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α-Lactalbumin mRNA in 4-day lactating rat mammary gland

(milk protein mRNAs/wheat germ system/cDNA·RNA hybridization)

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ABSTRACT **Rat α-Lactalbumin (α-LA) mRNA has been purified from the total RNA of 4-day lactating mammary gland by poly(U)-Sepharose chromatography and by fractionation of the poly(A)$^+$ RNA on sucrose gradients. α-LA mRNA was homogeneous as judged by electrophoresis in urea/agarose gel and in 1.5% agarose gel under glyoxal-denaturation conditions, which gave a molecular weight of 210,000. The chymotryptic fingerprints of the protein synthesized with this mRNA, in a translational system derived from wheat germ, were similar to those of purified rat α-LA. In rat milk or lactating gland, the content of M_r 42,000 casein is 6 times greater than that of M_r 29,000 casein and 10 times greater than that of α-LA. However, α-LA mRNA (M_r 210,000) and two casein mRNAs (M_r 460,000 and 390,000), coding for M_r 42,000 and 29,000 caseins, were present in equal proportions when measured with cDNA probes in the total RNA of 4- to 5-day lactating gland. Moreover, α-LA and total casein were synthesized in a ratio of 1:2.5 in a wheat-germ translational system using the total RNA of the lactating gland. The results suggest post-transcription controls in the synthesis of these proteins.**

Epithelial cells in the mammary gland proliferate extensively during pregnancy (1, 2) and synthesize large amounts of mammary-specific proteins—α-lactalbumin (α-LA) and casein—during gestation and lactation. The synthesis and secretion of these proteins are regulated by the multiple interactions of several peptide and steroid hormones (3–5). α-LA has been purified from rat milk (6, 7) and shown to exist in two forms—α-LA_1 and α-LA_2 (7). α-LA_1 migrates as M_r 21,500 and α-LA_2 as M_r 22,500 protein in sodium dodecyl sulfate/polyacrylamide gels but sedimentation equilibrium analysis showed M_rs of 21,000 for α-LA_1 and 16,000 for α-LA_2 (7). Both forms contain 7–9% carbohydrate and act as modifiers of galactosyltransferase, thus catalyzing the formation of lactose from UDP-galactose and glucose (8). Three species of casein with M_rs of 42,000, 29,000, and 25,000, respectively, also have been purified from rat milk (9).

Milk protein mRNAs have been isolated from various species (10–13) with the ultimate objective of understanding the mechanisms that regulate the synthesis of these proteins at the level of transcription and translation. As a contribution to this objective, we report that, in the 4-day lactating mammary gland of the rat, two casein mRNAs (M_r 460,000 and 390,000), coding for M_r 42,000 and 29,000 caseins, and α-LA mRNA (M_r 210,000) are present in equal proportions when measured with cDNA probes in the total RNA of the gland. We also report that, in a wheat-germ translational system, total RNA of 4- to 5-day lactating gland synthesizes α-LA and total caseins in a ratio of 1:2.5 (14). However, in the 4- to 5-day lactating gland, as well as in rat milk, the proportions of the proteins coded by these messages is markedly different; M_r 42,000 casein is 6-fold greater than M_r 29,000 casein and 10-fold greater than α-LA, which suggests post-transcription controls in the accumulation of these proteins.

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MATERIALS AND METHODS

Materials. Poly(U)-Sepharose was obtained from Pharmacia Fine Chemicals; wheat germ from General Mills, Inc., (Minneapolis, MN); chymotrypsin from Worthington Biochemical Corporation; S1 nuclease from Miles Laboratories; and reverse transcriptase from the Viral Oncology Services Branch of the National Institutes of Health. [^{3}H]Leucine and [α-^{32}P]GTP were purchased from ICN Pharmaceuticals. "StainsAll" was acquired through Eastman Kodak Company and acridine orange, from Matheson, Coleman and Bell.

Methods. Rat α-LA, casein, and their antisera were prepared as described (9, 13). Isolation of total poly(A)-bearing RNA, conditions for translation, immunoprecipitation of *in vitro* synthesized protein, and $NaDodSO_4$/polyacrylamide gel analysis of the trichloroacetic acid-precipitated and immunoprecipitated proteins have been described (13).

Poly(A)$^+$ RNA was fractionated in linear 4–20% sucrose gradient containing 70% formamide, 10 mM Tris (pH 7.0), 0.1 M NaCl, and 1 mM EDTA by centrifugation at 35,000 rpm for 36 hr in a Beckman SW 41 rotor at 9°. Gel electrophoresis of the RNA was carried out under glyoxal-denaturation conditions or in agarose/urea gels as described (13, 15).

