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# Tissue-specific expression of the rat β-casein gene in transgenic mice

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ABSTRACT

The rat β-casein gene is a member of a small gene family, encoding the principal milk proteins. In order to understand the mechanisms by which its stage- and tissue-specific expression are regulated, initially, a 14 kb genomic clone containing the entire 7.5 kb rat β-casein gene with 3.5 kb of 5' and 3.0 kb of 3' flanking DNA was microinjected into the germline of mice. Eight F0 transgenic mice were generated with copy numbers ranging from 1-10; five transmitted the transgene to their offspring in a Mendelian pattern. A specific RNase protection assay was developed to quantitate the level of expression of the rat β-casein transgene as compared to the endogenous mouse β-casein gene. Using this assay expression was demonstrated predominantly in the lactating mammary gland of transgenic mice at a level of 0.01-1% of the endogenous mouse β-casein gene. The transgene employed the authentic transcription initiation site observed previously in the analogous rat β-casein gene. In one line, a reduced level of expression of the transgene was also observed in the brain. The site of integration, therefore, plays an important role in influencing the level of expression of the transgene, but not its general pattern of tissue-specificity. The transgene appears to be developmentally-regulated in accordance with the endogenous mouse β-casein gene. These lines of mice generated carrying the rat β-casein transgene should provide useful models for studying the developmental and hormonal regulation of milk protein gene expression.

## INTRODUCTION

After conception, the mammary gland develops from the inactive state in the virgin animal into a fully functional tissue, synthesizing and secreting large quantities of proteins and other milk components. The caseins are the principal milk proteins encoded by a small gene family. Using cDNA probes corresponding to these proteins, the levels of different rat casein mRNAs during normal mammary gland development were found to increase from 100- to 3400-fold in response to both peptide and steroid hormones. For example, rat β-casein mRNA increases 250-fold from virgin to day 8 of lactation, at which time it is the predominant milk protein mRNA, comprising approximately 20% of the poly(A)$^+$ mRNA (1).

In order to elucidate the mechanisms by which expression of the rat β-casein gene is regulated in a tissue- and stage-specific fashion, initially,

the corresponding genomic sequences were isolated and characterized (2). To further define the *cis*-acting DNA sequences within or proximal to the gene, and eventually *trans*-acting factors, involved in the regulation of rat β-casein gene expression, gene transfer experiments in cell culture have been carried out in our laboratory. However, at present this approach has failed to generate either cell-type specific or hormonally-regulated expression, and instead constitutive expression has been observed (3). Transgenic mice, developed from eggs in which foreign DNA has been stably integrated, provide an excellent approach for addressing questions of tissue- and stage-specificity, allowing a detailed analysis of regulated gene expression in all tissues throughout development. Furthermore, using this approach, DNA fragments can be introduced as linear DNA fragments free of any vector and viral enhancer sequences, and following germ line integration may be assembled into the appropriate higher order chromatin structure during mammary development. A number of cellular genes or their fusion genes have been introduced into mice, and most of them resembling their mouse counterparts were expressed and regulated properly (for review, ref. 4). It has been shown by gene transfer into cell culture and mouse embryo experiments that the *cis*-acting DNA sequences responsible for the tissue-specific expression of several cellular genes can reside in the 5' flanking region, e.g., insulin, elastase, αA-crystallin, actin, collagen, class I histocompatibility antigen and whey acidic protein genes (5-11), introns, e.g., immunoglobulin (12,13), or even in 3' flanking DNA, e.g., β-globin gene (14), or a combination of the above (15). Therefore, initially, a clone containing the entire rat β-casein gene with 3.5 kb of 5' and 3.0 kb of 3' flanking DNA sequences was introduced into the germline of mice. Furthermore, it has been demonstrated that the level of regulation of casein gene expression is both transcriptional and post-transcriptional (16; Eisenstein and Rosen, in preparation). Interestingly, this construction contains a conserved sequences between -110 and -140 bp that has recently been identified as a milk protein gene consensus sequence (17), and may be involved in tissue-specific interaction with mammary nuclear proteins (18). Thus, by contrasting results obtained with casein promoter-reporter gene constructs in transgenic mice and explant cultures derived from these lines with those obtained with the whole gene, it should be possible to delineate the boundaries of DNA elements required to elicit both tissue-specific and hormonal regulation of the β-casein gene. As an initial step in this approach, we have established lines of transgenic mice carrying the entire rat β-casein gene, which express the transgene in a tissue- and stage-specific manner.

