Contribution of polyadenylate sequences to the translational efficiency of globin messenger RNAs

Antonio PARETS SOLER,* Daniel GOZALBO, Jesús ZUECO and Rafael SENTANDREU

Departament de Microbiologia, Facultat de Farmàcia, Universitat de València, Avda. Blasco Ibañez 13, 46010 Valencia, Spain

mRNAs from reticulocyte polysomes were fractionated by chromatography on poly(U)–Sephadex and thermal elution. The molar ratio of α - to β -globin mRNA was found to be 2:1 and 1:1 respectively in short- and long-poly(A) size classes. Translational analyses indicated that the globin mRNAs containing long poly(A) tracts (with a mean length of about 70 nucleotides) directed protein synthesis with higher rates than did mRNA containing short poly(A) tracts (15–35 nucleotides). Experiments performed with sub-saturating mRNA concentrations showed that the digestion with RNAase H induced a decrease in the translational capacity of both globin mRNAs and an increase in the α - to β -globin synthesis ratio. No correlation was observed between the size of the poly(A) tail in mRNA and the optimal K^+ requirement for translation.

INTRODUCTION

The poly(A) sequences found at the 3'-end of most eukaryotic mRNAs have been implicated in mRNA processing and transport, in mRNA stability and in mRNA translation. In relation to mRNA stability, globin mRNA containing poly(A) tracts of less than 30 nucleotides was much less stable after microinjection into *Xenopus* oocytes than was globin mRNA with longer poly(A) tracts (Nudel *et al.*, 1976). Nevertheless, studies of other mRNAs have not correlated mRNA stability with relative poly(A) content (Deshpande *et al.*, 1979; Palatnik *et al.*, 1980; Soreq *et al.*, 1981), and it has been suggested that this role may be indirect. Hence the degradation of de-adenylated rabbit α -globin mRNA in *Xenopus* oocytes was associated with its translation (Huez *et al.*, 1977).

The possibility that poly(A) sequences might function in mRNA translation has been extensively tested. Results showing that the translational efficiency of some poly(A)-containing mRNAs was similar to that of their de-adenylated counterparts argued against a role for poly(A) in protein synthesis (Sippel *et al.*, 1974; Soreq *et al.*, 1974; Williamson *et al.*, 1974). However, this lack of correlation between poly(A) length and mRNA efficiency might be attributed to the utilization of inefficient translation systems. Hence, poly(A)-containing ovalbumin mRNA was translated more efficiently than its de-adenylated counterpart in reticulocyte lysates (Doel & Carey, 1976), and similar results were obtained in *Xenopus* oocytes with α_{2u} -globulin mRNA (Deshpande *et al.*, 1979). It has more recently been reported that purified poly(A) might competitively inhibit protein synthesis *in vitro* in reticulocyte lysates (Jacobson & Favreau, 1983), and a close correlation between changes in poly(A) tail length and translational efficiency *in vivo* has been observed during *Dictyostelium* development (Palatnik *et al.*, 1984). A similar correlation between the adenylation status of some mRNAs and their translational activity was found after fertilization of *Spisula* oocytes, but the behaviour of other mRNAs was the opposite, indicating that polyadenylation was not the

sole factor controlling translational activation of maternal mRNAs (Rosenthal *et al.*, 1983). Despite the conflicting results reported in this field, there is a constant flow of data suggesting a function of poly(A) sequences in regulating protein synthesis (Bird *et al.*, 1985; Schönfelder *et al.*, 1985; Muschel *et al.*, 1986). In the present work, it is reported that α - and β -globin mRNAs containing long poly(A) tracts were translated more efficiently than their de-adenylated counterparts in reticulocyte lysates.

EXPERIMENTAL

Thermal chromatography on poly(U)–Sephadex

Polysomes, isolated from reticulocytes of phenylhydrazine-treated rabbits (Schreier *et al.*, 1977), were resuspended in buffer A [20 mM-Tris/HCl (pH 7.5)/10 mM-EDTA/0.2% (w/v) sodium *N*-lauroylsarcosine] containing 0.5 M-NaCl with a Dounce homogenizer, and RNA was precipitated by adding 1 vol. of ethanol. After 16 h at -20°C , the precipitate was collected by centrifugation at 16000 *g* for 10 min. The pellet was dried in a stream of nitrogen, dissolved in buffer A/0.5 M-NaCl and loaded on a poly(U)–Sephadex column (Pharmacia) at 25°C . The column was washed with 10 vol. of buffer A/0.5 M-NaCl and then with buffer A/0.7 M-NaCl. RNAs containing short poly(A) tracts were eluted by washing the column with buffer B [10 mM-Tris/HCl (pH 7.5)/10 mM-EDTA] at 25°C , and RNAs with longer poly(A) tracts were eluted with buffer B by raising the temperature to 55°C (Palatnik *et al.*, 1979; Bynum & Volkin, 1980).