Synthesis of cDNA was carried out in a final volume of 0.1 ml and the mixture consisted of 60 μg of mRNA, 700 units of reverse transcriptase and 20 μg of oligo dT_{12-18} per ml, 100 μM each of the triphosphates (600 μM for casein cDNA synthesis), [α-^{32}P]dGTP (25 Ci/mmol), 140 mM KCl (omitted for casein cDNA synthesis), 7 mM $MgCl_2$, and 100 μg of actinomycin D per ml. The mixture was incubated for 1 hr at 40° and cDNA was purified on alkaline 10–30% sucrose gradients. The size of ^{32}P-labeled cDNA was measured by the procedure of Hayward (16) by electrophoresis in an alkaline agarose slab gel. ^{32}P label was monitored by exposing the gel to x-ray film RP/R_2 from Kodak for 1½ hr.

Chymotryptic Digestion and Peptide Patterns. Three milligrams of rat α-LA_1 was mixed with trichloroacetic acid-precipitated *in vitro* synthesized protein, reduced, and alkylated. The chymotryptic digestion and the two-dimensional combination of high-voltage paper electrophoresis and chromatography were carried out as described (17). Fluoroautoradiography was carried out according to the method of Bonner and Laskey (18).

RESULTS

Purification and Characterization of α-LA mRNA. The purification of α-LA and casein mRNAs was followed by translating the RNA in the wheat-germ translational system and immunoprecipitating the *in vitro* synthesized protein by using antisera prepared against purified rat α-LA and against a mixture of purified M_r 42,000, 29,000, and 25,000 caseins (13). *In vitro* synthesized, immunoprecipitated α-LA and caseins were identified on sodium dodecyl sulfate/polyacrylamide gels.

Abbreviation: α-LA, α-lactalbumin.

Twenty five to 30% of the total protein synthesized *in vitro*, either for 60 or 120 min, from the total cellular RNA of 4- to 5-day lactating gland, with either [^{3}H]leucine or [^{35}S]methionionine, precipitated as α-LA and 60–65% precipitated as casein (Table 1). Direct comparison on sodium dodecyl sulfate/polyacrylamide gels of the total *in vitro* synthesized protein versus immunoprecipitated materials showed that the material synthesized *in vitro*, using mRNA from the 4- to 5-day lactating gland, was mainly composed of the α-LA and casein proteins. However, the ratio of α-LA to casein synthesis remains about 1:2.2 in the RNAs extracted from the gland only from the first to fourth days of lactation and thereafter the ratio changes, showing a relative increase in casein mRNA activity and the emergence of other mRNA activities (unpublished data).

When isolated on poly(U)-Sepharose columns, the poly(A)$^+$ RNA synthesized 50% of the total protein as α-LA and the remainder as casein (Table 1). Poly(A)$^+$ RNA obtained from dT-cellulose columns synthesized more casein protein than α-LA. This suggests that the casein mRNA has shorter poly(A) tails than α-LA mRNA because RNAs with shorter and longer poly(A) tails are equally well selected on dT-cellulose columns but poly(U)-Sepharose shows poly(A) length dependence.

Poly(A)$^+$ RNA, purified twice on a dT-cellulose column, showed four main bands when electrophoresed as glyoxal-denatured RNA in 1.5% agarose gels (Fig. 1 *left inset*). Materials migrating as 28S RNA and two small, closely spaced RNA bands were also detectable on the gels. By comparison against *Hae* III-digestion fragments of φX174RF DNA and against 23S, 16S, and 5S *E. coli* RNA markers, the M_rs of the major bands were 460,000, 390,000, 290,000, and 210,000. Sedimentation of the poly(A)$^+$ RNA, previously purified on the poly(U)-Sepharose column, showed a peak of absorbance around 8.3 S (fractions 40–46, Fig. 1). This RNA has been previously shown to contain α-LA mRNA activity (13). The RNA sedimenting between 11 and 16 S (fractions 15–38) shows casein mRNA activity (13). Gel analysis, under glyoxal-denaturation conditions, of the RNA sedimented on the gradient and the pooled fractions is shown in Fig. 1 *right inset*. Fractions 40–46 showed a major RNA band of M_r 210,000 corresponding to α-LA mRNA and a smaller RNA species below it. Fractions 24–34 showed three main RNA bands corresponding to M_rs of 460,000, 390,000, and 290,000. Fractions 14–23 showed three RNA bands; the top band had the mobility of 18S rRNA and the two others corresponded to M_r 460,000 and 390,000 casein mRNAs. The slower mobility of M_r 390,000 RNA in this sample may be due to incomplete denaturation of the RNA.

