The poly(A) tail length of casein mRNA in the lactating mammary gland changes depending upon the accumulation and removal of milk

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The length of casein mRNA from the lactating mouse mammary gland, as assessed on Northern blots, is shorter after weaning, but is elongated following the removal of milk. In order to investigate this phenomenon, the molecular structures of β- and γ-casein mRNAs were analysed. The coding and non-coding regions of the two forms were the same length, but the long form of casein mRNA had a longer poly(A) tail than the short form ($P < 0.05$). In order to examine the stability of casein mRNA under identical conditions, casein mRNAs with the long and short poly(A) tails were incubated in the rabbit reticulocyte lysate (RRL) cell-free translation system. Casein mRNA with the long poly(A) tail had a longer half-life than that with the short tail ($P < 0.05$). The β- and γ-casein mRNAs were first degraded into 0.92 and 0.81 kb fragments respectively. With undegraded mRNA, the poly(A) tail shortening by exoribonuclease was not observed until the end of the incubation. Northern blot analysis showed that casein mRNA with the long poly(A) tail was protected efficiently from endoribonucleases. We conclude that the length of the poly(A) tail of casein mRNA in the lactating mammary gland changes depending upon the accumulation and removal of the gland's milk, and we show that the longer poly(A) tail potentially protects the mRNA from degradation by endoribonucleases.

Key words: lactation, mRNA half-life, mouse mammary gland.

INTRODUCTION

Caseins are the major milk proteins produced by the lactating mammary gland, and are present in the micellar complex. In mouse milk, the casein micelle consists of α-, β-, γ- and κ-caseins, and the casein genes are mapped to chromosome 5 [1]. Nucleotide sequence analysis of the signal peptide reveals that the mouse α-, β-, γ- and κ-casein genes have high sequence identity with the bovine α_{s1}-, β-, α_{s2}- and κ-casein genes respectively [2]. Casein synthesis is regulated by prolactin in the presence of insulin and glucocorticoids *in vitro* [3]. Prolactin regulates casein synthesis by binding to the long form of the prolactin receptor (PRL-R_L) [4,5]. PRL-R_L mRNA is expressed acutely approx. 12 h before parturition [6].

The accumulation and removal of milk results in dramatic changes in mammary gland function. Milk synthesis is slowed by the accumulation of milk in lactating mammary glands. The level of casein mRNA declines following weaning in rats [7] and mice [8]. This phenomenon is partly explained by the decreases in PRL-R_L protein and mRNA [9]. It is generally accepted that the mRNA level is determined by the balance between synthesis and degradation. Post-transcriptional regulation of casein mRNA has been implicated, with evidence that casein mRNA stability, in terms of its half-life ($t_{\frac{1}{2}}$), changes in response to a variety of stimuli *in vitro* [10–13]. However, the post-transcriptional regulation of casein mRNA *in vivo* has not been elucidated. Kim et al. [8] reported that the lactating mammary gland reversibly resumed casein mRNA synthesis when milk was removed by suckling. mRNA stability was examined using at least two types of mammary glands: one that was maximally filled with milk due to weaning, and the other that was emptied of milk by suckling after weaning. The structure of mRNA is related to mRNA stability [14–17]. The relative stabilities of mRNAs in the rabbit reticulocyte lysate (RRL) cell-free translation system are known to correlate well with their relative stabilities determined in whole-cell systems [18,19]. Using this RRL system, the structure and stability of casein mRNA from these glands were compared with those from normally lactating mammary glands. cDNA clones for β- and γ-casein have been sequenced [20,21]. In the present study, β- and γ-casein mRNAs were analysed, and mRNA for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a control.

MATERIALS AND METHODS

Animals

ICR and C3H/He mice were used (Clea, Tokyo, Japan). Most experiments were carried out using ICR mice. Animals were kept in an air-conditioned and well-ventilated room (23 ± 1 °C; relative humidity 60–70 %; lights on 05:00–19:00 h), with food and water available *ad libitum*. The day of parturition was counted as day 0, and the litter size was adjusted to 10 pups per dam on day 0. The pups were separated from their mothers on day 8 (W0). At 24 h after weaning (W24), some mothers were allowed to nurse 10 foster pups per head for 12 h (W24/N12), or for up to 24 h Foster pups were the same age (days) as the mother's own pups, and had been separated from their mothers for 8 h.

