The Ribonucleic Acids of the Mammary Gland of the Guinea Pig

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1. ^{32}P -labelled polyribosome preparations were made from the mammary glands of lactating and late-pregnant guinea pigs after injection of $[\text{}^{32}\text{P}]\text{P}_i$ into the animals.
2. The RNA of polyribosomes, ribosomal subunits and that released from polyribosomes by EDTA were analysed by zone velocity centrifugation and by polyacrylamide-gel electrophoresis.
3. RNA species which have the physical properties expected for the milk protein mRNA were detected. RNA species of a size which could code for the caseins were present in lactating but not in pre-lactating mammary-gland polyribosomes.

The mammary gland undergoes a process of differentiation at about the time of parturition and commences the synthesis of milk proteins. Since these proteins are novel to the mammary gland of the primiparous animal they represent the expression of a previously repressed genetic potential. With this in mind we have studied the species of RNA present in the mammary gland of the guinea pig when the animal is in the pre-partum and post-partum states. Among our objectives was the characterization of the mRNA that directs the synthesis of milk proteins and in particular that of α -lactalbumin which is virtually the sole protein in the whey of this animal.

Green *et al.* (1971) compared the pattern of incorporation of $[\text{}^3\text{H}]\text{uridine}$ into the total RNA of virgin mouse mammary explants incubated in media without supplementation with hormones, with insulin and cortisol added and with insulin, cortisol and prolactin added. It had previously been shown that all three hormones were necessary for the synthesis of casein by the explants (Turkington, 1968). Insulin and cortisol mediated an increase in the proportion of $[\text{}^3\text{H}]\text{uridine}$ -labelled high-molecular-weight (5×10^6 – 10×10^6) RNA. Prolactin potentiated this increase. They did not detect any changes in the labelling of RNA species which could have been mRNA but suggested that such species might be detected if polyribosomal RNA were analysed.

We have chosen to use the guinea pig for our studies because of the ease with which polyribosomes may be prepared from the mammary gland of the lactating animal (Fairhurst *et al.*, 1971). Campbell *et al.* (1973a) have demonstrated that RNA isolated from the polyribosomes of lactating mammary gland can direct the synthesis of α -lactalbumin in the *Xenopus* oocyte. Although the synthesis of casein has not been studied in our

laboratory, Gaye *et al.* (1973) have demonstrated that an RNA fraction isolated from the membrane-bound polyribosomes of rabbit lactating mammary gland can direct the synthesis of α_2 -casein in a cell-free protein-synthesizing system.

A preliminary account of some aspects of this work has already appeared (Campbell *et al.*, 1973b).

Experimental

Materials

Chemicals were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. and were of AnalaR Grade wherever possible. The scintillators were purchased from Nuclear Enterprises (G.B.) Ltd., Sighthill, Edinburgh, U.K.; puromycin dihydrochloride was from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.; poly(U) was from Miles Laboratories Inc., Kankakee, Ill. 60901, U.S.A.; and Sephadex was from Pharmacia Fine Chemicals, Uppsala, Sweden. Diethyl pyrocarbonate (also known as diethyl oxydiformate) and tri-iso-propyl naphthalene-sulphonate were purchased from Kodak Ltd., Liverpool, U.K. Other chemicals were as specified by Campbell *et al.* (1967).

Radiochemicals

The following radiochemicals were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.: L-[U- ^{14}C]leucine (sp. radioactivity 331 mCi/mmol), L-[U- ^{14}C]phenylalanine (sp. radioactivity 513 mCi/mmol) and sodium $[\text{}^{32}\text{P}]\text{phosphate}$ Injection B.P. (5 mCi/ml).

Treatment of solutions with diethyl pyrocarbonate

The solutions used for the analysis of ribosomes and nucleic acids were made 0.1% (v/v) in diethyl

pyrocarbonate to inactivate endogenous RNAases* (Humm *et al.*, 1971). Diethyl pyrocarbonate was decomposed to ethanol and CO₂ by heating at 90°C for 1–2 h.