Table 1. α-LA and casein mRNA activity

RNA	Synthesis time, min	Total mRNA activity, cpm/μg RNA × 10^{-3}	mRNA activity, % of total α-LA	mRNA activity, % of total Casein
4-day lact. total mRNA	60	28.97	29.7	65.1
	120	50.14	24.6	60.9
Poly(A)$^+$ RNA:				
dT-Cellulose	60	9.23	54.4	59.0
	120	30.6	35.6	45.8
Poly(U)-Sepharose	60	17.56	50.6	50.5
	120	39.86	52.8	49.2

RNA was translated in a wheat-germ translational system as described (13). α-LA and casein mRNA activities were measured as the counts precipitated with the corresponding antiserum from the synthesized protein. The results are expressed as percentage of the total *in vitro* synthesized protein.

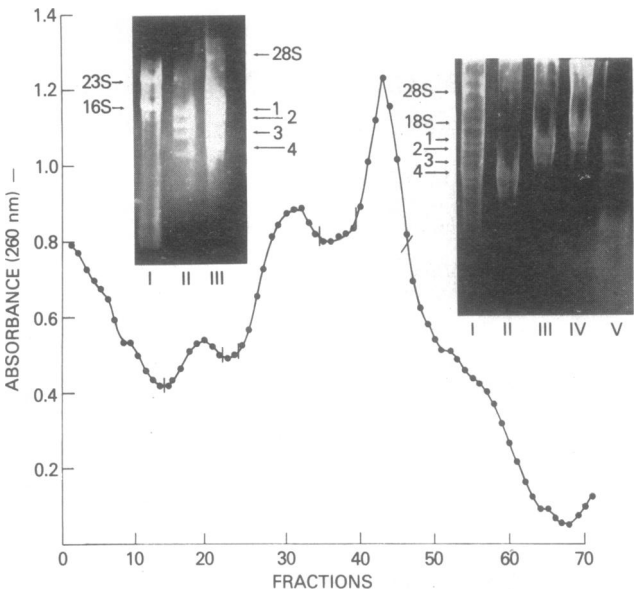

FIG. 1. Sedimentation of poly(U)-Sepharose-purified poly(A)$^+$ RNA from 4-day lactating rat mammary gland on 4–20% sucrose gradients (500 μg of RNA per gradient). (*Insets*) Gel electrophoresis of glyoxal-denatured RNA and DNA in 1.5% agarose gels. (*Left Inset*) Lane I: 23S, 16S, 5S, and 4S *Escherichia coli* RNA markers. Lane II: 1342-, 1078-, 872-, and 606-nucleotide-long fragments of φX174 RF DNA. Lane III: 30 μg of poly(A)$^+$ RNA purified on dT-cellulose column. Bands 1, 2, 3, and 4 correspond to poly(A)$^+$ RNA of M_rs 460,000, 390,000, 290,000, and 210,000, respectively. (*Right Inset*) Lane I: 20 μg of poly(A)$^+$ RNA purified on poly(U)-Sepharose column. Lane II: RNA from fractions 40–46. Lane III: fractions 24–34. Lane IV: fractions 14–23. Lane V: *Hae* III-digested fragments of φX174 DNA.

Pooled fractions from the first gradient containing α-LA mRNA (fractions 40–46, Fig. 1) and casein mRNA (fractions 24–34, Fig. 1) were resedimented on 4–20% sucrose in 70% formamide (Fig. 2). The peak (fractions 25–28) in the α-LA mRNA gradient (Fig. 2*A*) were analyzed on urea/agarose gels (Fig. 2*A*, *right inset*) and as glyoxal-denatured RNA in 1.5% agarose gels (Fig. 2*A*, *left inset*). The RNA electrophoresed as a single band with a M_r of about 210,000. Individual casein mRNAs were not well separated under these conditions (Fig. 2*B*). However, RNA from fractions 6–10 gave a relatively pure band of RNA electrophoresing as 16 S when analyzed by the urea/agarose gels (Fig. 2*B inset*, lanes 1 and 2). Under glyoxal-denaturation conditions, this RNA showed two RNA species with M_rs of 460,000 and 390,000 (Fig. 2*B inset*, lanes 3 and 4). Henceforth, this fraction is called 16S doublet RNA. RNA sedimenting between fractions 25 and 30 (Fig. 2*B*) showed predominately 12S casein mRNA (M_r 290,000) and also contained some 16S mRNA (data not shown).