De-adenylation of mRNAs

For selective digestion of poly(A) tracts, the reaction mixtures contained the following, in a final volume of 50 μl : 20 μg of mRNA and 5 μg of d(T)₁₅ (Boehringer) in 20 mM-Hepes/KOH (pH 8)/100 mM-KCl/10 mM-MgCl₂/1 mM-dithiothreitol. Samples were heated to 65°C for 2 min, incubated on ice for 5 min, and 2.7 units of RNAase H (Boehringer) was added (1 unit produces

* To whom correspondence should be addressed.

50 μ mol of acid-soluble ribonucleotides from poly(A) $\cdot$ poly(dT)/min at pH 8 and 37 °C). After 30 min of incubation at 37 °C, 50 μ l of 25 mM-sodium citrate (pH 7)/7.5 M-guanidine hydrochloride/0.5% sodium *N*-lauroylsarcosine/5 mM-dithiothreitol was added. Samples were vigorously mixed and kept for 42 h at -20 °C in the presence of 400 μ l of ethanol. The RNA precipitate was collected by centrifugation at 10000 *g* for 45 min, dispersed in 600 μ l of ethanol, and kept at -20 °C for 5 days. The final RNA precipitate, collected by centrifugation as above, was dried and dissolved in water.

Protein synthesis in reticulocyte lysates

Rabbit reticulocytes were lysed with 1 vol. of ice-cold water and centrifuged for 15 min at 25000 *g*. The supernatant was supplemented with 25 μ M-haemin, 50 μ g of creatine kinase/ml and 2 mM-dithiothreitol, and treated with micrococcal nuclease (Pelham & Jackson, 1976). Reaction mixtures contained, in a final volume of 30 μ l: 10 μ l of nuclease-treated lysate, 1 mM-ATP, 0.2 mM-GTP, 10 mM-phosphocreatine, 60 μ g of creatine kinase/ml, 40 μ M of each of the amino acids except leucine, 0.33 mCi of [3 H]leucine (50 Ci/mmol)/ml, 0.5 mM spermidine, 0.6 mg of yeast tRNA/ml, 20 mM-Hepes/KOH (pH 7.5), 0.25 mM-magnesium acetate, and the indicated concentrations of the different globin-mRNA preparations. Unless otherwise indicated, reaction mixtures contained 70 mM-KCl, and protein synthesis was stopped by dilution with 30 μ l of ice-cold water after 70 min of incubation at 35 °C. To determine the radioactivity incorporated into acid-insoluble material, samples of reaction mixture were adjusted to 300 mM-NaOH, 1.7% (v/v) H_2O_2 and 2 mM-leucine. After 15 min at 35 °C, the mixture was precipitated with 1 ml of 25% (v/v) trichloroacetic acid at 4 °C. To quantify the synthesis of globin chains, samples of the reaction mixtures were electrophoresed on urea/polyacrylamide gels (Rovera *et al.*, 1978). Gels were stained with Coomassie Brilliant Blue, destained and dried. The α - and β -globin bands were cut out, hydrated with 200 μ l of water, digested with 2 ml of NCS tissue solubilizer (Amersham) at 50 °C for 2 h, and the radioactivity was determined by liquid-scintillation counting in 10 ml of 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.1 g/l) and 2,5-diphenyloxazole (5 g/l) (Packard).

Determination of the size of the poly(A) tract of RNA

RNA (15 μ g) was digested by RNAases A (15 μ g/ml) and T_1 (15 units/ml) (1 unit releases acid-soluble oligonucleotides from yeast RNA equivalent to 1 A_{260} unit/ml in 15 min at pH 7.5 and 37 °C) in 10 mM-Tris/HCl (pH 7.4)/1 mM-EDTA/300 mM-NaCl (final volume 50 μ l). After 90 min incubation at 37 °C, 50 μ l of 25 mM-sodium citrate (pH 7)/7.5 M-guanidine hydrochloride/0.5% sodium *N*-lauroylsarcosine/5 mM-dithiothreitol was added, and the remaining poly(A) was precipitated with 250 μ l of ethanol at -20 °C in the presence of 10 μ g of yeast tRNA. The precipitate was recovered by centrifugation at 10000 *g* for 45 min, washed in ethanol, dried and dissolved in 30 μ l of 40 mM-Tris/HCl (pH 7.3)/20 mM-sodium acetate/1 mM-EDTA/0.2% SDS/10% (w/v) sucrose. The samples were then subjected to electrophoresis on 10% -polyacrylamide gels (Palatnik *et al.*, 1979) and the gels were sliced into 5 mm sections. Each slice was then incubated in 0.5 ml

