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Role of the Polyadenylate Segment in the Translation of Globin Messenger RNA in *Xenopus* Oocytes

[poly(A)/functional stability/Krebs II ascites tumor]

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ABSTRACT **The translations of native messenger RNA for rabbit globin and that of poly(A)-free globin messenger RNA have been compared after injection into *Xenopus* oocytes. The initial rate of translation of poly(A)-free mRNA is close to that found with intact mRNA. However, at longer incubation periods, the rate of globin synthesis with poly(A)-free mRNA is considerably lower than with native mRNA. Similar differences in the template activity of the two mRNA preparations were found with a cell-free extract of Krebs II ascites tumor. It is concluded that the presence of the 3′ poly(A)-rich sequence in mRNA is required to ensure high functional stability.**

The existence of poly(A)-rich sequences at the 3′-OH terminus of eukaryotic mRNAs is well documented (1). It seems that the poly(A)-rich segments become shorter with aging of mRNA (2, 3). However, their precise role remains, so far, obscure. Since the mRNA species coding for histones do not appear to contain poly(A) sequences (4), it seems unlikely that presence of the poly(A) sequences is a prerequisite for translation.

It is interesting to consider the hypothesis that the length of the poly(A) segment is directly related to the translational half-life of the mRNA in a given system. One would therefore predict that mRNA molecules that lack poly(A) regions would have a much shorter functional half-life than those that contain poly(A) segments.

Specific methods have been developed for the removal of poly(A)-rich sequences without degrading the rest of the mRNA molecule. Williamson *et al.* (6) have shown for mouse and we have shown for rabbit globin (14) that poly(A)-free globin mRNA can still be translated in a Krebs II ascites tumor cell-free extract. In our work, we have, however, observed that, at longer periods of incubation, the rate of protein synthesis had a tendency to level off more strongly with poly(A)-free mRNA than with native poly(A)-containing mRNA (14). However, it should be noted that the *in vitro* cell-free systems are far less efficient in translation than an *in vivo* system such as frog oocytes (5). Reinitiations occur at a much greater rate in oocytes and the translation of globin mRNAs injected into frog oocytes proceeds for several days as efficiently as with the endogenous messages. We have, therefore, used *Xenopus* oocytes to compare the rate and extent of translation of both intact and poly(A)-free globin mRNA.

MATERIALS AND METHODS

Preparation of Rabbit Globin mRNA and Rabbit Reticulocyte Ribosomal Wash Fluid. Rabbit reticulocyte polysomes were obtained from phenylhydrazine-treated animals (7). Globin mRNA was prepared from 0.5 M KCl washed polysomes (8). The supernatant obtained after washing the reticulocyte polysomes with 0.5 M KCl served as a source of crude initiation factors (9).

Preparation of Poly(A)-Free Globin mRNA. Poly(A)-free globin mRNA was prepared by limited phosphorolysis of globin mRNA at 0° with excess amounts of highly purified (250 units/mg) *Escherichia coli* polynucleotide phosphorylase (polyribonucleotide nucleotidyltransferase EC 2.7.7.8). As previously shown, under these conditions, the poly(A)-rich segment of globin mRNA is removed without degradation of the rest of the molecule. The phosphorolysis product migrates as a single band on polyacrylamide gel electrophoresis. The apparent molecular weight of the poly(A)-free mRNA was estimated to be 1.8×10^5, as compared with 2.3×10^5 for the intact globin mRNA. In addition, the poly(A)-free mRNA fails to bind to the oligo(dT)-cellulose column (14).

Cell Free Protein Synthesis. The template activity of mRNAs for protein synthesis was tested using Krebs II ascites cell free system (10) containing *N*-*2*-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes) buffer instead of Tris·HCl (11).

Injection Into Oocytes. The whole experiment was done with oocytes from the same *Xenopus laevis* female. Oocytes were injected with 50 nl of a globin mRNA or poly(A)-free mRNA dissolved in water and adjusted to a concentration of 140 μg/ml. The injection procedure and the culture of the oocytes have been described by Gurdon *et al.* (5).

It is important to note that the concentrations used here are well below those required for messenger saturation in the oocytes (12).