Pooled fractions of α-LA mRNA (Fig. 2*A*, fractions 25–28) and of casein mRNA (Fig. 2*B*, fractions 15–20) were used in the wheat germ translational system for *in vitro* protein synthesis. Table 2 shows the immunospecificity of the *in vitro* synthesized proteins. Ninety-five percent of the proteins synthesized *in vitro* with α-LA mRNA fractions precipitated with α-LA antiserum and about 13% with the casein antiserum. Normal serum, however, precipitated about 5% of the counts. Unlabeled α-LA protein, but not the mixture of M_r 42,000, 29,000, and 25,000 caseins competed for this immunoprecipitation. Proteins synthesized with a 16S and 12S casein mRNA mixture were quantitatively precipitable with the casein antiserum and competed only with the mixture of M_r 42,000, 29,000, and 25,000 unlabeled caseins. After sodium dodecyl sulfate/polyacrylamide electrophoresis, both trichloroacetic acid-insoluble and immunoprecipitated products of α-LA

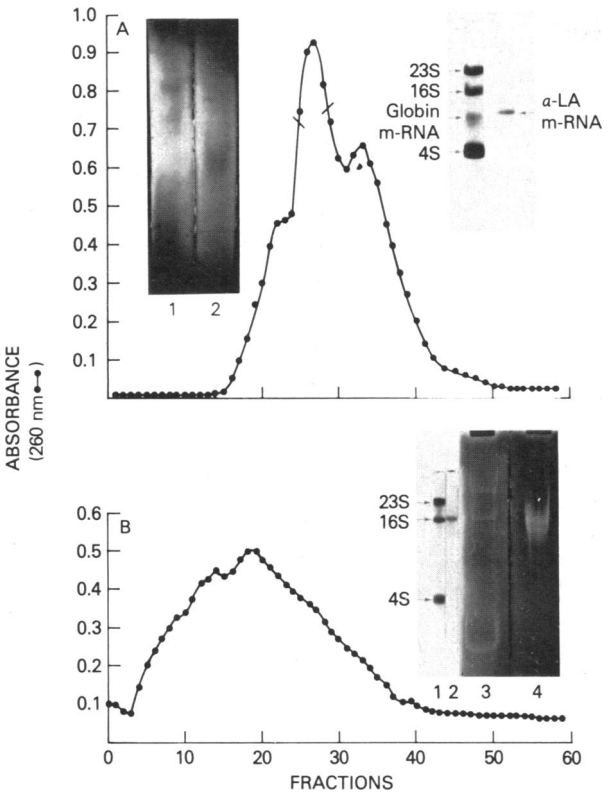

FIG. 2. (*A*) Sedimentation of 200 μg of poly(A)$^+$ RNA, from the pool of fractions 40–46 of Fig. 1, on 4–20% sucrose in 70% formamide. (*Left Inset*) Electrophoresis of glyoxal-denatured 23S and 16S *E. coli* rRNA markers in 1.5% agarose gel (lane 1) and of 9 μg of α-LA mRNA from combined fractions 25–28 (lane 2). (*Right Inset*) Electrophoresis in urea/agarose gels (7 μg of α-LA mRNA). (*B*) Sedimentation of 170 μg of poly(A)$^+$ RNA, from the pool of fractions 24–34 of Fig. 1 on 4–20% sucrose gradients in 70% formamide. (*Inset*) Electrophoresis of 23S, 16S, and 4S *E. coli* RNA (lanes 1 and 3) and 10 μg of RNA from fraction 10 (lanes 2 and 4). Lanes 1 and 2 were urea/agarose gels; lanes 3 and 4 were run under glyoxal-denaturation conditions.

