextran, Polybrene, or protamine were tested. We found that transfection using the calcium phosphate-precipitation procedure produced high-efficiency and more reproducible results among the methods tested. In addition, transfection was most efficient when PMME were cultured on plastic rather than on collagen gel or Matrigel. Transfection treatment produced no apparent changes in cell morphology of cultured PMME (data not shown). As shown below, however, plastic dishes were not suitable for hormonal induction of the transfected gene expression in PMME. Thus, we employed a

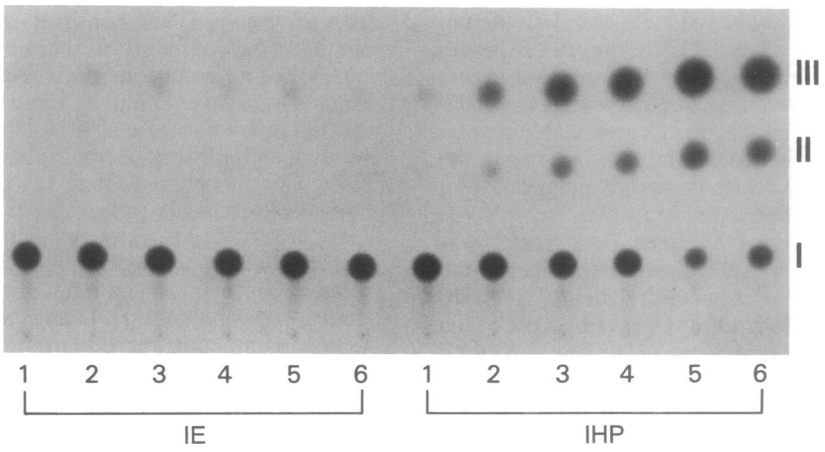

FIG. 2. Transient expression of pCsn(−5300,+7)CAT/3'Csn in PMME. PMME were transfected and then incubated on Matrigel in the presence of insulin and EGF (IE) or in the presence of insulin, hydrocortisone, and prolactin (IHP) for 6 days. At indicated times (in days), cells were harvested and CAT activity was assayed as described. Cell extracts used were 1.0 μg of protein per assay; incubation time was 2 hr. An autoradiogram of a representative experiment is shown; I, chloramphenicol; II, 1-acetylchloramphenicol; and III, 3-acetylchloramphenicol.

procedure involving transfection with calcium phosphate on plastic and transfer of cells onto appropriate substrata for hormonal induction.

When cells were transfected with pCsn(−5300,+7)CAT/3'Csn and cultured on Matrigel in the presence of insulin, hydrocortisone, and prolactin, CAT activity gradually increased with time and reached a maximum at day 5 during 6-day incubation: CAT activity at day 1, 2, 3, 4, 5, and 6 was 0.7, 5.0, 15.2, 25.1, 71.4, and 55.8, respectively. In the presence of insulin and EGF, CAT activity stayed at low levels, 0.2–0.7, during 6-day incubation (Fig. 2).

Effect of Transfection Treatment on Expression of the Endogenous β-Casein Gene. In nontransfected PMME, the level of endogenous β-casein RNA was increased ≈15-fold in the presence of insulin, hydrocortisone, and prolactin when compared with the insulin and EGF system (Fig. 3). The level of β-casein RNA and its extent of induction in the cells transfected with either pBS(+) or pCsn(−5300,+7)CAT/3'Csn were similar to those in nontransfected cells, indicating that the transfection treatment had negligible effect on hormonal induction of β-casein gene expression in this assay system.