## MATERIALS AND METHODS

### Enzymes and Plasmids

All restriction enzymes were purchased from Bethesda Research Laboratories (Bethesda, MD). For Sp6 riboprobe synthesis and RNase protection assays, Sp6 polymerase, Sp65 plasmid, and DNase I were purchased from Promega (Madison, WI). RNase A was purchased from Sigma (St. Louis, MO). Enzymes were used according to the suppliers' specifications.

### Screening of Rat Genomic Library and Preparation of DNA for Microinjection

A Charon 35 rat genomic DNA library (gift of Dr. C. Kasper, University of Wisconsin, Madison, WI) was screened by using probes described previously (2). A 14 kb clone containing the entire rat β-casein gene and 3.5 kb of 5' and 3.0 kb of 3' flanking DNA was isolated (Fig. 1). The clone can be released in its entirety from the Charon 35 vector by digestion with *Sma*I. The vector-free 14 kb fragment (designated as B14) used for microinjection was purified by a low-melting agarose procedure (19) and dissolved in 10 mM Tris-HCl (pH 7.4), 0.25 mM EDTA according to Brinster *et al.* (20).

### Microinjection and Transfer of Mouse Embryos

Transgenic mice were generated essentially as described by Hogan *et al.* (21), as modified by DeMayo *et al.* (22). All mice were purchased from Sprague Dawley (Houston, TX). Superovulated female ICR donor mice (4-5 weeks of age) were mated with 3-12 month old B6C3F1 males. Embryos were flushed from the oviduct of donor mice, and the male pronuclei were microinjected with the rat B14 DNA fragment. Viable injected embryos were transferred to ICR recipient mice mated with vasectomized mice.

### Isolation and Hybridization of DNA

Two-cm of mouse tail was resected and homogenized using a Polytron (Brinkman) in a 4 ml solution containing 0.15 M NaCl, 1 mM EDTA and 20 mM Tris-HCl (pH 7.8). Sodium dodecyl sulfate (SDS) and proteinase K (Boehringer Mannheim, Indianapolis, IN) were added at final concentrations of 1% and 0.8 mg/ml, respectively, and the homogenates were incubated at 37°C overnight. High molecular weight DNA was isolated from the homogenates by extraction with an equal volume of phenol/chloroform/isoamyl alcohol (24:24:1), followed by spooling the DNA twice in the presence of 0.3 M NaOAc (pH 5.4) and 2 volumes of cold ethanol. DNA was dissolved in 10 mM Tris (pH 7.4) and 1 mM EDTA.

Approximately 10 μg of DNA from each sample was digested with *Eco*RI in the presence of 5 mM spermidine overnight (23), fractionated in a 1% agarose gel, transferred to nitrocellulose filters, and hybridized to a nick-translated 1.9 kb *Eco*RI probe (Fig. 1) with a specific activity of 2-8 X $10^8$ cpm/μg as described (19). It hybridizes to a 1.9 kb EcoRI rat genomic DNA

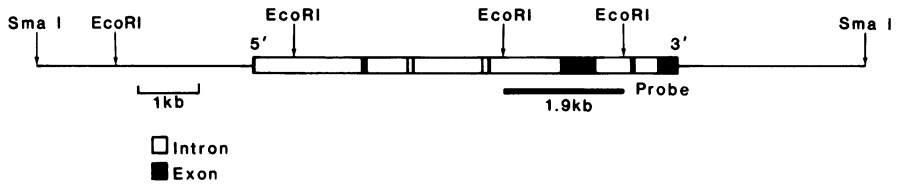


Figure 1. Structure of the transferred rat β-casein gene. The 14 kb clone contains the entire rat β-casein gene (3) plus 3.5 kb of 5' and 3.0 kb of 3' flanking DNA. The 1.9 kb of *Eco*RI fragment was used to screen the positive transgenic mice (in Fig. 2).