Each type of mRNA preparation was injected into 60 oocytes. At different times, 10 oocytes were removed from each batch and incubated in 100 μl of Barth medium containing 1 mCi/ml of [^{3}H]histidine (50 Ci/mmole) for various periods of times (see *Results*). At the end of the incubation, the oocytes were washed with Barth medium and stored at −80° until analyzed.

Analysis of the synthesized proteins was carried out by gel filtration on Sephadex G-100 columns (12).

RESULTS

Cell Free Translation of the Poly(A)-Free mRNA. As previously shown, both globin mRNA and poly(A)-free globin mRNA stimulate amino-acid incorporation in a preincubated Krebs II ascites cell-free system. The product of the cell-free system comigrates with rabbit globin on polyacrylamide–

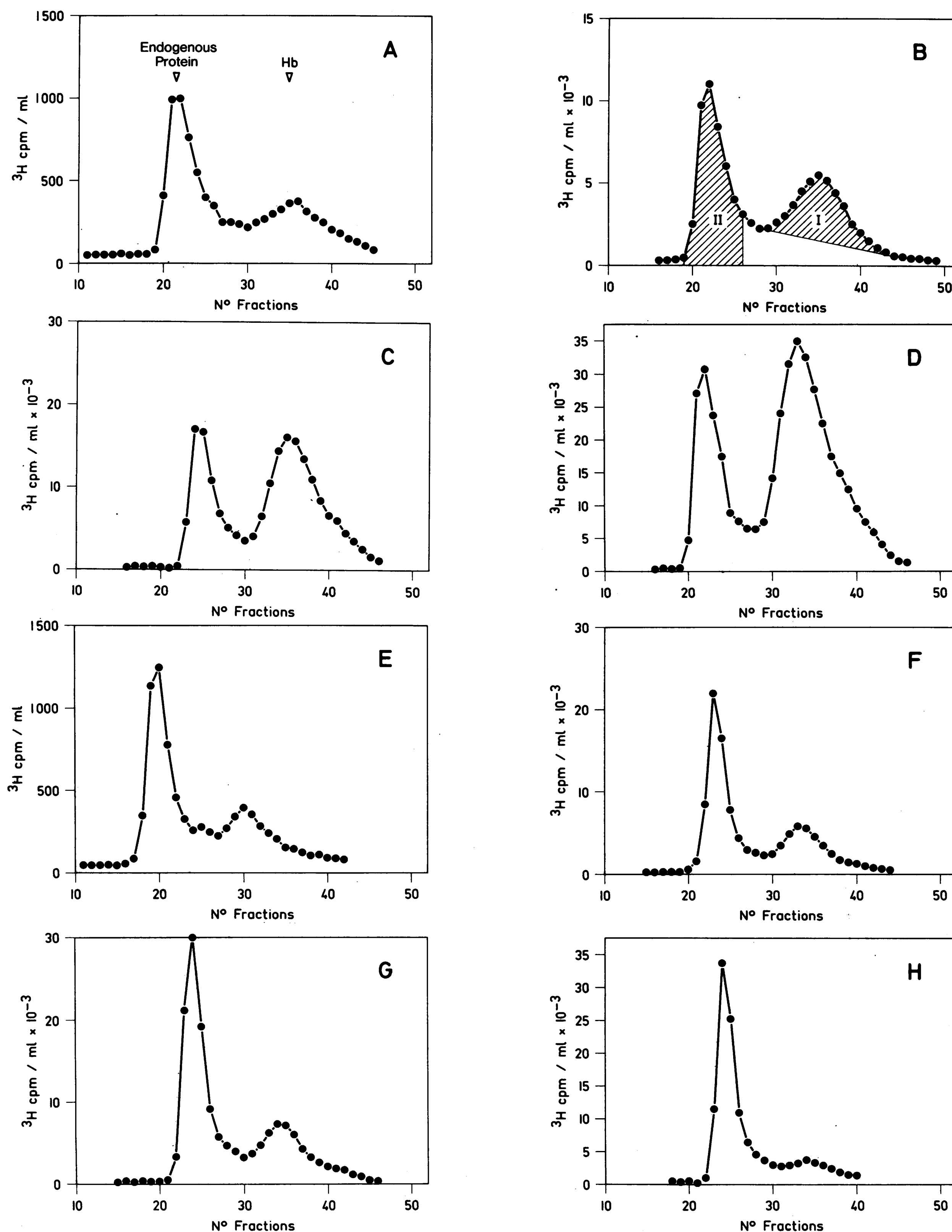

FIG. 1. Hemoglobin synthesis directed by globin mRNA with and without poly(A). Oocytes (120) from a single *Xenopus laevis* female were injected with 50 nl each of 140 μg/ml of mRNA aqueous solutions. Sixty of them received native globin mRNA, the other 60 received globin mRNA lacking poly(A). At different times after the injection, 10 oocytes of either batch were incubated in 100 μl of Barth medium containing 1 mCi/ml of [^{3}H]histidine (50 Ci/mmole) at 19°. At the end of the incubation, oocytes were washed and homogenized. Cell debris was removed by low speed centrifugation and the supernatant was analyzed by gel filtration on 70 cm × 1 cm Sephadex G-100 columns (12). The radioactivity of aliquots of the eluted fractions was measured in a toluene–Triton (X-100) mixture using a scintillation spectrophotometer. Samples *A* to *D*: oocytes injected with native mRNA. Samples *E* to *H*: oocytes injected with mRNA lacking poly(A). Incubation periods: *A* and *E*: 0–1 hr; *B* and *F*: 1–5 hr; *C* and *G*: 5–20 hr; *D* and *H*: 20–48 hr.

sodium dodecyl sulfate gels (14). The ratio between α and β globin chains synthesized in the cell-free system, and the ratio between complete and incomplete chains are about the same for the two mRNA preparations (unpublished observations).

As shown in Table 1, the initial rate of translation is the same for both mRNA preparations, and the amino acid incorporation proceeds linearly up to 30–45 min of incubation. However, upon longer periods of incubation, intact mRNA is more active than poly(A)-free mRNA. Table 1 shows that the rate of protein synthesis levels off more rapidly with poly(A)-free mRNA than with the intact mRNA. This suggests that the removal of the terminal poly(A)-segment shortens the time period during which the mRNA functions as a template in the cell-free system.