Injection of guinea pigs with [³²P]P_i

The guinea pigs were of the Hartley strain. The pregnant animals were estimated to be within 1 week of parturition, the fetuses being fully formed with their eyes open. The lactating animals were within the first week after parturition; the duration of lactation is indicated more precisely when known.

Intraperitoneal injection of [³²P]P_i into lactating animals proved satisfactory in that all major species of RNA in the mammary gland became labelled. In the case of the pre-lactating animals only the tRNA of the mammary gland became labelled after intraperitoneal injection. Intravenous injection of the radioactive isotope, however, was more successful. For this the femoral vein was exposed under deep ether anaesthesia and up to 1 ml of a solution of the radioactive isotope was injected. The incision was closed with Michel suture clips. The operation proved difficult because of the toughness of the muscle fibres surrounding the vein and its small diameter.

Preparation of polyribosomes from guinea-pig mammary gland

The animals were killed at the stated times after injection with [³²P]P_i. Polyribosomes were isolated from the tissue as described by Fairhurst *et al.* (1971). The polyribosomal pellet was resuspended in 0.25 M-sucrose in medium A [25 mM-KCl–50 mM-Tris buffer (adjusted to pH 7.8 with HCl)–10 mM-MgSO₄], and centrifuged a second time through a discontinuous sucrose gradient (4 ml of 0.5 M-sucrose in medium A on a cushion of 3 ml of 2 M-sucrose in medium A).

Dissociation of polyribosomes into ribosomal subunits

The polyribosomes were suspended in ice-cold medium B [50 mM-Tris buffer (adjusted to pH 7.8 with HCl)–150 mM-KCl–1 mM-MgSO₄]. The ribosome concentration was about 15 mg/ml as measured on the basis of $E_{260}^{1\%} = 134$ at 260 nm. The suspension was incubated first at 0°C with 0.1 vol. of 10 mM-puromycin and 0.1 vol. of 5 mM-GTP for 15 min, secondly at 37°C for 15 min and then cooled in ice. Samples (1 ml) were loaded on to 36 ml linear gradients of 15–35% (w/v) sucrose in medium B. Centrifugation was for 10 h at 2°C and 95 000g_{av} in the SW 27 rotor of the Beckman L2-65B centrifuge. The E_{260} was measured in a Gilford model 2000 recording spectrometer by pumping the gradients

from the bottom through a flow cell at a constant flow rate of about 1 ml/min. The subunit peaks were collected and pooled in chilled tubes. The subunits were pelleted by centrifugation for 4 h at 2°C and 165 000g_{av} in the Ti 50 rotor of the Beckman L2-65B centrifuge. The pellets were stored at –15°C.

Treatment of polyribosomes with EDTA

The polyribosome pellets were treated with EDTA as described by Huez *et al.* (1967). Samples (1 ml) were layered on to 36 ml linear gradients of 15–30% (w/v) sucrose in 10 mM-Tris buffer (adjusted to pH 7.4 with HCl)–10 mM-KCl. The gradients were centrifuged for 29 h at 2°C at 95 000g_{av} in the SW 27 rotor of the Beckman L2-65B centrifuge. The gradients were analysed and fractionated as described above. The fractions were overlaid with buffer and centrifuged for 13 h at 2°C and 165 000g_{av} in the Ti 50 rotor of the Beckman L2-65B centrifuge. The pellets were stored at –15°C.

Purification of polyribosomal RNA

Polyribosomal RNA was purified by the method of Forrester *et al.* (1970).

Analysis of nucleic acids by rate zonal sedimentation

The nucleic acids or ribonucleoproteins were suspended in a medium containing 3% (w/v) sodium dodecyl sulphate, and 0.5 ml samples were layered on to 36 ml gradients of 0.5–1.1 M-sucrose. The gradient was made in either 50 mM-Tris buffer (adjusted to pH 7.5 with HCl)–0.5 M-NaCl (Blobel, 1971) or in 0.1 M-sodium acetate (Kirby *et al.*, 1969).