RNA extraction

Whole mammary glands were collected immediately after cervical dislocation. Total RNA was isolated from fresh glands by the acidic guanidinium thiocyanate/phenol/chloroform method [23]. The concentration and purity of RNA were determined by

Abbreviations used: RRL, rabbit reticulocyte lysate; PRL-R_L, long form of the prolactin receptor; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; DIG, digoxigenin; cRNA, complementary RNA; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription–PCR; ANOVA, analysis of variance; PABP, poly(A)-binding protein.

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measuring the absorbance (A) at 260 nm and the A_{260}/A_{280} ratio respectively, using a spectrophotometer.

Northern blot analysis

RNA (0.5–2.0 μg) was separated by electrophoresis on a 1.2 % (w/v) agarose gel containing 2.2 M formaldehyde, and then transferred to a Hybond-N$^+$ membrane (Amersham, Aylesbury, Bucks., U.K.). RNA blots were prehybridized with salmon sperm DNA (Gibco BRL, Grand Island, NY, U.S.A.) at 65 °C for 3 h and then hybridized with a digoxigenin (DIG)-labelled complementary RNA (cRNA) probe at 65 °C for 16 h. The cRNA probe concentrations were 30 ng/ml for β- and γ-casein mRNAs, and 100 ng/ml for G3PDH mRNA. The membrane was processed according to standard procedures [24]. Signals were recorded on X-ray film using a DIG Luminescent Detection kit (Boehringer Mannheim, Mannheim, Germany). The mobility of mRNA was compared with that of RNA size makers (Gibco BRL). Data were analysed using a GS-670 densitometer with Molecular Analysis software (Bio-Rad, Hercules, CA, U.S.A.).

Preparation of DIG-labelled antisense cRNA probe

cDNA, prepared from lactating mammary gland RNA, was amplified using a TP2000 thermal cycler (TaKaRa, Kyoto, Japan) in the presence of *Taq* DNA polymerase (Gibco BRL), primers (Toa Synthesis, Tokyo, Japan) and dNTPs (Gibco BRL) [6]. Sense and antisense primers were as follows: for β-casein, 5′-GCTGGACAATAGCGTGTTCTTCC-3′ and 5′-GGGAAGGTGTAATCCTTACTGGG-3′ respectively; for γ-casein, 5′-GACTACATTTACTGTATCCTCTGAG-3′ and 5′-GGGAAGGTGTAATCCTTACTGGG-3′ respectively; for mouse G3PDH [22], 5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′ and 5′-CATGTAGGCCATGAGGTCCACCAC-3′ respectively. The PCR products were inserted into the plasmid using a TA cloning kit (Invitrogen, San Diego, CA, U.S.A.). The plasmid DNA was amplified and isolated according to the manufacturer's instructions. The plasmid DNA was linearized by digestion with *Eco*RV, *Spe*I or *Bam*HI (Toyobo, Osaka, Japan) for β-casein, γ-casein and G3PDH DNA clones respectively, and cRNA probes were synthesized in the presence of DIG-labelled UTP by SP6 or T7 RNA polymerase using a DIG-labelling kit (Boehringer Mannheim).

Determination of poly(A) tail length by RNase H digestion

The poly(A) tail was digested by RNase H [25]. RNA (30 μg) was dissolved in 1 mM EDTA, heated to 100 °C for 5 min and immediately chilled on ice. RNA samples were incubated with or without 25 μg/ml oligo(dT)$_{12–18}$ (Gibco BRL) for 10 min at 25 °C. KCl was adjusted to 50 mM and hybridization was continued for another 10 min. The hybrids were incubated at 37 °C for 30 min with 0.6 unit/40 μl RNase H (TaKaRa) in 0.5 mM EDTA, 25 mM KCl, 28 mM $MgCl_2$ and 20 mM Tris/HCl (pH 8.0). RNA was used for Northern blots.

5′-, 3′-Rapid amplification of cDNA ends (RACE) and reverse transcription–PCR (RT-PCR)