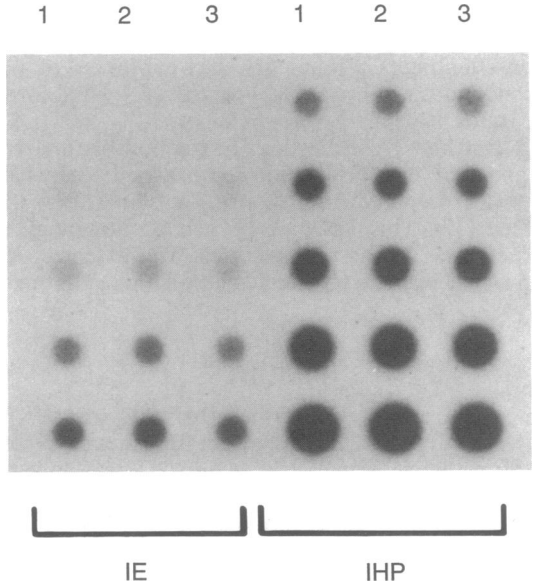

FIG. 3. Effect of transfection treatment on the level of the endogenous β-casein RNA in PMME. PMME were transfected with either pBS(+) (columns 1) or pCsn(−5300,+7)CAT/3'Csn (columns 2) or received no transfection (columns 3) and were then incubated on Matrigel in the presence of insulin and EGF (IE) or in the presence of insulin, hydrocortisone, and prolactin (IHP). Total cellular RNA was extracted, dotted onto nitrocellulose, hybridized with β-casein anti-mRNA probe, and the extent of hybridization was determined. The bottom dot is 400 ng of RNA; the other dots are sequential 1:2 dilutions.

Synergistic Actions of Lactogenic Hormones on Expression of Transfected β-Casein–CAT Chimeric Gene. pCsn(−5300,+7)CAT/3'Csn was transfected into PMME, and CAT activity was examined under various hormonal conditions (Table 1). When insulin or hydrocortisone or prolactin was added alone to the culture, ≈2-fold increase of CAT activity was seen when compared with the no-hormone system. When two of the three hormones were added together, CAT activity was generally higher than that of the cells incubated with single hormone, and the combination of insulin and prolactin produced the highest extent of induction (5.8-fold). When the three hormones were added simultaneously, the extent of induction increased to 13-fold.

Effect of Substratum on Expression of Transfected Gene. Functional differentiation of mammary epithelial cells depends on not only the synergistic action of hormones but also cell-substratum interaction (2, 3). To examine the effect of substratum on the expression of transfected gene, cells were cultured on either Matrigel or type I collagen gel or plastic after transfection (Fig. 4, Table 2). When cells transfected with pCsn(−5300,+7)CAT/3'Csn were cultured on Matrigel

Table 1. Effect of hormones on expression of pCsn(−5300,+7)CAT/3'Csn

Hormone	CAT activity	Induction, -fold
None	0.076 ± 0.003	1
Insulin	0.161 ± 0.025	2.1
Hydrocortisone	0.133 ± 0.013	1.7
Prolactin	0.125 ± 0.033	1.6
Insulin, hydrocortisone	0.213 ± 0.001	2.8
Insulin, prolactin	0.441 ± 0.075	5.8
Hydrocortisone, prolactin	0.329 ± 0.040	4.3
Insulin, hydrocortisone, prolactin	0.991 ± 0.033	13.0

PMME were transfected and then incubated on Matrigel with the indicated hormones. CAT activity was expressed as percent conversion of the substrate per hr per μg of protein. Values are means ± SEM of the results from three transfections in which the same batch of cell preparation was used.

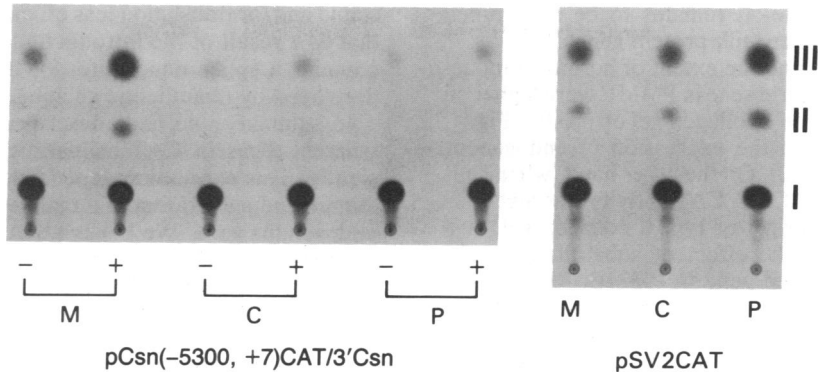

FIG. 4. Effect of substratum on expression of pCsn(−5300,+7)CAT/3'Csn and pSV2CAT in PMME. PMME were transfected and then incubated on either Matrigel (M) or type I collagen gel (C) or plastic (P). For pCsn(−5300,+7)CAT/3'Csn, basal activity of CAT (−) was determined in cells cultured in the presence of insulin and EGF, and induced activity (+) was determined in cells cultured in the presence of insulin, hydrocortisone, and prolactin. The amount of cell extracts used was 0.6 μg of protein per assay; incubation time was 4 hr. For pSV2CAT, CAT activity was determined in cells cultured with insulin and EGF. Amount of cell extracts was 1.0 μg of protein per assay, and incubation time was 1 hr. I, chloramphenicol; II, 1-acetylchloramphenicol; and III, 3-acetylchloramphenicol.

in the presence of insulin, hydrocortisone, and prolactin, a high level of induction (10.9 fold) of CAT activity was observed. On the other hand, cells cultured on either type I collagen gel or plastic showed much lower levels of both basal and induced CAT activity.