fragment (Fig. 2, lane A). The filter was washed in 2X SSC (1X SSC is a 0.15 M NaCl plus 0.015 M sodium citrate) and 0.5% SDS, followed by 0.1X SSC and 0.5 % SDS at 68°C for 2 h each. Autoradiography was performed using Kodak XAR or Fuji film with a DuPont intensifying screen at -80°. To determine the copy number of the transgene, variable inputs of rat DNA measured by a fluorometric assay were used and the intensity of the 1.9 kb EcoRI fragment quantitated by scanning densitometry of several autoradiographic exposures and compared to the same size fragments in a known amount of transgenic mouse DNA. Densitometry was performed with a fiber optic scanner (Kontes, Model 800).

RNA Isolation

Total RNA was isolated by homogenization of tissues in a guanidium isothiocyanate solution followed by CsCl fractionation according to Chirgwin *et al.* (24).

RNase Protection Assays

Since the mouse and rat β-casein mRNA share greater than 80% sequence homology, are of the same length (25), and have a high A and T content, a specific RNase protection assay, which can recognize and cleave a single mismatched basepair, was developed in order to distinguish the mouse and the rat β-casein mRNAs. This was performed essentially as described by Melton *et al.* (26) with the following modifications. A 3' end *Pst*I fragment of the β-casein cDNA was cloned into an Sp65 vector and linearized with *Pvu*II (Fig. 3). *In vitro* transcription of the Sp65 template was performed according to Melton *et al.* (26). A 650 nucleotide (NT) full-length probe was isolated from a 5% 8 M urea polyacrylamide gel by homogenization in a solution containing 500 mM $NH_4OAc$, 1 mM EDTA and 0.5% SDS. RNA isolated from lactating mammary glands of rats (0.5 µg) made up to 50 µg with yeast tRNA and 50 µg of RNA isolated from lactating mammary glands as well as other tissues of mice were dissolved in 30 µl of hybridization buffer containing 60% formamide, 3 M NaCl, 10 mM EDTA, 50 mM HEPES (pH 7.5) and 1.3-2 X $10^6$ cpm of the 650 NT riboprobe.

The reaction mixture was denatured at 95°C for 5 min and incubated at 55°C overnight. RNase digestion buffer (300 µl) containing 50 mM Tris-HCl (pH 7.5), 25 mM EDTA, 1.5 M NaCl, and 35 µg/ml of RNase A was added to the reaction mixture, and the incubation performed at 25°C for 15 min. The RNase digestion was stopped by adding 40 µg of proteinase K and 20 µl of 10% SDS and incubated at 25°C for 15 min. The protected fragments were isolated by phenol/chloroform/isoamyl alcohol extraction, ethanol precipitation and analyzed on a 5%/8 M urea polyacrylamide gel. A 450 NT fragment protected by the rat β-casein mRNA was observed (Fig. 3). Densitometric scanning of the autoradiographs with different exposures was employed to quantitate the level of expression.

For mapping the site(s) of initiation of the transgene in transgenic mice, a specific RNase protection assay was also developed. An 800 NT of *Eco*RI-*Hin*dIII genomic fragment containing the first exon (43 NT) of the rat β-casein gene, and 535 NT of intron A was cloned into the Sp65 vector, linearized with *Hin*dIII, and transcribed as described above (Fig. 5). The RNase protection conditions were essentially the same as above with the following exceptions: *(1)* 80% formamide was used for hybridization buffer; *(2)* 100 µg/ml of RNase A was used, and the reaction mixture was digested at 37°C for 30 min; *(3)* the protected fragments were analyzed on a 10%/8 M urea polyacrylamide gel.

## RESULTS

### Generation of Mice Containing the Rat β-Casein Gene

Although the rat β-casein gene had been isolated and characterized previously from a Charon 4A genomic library (2), the entire gene containing 5' and 3' flanking sequences could not be excised conveniently free of vector sequences because of numerous *Eco*RI sites within and flanking the gene. Accordingly, a homologous 14 kb genomic clone containing the entire 7.5 kb of rat β-casein gene and 3.5 kb of 5' and 3.0 kb of 3' flanking DNA sequence was isolated and characterized from a Charon 35 rat genomic library containing a multiple cloning cassette (27). This 14 kb fragment could be excised completely from the vector sequence by a blunt-end cutter, *Sma*I. It has been reported previously that vector sequences can influence the tissue-specificity and levels of transgene expression in transgenic mice carrying human β-globin (28) and actin-globin genes (8). The vector-free 14 kb genomic DNA fragement was used, therefore, to generate lines of transgenic mice. Figure 2 is an example of the screening results. Positive mice were screened using a genomic DNA tail blotting technique. A 1.9 kb repeat-free *Eco*RI genomic fragment was nick-translated as the probe (Fig. 1). It hybridizes to a 1.9 kb *Eco*RI rat