The gradients were centrifuged in the SW 27 rotor of the Beckman L2-65B centrifuge; the time, temperature and force of sedimentation is given in the legends to the Figures.

The distribution of radioactivity on gradients was determined by collecting the effluent from the recording spectrophotometer into scintillation vials. To each fraction (0.4–0.5 ml) 1 ml of water was added, then 10 ml of a scintillation liquid {7 parts of toluene-PPO (diphenyloxazole; 10 g/l)–POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene; 100 mg/l] to 6 parts of Triton X-100}. The contents of the vials were mixed by vigorous shaking. The radioactivity of the samples was determined in a Beckman model LS 200B liquid-scintillation spectrophotometer.

Analysis of nucleic acids by polyacrylamide-gel electrophoresis

The apparatus used was based on the vertical-tube design of Davis (1964) but had been fitted with platinum electrodes. The apparatus held eight

* Abbreviation: RNAase, ribonuclease.

siliconized glass tubes each of which was 17 cm long and had an internal diameter of 10 mm. The buffer compartments had a capacity of 1.5 l. This apparatus was always used in a cold-room at 5°C. The electrophoresis and the staining of the gels were carried out as described by von der Decken *et al.* (1970).

The gels were cut into 1 mm slices with a Mickel gel slicer as described by Loening (1968a). The slices were put into scintillation vials with 0.5 ml of Soluene T.M. 100. The vials were capped and left overnight at 60°C. The addition of 10 ml of a toluene-based scintillation fluid caused the slices to swell and become transparent; over 96% of the radioactivity in the gel was released into the fluid.

The radioactivities of the samples were determined in a Beckman model LS 200B liquid-scintillation spectrometer, the window being used for ^{32}P in the presence of ^3H .

The molecular weights of RNA species were estimated by interpolation on plots of log (molecular weight) versus mobility of the electrophoresed RNA (Loening, 1969).

The marker RNA species were rRNA and tRNA. The major rRNA species (28S and 18S) were taken to have molecular weights of 1.75×10^{-6} and 0.7×10^{-6} (Loening, 1968b). The molecular weight of 5S rRNA was taken as 44 000 and that of tRNA, 23 000–28 000 (Forget & Weissman, 1967; Brown, 1963).

Results

Analysis of ^{32}P -labelled polyribosomes from the mammary gland of lactating animals

After injection of ^{32}P into lactating animals a polyribosome preparation was made by the method of Fairhurst *et al.* (1971). Analysis of the preparation on a sucrose density gradient indicates that the ^{32}P -labelled RNA profile follows that of the E_{260} due to the polyribosomes, except at the top of the gradient (Fig. 1). This lower-molecular-weight ^{32}P -labelled material was removed by the second centrifugation through a discontinuous sucrose gradient (see the Experimental section).

Analysis of ^{32}P -labelled RNA from polyribosomes of the mammary gland of lactating animals

The polyribosome pellet was treated with 3% (w/v) sodium dodecyl sulphate and layered on to sucrose density gradients. As shown in Fig. 2(a) not only is the rRNA labelled but so also is the RNA appearing between 5S and 18S RNA species. When ribosomal subunit RNA was similarly analysed this latter RNA is not present (Figs. 2b and 2c). We do not claim that the single highly labelled fraction is significant for it was not reproducibly present. Since the ribosomal subunits were prepared by a method

which should eliminate mRNA they should contain only rRNA, degraded rRNA and some tRNA (Blobel & Sabatini, 1970). The degradation products of the 28S rRNA are seen at the high-molecular-weight end of the profile in Fig. 2(b); the RNA of the small subunit includes a species which may be a degradation product of the 18S rRNA.

The 18S rRNA was approx. 40% dimerized at 0°C in a buffer containing 0.5 M-NaCl. The dimer was not present when the RNA was sedimented at 18°C in 0.2 M-sodium acetate. Kirby *et al.* (1969) demonstrated that the latter conditions both prevented and reversed the aggregation of rat kidney tumour cell rRNA.