Translation of Intact and Poly(A)-Free Globin mRNA in Xenopus Oocytes. Translation of globin mRNA in the Krebs S-30 extract is active for about 90 min and then stops. The low efficiency and the poor stability of the cell-free system probably result from changes in the relative concentrations of cell components during the extraction procedure and cell-free protein synthesis. To overcome the time limitation of this system, we decided to compare the translation of intact globin mRNA and poly(A)-free mRNA in the *Xenopus* oocyte system. This permits the use of a living cell to compare the translational efficiency of the two mRNA preparations for longer periods and to draw conclusions on the role of poly(A), which could not be obtained in the Krebs ascites cell-free system.

Analysis of the proteins synthesized at different periods of time in oocytes injected with intact globin mRNA are given in Fig. 1*A* to *D*. Corresponding data for oocytes injected with poly(A)-free mRNA are given in Fig. 1*E* to *H*. In the case of native mRNA there is a progressive increase of the rate of Hb synthesis. This is in good agreement with previous observations (5).

By comparison of Fig. 1*A* and *E*, it appears that during the first hour after the injection of the mRNA samples, Hb synthesis is roughly the same for native mRNA and poly(A)-free mRNA. However, after 20 hr of incubation, a marked difference in the rate of Hb synthesis is noticed. At that time, Hb synthesis represents 142% of endogenous protein synthesis in oocytes injected with native mRNA, while it amounts to only 24% with the system programmed with poly(A)-free mRNA. The results are summarized in Fig. 2, where the ratio of Hb synthesis to endogenous protein synthesis has been plotted for each time period. The way in which the ratio has been arbitrarily estimated is explained in the *legend* of Fig. 2 and illustrated in Fig. 1*B*.

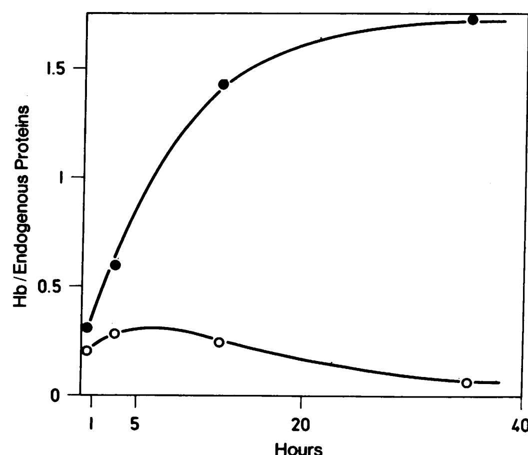

FIG. 2. The time course of the rate of Hb synthesis relative to that of endogenous protein synthesis. The ratios of the two rates of synthesis have been deduced from the data in Fig. 1, using the method of computation indicated in Fig. 1*B*. The peaks I and II (indicating Hb and endogenous protein synthesis, respectively) have been integrated and arbitrarily chosen as representative of the amounts of Hb and endogenous proteins synthesized during the periods of time indicated.