To examine whether the effect of substratum is specific to the expression of β-casein–CAT chimeric gene, PMME were transfected with pSV2CAT in which CAT gene was driven by the early promoter of simian virus including enhancer element (22). In the presence of insulin and EGF, the level of CAT activity was similar in cells cultured on either Matrigel or collagen gel (Fig. 4). On the other hand, CAT activity was 3-fold higher in cells cultured on plastic. Similar results were obtained in culture systems employing insulin, hydrocortisone, and prolactin (data not shown).

Analysis of β-Casein Gene Sequences Affecting Expression of CAT Activity. As initial attempts to examine the sequence requirements for the expression of β-casein–CAT chimeric gene, transfection was carried out by using other constructs (see *Materials and Methods*), and their expression was compared with that of pCsn(−5300,+7)CAT/3'Csn (Table 3). When pCsn(−381,−5300/−380,+7)CAT/3'Csn was used, both the basal and induced levels of CAT activity increased ≈2-fold, and the extent of induction (12.7-fold) was similar to that of pCsn(−5300,+7)CAT/3'Csn (11.9-fold). When pCsn(−545,+7)CAT/3'Csn was used, the basal and induced levels increased 3.3- and 2.3-fold, respectively, and the extent of induction (8.3-fold) was slightly lower. When pCsn(−5300,+91)CAT/3'Csn was used, both the basal and induced levels of CAT activity decreased to <3% of those of pCsn(−5300,+7)CAT/3'Csn, but the level of induction (12.2-fold) was virtually the same as in the case of pCsn(−5300,+7)-CAT/3'Csn. In contrast to the β-casein–CAT chimeric gene, pSV2CAT showed a high level of CAT activity regardless of the hormonal milieu, suggesting that the expression from simian virus 40 early promoter was hormone-independent.

Table 2. Effect of substratum on expression of pCsn(−5300,+7)CAT/3'Csn

Substratum	CAT activity		Induction, -fold
	Basal	Induced	
Matrigel	0.38 ± 0.14	4.18 ± 0.83	10.9
Collagen	0.16 ± 0.04	0.29 ± 0.03	1.8
Plastic	0.15 ± 0.06	0.32 ± 0.06	2.1

PMME were transfected, then transferred, and cultured on the indicated substratum. Basal activity of CAT was determined in cells cultured in the presence of insulin and EGF, and induced activity was determined in cells cultured in the presence of insulin, hydrocortisone, and prolactin. CAT activity was expressed as described for Table 1. Values are means ± SEM of the results from three transfections in which the same batch of cell preparation was used.

DISCUSSION

In this study, we showed that PMME could be used as recipient cells for transfection of DNA to study hormonal regulation of milk protein gene expression. The successful transient expression of β-casein–CAT chimeric gene in PMME depended on a two-step culture employing two different substrata wherein the structural and functional properties of PMME were differentially altered (2). On plastic, PMME do not respond to hormonal stimuli but form a spread monolayer which is suitable for transfection of DNA coprecipitated with calcium phosphate. On a reconstituted basement membrane, PMME express differentiated functions and form a multicellular structure resembling secretory alveoli (2). In our standard procedure, PMME were transfected on plastic and subsequently transferred onto Matrigel so that we could take advantage of both substrata.

In PMME prepared from pregnant mice, the expression of β-casein–CAT chimeric gene was maximally induced in the presence of insulin, hydrocortisone, and prolactin (Table 1). CAT activity from the transfected gene was increased with time for at least 5 days (Fig. 2). On the other hand, the CAT activity in cells transfected with pSV2CAT was unaffected by alterations in the hormonal milieu. Thus, these results indicated that hormonal induction of transfected genes in PMME

Table 3. Effect of β-casein gene sequences and simian virus 40 promoter on expression of CAT activity

CAT constructs	CAT activity		Induction, -fold	*n*
	Basal	Induced		
−5300,+7	0.65 ± 0.11	7.69 ± 1.01	11.9	5
−381,−5300/−380,+7	1.23 ± 0.11	15.53 ± 2.87	12.7	3
−545,+7	2.15 ± 0.63	17.71 ± 2.97	8.3	3
−5300,+91	0.03 ± 0	0.36 ± 0.06	12.0	2
pSV2CAT	20.23 ± 5.93	26.61 ± 4.38	1.3	3