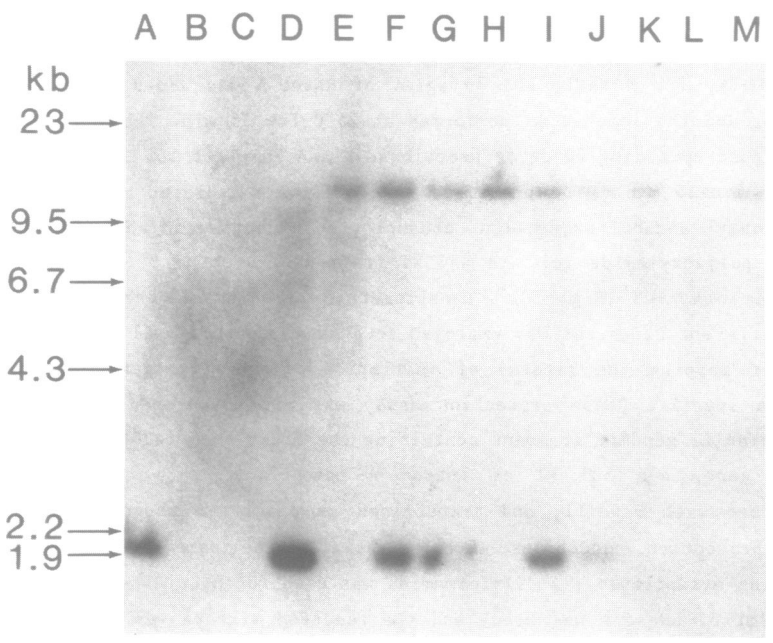


Figure 2. An example of screening of positive transgenic mice. Approximately 10 µg of DNA from each sample was digested with *Eco*RI, separated on a 1% agarose gel, transferred to a nitrocellulose filter, and hybridized with a nick-translated 1.9 kb probe as indicated in Fig. 1. Lane A, rat; M, normal mouse; B, blank; C-L, mice derived from injected embryos. This probe hybridized a 1.9 kb band as an indication for positive transgenic mice. The 10 kb band shown in mouse samples represents the endogenous mouse gene which cross-reacted with the rat probe.

genomic DNA fragment (Fig. 2, lane A), while the 10 kb band observed (Fig. 2, lane C-M) represents the endogenous mouse β-casein gene. Thus, the transgene and endogenous β-casein gene can be easily distinguished. By such analysis, eight transgenic mice were generated with copy numbers ranging from 1 to 10 (data not shown). Using our conditions of microinjection (22), we have observed a 1.3% efficiency in generating positive transgenic mice in terms of per embryo injection with this construction. Furthermore, although the DNA construct used for microinjection is blunt-ended, it gave a 50% efficiency in generating positive transgenic mice for the number of mice screened, but no copy numbers greater than ten. Five of the eight founder mice are female, and five of the eight mice transmitted the transgene to their offspring in a Mendelian fashion. Several homozygous lines of transgenic mice have been established which will be used for developmental and hormonal studies.