Primers used were as follows: primer A, 5′-CTGTACTTTCTGCAGCAAGTAGCAC-3′ (sense; 620–644 nt, β-casein); primer B, 5′-CCCAGTAAGGATTACACCTTCCC-3′ (sense; 561–583 nt, γ-casein); primer C, 5′-P+GTGCTACTTGCTGCA-3′ (antisense; 630–644 nt, β-casein); primer D, 5′-P+GGGAAGGTGTAATCC-3′ (antisense; 569–583 nt, γ-casein); primer E, 5′-AACTCTGAAACTGTGCTCCG-3′ (sense; 446–465 nt, β-casein); primer F, 5′-GTGAACTTTAGCCTGGAGCA-3′ (antisense; 225–244 nt, β-casein); primer G, 5′-AGATACCTGTGAGCTACTGG-3′ (sense; 538–557 nt, γ-casein); primer H, 5′-CTGGTGAAGAGGTGTGCAAC-3′ (antisense; 343–362 nt, γ-casein); primer I, 5′-CTCAGAGGATACAGTAAATGTAGTC-3′ (antisense; 106–130 nt, β-casein); primer J, 5′-GGAAGAACACGCTATTGTCCAGC-3′ (antisense; 170–192 nt, γ-casein); primer K, 5′-GACTACATTTACTGTATCCTCTGAG-3′ (sense; 106–130 nt, β-casein); primer L, 5′-GTGCTACTTGCTGCAGAAAGTACAG-3′ (antisense; 620–644 nt, β-casein); primer M, 5′-GCTGGACAATAGCGTGTTCTTCC-3′ (sense; 170–192 nt, γ-casein); primer N, 5′-GGGAAGGTGTAATCCTTACTGGG-3′ (antisense; 560–583 nt, γ-casein).

3′-RACE was performed using a 3′-Full RACE Core Set (TaKaRa) [26]. RNA was reverse-transcribed to cDNA by AMV (avian myeloblastosis virus) reverse transcriptase in the presence of oligo(dT)-3′ site adaptor primer using a sequential programme of 30 °C for 1 min, 50 °C for 30 min and 95 °C for 5 min, according to the manufacturer's instructions. cDNA was amplified by PCR with 0.2 μM primer A for β-casein (primer B for γ-casein) and 0.2 μM oligo(dT)-3′ site adaptor primer. 5′-RACE was performed using a 5′-Full RACE Core Set (TaKaRa) [27]. RNA was reverse-transcribed to cDNA with phosphorylated primer C for β-casein (primer D for γ-casein). After digestion with RNase H at 30 °C for 60 min, cDNA in 12 μl of water was incubated for 17 h at 16 °C with 1 μl of T4 RNA ligase (40 units), 8 μl of 5 × RNA ligation buffer and 20 μl of 40 % poly(ethylene glycol) 6000 to ligate the 5′-end to the 3′-end. Circular cDNA was used as the template. In the first PCR, circular cDNA was amplified with 0.2 μM each of primers E and F for β-casein (primers G and H for γ-casein). In the second PCR, primers A and I were used for β-casein (primers B and J for γ-casein). RT-PCR was performed using cDNA prepared for 3′-RACE. cDNAs for β- and γ-casein were amplified with primers K and L and with primers M and N respectively. PCR products separated through a 2.0 % (w/v) agarose gel were stained with ethidium bromide. The size of DNA was estimated by comparison with a 100 bp ladder (Pharmacia, Uppsala, Sweden).

Decapping of RNA

In order to remove the 5′-end cap (m^{7}GpppN), 60 μg of RNA was digested with 15 units of tobacco acid pyrophosphatase (Wako, Osaka, Japan) at 37 for 60 min in 120 μl of a solution containing 50 mM sodium acetate (pH 5.5), 10 mM 2-mercaptoethanol, 1 mM EDTA and 120 units of RNase inhibitor [28].

Degradation of mRNA in RRL system

Degradation of mRNA was performed for nuclease-treated RRL (Promega, Madison, WI, U.S.A.) [18]. The reaction mixture consisted of 0.7 vol. of RRL and 0.3 vol. of a solution containing 200 μg/ml RNA and a kit-supplied amino acid mixture. The reactions were carried out at 30 °C for 160 min. RNA in an aliquot was extracted with ISOGEN (Nippon Gene, Osaka, Japan) according to the kit-supplied protocol. Signals on Northern blots were scanned by a densitometer. The $t_{\frac{1}{2}}$ was calculated from the first-order kinetics.

Statistics

Data are expressed as means ± S.E.M. Statistical analyses were carried out using Student's t-test, linear regression analysis and analysis of variance (ANOVA) (Multistat; Biosoft, Cambridge, U.K.). Differences were considered to be significant at $P < 0.05$.