DISCUSSION

It is clear that *Xenopus* oocytes constitute an excellent system for testing the functional lifetime of natural mRNAs. It has been shown that globin mRNA enjoys a higher stability in some oocyte batches than in reticulocytes, where it is normally translated (13).

In our experimental conditions, native globin mRNA has been translated with a high efficiency at least for 48 hr. The increase in efficiency of translation of native mRNA during the first hours of incubation could be due to the delay required for

TABLE 1. *Time course of amino-acid incorporation in response to poly(A)-free mRNA and intact mRNA in Krebs ascites cell-free system*

	Incorporation			Incorporation at 15-min intervals		
Time (min)	mRNA (cpm)	Poly(A)-free mRNA (cpm)	Time interval (min)	mRNA (cpm)	Poly(A)-free mRNA (cpm)	Ratio of mRNA/ poly(A)-free mRNA
15	1,590	1,890	0–15	1,590	1,890	0.84
30	4,850	5,480	15–30	3,260	3,590	0.91
45	7,030	7,320	30–45	2,180	1,840	1.18
60	8,620	7,880	45–60	1,590	560	2.84
75	10,100	8,350	60–75	1,480	470	3.15

The reaction mixture (0.2 ml) contained 20 μl of preincubated Krebs S-30 (a preparation of soluble proteins, 60 A_{260} units/ml); 30 mM Hepes buffer, pH 7.5; 70 mM KCl; 3 mM $MgCl_2$; 7 mM 2-mercaptoethanol; 1 mM ATP; 0.25 mM GTP; 5 mM creatine phosphate; 7.5 μg of creatine kinase; 2 μCi of ^{14}C-labeled algal protein hydrolysate (Amersham, 57 Ci/mole of C); supplemented with the four missing unlabeled amino acids at a concentration of 0.1 mM; and 100 μg of crude reticulocyte ribosomal wash fluid. The mRNA concentrations used were: 0.53 μg of mRNA or 0.47 μg of poly(A)-free mRNA per 0.2 ml of reaction mixture. At the indicated time intervals 15-μl aliquots were removed and applied onto Whatman no. 3 filter paper discs and processed for measurement of amino acid incorporation into proteins (10).

the injected message molecules to diffuse into the cytoplasm and to be translated by the free ribosomes of the cell. In the case of poly(A)-free mRNA the same phenomenon is observed, but it is rapidly overcome by a decrease in the rate of translation.

From the results obtained one can estimate that the functional half-life of poly(A)-free mRNA is from 5 to 10 hr. (This approximate value could of course be different if the poly(A)-free mRNA was translated in living reticulocytes.) We conclude from our experiments that the presence of the poly(A)-segment in the mRNA molecule is not necessary for its translation, but is required to sustain extensive translation. The data obtained in the Krebs cell-free system support this conclusion. In this system, the poly(A)-free mRNA is translated normally for the first 30–45 min; but at 60 min the rate of its translation is already much lower than that of the intact globin mRNA. It is not known whether the data presented here are related to the fact that the poly(A)-segments of mRNA become shorter during aging (2, 3). However, the results are in good agreement with the fact that prokaryote messages and histone messages in eukaryotes, neither of which contain poly(A) segments, have a very short half-life. The mechanism by which prolonged translation of poly(A)-free mRNA is prevented is not known yet. One possibility is that the poly(A)-free mRNA is less stable during translation. Poly(A) itself or the proteins which are known to bind to the poly(A) segment of mRNA as part of the messenger ribonucleoprotein (2) may have a role in protecting mRNA against degradation (14). However, another possibility, which cannot be excluded, is that, in the absence of the poly(A) segment, the translation process itself is slowly blocked due to a less efficient recycling of ribosomes.

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