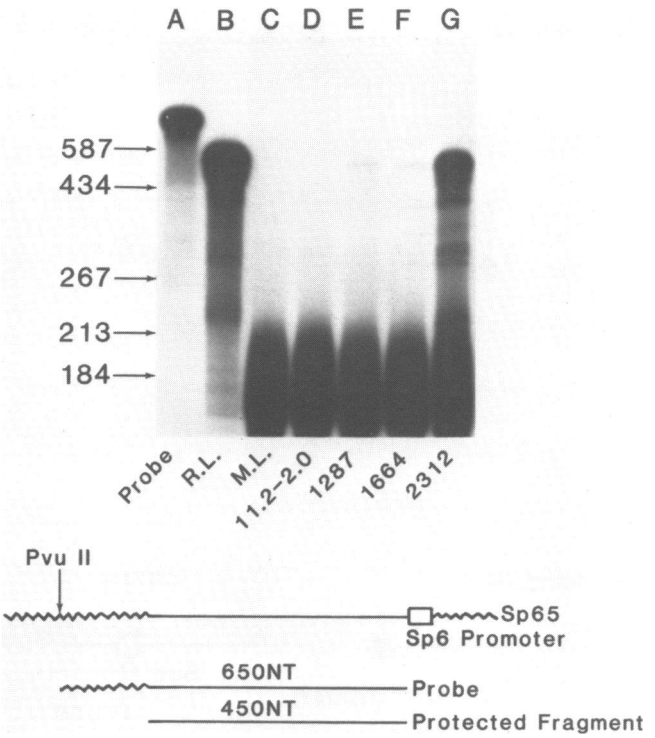


Figure 3. Expression of the rat β-casein gene in lactating tissue. The RNase protection assay was performed as described in the *Materials and Methods* section. Lane A, probe alone; RNA isolated from lactating mammary glands of rats (B), mice (C), transgenic mouse 1285 (D), 1287 (E), 1664 (F) and 2312 (G), respectively. Note that 2312 is a female F1 from F0 male founder mouse 1290, the all others are F0 female mice (see text). End-labeled pBR322-*Hae*III-digested fragments were used as size markers.

Expression of the Transgene in Lactating Tissue

For the female founder mice, we performed mammary gland biopsies during lactation to investigate the expression of the transgene. This method permitted the rapid screening of transgene expression. The fourth mammary gland was surgically removed at lactation (day 7-10) from mice under anesthesia. Total RNA was isolated and the expression of the transgene was assayed by the RNase protection protocol described in *Materials and Methods*. Male founder mice were mated to generate female F1 mice for the same examination. A 650 NT probe was used which generated a 450 NT protected fragment of the 3' end of rat β-casein mRNA (Fig. 3, lane B). In contrast, smaller fragments were protected with mouse β-casein mRNA (Fig. 3, lane C), representing

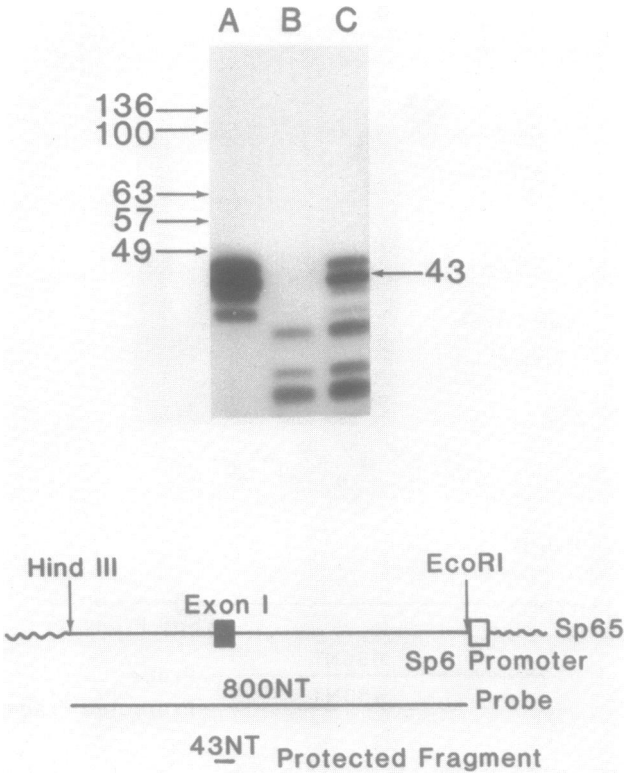


Figure 4. Correct initiation of the rat β-casein gene in transgenic mice. The RNase protection assay as described in *Materials and Methods* was employed to map the initiation of the rat β-casein gene in transgenic mice. RNA from lactating mamary gland of rat (A) normal mouse (B) and mouse 5067 (c). End-labeled pBR322-*Alu*I-digested fragments were used as size markers, demonstrating that mouse 5067 had the same protected fragments (C) as rat (A) beside the protected mouse fragments (B).

smaller regions of homology between the mRNAs and degradation products, since RNase A can cleave mismatched sites between the probe and mouse β-casein mRNA. Of five lines of mice examined, three mice (1287, 1664 and 2312, the female offspring of male founder mouse 1290) showed markedly different levels of expression of the transgene in lactating mammary tissue (Fig. 3, lane E-G).