RESULTS

Northern blot analysis of β- and γ-casein mRNAs

Pups were removed from lactating mice for 24 h (weaning). At the end of the 24 h weaning period, some mice were again allowed to nurse foster pups for 24 h. At the times indicated in Figure 1, β- and γ-casein mRNAs were analysed by Northern blot analysis. The β-casein mRNA was approx. 1.3 kb in size at 0 h of weaning. After 12 and 24 h of weaning, the β-casein mRNA had moved on the blot, and was shorter by approx. 50 nt. Upon returning the pups, the position of the β-casein mRNA on the blot moved slowly, until it was approx. 100 nt longer than that at 24 h of weaning. At 6 h of nursing, the band of β-casein mRNA consisted of two mRNA species with different mobilities. γ-Casein mRNA was approx. 1.2 kb in length at 0 h of weaning. The length of the γ-casein mRNA changed in a similar manner as for the β-casein mRNA both after weaning and upon returning the pups for nursing.

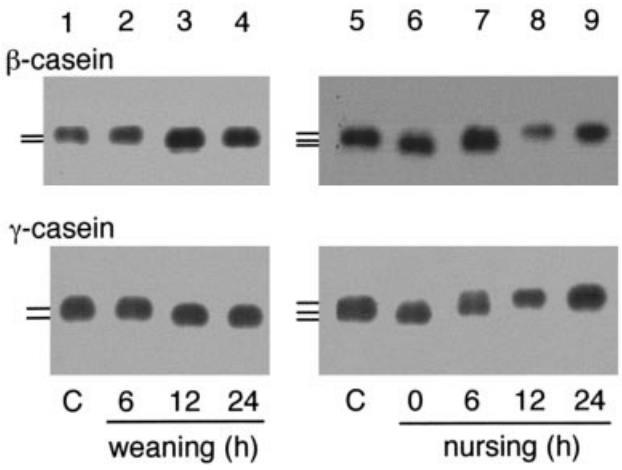


Figure 1 Northern blots of β- and γ-casein mRNAs

Lactating mice (C) had their pups removed for up to 24 h (weaning; left panels), after which some mice were allowed to nurse for up to a further 24 h (right panels). The upper and lower panels show β- and γ-casein mRNAs respectively. The amount of RNA applied was 0.5 μg (lanes 1, 2, 5, 8 and 9), 1 μg (lanes 3 and 7) or 2.0 μg (lanes 4 and 6). The horizontal lines at the left of each panel indicate the peaks of representative mRNA species.

Structures of β- and γ-casein mRNAs

The long and short forms of casein mRNAs were obtained from the W0 (0 h of weaning) and W24 (24 h of weaning) groups respectively. The structures of these mRNAs were analysed by 5′ and 3′ RACE, RT-PCR and RNase H digestion. The expected sizes of PCR products are shown in Figure 2(A). Differences in mobility between the long and short forms were not observed by 5′ RACE, RT-PCR or 3′ RACE (Figure 2B), indicating that, in any form, β-casein mRNAs from the 5′ to the 3′ end [excluding the poly(A) tails] consist of 1120 nt, i.e. the expected size. The poly(A) tail lengths of casein mRNAs were determined by RNase H digestion. Northern blot analysis revealed that β-casein mRNA preincubated with oligo(dT) migrated faster than that preincubated without oligo(dT) (Figure 2C). β-Casein mRNAs from the W0 and W24 groups had the same mobility after preincubation with oligo(dT), with a size of approx. 1.1 kb. The results thus indicated that the two β-casein mRNAs were