PMME were transfected with pCsn(−5300,+7)CAT/3'Csn, pCsn(−381,−5300/−380,+7)CAT/3'Csn, pCsn(−545,+7)CAT/3'Csn, pCsn(−5300,+91)CAT/3'Csn, or pSV2CAT. PMME were cultured on Matrigel either in the presence of insulin and EGF or in the presence of insulin, hydrocortisone, and prolactin to determine basal and induced levels of CAT activity, respectively. Values are means ± SEM of the results from the indicated number (*n*) of transfection experiments in which the same batch of cell preparation was used.

is specific for β-casein gene. It remains to be determined whether this is true for other milk protein genes.

The level of expression and the extent of hormonal induction of β-casein–CAT chimeric gene in PMME were higher on Matrigel than on either type I collagen gel or plastic (Fig. 4, Table 2). This is similar to the expression of endogenous β-casein gene, as reported (2). On the other hand, when cells were transfected with pSV2CAT, CAT activity was higher on plastic than on either Matrigel or type I collagen gel, suggesting the specificity of the effect of substratum on the expression of the transfected gene in PMME. Mechanisms whereby various substrata influence mammary cell differentiation and hormone-responsiveness remain to be elucidated.

The transfection treatment appeared to have negligible effect on expression of the endogenous β-casein gene (Fig. 3). Although assay procedures were different, we have found a similar level of hormonal induction for expression of both the endogenous β-casein gene and transfected pCsn(−5300,+7)-CAT/3'Csn, suggesting that this β-casein–CAT construct contains most, if not all, essential sequence elements for hormonal induction of β-casein gene expression. Using pCsn(−5300,+7)CAT/3'Csn as a starting material, we can explore further the regulatory sequence elements responsible for casein gene expression.

It has been shown, using transgenic animals, that the 5'-flanking sequence of rat β-casein gene is sufficient for tissue-specific expression of β-casein–CAT chimeric genes but not for hormonal induction of their expression (10). These results suggested that additional sequence elements in an intragenic and/or 3'-flanking region are required for hormonal regulation. The structure and sequence of β-casein gene are highly conserved between rat and mouse (8). β-casein–CAT constructs used in this study showed hormonally induced expression, the extent of which was similar to that of the endogenous β-casein gene. These constructs contained parts of exon 9 and 3'-flanking sequences that were not included in the CAT constructs used in the previous study of transgenic mice (10). Thus, by taking account of these findings together, it is possible that the exon 9 and 3'-flanking sequence used in our constructs contain regulatory sequence elements responsible for hormonal induction. These elements could be a hormone-inducible enhancer and/or a part of transcript involved in regulation of its stability. Enhancer elements locating in the 3'-flanking region have been described for other genes (24–27). The sequence elements responsible for regulation of mRNA stability have been characterized for the transferrin receptor gene (28, 29).

In addition to the sequence elements responsible for hormonal induction, there may be other types of sequence element important in determining the magnitude of the chimeric gene expression. Our data indicated that inversion of the 5'-upstream sequence of 4.9 kb (nt −5300 to −381) or removal of the 5'-upstream sequence of 4.75 kb (nt −5300 to −546) in the β-casein chimeric gene caused >2-fold increase of CAT activity without affecting the extent of hormonal induction appreciably (Table 3). These results suggested that there are sequence elements between nt −5300 and −545 that negatively affect the transcription. Expression of pCsn-(−5300,+91)CAT/3'Csn was <3% of that of pCsn(−5300,+7)-CAT/3'Csn, whereas it retained the same level of induction as pCsn(−5300,+7)CAT/3'Csn. This could be explained by the introduction of a sequence element that suppresses the transcription. However, it is also possible that the presence of the additional 84-bp sequence makes the transcript less stable and/or translation less efficient. Another possibility is that as a result of the introduction of a 84-bp sequence that contains a splice donor site (8), the CAT reading frame is destroyed by unauthentic splicing.

In summary, we have described transfection in PMME, wherein β-casein–CAT chimeric genes are expressed transiently. This expression depended on appropriate hormonal stimuli and substratum in a manner analogous to that of the endogenous gene. We believe that the gene-transfer system described in this report will provide a valuable tool for studying the molecular mechanism of the expression of developmentally and hormonally regulated genes.

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