However, the RNase protection assay used was not done under conditions of probe excess, and there is extensive homology between the mouse and rat β-casein mRNAs (25). Therefore, in order to quantitate the levels of expression of the transgene versus the endogenous mouse β-casein gene, different concentrations of RNA isolated from lactating mammary glands of rats were mixed with

RNA isolated from lactating mammary glands of mice up to a final amount of 50 µg and subjected to the same RNase protection assay. The relative level of expression was calculated based on the intensity of the 450 NT band as determined by densitometric scanning. Mouse 2312 had 1% of the level of expression of the endogenous mouse β-casein gene, and mouse 1287 and 1664 each had 0.01% (data not shown).

Initiation of the Transgene in Transgenic Mice

In transgenic mice, it usually has been shown that the transgenes can be initiated by RNA polymerase at authentic transcriptional start sites (8,10,29-32). Another RNase protection assay was employed, therefore, to determine if the rat β-casein gene also is transcribed correctly, initiating at the authentic start site in transgenic mice. An 800 NT riboprobe containing the first exon and part of 5' flanking and intron A sequences (Fig. 4) was employed. The data shown in Figure 4 illustrate that the rat β-casein mRNA from mouse 5067 (lane 3) utilized the same transcriptional start sites as RNA isolated from the rat lactating gland (lane 1). A predominant band, 43 NT in size, was observed in both samples, as well as several other less intense bands, but these were not seen in RNA samples isolated from the lactating mammary gland of non-transgenic mice (lane 2). Therefore, transcription appears to be initiated at the authentic site. We concluded that the rat β-casein gene was both expressed and correctly initiated during lactation in the mammary glands of transgenic mice.

Expression of the Transgene in Various Tissues of Transgenic Mice

In order to examine if the DNA construct integrated into the mouse genome contains sufficient information to elicit tissue-specific expression, total RNA was isolated from various tissues of transgenic mice at day 7 of lactation, and subjected to the RNase protection assay as described above (Fig. 3). The results are shown in Figure 5. In panel A, mouse 5067, the female F1 offspring of mouse 1290, was sacrificed at day 7 of lactation. The transgene was expressed exclusively in the mammary gland (lane 3) but not in other tissues assayed. In panel B, mouse 2567, the female F1 offspring of mouse 1287, expressed the transgene predominantly in the mammary gland (lane 4). The 5-fold longer autoradiographic exposure shown (Fig. 5B) indicates that the transgene is also expressed in the brain (lane 7) but at a much reduced level (approximately 30-fold less than that in mammary gland). Such expression was not observed in mouse 5067, even after long exposures. Furthermore, expression of the transgene was not detected in several other tissues including the salivary gland and thymus of mouse 5067, and in all tissues assayed of mouse 1956 (the female F1 offspring of negative F0 mouse 1285; see Fig. 3 and text)

