ABSENCE OF POLY(A) IN A LARGE PART OF NEWLY SYNTHESIZED CASEIN mRNAs

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1. Introduction

A poly(A) fragment proved to be covalently bound to the mRNA in eucaryotes [1,2], in procaryotes [3] and in virus [4]. However, this ubiquity is not absolute and several mRNAs were demonstrated to be devoid of this sequence. It is admitted that the mRNAs for histones have no poly(A) [5,6]. In sea urchin embryos [7] and in HeLa cells [8], the co-existence of two types of mRNAs containing and lacking poly(A) was observed, these two types of mRNAs exhibiting no common sequences. By contrast, several mRNAs identified by translation into specific proteins, could not be retained quantitatively by oligo(T)-cellulose or poly(U)-Sepharose, suggesting that a variable proportion of these active mRNAs lacks the poly(A) sequence [9–19]. The poly(A) is normally added to the coding part of the mRNA, partly during transcription and partly later [20]. The length of the poly(A) and possibly its presence may vary during the life of the mRNA: it may be shortened with aging [21,22] or on the contrary, lengthened at the time of some cellular events [23–25].

In lactating ewe [26], rat [27] and rabbit [28] mammary gland, half of the mRNAs for casein is devoid of poly(A). Lactation represents a steady state and the absence of poly(A) may be the result of an equilibrium between the newly synthesized mRNAs containing the poly(A) and the aged mRNAs having lost the poly(A). Prolactin injected into pseudo-pregnant rabbits is capable of inducing in a few hours the synthesis of the mRNAs for casein [29,30]. In the present work, the proportion of the poly(A) containing mRNAs for casein was estimated by hybridization with casein cDNA during induction of mRNA synthesis by lactogenic hormones. This experiment is susceptible to determine whether the absence of poly(A) in part of the casein mRNAs is the result of a removal of this sequence or whether part of the mRNA is synthesized without the further addition of poly(A).

2. Materials and methods

Induction of casein mRNA synthesis was obtained after intramuscular injections (100 IU/injection) of ovine prolactin (Byla) to pseudopregnant rabbits every 12 h as previously described [29]. Four animals were subjected to the same treatment in each group. One rabbit killed after 3 h of hormonal treatment had been injected with prolactin intravenously (100 IU) and intramuscularly (100 IU), and with hydrocortisone acetate (Roussel) intramuscularly (10 mg) to obtain a maximum response within the shortest time [31].

Total polysomes were prepared by homogenizing the tissue directly in 50 mM Tris-HCl, pH 7.5, 150 mM KCl, 5 mM $MgCl_2$, 500 μg/ml heparin, 0.25% sodium deoxycholate, 4% Triton. The 40 000 *g* supernatant of the homogenate was centrifuged overnight at 70 000 *g* over a 1.5 M sucrose cushion. The resulting pellet was dissolved in 100 mM Tris-HCl, pH 9, 0.5% SDS and the polysomal RNA was extracted by the phenol method [29]. Equal amounts of mammary gland from each animal in each group were pooled before homogeneization.

Casein mRNA was purified from membrane-bound polysomes by immunoprecipitation of polysomes synthesizing casein with anti-casein antibody as previously described [30,32].

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DNA complementary to purified casein mRNA was synthesized with the Klenow subfragment of *E. coli* DNA polymerase I (Boehringer). The cDNA formed contained 200 nucleotides as determined by polyacrylamide gel electrophoresis in formamide [28].

Hybridization of cDNA with RNA in excess was performed essentially as previously described [28]. Total polysomal RNA (10^{-4} to 5 A_{260}) was incubated with cDNA for 24 h at 65°C in a buffer containing 0.6 M NaCl. At the end of incubation, the percentage of DNA hybridized with casein mRNA was determined after digestion of the non-hybridized cDNA with S_1 nuclease. Results are expressed in standard conditions 0.12 PB proposed by Britten and Smith [33]. Casein mRNA was estimated in total polysomal RNA containing all the polysomal mRNA and in an aliquot of this fraction devoid of the poly(A)-containing mRNA by chromatography through poly(U)-Sepharose.