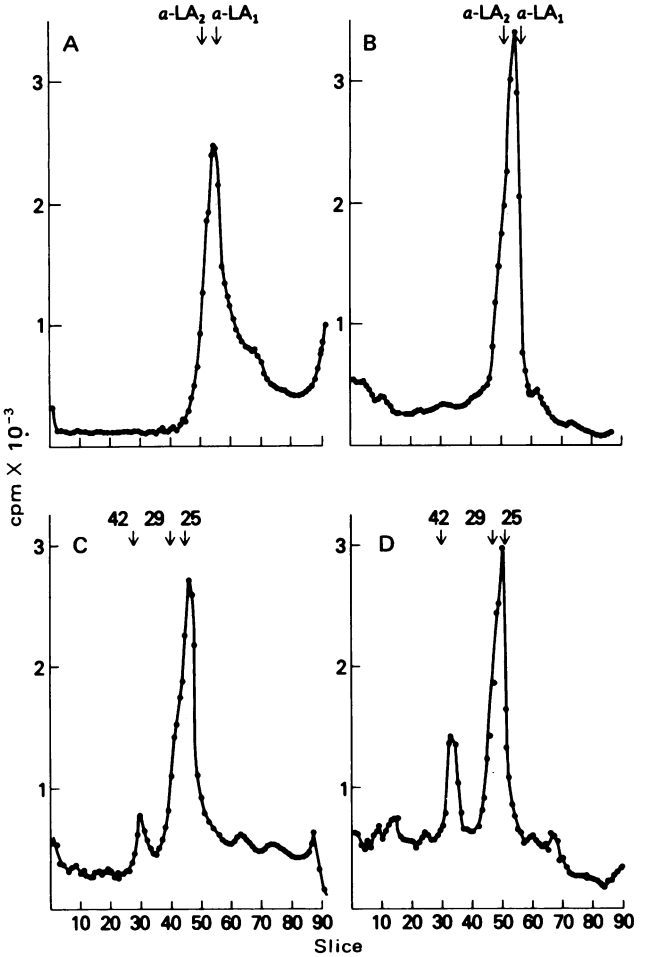

FIG. 3. Sodium dodecyl sulfate/polyacrylamide (12.5%) gel analysis of trichloroacetic acid-insoluble (*A* and *C*) and immunoprecipitated (*B* and *D*) translation products of α-LA mRNA (*A* and *B*) and a mixture of 16S doublet and 12S casein mRNAs (*C* and *D*). In *C* and *D*, arrows indicate positions of M_r 42,000, 29,000, and 25,000.

mRNA translation migrated very close to the rat α-LA marker position (Fig. 3 *A* and *B*), and the products of 16S and 12S casein mRNA migrated close to the M_r 42,000, 29,000, and 25,000 casein marker positions (Fig. 3 *C* and *D*).

The trichloroacetic acid-precipitated product from α-LA mRNA translation was subjected to chymotryptic digestion and analyzed for peptides; the maps were compared to those derived from α-LA$_1$. [^{3}H]Leucine-labeled *in vitro* synthesized protein was reduced and carboxymethylated along with unlabeled α-LA$_1$ carrier protein. After chymotryptic digestion, two-dimensional chromatography, and fluororadiography of the chromatogram, the autoradiogram (Fig. 4*A*) was compared to the ninhydrin spots in the chromatogram derived from α-LA$_1$ (Fig. 4 *B* and *C*). All of the [^{3}H]leucine-labeled peptides were present in the α-LA$_1$ fingerprints (compare *A* to *C*). Because the *in vitro* synthesized protein was labeled with [^{3}H]-leucine, the absence of certain spots in the fluoroautoradiogram compared to the ninhydrin spots in the chromatogram might suggest the absence of leucine in these peptides. The chromatogram that was treated with 2,5-diphenyloxazole for fluoroautoradiography gave less-intense ninhydrin spots of the peptides derived from carrier α-LA$_1$; for clarity, the latter were circled with pencil (Fig. 4*B*). Nevertheless, the spots were comparable to the spots in the autoradiogram (Fig. 4 *B* and *A*).

Table 2. Immunoprecipitation specificity of *in vitro* synthesized protein from α-LA and casein mRNA

mRNA	Antiserum	Competitor	% of insoluble counts*
α-LA	α-LA	None	94.8
		α-LA, 20 μg	5.7
		Casein, 20 μg	89.3
	Casein	None	12.6
	Normal serum	None	4.8
16S and 12S casein	Casein	None	98.0
		Casein, 20 μg	6.5
		α-LA, 20 μg	97.2
	α-LA	None	11.2
	Normal serum	None	3.2

* Precipitated with trichloroacetic acid. For α-LA and caseins, 112,000 and 320,000 cpm, respectively, were used for immunoprecipitation.

Quantitation of α-LA and 16S Casein mRNA with cDNA Probes. cDNA Probes were synthesized from α-LA mRNA peak fractions (Fig. 2*A*) and from 16S doublet casein mRNA (Fig. 2*B*, fractions 6–10). The ^{32}P-labeled cDNAs used for hybridizations were characterized on alkaline/agarose gels (Fig. 5 *inset*). α-LA and casein cDNAs were about 450 and 750