The polyribosomal pellet was next analysed by polyacrylamide-gel electrophoresis. The pattern of staining and of ^{32}P radioactivity in the gels is shown in Figs. 3(a) and 3(b).

The three stained bands which migrate between the 28S and the 18S rRNA were present when the RNA of isolated large ribosomal subunits was analysed. They were considered to be products of the degradation of 28S rRNA. Similar RNA species have been observed in preparations from rat liver and rat muscle (von der Decken *et al.*, 1970). Aaij *et al.* (1971) have suggested that these RNA species are intermediates in the degradation of rRNA *in vivo*.

The zone of labelled material (Fig. 3b) which migrates between the 5S and the 18S rRNA is not associated with any stained band. It follows that the labelled material, if it is RNA, has a specific radioactivity at least 40 times greater than that of the rRNA. The zone was present in polyribosomal RNA purified by the deproteinization procedure of

Fig. 1. Sedimentation profile of polyribosomes from lactating mammary gland

A guinea pig that had been lactating for 3 days was injected with 10 mCi of [^{32}P]P_i 4 h before it was killed. Polyribosomes (0.3 mg) were loaded on to a 29 ml gradient of 15–50% (w/v) sucrose in medium A. The gradients were centrifuged for 4 h at 2°C and 63 000 g_{av} in the Beckman SW 25.1 rotor. The E_{260} of a blank gradient has been subtracted from that of the polyribosome gradient. . . . , E_{260} ; —, ^{32}P radioactivity (c.p.m.).

Fig. 2. Sedimentation profile of the RNA from polyribosomes and from ribosomal subunits prepared from mammary glands of lactating animals

Two guinea pigs that had been lactating for 3 days were injected with 2.5 mCi of $[^{32}\text{P}]\text{P}_i$. After 17 h polyribosomes and ribosomal subunits were prepared. The pellets were taken up in 50 mM-Tris (adjusted to pH 7.5 with HCl), 3% sodium dodecylsulphate and layered on to 35 ml gradients of 0.5–1.1 M-sucrose in the same buffer. The gradients were centrifuged for 45 h at 2°C and $95\,000g_{\text{av}}$ in the Beckman SW 27 rotor. $\cdots$, E_{260} ; —, ^{32}P radioactivity (c.p.m.). (a) RNA of polyribosomes; (b) RNA of the large ribosomal subunit; (c) RNA of the small ribosomal subunit.

Forrester *et al.* (1970). It is not therefore a phosphoprotein nor is it likely to be adventitiously associated with the RNA. The radioactivity of this zone is

decreased when the polyribosomes are briefly incubated with RNAase before being deproteinized. Labelled material is then found to be distributed

Fig. 3. Gel electrophoresis of polyribosomal RNA

The polyribosomes were prepared from the mammary glands of a guinea pig which had been lactating for 3 days; the animals were injected with 10mCi of [^{32}P]P_i 3h before being killed. (a) An amount corresponding to $E_{260} = 0.43$ was applied to a 2.4% polyacrylamide gel (5mm $\times$ 110mm); electrophoresis was for 2.25h at 5mA per tube and at 4°C. The gel was stained with Toluidine Blue. (b) An amount corresponding to $E_{260} = 4.5$ was applied to a 2.2% polyacrylamide gel (10mm $\times$ 170mm); electrophoresis was for 4h at 5mA per tube and at 4°C. The radioactivity of 1mm slices of the gel was measured. —, Intensity of Toluidine Blue staining; ----, ^{32}P radioactivity (c.p.m.).

uniformly along the 5S–18S RNA zone and to be associated with stained bands of RNA. Incubation of polyribosomes with RNAase for periods up to 30min at 0°C showed that extensive degradation of rRNA occurred. The stained labelled material observed between the 18S RNA and 5S RNA is probably degraded rRNA. It is concluded that the labelled zone of undegraded polyribosomes is indeed RNA of higher specific radioactivity than the rRNA.