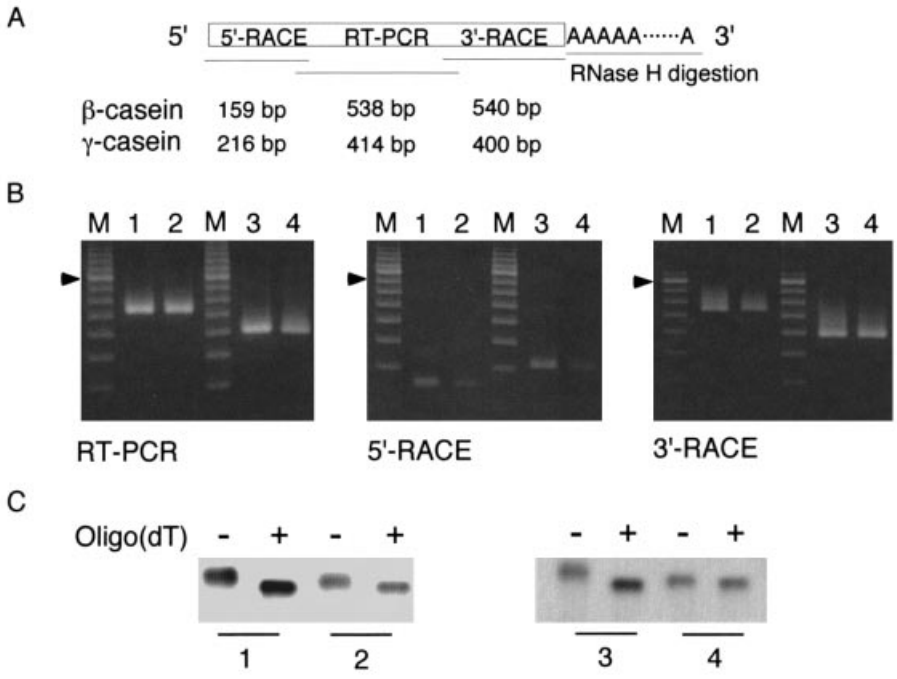


Figure 2 Structures of β- and γ-casein mRNAs from the W0 and W24 groups

(**A**) The schematic strategy of analysis and the expected lengths of PCR products are shown. (**B**) Lanes 1 and 2, β-casein mRNA; lanes 3 and 4, γ-casein mRNA; lanes 1 and 3, mRNA from W0 animals; lanes 2 and 4 mRNA from W24 animals. Lanes M contain 100 bp DNA size markers, and the arrowhead shows the 800 bp marker. (**C**) RNA preincubated with (+) or without (−) oligo(dT) was digested by RNase H. Left panel, β-casein mRNA; right panel, γ-casein mRNA. Lane numbers correspond to those in (**B**).

Table 1 Poly(A) tail lengths of β- and γ-casein mRNAs

The assay conditions are described in the text. As controls, RNA samples prepared from mammary glands of C3H/He mice were similarly analysed. Values are means ± S.E.M. ($n = 5$). Significance of differences: $^{*}P < 0.05$; $^{**}P < 0.01$ compared with W0; †$P < 0.05$; ††$P < 0.01$ compared with W24.

	Poly(A) tail length (nt)		
mRNA	W0	W24	W24/N12
ICR mice			
β-Casein	87 ± 6	31 ± 7*	155 ± 3††
γ-Casein	72 ± 15	20 ± 12**	126 ± 44†
G3PDH	39 ± 9	24 ± 2	19 ± 7
C3H/He mice			
β-Casein	95 ± 9	37 ± 3**	118 ± 17††
γ-Casein	64 ± 10	23 ± 9*	95 ± 11††

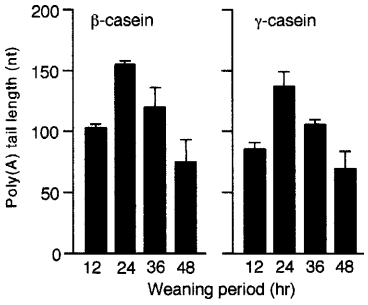


Figure 3 Effect of the weaning period on the change in the poly(A) tail length of β- and γ-casein mRNAs

Young were removed for 12, 24, 36 or 48 h (weaning period), and then returned for 12 h (nursing). Values are means ± S.E.M. ($n = 3$).

the same length from the 5′ to the 3′ end, but that the length of the poly(A) tail was different. The results with γ-casein mRNA were identical with those observed for β-casein mRNA. Taken together, the difference in mRNA mobility on Northern blots (Figure 1) could be entirely explained by the difference in the poly(A) tail length of the mRNAs.

Poly(A) tail lengths of β- and γ-casein mRNAs during weaning and during nursing

The poly(A) tail lengths for β-casein, γ-casein and G3PDH mRNAs are shown in Table 1. For β-casein mRNA, the length of the poly(A) tail decreased from 87 to 31 nt during the 24 h weaning period ($P < 0.05$), but increased to 155 nt during the subsequent 12 h nursing period ($P < 0.01$). For γ-casein mRNA, the length of the poly(A) tail decreased during the 24 h weaning period ($P < 0.05$), but increased during the subsequent 12 h nursing period ($P < 0.01$). The poly(A) tail length of the G3PDH mRNA did not change. In C3H/He mice also, the length of the poly(A) tail of each casein mRNA decreased and increased during the weaning and nursing periods respectively.