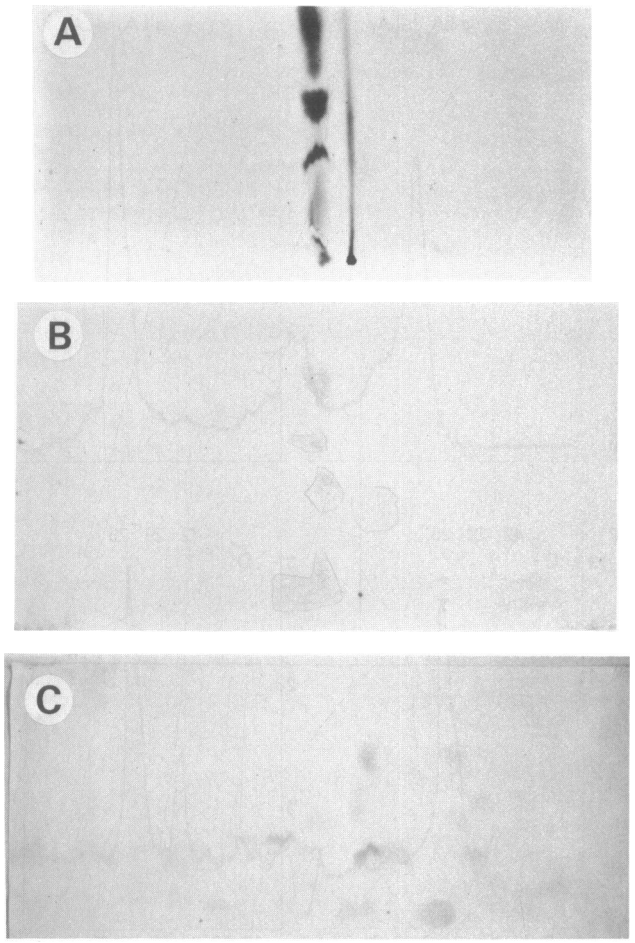

FIG. 4. (*A*) Fluoroautoradiogram of the chymotryptic digest of [^{3}H]leucine-labeled translational product of α-LA mRNA and unlabeled α-LA$_1$ carrier. (*B*) The same chromatogram developed with ninhydrin. (*C*) The chromatogram of chymotryptic fingerprints of α-LA$_1$ developed with ninhydrin but without 2,5-diphenyloxazole treatment.

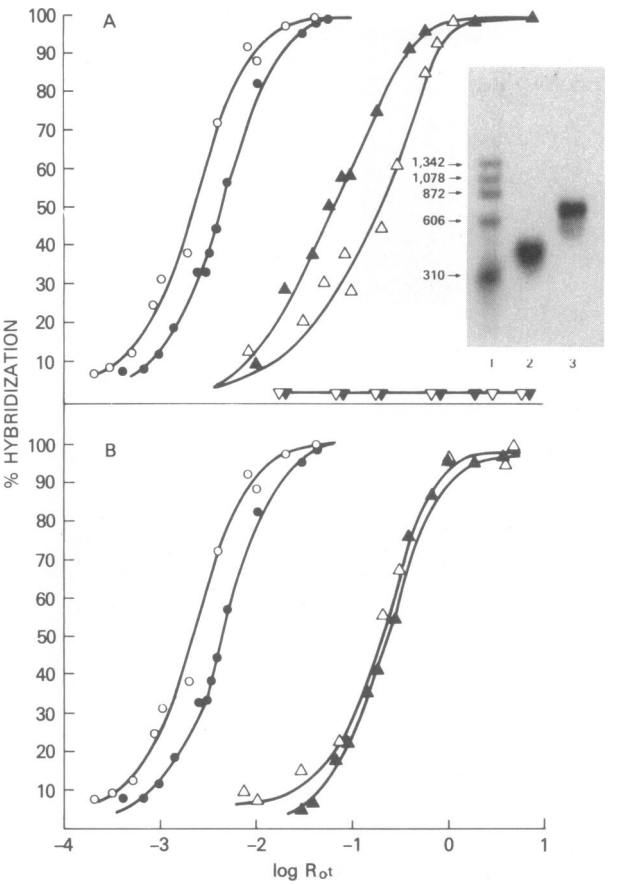

FIG. 5. Specificity of ^{32}P-labeled α-LA and casein cDNAs. Hybridization of excess RNA to cDNA was performed in a final volume of 50 μl containing 0.6 M NaCl, 0.01 M Tris·HCl (pH 7.0), 2 mM Na_2EDTA, 200 μg of yeast RNA per ml, 0.1% sodium dodecyl sulfate, about 1500 cpm of either cDNA and varying amounts of RNA. The extent of hybridization was assessed by S1 nuclease digestion. (*A*) RNA excess hybridization of α-LA cDNA with α-LA mRNA (○), casein M_r 460,000 and 390,000 mRNA (△), and rat liver total RNA (▽). Hybridization of casein cDNA with casein 16S doublet M_r 460,000 and 390,000 mRNA (●), α-LA mRNA (▲), and rat liver total RNA (▼). (*Inset*) Alkaline/agarose gel analysis of ^{32}P-labeled *Hae* III-digested fragments from φX174 RF DNA (lane 1), ^{32}P-labeled α-LA (lane 2), and casein cDNAs (lane 3). (*B*) Hybridization of total RNA from 4-day lactating rat mammary gland with α-LA (△) and casein (▲) cDNA probes. Also shown is hybridization of α-LA cDNA with α-LA mRNA (○) and casein cDNA with casein mRNA (●).