We consider that a polyribosomal RNA which was not derived from the rRNA and was not adventitiously associated with the ribosomes might be a mRNA. We therefore examined the RNA released from the polyribosomes by EDTA to see if our high specific radioactivity RNA was present. Marbaix & Burny (1964) have shown that treatment of reticulocyte polyribosomes with EDTA releases ribonucleoprotein particles which have proved to contain the globin mRNA. Lebleu *et al.* (1971) demonstrated that if the polyribosomes were exposed to deoxycholate before treatment with EDTA then the naked globin mRNA is released. The method has been used for the preparation of putative mRNA fractions from a variety of tissues.

The RNA of EDTA-derived ribosomal subunits and that released from polyribosomes of three or more ribosomes was analysed by polyacrylamide-gel electrophoresis. The results are shown in Figs. 4(a), 4(b) and 4(c). The EDTA-released RNA was concentrated by differential centrifugation which was designed to give minimal yields of 4S RNA. In this way we were able to avoid loading the gel with large quantities of labelled tRNA.

It is seen that the RNA of the large subunit (Fig. 4a) contains species which correspond to the products of degradation of 28S rRNA previously observed. There is very little labelled RNA migrating in the zone between the 5S and the 18S rRNA. The RNA of the small subunit (Fig. 4b) contained only the 18S rRNA and the RNA species seen also in Fig. 2(b) which may be a degradation product of the 18S rRNA.

The RNA released from the polyribosomes sediments more slowly than does the 18S RNA of purified rRNA on similar gradients. There was no evidence of any RNA sedimenting in the size range of rRNA precursor molecules. There was a little very rapidly sedimenting material which could have been aggregates of RNA or ribonucleoprotein. We looked for such large molecules because of the report that nuclear RNA is a possible contaminant of polyribosome preparations (Plagemann, 1969).

The major zone of labelled RNA released by EDTA migrates with an apparent molecular weight in the range 1.6×10^5 – 3.2×10^5 ; the lighter zone is in the range 0.6×10^5 – 1.6×10^5 . These may be compared with the species seen in Fig. 3(b) in which the major zone of labelled RNA is in the range 1.75×10^5 – 2.45×10^5 , and the lighter shoulder is in the range 0.8×10^5 – 1.6×10^5 .

Analysis of ^{32}P -labelled polyribosomes from the mammary gland of late-pregnant animals

After intravenous injection of [^{32}P]P_i into late-pregnant animals polyribosomes were prepared from the mammary glands and analysed on a sucrose

Fig. 4. Gel electrophoresis of the RNA released from polyribosomes by EDTA and of the RNA of EDTA-derived ribosomal subunits

The polyribosomes were prepared from the mammary glands of a guinea pig which had been lactating for 3 days; the animal was injected with 10mCi of $[^{32}\text{P}]\text{P}_i$ 4h before being killed. The RNA was applied to a 4% polyacrylamide gel (10mm $\times$ 170mm); electrophoresis was for 3h at 10mA and at 4°C. The radioactivity of 1mm slices of the gel was measured. (a) RNA of the large EDTA-derived ribosomal subunit; (b) RNA of the small EDTA-derived ribosomal subunit; (c) RNA released from the polyribosomes by treatment with EDTA.

Fig. 5. Sedimentation profile of polyribosomes from pre-lactating mammary gland

Two late-pregnant guinea pigs were each injected with 3.5 mCi of [^{32}P]P_i 4 h before being killed. Polyribosomes (0.15 mg) were loaded on to a 39 ml gradient of 15–50% sucrose in medium A. The gradients were centrifuged for 4 h at 2°C and 63 600 g_{av} in the Beckman SW 25.1 rotor. The E_{260} of a blank gradient had been subtracted from that of the polyribosome gradient. —, E_{260} ; ----, ^{32}P radioactivity (c.p.m.).