The effect of the weaning period on poly(A) tail length was determined by separating pups from the mothers for 12, 24, 36 or 48 h; dams were then allowed to nurse foster pups for 12 h. For both the β- and γ-casein mRNAs, the poly(A) tail length increased during the 12 h nursing period ($P < 0.05$) (Figure 3).

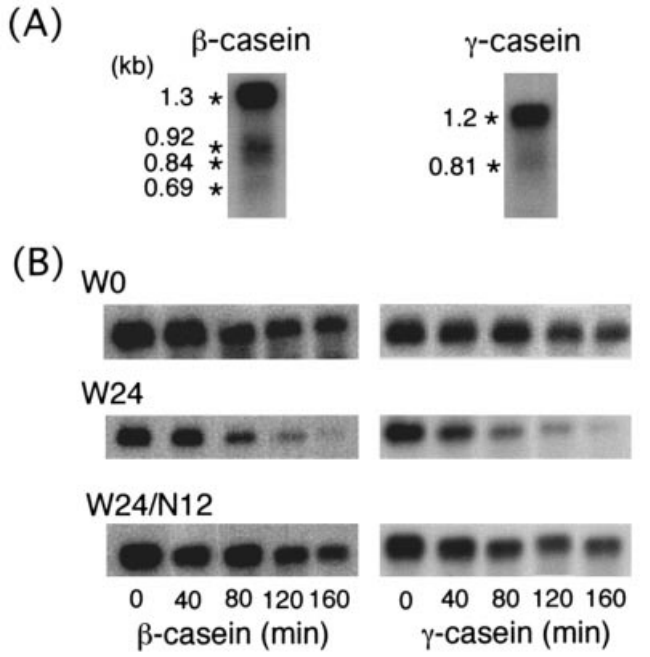


Figure 4 Degradation of β- and γ-casein mRNAs from W0, W24 and W24/N12 animals in the RRL system

At the times indicated, mRNAs were detected by Northern blot analysis. (**A**) RNA samples from W0 mice were incubated in the RRL system for 120 min. For each panel, the top species is the undegraded mRNA, and the lower species are degradation products, with positions marked by asterisks. (**B**) The left- and right-hand panels represent time courses of degradation of β- and γ-casein mRNAs respectively.

The longest poly(A) tail was observed in the W24/N12 group. For weaning periods longer than 24 h the increase in poly(A) tail length during nursing was less, i.e. its degree was dependent on the weaning period (one-factor ANOVA; $P < 0.05$). In the weaned, non-nursing controls, the poly(A) tail length remained at approx. 30 nt for β-casein mRNA and approx. 20 nt for γ-casein mRNA.

Stability of β- and γ-casein mRNAs during weaning and nursing

Using RNAs obtained from the W0, W24 and W24/N12 groups, the stability of β- and γ-casein mRNAs was determined by incubation in an RRL system. During incubation, β-casein mRNA was degraded into three mRNAs, with mobilities of 0.92, 0.84 and 0.69 kb, and γ-casein mRNA was degraded into one mRNA with a mobility of 0.81 kb (Figure 4A). The band intensities of undegraded and degraded mRNAs became weaker and stronger respectively as the incubation progressed. On autoradiograms, both the β- and γ-casein mRNAs decreased; in particular, those from the W24 group disappeared very rapidly (Figure 4B).

The amount of intact mRNA after various incubation periods was compared with that at 0 min of incubation (Figure 5). For the W24 group, the relationships were linear ($r = -0.95$ to -0.97). For the W0 and W24/N12 groups, the amounts of intact β- and γ-casein mRNAs also decreased with time in a linear fashion ($r = -0.89$ to -0.95), but degradation was slower. The $t_{\frac{1}{2}}$ values for β-casein, γ-casein and G3PDH mRNAs are shown in Table 2. For β-casein mRNA, the $t_{\frac{1}{2}}$ decreased from 120 to 70 min during the 24 h weaning period ($P < 0.05$), and

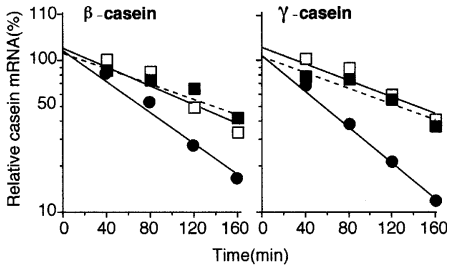


Figure 5 Time-dependent degradation of β- and γ-casein mRNAs in the RRL system

Autoradiograms shown in Figure 4 were densitometer-scanned. The absorbance at 0 min was taken as the 100% value. □, W0; ●, W24; ■, W24/N12 (broken line). Values are means of five different samples.