3. Results and discussion

Quantification of casein mRNA sequence in total polysomal RNA and in the same fraction from which the poly(A)-containing mRNA has been removed by chromatography with poly(U)-Sepharose, reveals that in the lactating animal, a large fraction of casein mRNA has no poly(A) (fig.1A and 1B). The proportion of casein mRNA lacking poly(A) in the rabbit was 25% as determined here by hybridization instead of about 50% as previously determined in the ewe and the rat by translation [26,27].

The proportion of casein mRNAs containing the poly(A) is similar before activation of casein genes and at different time of the induction (fig.1A and 1B). The results summarized in fig.2 argue strongly against a removal of poly(A) from mRNA with aging since the mRNA content of mammary gland was multiplied by 8, 30 and 170 respectively at 6, 18 and 48 h of the prolactin treatment [30]. On the contrary they suggest that the processing of casein mRNA synthesis does not include a systematic addition of poly(A) to all the mRNAs.

The absence of poly(A) in mRNA cannot be attributed to phenol extraction since the same result was obtained without extraction the RNA [26,28]. It is not due to a removal of poly(U) from poly(U)-Sepharose during elution of mRNA since the eluted mRNA was still capable of being hybridized to poly(U)

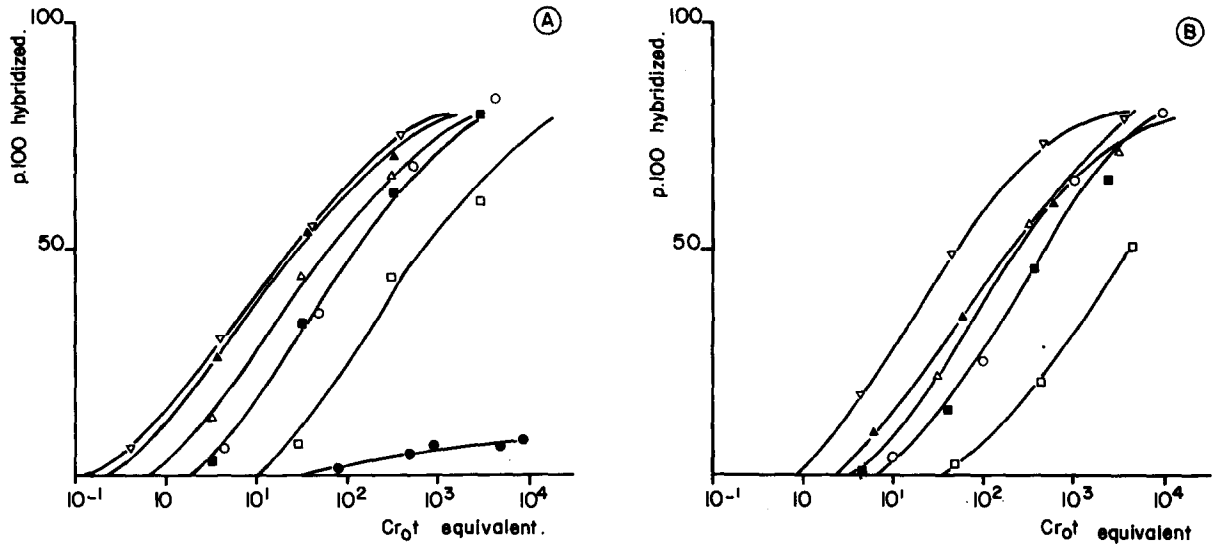

Fig.1. Kinetic hybridization curves of casein cDNA with polysomal RNA. (A) Total polysomal RNA. (B) Total polysomal RNA minus poly(A) containing mRNA. (▽——▽) Lactating rabbit. (▲——▲) Pseudopregnant animal after 48 h of prolactin treatment. (△——△) Prolactin 18 h. (■——■) Prolactin 6 h. (○——○) Prolactin + hydrocortisone acetate 3 h. (□——□) Untreated pseudopregnant rabbit. (●——●) Total rabbit liver RNA and ribosomal rabbit reticulocyte RNA.