Fig. 6. Gel electrophoresis of polyribosomal RNA

The preparation from late-pregnant guinea pigs is that illustrated in Fig. 5. An amount corresponding to $E_{260} = 3.0$ was applied to a 2.4% polyacrylamide gel (10 mm $\times$ 170 mm); electrophoresis was for 4 h at 5 mA per tube and at 4°C. The gels were stained with Toluidine Blue and the radioactivity of 1 mm slices was measured. —, Intensity of Toluidine Blue staining; ----, ^{32}P radioactivity (c.p.m.).

density gradient. The ^{32}P -labelled RNA profile follows that of the E_{260} due to the polyribosomes, except at the top of the gradient (Fig. 5). As with the

preparation from lactating glands the low-molecular-weight labelled material was removed by washing the polyribosomes. The results agree with those of Fairhurst *et al.* (1971) in that the ratio of polyribosomes to monomeric ribosomes is much lower than that for the preparation from lactating animals.

Analysis of ^{32}P -labelled RNA from polyribosomes of the mammary gland of late-pregnant animals

The difficulty of the preparation of labelled polyribosomes from late-pregnant animals and the small yields obtained precluded a complete comparison of their RNA with that prepared from the lactating animal. The polyribosomal pellets were analysed by polyacrylamide-gel electrophoresis (Fig. 6). The pattern of staining of the gels generally resembled that of the preparation from lactating animals. The stained bands which migrate between the 5S and the 18S rRNA in Fig. 6 were not usually seen. The pattern of labelled RNA other than rRNA differed from that of the preparation from lactating animals. In particular the major zone of labelled RNA (see Fig. 3b) containing species of estimated molecular weight 1.7×10^5 – 2.4×10^5 , which could code for the caseins, was not present.

Discussion

The major milk proteins synthesized by the lactating guinea-pig mammary gland are the caseins and α -lactalbumin (Brew & Campbell, 1967). The serum albumin and immunoglobulins of the milk are transferred from the serum to the milk (McKenzie, 1967; Jordan & Morgan, 1970). Since the gland is growing during the first days of lactation it will be synthesizing proteins other than those of the milk. The histones are a major class of protein associated with the growth of the gland. Stellwagen & Cole (1969) have shown that the rate of synthesis of histones by rat mammary gland increases just before parturition, is maximal 1 day after parturition and declines to that of the pre-lactating gland after 6 days. It is likely that the milk proteins are the major class of protein synthesized by the gland after an initial period of rapid growth.

The molecular weights of the milk proteins, the histones and the minimum molecular weights of the mRNA species which could code for them are summarized in Table 1. It is seen that if the milk protein mRNA species are of the minimum necessary size they will be found between the 5S and the 18S rRNA when polyribosomal RNA is fractionated on the basis of size. The RNA species found in the mammary gland (Table 2) are comparable in size with those listed in Table 1.

Two sources, which are artifacts of polyribosomal RNA, with a specific radioactivity higher than that of the rRNA have been reported,

Table 1. *Some proteins synthesized by the lactating mammary gland and the estimated minimum molecular weights of the mRNA species which would code for them*

All of the proteins are known to be single polypeptide chains.

Protein	Molecular weight	Number of residues	$10^{-5} \times$ Minimum mol.wt. of mRNA	Maximum size of polyribosome (ribosomes)	% of protein in guinea-pig milk protein
α -Casein	27000		2.41	9	79
β -Casein	19500		1.73	6	
K-Casein	23900	209	2.13	8	
α -Lactalbumin	16200	123	1.26	4	5.6
Lactose synthetase A protein	44000 (Glycoprotein)	330	3.34	12	—
Histones I	19 500–21 000	212	2.16	8	Nil
II, III	13 000–17 000	125–157	1.27–1.60	4–5	Nil
IV	11 200	102	1.04	3	Nil

Table 2. *Estimated molecular weights of ^{32}P -labelled RNA species present in the polyribosomes of the lactating guinea pig*

The rRNA species have been omitted. The methods by which RNA was prepared were (1) solubilization of polyribosomes in buffer containing 4% sodium dodecyl sulphate, (2) deproteinization of polyribosomes by the methods of Forrester *et al.* (1970), (3) release from the polyribosomes by treatment with EDTA.