nucleotides long, respectively. α-LA cDNA and casein cDNA hybridized with their own mRNAs as a single component with a $R_0t_{1/2}$ of 2.3×10^{-3} and 4.5×10^{-3} mol·sec/liter, respectively (Fig. 5*A*). The hybridizations occurred within 1½ orders of magnitude, as expected of pseudo-first-order reactions. Hybridization of α-LA [^{32}P]cDNA with its own mRNA at $R_0t = 5 \times 10^{-3}$ gave nearly 100% S1-resistant α-LA cDNA. The casein [^{32}P]cDNA hybridized with its own mRNA with only 85% efficiency even at high R_0t values and was normalized to 100% so that it could be compared with α-LA cDNA hybridization. ^{32}P-Labeled α-LA cDNA probe required a 100-fold excess of 16S casein mRNA to obtain comparable hybridization efficiencies. This indicates about 1% contamination of casein mRNA sequences in the α-LA mRNA. Similar analysis of the casein cDNA probe, however, showed 6% contamination of α-LA mRNA in casein mRNA (Fig. 5*A*). The total RNA of 4-day lactating gland was hybridized with these probes (Fig. 5*B*). α-LA and 16S casein mRNA sequences were quantitated in this total RNA ($100 \times R_0t_{1/2}$ pure mRNA ÷ $R_0t_{1/2}$ given RNA). The α-LA mRNA sequences were 0.9% and the casein doublet mRNA sequences were 1.8% of the total RNA (Table 3). α-LA protein and the individual caseins were quantitated by a radioimmunoassay (19) in 4-day lactating gland. α-LA was about 0.6% of the total protein of the gland and M_r 42,000, 29,000, and 25,000 caseins were about 6.6, 1.0, and 1.8%, respectively. α-LA and caseins were also measured in the milk and were present in similar proportions as in the gland.

Table 3. α-LA and casein proteins and their mRNAs in 4- to 5-day lactating rat mammary gland

		Caseins		
	α-LA	42,000	29,000	25,000
Proteins*	0.60	6.6	1.0	1.8
mRNA†	0.90		1.8‡	

* α-LA and individual caseins were measured by a radioimmunoassay and are expressed as percentage of the total protein in the gland.
† α-LA mRNA and 16S doublet casein mRNA quantities, determined by hybridization with their cDNA probes, are expressed as percentage of the total RNA.
‡ 16S doublet.

DISCUSSION

In the milk from 5- to 8-day lactating rat, α-LA represents about 4% of the total protein quantitated either by radioimmunoassay or by enzymatic assay. Casein comprises about 50% of the total protein of milk and, when separated into individual caseins by DEAE-cellulose column chromatography, shows a ratio of 5:1:2 of M_r 42,000, 29,000, and 25,000 caseins, respectively (ref. 9; unpublished results). These proteins were found in similar ratios in milk analyzed by radioimmunoassays. Because the ratio of α-LA to individual caseins in the gland remains approximately the same (Table 3) as in the milk, the results suggest that there is no preferential secretion of any of these proteins during this period of lactation. However, in a wheat germ translational system, the RNA extracted from the same gland synthesized 25–30% α-LA and 60–65% casein, giving a ratio of about 1:2.2 of α-LA to casein mRNA activities. The absence of other mRNA activities during the first to fourth days of lactation may in part be due to lack of proper conditions for their translation in this system. However, it is possible that during this period the gland is at an unique stage of development and geared to produce α-LA and casein because one can measure the emergence of other mRNA activities after the fourth to fifth day of lactation (unpublished data).

Under our conditions of synthesis in this cell-free system, M_r 42,000 protein is translated with less efficiency than are M_r 29,000 and 25,000 caseins (9, 10) (see Fig. 3 *C* and *D*) and thus this assay is not an absolute measure of the total casein mRNAs. When electrophoresed as glyoxal-denatured RNA, poly(A)$^+$ bearing RNA shows nearly equal proportions of all four species of RNA that code for α-LA and M_r 42,000, 29,000 and 25,000 caseins. This suggests that these mRNAs in the total RNA are present in nearly equal proportions. However, this also could be misleading because poly(U)-Sepharose or dT-cellulose columns select RNAs based on the length of their poly(A) sequences and certain poly(A)$^+$ RNAs do not bind well with these materials (20).

The absolute levels of mRNA sequences can be quantitated with the cDNA probes. The use of these probes as an assay for mRNA levels depends on the purity and specificity of the mRNA from which the DNA was transcribed. In previous experiments, rat α-LA mRNA was partially purified from the total RNA of 4-day lactating mammary gland (13) and now has been further purified, to free it of contaminating RNA, by sedimentation on a sucrose gradient in 70% formamide. The protein synthesized with this RNA in the wheat germ translational system was quantitatively precipitated only with rat α-LA antiserum and not by casein antiserum. The synthesized product was identical with rat α-LA_1, both by sodium dodecyl sulfate/polyacrylamide gel analysis and by chymotryptic fingerprint analysis. The RNA migrates as a single band when electrophoresed as glyoxal-denatured RNA in 1.5% agarose. Thus, as judged by its capability to direct synthesis of α-LA protein in a cell-free translational system (derived from wheat germ) and by its homogeneity under full denaturation conditions, α-LA mRNA is relatively homogeneous.