of 30 mM-sodium citrate/300 mM-NaCl/0.1% SDS for 60 h at 37 °C, and the poly(A) content was assayed as described by Rosbash & Ford (1974), with some modifications. Briefly, 0.1 ml of the solution containing the extracted poly(A) was mixed with 0.1 ml of 10 mM-Tris/HCl (pH 7.4)/700 mM-NaCl and 55000 d.p.m. of [3 H]poly(U) (3.6×10^6 d.p.m./ μ g) (Amersham). After incubation at 41 °C for 1 h, the reaction mixtures were treated with 50 units of nuclease S1 (Boehringer) (1 unit releases 1 μ g of acid-soluble deoxynucleotides from denatured DNA/min at pH 7.5 and 37 °C), and the radioactivity present in the acid-insoluble material was measured. Poly(A) standards of 30, 70 and 200 average monomer length were electrophoresed in parallel. The size distribution of the unknown poly(A) tracts was then determined from the relationship between relative mobility and logarithm of the number of nucleotides.

Preparation of the oligodeoxyribonucleotides

The oligodeoxyribonucleotides were prepared by the phosphoramidite method (Beaucage & Caruthers, 1981; Mateucci & Caruthers, 1981) with an Applied Biosystems DNA synthesizer model 381A.

Synthesis of cDNA

Hybridizations were made in a 10 μ l final volume of 50 mM-Pipes (pH 6.4)/2 M-NaCl containing 0.2 μ g of mRNA from either poly(U)-Sephadex fraction I or II and 30 μ g of oligodeoxyribonucleotide. After incubation at 55 °C for 6 h, the reaction mixtures were adjusted to 100 μ l, and contained 50 mM-Tris/HCl (pH 8.3), 10 mM-magnesium acetate, 50 mM-KCl, 3 mM-dithiothreitol, 0.1% Nonidet P40, 0.5 mM each of dCTP, dATP and dTTP, 10 μ Ci of [3 H]dGTP (4.7 Ci/mmol) and 15 units of reverse transcriptase isolated from Rous-associated virus 2 (Amersham) (1 unit catalyses the incorporation of 0.1 nmol of dTMP into acid-insoluble product/min at pH 8.3 and 37 °C). After 70 min of incubation at 37 °C, the nucleic acids were precipitated in the presence of 50 μ g of DNA (from herring sperm) by adding 300 μ l of ethanol. The precipitate was resuspended in 50 μ l of 1 mM-EDTA, and 5 μ l of 3 M-NaOH was added to hydrolyse the mRNA. After 30 min of incubation at 60 °C, 3 M-HCl (5 μ l) and 1 M-Tris/HCl, pH 7.5 (10 μ l), were added and DNA was precipitated in the presence of ethanol. The precipitate was washed once with ethanol and dried. For electrophoretic analyses, the pellet was dissolved in 50 μ l of 40 mM-Tris/HCl (pH 7.5)/20 mM-sodium acetate/1 mM-EDTA/0.2% SDS/10% sucrose and loaded on to a 10% -polyacrylamide gel (Palatnik *et al.*, 1979). After running, the gel was sliced into 5 mm sections, each slice was digested at 50 °C for 2 h with 2 ml of NCS tissue-solubilizer/water (9:1, v/v), and the radioactivity was measured. For hybridization analyses, the pellet was dissolved in 80 μ l of 10 mM-Tris/HCl (pH 7.5)/100 mM-NaCl/1 mM-EDTA.

Hybridization of globin mRNA with cDNA

Hybridizations were carried out in reaction mixtures (100 μ l) containing 40 mM-Pipes, pH 6.4, 450 mM-NaCl, 1 mM-EDTA, 50% (v/v) formamide, 0.66 μ g of mRNA and the radiolabelled cDNA specific for α -globin mRNA (6.6×10^5 c.p.m.) or β -globin mRNA (10.7×10^5 c.p.m.). For hybridization experiments, the radiolabelled cDNA

Fig. 1. Thermal chromatography on poly(U)-Sepharose of RNA from reticulocyte polysomes

mRNAs containing short poly(A) tracts were eluted by washing the column with elution buffer at 25 °C (fraction I) and mRNAs with long poly(A) tracts were eluted at 55 °C (fraction II). The upper panel shows an electrophoretogram of the α - and β -globin chains synthesized in a reticulocyte lysate programmed with 10 μ g of the mRNA preparation from either fraction I or II/ml. The band of β -globin encoded by the mRNA from fraction I is under-represented, since pre-flashed film was not used for fluorography (the radioactivity present in each band is given in the text).

was synthesized with as template the globin mRNA preparation from poly(U) Sepharose fraction II.