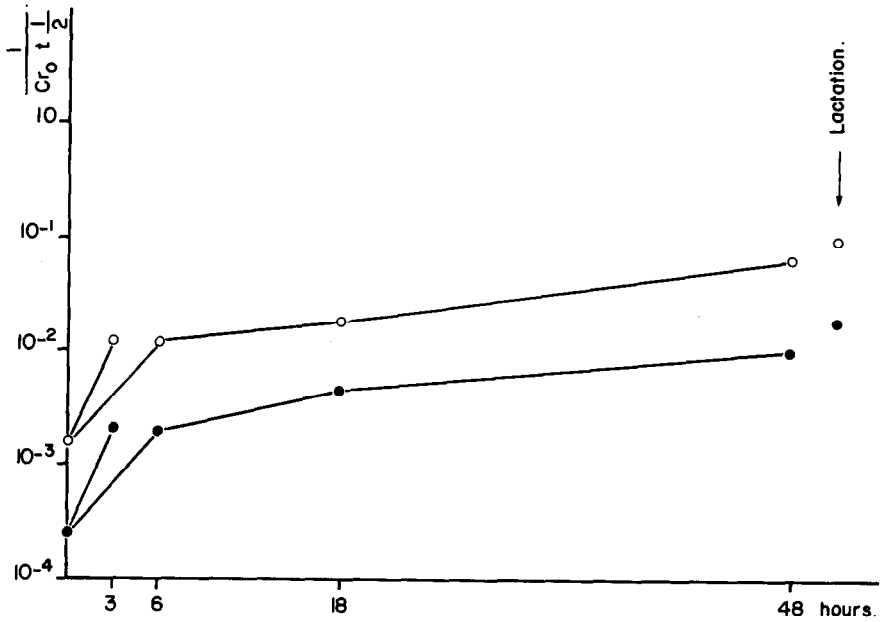

Fig.2. Variations of the concentration in casein mRNA containing and lacking poly(A) during activation of casein gene with prolactin. Cr_0t ½ are the transition points of the kinetic curves in fig.1. (o——o) Total polysomal RNA. (•——•) Total polysomal RNA minus poly(A)-containing mRNA. 6 – 18 – 48 h: prolactin. 3 h: prolactin + hydrocortisone acetate.

[26] and since the column was used for more than two years without any loss in its capacity to retain mRNA. Moreover oligo(T)-cellulose was less efficient than poly(U)-Sepharose for casein mRNAs and a poly(U)-Sepharose chromatography in 0.5 M NaCl susceptible to retain mRNA containing poly(A) too short to form a stable hybrid with poly(U) at 0.12 M NaCl, did not improve the yield of mRNA recovery.

Poly(A) proved to increase the stability of mRNA towards nucleases [34–36]. However, in sea urchin embryo the mRNAs lacking poly(A) exhibited a stability similar to those containing poly(A) [37]. A possible difference in stability of casein mRNA lacking poly(A) remains to be established.

mRNA in which the poly(A) has been experimentally removed can be translated in vitro with an equal efficiency [14,38,43]. However a role for poly(A) cannot be excluded [44–48]. It might be of interest to determine whether casein mRNA lacking poly(A) can be translated with the same efficiency as those containing poly(A).

No simple explanation for the absence of poly(A) in part of casein mRNAs in relation to their function in mammary gland can be formulated. It is noteworthy that 30% of the mRNA for ovalbumin, synthesized massively like mRNA for casein, are devoid of poly(A) [13]. The processing of poly(A) addition to mRNA might simply be non-quantitative when large amounts of mRNA are synthesized.

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