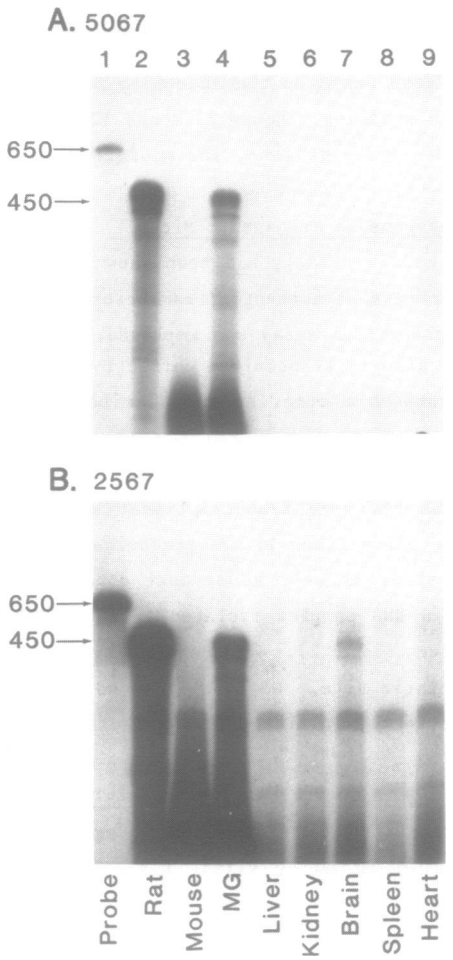


Figure 5. Tissue-specific expression of the rat β-casein gene in transgenic mice. The same RNase protection assay in Fig. 3 was used. In both A and B panels, lane 1, probe; lane 2, RNA isolated from lactating mammary gland of rats; lane 3, RNA isolated from lactating mammary glands of normal mice; lane 4, RNA isolated from mammary gland (MG) of lactating transgenic mice; lane 5-9, RNA from various tissues as indicated. Note the exposure time for the film was 12 h and 60 h for A and B, respectively. Mouse 5067 and 2567 are female $F_1$ of founder mouse 1290 and 1287, respectively (see text and Fig. 3).

(not shown). These data demonstrate that the 14 kb transgene contains sufficient information to direct the tissue-specific expression of the rat β-casein gene in the mammary gland.

Developmental Expression of the Transgene in the Mammary Gland

Rat β-casein gene expression is developmentally regulated (1). In order

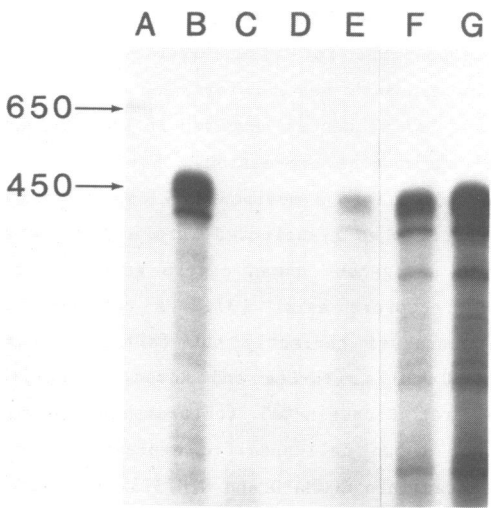


Figure 6. Developmental expression of the rat β-casein gene in transgenic mice. Lane A, probe; lane B-F, RNA isolated from mammary gland of lactating rats (B), lactating normal mice (C), and transgenic mice at various stages of development from virgin (D), mid-pregnancy (E), late-pregnancy (F) to 7 days of lactation (G), respectively.

to determine if the transgene contains sufficient information to elicit developmental regulation, RNA was isolated from the mammary gland of the offspring of mouse 1290 at various stages of development from virgin, mid-pregnancy, late-pregnancy to 7 days of lactation (Fig. 6, lane D-G, respectively), and subjected to the RNase protection assay as described above. The intensity of the 450 NT protected band is increased 10-fold from mid-pregnancy (lane E) to lactation (lane G), demonstrating that the transgene is also developmentally-regulated in accordance with the endogenous mouse β-casein gene.

## DISCUSSION

The expression of milk protein genes in mammary epithelial cells is regulated by a variety of factors, including peptide and steroid hormones, cell-cell and cell-substratum interactions (33,34). Hormone regulated mouse β-casein gene expression has been observed in primary mammary epithelial cells when they are co-cultured with 3T3-L1 adipocytes or grown on floating type I collagen or collagen-extracellular matrix gels (33). Casein gene expression was also enhanced in a mammary-derived cell line, COMMA-D, when cultured on a hydrated collagen gel (34) in response to hormones, namely, insulin, hydrocortisone and prolactin. Although DNA-mediated gene transfer into cell