Table 2 Half-lives of β- and γ-casein mRNAs in the RRL cell-free translation system

The assay conditions are described in the text. The 5′-end cap was removed by pyrophosphatase digestion (decapped). ND, not determined. Values are means ± S.E.M. ($n = 5$). Significance of differences: *$P < 0.05$ compared with W0; †$P < 0.05$ compared with W24.

	$t_{\frac{1}{2}}$ (min)		
mRNA	W0	W24	W24/N12
β-Casein	120 ± 13	70 ± 12*	137 ± 10†
Decapped	137 ± 15	77 ± 2*	ND
γ-Casein	139 ± 26	57 ± 8*	121 ± 15†
Decapped	138 ± 19	74 ± 10*	ND
G3PDH	102 ± 9	103 ± 14	ND

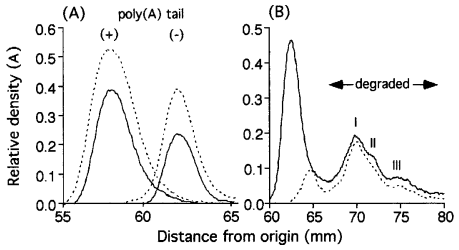


Figure 6 Densitometry of β-casein mRNA

(**A**) RNA samples from W24/N12 mice, preincubated for 0 min (broken line) or 120 min (solid line) in the RRL system, were incubated with RNase H in the presence (right) or absence (left) of oligo(dT). (**B**) RNA samples from W0 (solid line) or W24 (broken line) animals were incubated in the RRL system for 160 min. Distinct bands are indicated by roman numbers.

increased again to 137 min during the 12 h nursing period ($P < 0.01$). The $t_{\frac{1}{2}}$ values for decapped mRNAs were the same as those for capped mRNAs. For γ-casein mRNA, the results were essentially the same as those seen with β-casein. The $t_{\frac{1}{2}}$ of G3PDH mRNA was not changed after 24 h of weaning.

Northern blots were densitometer-scanned, and representative results for β-casein mRNA are shown in Figure 6. Throughout incubation in the RRL system, the distance to the peak position and the absorbance profile were the same as those of the 0 min control (Figure 6A). Three distinct bands of degraded mRNAs, with mobilities of 0.92 (I), 0.84 (II) and 0.69 (III) kb, were detected (Figure 6B). The 0.92 kb fragment was detected after 40 min of incubation and remained dominant until the end of the incubation. For γ-casein mRNA, the results were essentially the same as seen for β-casein mRNA, except that one distinct band of degraded mRNA of size 0.81 kb was detected (see Figure 4A).

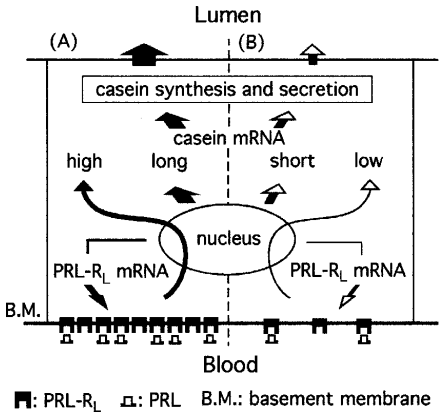
DISCUSSION

We have observed that, without alterations in the coding and non-coding regions of casein mRNA, the poly(A) tail of this mRNA, but not of G3PDH mRNA, is shortened and elongated in the lactating mammary gland depending upon the accumulation and removal respectively of the gland's milk. It has been reported that the lengths of the poly(A) tails of mRNAs for metallothionein [23], glucose transporter-1 [29], c-*myc* [30], albumin [31], arginine-vasotocin [32] and vasopressin [33] can be shortened under both normal and artificially regulated conditions. The poly(A) tails of M1 and M2 pyruvate kinase mRNAs are elongated and shortened respectively during spermatogenesis [34]. In the present experiments, the poly(A) tail of G3PDH mRNA was not altered in length, confirming previous results [29,30].