The cDNA probes synthesized from the purified α-LA mRNA and from 16S doublet casein mRNA were used for the quantitation of these mRNAs in the total RNA of the gland. The 16S doublet contained, in equal amounts, M_r 460,000 and 390,000 mRNAs as judged on gels under glyoxal-denaturation conditions, and the DNA transcript from this message hybridized to its own mRNA with pseudo-first-order kinetics. mRNA measured with these probes gave a ratio of 1:2 of α-LA to casein 16S doublet mRNA in the total RNA of 4- to 5-day lactating gland. If the cDNA from 16S mRNA represents the two messages in equal amounts, then α-LA mRNA is in equal proportion to each of these messages. If only one of the 16S messages is transcribed, then α-LA mRNA could represent only half the amount of 16S mRNA. However, α-LA protein and M_r 42,000 and 29,000 casein coded for by these messages are present disproportionately in the gland or milk: there is 10 times more M_r 42,000 casein than α-LA and 6–7 times more than M_r 29,000 casein. These results suggest that: (*i*) these mRNAs are translated *in vivo* with different efficiencies, (*ii*) some of the mRNA is present in the form not accessible for translation (localized in the nucleus or as ribonucleoprotein particles), or (*iii*) the degradations of these proteins occur with different rates. Several mRNAs in eukaryotic cells have been shown to exist as nonpolysomal messenger ribonucleoprotein complexes (21, 22). Now, with the availability of the rat α-LA cDNA probe, it should be possible to investigate the form and location of the α-LA message in the cell and the nature of the primary transcript.

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1. Griffith, D. R. & Turner, C. W. (1961) *Proc. Soc. Exp. Biol. Med.* **106**, 448–450.
2. Munford, R. E. (1963) *J. Endocrinol.* **28**, 1–15.
3. Oka, T. & Topper, Y. J. (1971) *J. Biol. Chem.* **246**, 7701–7707.
4. Topper, Y. J. (1970) *Recent Prog. Horm. Res.* **26**, 287–308.
5. Denamur, R. (1974) *Lactation*, eds. Larson, B. L. & Smith, V. R. (Academic, New York), Vol. 1, pp. 413–465.
6. Qasba, P. K. & Chakrabarty, P. K. (1978) *J. Biol. Chem.* **253**, 1167–1173.
7. Brown, R. C., Fish, W. W., Ludson, B. G. & Ebner, K. E. (1977) *Biochim. Biophys. Acta* **491**, 82–92.
8. Brew, K. & Hill, R. L. (1975) *Rev. Physiol. Biochem. Pharmacol.* **72**, 105–158.
9. Rosen, J. M., Woo, S. L. C. & Comstock, J. P. (1975) *Biochemistry* **14**, 2895–2903.
10. Rosen, J. M. (1976) *Biochemistry* **15**, 5263–5271.
11. Houdebine, L. M. & Gay, P. (1975) *Eur. J. Biochem.* **63**, 9–14.
12. Craig, K. R., Brown, A. P., Harrison, S. O., McIlreavy, P. & Campbell, N. P. (1976) *Biochem. J.* **160**, 57–74.
13. Chakrabarty, P. K. & Qasba, P. K. (1977) *Nucleic Acids Res.* **4**, 2065–2074.
14. Qasba, P. K. & Gullino, P. M. (1977) *Cancer Res.* **37**, 3792–3795.
15. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835–4838.
16. Hayward, G. S. (1972) *Virology* **49**, 342–344.
17. Kalan, E. B., Greenberg, R. & Thompson, M. P. (1966) *Arch. Biochem. Biophys.* **115**, 468–477.
18. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
19. Qasba, P. K., Chakrabarty, P. K. & Adler, R. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 3475 (abstr.).
20. Deeley, R. G., Gordon, J. I., Burns, H. T. A., Mullinix, P. K., Bina-Stein, M. & Goldberger, R. F. (1977) *J. Biol. Chem.* **252**, 8310–8319.
21. Spirin, A. S. (1972) in *The Mechanism of Protein Synthesis and Its Regulation*, ed. Bosch, L. (North Holland, Amsterdam, Netherlands), p. 515.
22. Baglioni, C. (1974) *Ann. N. Y. Acad. Sci.* **24**, 183–190.