Preparation of the RNA	Composition of the gels		Zones of ^{32}P -labelled RNA ($10^{-5} \times$ mol.wt.)	Distinct peaks of ^{32}P -labelled RNA ($10^{-5} \times$ mol.wt.)
	Acrylamide % (w/v)	Agarose % (w/v)		
(1)	2.0	0.5	0.87–1.6 7.75–2.45 2.7 –5.25	2.0
(1)	2.2	0.5	0.7 –1.7 1.7 –2.6 2.6 –5.7	2.0
(1)	2.4	—	1.05–2.3	1.9 12.3 2.4
(2)	2.4	—	1.66–2.3	9.8
(1)	4	—	0.5 –3.8	0.58 1.05 1.90
(3)	4	—	1.15–2	0.66 0.87 1.74 2.18 3.72

First, Plagemann (1969) has shown that RNA which leaks from the nuclei during the homogenization of rat hepatoma cells can become associated with the polyribosomes. The RNA species listed in Table 2 are resistant to washing of the polyribosomes with solutions containing any of the following: 25 mM-KCl–5 mM-Mg²⁺, 150 mM-KCl–1 mM-Mg²⁺ or 500 mM-KCl–5 mM-Mg²⁺. The washing does, however, remove some labelled material from the polyribosome preparation. Incubation of the

polyribosomes with puromycin and GTP followed by isolation of ribosomal subunits from buffers containing either 150 mM-KCl–1 mM-Mg²⁺ or 500 mM-KCl–5 mM-Mg²⁺ removes the RNA species indicated in Table 2 from the ribosomes.

We would expect RNA species adventitiously associated with the polyribosomes to be removed by both treatments. Messenger-like RNA species should be removed only by the latter treatment (Blobel, 1971). The results support the view that we are

studying messenger-like RNA rather than RNA of nuclear origin associated with the polyribosomes.

Secondly, Dingman *et al.* (1970) have suggested that newly synthesized rRNA is less stable than the bulk of the rRNA. It follows that the products of degradation of rRNA could have a higher specific radioactivity than the intact rRNA if the period of labelling is significantly less than the half-life of the rRNA. The breaks in the rRNA will not be manifested until the ribosomal subunits are dissociated by sodium dodecyl sulphate. We have shown that the RNA species indicated in Table 2 are not associated with ribosomal subunits prepared by dissociation of polyribosomes with puromycin or with EDTA. We conclude that these RNA species are not products of degradation of rRNA.

We conclude that we have demonstrated the presence in lactating mammary gland of RNA species which have the following properties expected for milk protein mRNA. (1) They are associated with polyribosomes. (2) They cannot be washed off the polyribosomes by solutions containing up to 0.5M-KCl unless the polyribosomes are first incubated with puromycin. (3) They are not rRNA species, nor are they products of the degradation of rRNA. (4) They are released from the polyribosomes by treatment with EDTA. (5) They make up less than 1% of the total polyribosomal RNA but are of much higher specific radioactivity than the rRNA. (6) They appear to be very sensitive to treatment of the c-ribosomes with RNAase although the results are confused by degradation of the rRNA by the enzyme. (7) The major component of the RNA isolated from the lactating mammary gland is of a size which could code for the caseins, the major proteins of milk. This zone of labelled RNA which could code for the caseins was not detected in RNA extracted from pre-lactating mammary-gland polyribosomes although lower-molecular-weight RNA species were present. Further characterization of these RNA species must await the demonstration that they direct the synthesis of milk proteins in a heterologous protein-synthesizing system. Experiments currently in process in our laboratory have demonstrated that an RNA fraction can be isolated from the mammary gland of lactating guinea pigs that has a poly(A) tail and that such RNA stimulates protein synthesis in a heterologous system.

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