culture has been a powerful approach for identifying *cis*-acting DNA sequences for several genes responsible for tissue-specific expression and hormonal regulation (35), this approach has so far been refractory when applied to defining the *cis*-acting DNA sequences involved in tissue-specific and hormonal regulation of rat β-casein gene expression. For example, a genomic rat β-casein gene sequence cloned into a bovine papilloma virus (BPV) vector failed to be regulated correctly when transfected into a human mammary derived cell line, T47-D, in which endogenous human casein genes are not expressed, but functional prolactin receptors exist (3). A rat α-casein minigene in a similar BPV vector expressed correctly but failed to respond to hormonal stimulation in COMMA-D cells (Yu-Lee and Rosen, unpublished observation). Furthermore, several rat β-casein-CAT (chloramphenicol acetyl transferase) fusion genes have not shown cell-specific expression and hormonal responsiveness when transfected into COMMA-D and NIH/3T3 cells (36). The failure of these gene transfer experiments in cell culture may be due to: *(1)* the lack of important *cis*-acting DNA sequences or the presence of vector sequences, e.g., BPV, in those constructions preventing the normal expression of the transferred genes; *(2)* the lack of appropriate recipient cells for gene transfer; or perhaps *(3)* the transferred genes need to be processed through germ line "imprinting" in order to acquire the necessary developmentally-regulated chromatin structure and DNA modifications required for normal expression. Transgenic mice, therefore, provided an excellent alternative approach for addressing the question of regulation of rat β-casein gene expression because the "manipulated" genes free of vector sequences could be introduced into the germ line to mimic the *in vivo* environment and subsequently to examine the expression of the transgenes in various tissues throughout development. Furthermore, mammary gland explant cultures derived from lines of transgenic mice and grown in a chemically-defined medium (37) can be employed to examine hormonal responsiveness, eventually facilitating the definition of *cis*-acting DNA sequences responsible for hormonal regulation.

The results presented in this manuscript demonstrate that the 14 kb genomic clone containing the entire rat β-casein gene and 3.5 kb of 5' and 3.0 kb of 3' flanking DNA sequences is correctly initiated at the correct transcriptional start site and is expressed in a tissue-specific fashion. The transgene also appears to be developmentally-regulated in accordance with the endogenous mouse β-casein gene. From a variety of gene transfer experiments with different genes in both cell culture and/or transgenic mice, it has been suggested that tissue-specific gene expression may require that the *cis*-acting DNA sequences be packaged into a higher order chromatin structure which can be

modulated both positively and negatively by *trans*-acting factors in response to environmental signals (4,38). Since not every transgenic mouse expressed the β-casein transgene (Fig. 3), it appears that the site of integration may influence the level of expression, but not the general pattern of tissue-specificity of expression in transgenic mice. Furthermore, the level of expression of the rat β-casein gene is only 0.01-1% of the level of the endogenous mouse gene. These results are consistent with data obtained from transgenic mice carrying other cellular genes, in which the site of integration has been suggested to influence the accessibility of *trans*-acting factors to the *cis*-acting DNA sequences (4). Alternatively, it is possible that there are other sequences further upstream and/or downstream of the rat β-casein gene that influence the level of expression but are not present in the construction. Additionally, in one transgenic mouse 2567, a low level of expression of the transgene was observed in the brain, consistent with observations by others that expression of transgenes in inappropriate tissues is usually at a reduced level (4). Again, the site of integration may play an important role for such "leaky expression" since not every transgenic mouse expressed the rat β-casein transgene in the brain (Fig. 5, panel B, lane 7).

The studies reported herein are the first step in defining the *cis*-acting DNA sequences involved in the tissue- and stage-specific expression of the milk protein genes, specifically the rat β-casein gene. Their long range goals are: first, to help elucidate the molecular mechanisms by which milk protein gene expression is hormonally- and developmentally-regulated in a tissue-specific fashion; second, to provide a way to target various oncogene products for selective expression in the mammary gland as a means of facilitating our understanding of mammary tumorigenesis (11,40), and to target biomedically important proteins to be expressed in the mammary gland, secreted and recovered from milk (41). Recently, Simons *et al.* have reported that a sheep milk protein gene, β-lactoglobulin, is also expressed specifically in mammary gland of transgenic mice, and secreted into milk (42). Thus, the feasibility of these experiments seems assured. We are in the process of defining the minimal sequences responsible for the tissue-specific expression of the rat β-casein gene by using the same transgenic approach and casein-CAT fusion genes.

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