For c-*fos* mRNA, shortening of the poly(A) is translation-dependent, as it is slowed down by a translation block [30,35]. Similar results were reported for the thyrotropin β-subunit mRNA in TtT97 thyrotropic tumour cells [36]. However, in the present study the poly(A) tail lengths of the casein mRNAs began to be shortened under conditions of maximum milk accumulation; translational activity is low in this state. Conversely, elongation of the poly(A) tail length of casein mRNA occurred under conditions where the translational activity switched to high. When pups are returned to the mother at 24 h after weaning, the ability to synthesize casein mRNA [9] and to produce milk [37] are restored in the lactating mammary gland. It appears that our data are inconsistent with the evidence described above [30,35,36], but are consistent with the findings that stabilization of β-casein mRNA requires ongoing protein synthesis *in vitro* [12]. Taken together, in the present study shortening of poly(A) tail occurred when translational activity became low, whereas elongation was associated with a high level of translation.

Using the RRL cell-free translation system means that mRNA is able to form a complex with the ribosomes, as mRNA is normally present as polysomes in the cytoplasm. Thus this system should reflect the situation *in vivo*. It is known that the relative stabilities of various mRNAs in the RRL system correlate well with their relative stabilities in living cells [18,19].

Poly(A)-binding protein (PABP) binds to poly(A) at a ratio of 27 nt per one PABP molecule (including the space between two PABPs) [38], and is present in the cytoplasm at a concentration sufficient to bind all of the poly(A) of mRNA [39]. The poly(A)–PABP complexes at the mRNA 3′ terminus protect mRNA from rapid destruction *in vitro* [40]. The poly(A) tail inhibits the assembly of a 3′ to 5′ exoribonuclease, and stabilization of poly(A) requires the interaction of proteins with the poly(A) tail [41,42]. PABP-depleted c-*myc* mRNA is no longer resistant to RNase [43]. Although poly(A) tail shortening by exoribonuclease was not observed in the RRL system, distinct bands of degraded mRNAs were detected on Northern blots. The blots also showed that the long poly(A) tail efficiently protected mRNA from degradation by endoribonucleases. The mechanism for this is not known, but we speculate that the long poly(A) tail effectively protects mRNA from endoribonucleases,



Scheme 1 Schematic presentation of the regulation of milk synthesis in the lactating mammary gland

In (**A**), PRL-R$_L$ mRNA synthesis is active and the poly(A) tail of casein mRNA is long. In (**B**), PRL-R$_L$ mRNA synthesis is inactive and the poly(A) tail of casein mRNA is short. The mammary gland changes from (**A**) to (**B**) after stopping nursing, but returns to (**A**) reversibly on commencing nursing. Closed and open arrowheads indicate the signal as either strong or weak respectively. PRL, prolactin. Data for PRL-R$_L$ and casein mRNAs are from [8,9].

probably through the association of PABP with some protein(s) in the protein–mRNA complex at the 3′-end region. Recently it was shown that the association of PABP in the poly(A)–PABP complex with the PABP-interacting protein (PAIP-1) on mRNA stimulates translation in mammalian cells [44].

The mammary gland at mid-lactation actively synthesizes caseins, resulting in high levels of their mRNAs [2]. The accumulation of casein mRNA is due to an increase in the transcription rate (2–4-fold) and efficient stabilization (17–25-fold), as demonstrated *in vitro* [45]. The level of casein mRNA declines following weaning in rats and mice [8,9]. As the casein mRNA with the short poly(A) tail had a short half-life in the RRL system, we suggest that shortening of the poly(A) tail is involved in the disappearance of casein mRNA from the mammary gland during the weaning period. The effect of the poly(A) tail on translational efficiency depends on its length [46], via interaction with the 5′-end cap site of the mRNA [47,48]. The present data showed that the half-life of casein mRNA was not affected by either the presence or absence of the 5′-end cap.

In Nature, the lactating mother is not always able to nurse her young successfully, and on occasion she may be temporarily apart from her young. Under these circumstances, the mother must slow down the rate of milk synthesis within a short period in order to prevent over-accumulation of the gland's milk. Once reunited with her young, it is necessary for the mother to resume milk supply. With a longer weaning period, we know that the mammary gland would begin to regress. Based on the available evidence, the possible regulatory mechanisms that occur *in vivo* are shown in Scheme 1. However, this model should be evaluated using either a culture system suitable for the lactating mammary gland or a mammary gland cell-free translation system. Due to the high metabolic activity of the lactating mammary gland and the high RNase activity of mammary gland lysates, neither of these systems are yet available.

Received 20 September 1999/10 January 2000; accepted 15 February 2000

This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan, and from the Morinaga Hôshikai, Mishima Kaiun and Rhoshoku Kenkyukai Science Foundations of Tokyo.

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