Reaction mixtures were incubated for 10 min at 70 °C and then for 24 h at 45 °C. The extent of hybridization was determined by S1-nuclease digestion as described above. The percentage of [3 H]cDNA hybridization was considered to be equivalent to the percentage of S1-nuclease-resistant radioactivity.

RESULTS AND DISCUSSION

The poly(A)-containing RNAs from rabbit reticulocyte polysomes were fractionated by chromatography on a poly(U)-Sepharose column and thermal elution. RNAs with short poly(A) tracts were eluted at 25 °C (fraction I) and RNAs with the longest poly(A) tracts were eluted by raising the temperature to 55 °C (fraction II) (Fig. 1). Fig. 2 shows that the poly(A) tracts on the mRNA present in fraction I range from 15 to 35

Fig. 2. Size distribution of poly(A) tracts of the RNA

The mRNAs present in poly(U)-Sepharose fraction I (Δ) and fraction II ($\bullet$) were digested with RNAses A and T_1 , and the poly(A) tail length was then determined by electrophoresis with Bromophenol Blue as a reference marker. The gel was sliced, the poly(A) was eluted, and the relative mobility of the poly(A) tracts determined by hybridization to [3 H]poly(U). The mobility of the poly(A) standards relative to Bromophenol Blue ($R_F = 1.0$) was as follows: (rA) $_{200(av.)}$, 0.2; (rA) $_{70(av.)}$, 0.68; (rA) $_{30(av.)}$, 1.08.

nucleotides in length, whereas the poly(A) tail length of mRNA from fraction II was about 70 nucleotides.

In order to determine the relative content of the α - and β -globin mRNA present in the poly(U)-Sepharose fractions I and II, specific DNA probes for each globin mRNA were constructed. The oligodeoxyribonucleotides d(CATGGTGGTTCC) and d(CATTCTGTCTGT), complementary to the initiation-codon regions of α - and β -globin mRNA respectively (Fig. 3c), were used as primers for reverse transcriptase in order to synthesize a radiolabelled cDNA specific probe for either α - or β -globin mRNA. Blake *et al.* (1985), using these oligomers as primers for reverse transcriptase, showed that each oligomer can form only one completely base-paired duplex with its target mRNA. Figs. 3(a) and 3(b) show that the chain lengths of the transcripts resulting from priming by d(CATGGTGGTTCC) and d(CATTCTGTCTGT) were estimated to be approx. 38 and 52 nucleotides respectively, consistent with the expected chain lengths of 39 and 56 nucleotides respectively for the α - and β -globin-cDNA transcripts (Fig. 3c). When the mRNA from poly(U)-Sepharose fraction I was used as template for reverse transcriptase, the radioactivity incorporated into the α - and β -globin-cDNA transcripts was 5.44×10^6 and 5.26×10^6 c.p.m. respectively (Fig. 3a). In contrast, with the mRNA from poly(U)-Sepharose fraction II, the radioactivity incorporated into the α -globin-cDNA transcript was 2.8×10^6 c.p.m., whereas the β -globin-cDNA transcript incorporated 5.43×10^6 c.p.m. (Fig. 3b). In accord with the 5'-terminal nucleotide sequences of globin mRNAs (Fig. 3c), the theoretical number of incorporated guanylyl residues would be 8 for the α -globin-cDNA

Fig. 3. Polyacrylamide-gel electrophoresis of the cDNA transcripts

The oligodeoxyribonucleotides d(CATGGTGGTTCC) (●) and d(CATTCTGTCTGT) (□) were used as primers for reverse transcriptase in the presence of either poly(U)-Sephacrose fraction I (a) or fraction II (b). The cDNA transcripts were electrophoresed on a 10%-polyacrylamide gel, and the radioactivity was measured. The mobilities of restriction-endonuclease digests of λ -phage DNA are used as size markers (arrows): the fragments of 87 and 94 nucleotides are produced by the enzyme *Pst*I and that of 125 nucleotides by the enzyme *Hind*III. (c) Non-coding 5'-terminal nucleotide sequences of α -globin mRNA (Heindell *et al.*, 1978) and β -globin mRNA (Efstratiadis *et al.*, 1977); the sequences of the complementary oligodeoxyribonucleotides are shown below each mRNA sequence. The initiator AUG is enclosed in a 'box'.

transcript and 13 for the β -globin-cDNA. However, since the exact chain length of the cDNA transcripts was not determined, these values were not taken into consideration for the estimation of the molar ratio of α - to β -globin mRNA. Nevertheless, the results shown in Figs. 3(a) and 3(b) indicated that the α - to β -globin-mRNA ratio from fraction I was about 2-fold higher than that ratio from fraction II. In fact, the ratio of the two globin mRNAs from each poly(U)-Sephacrose fraction was estimated (as indicated in the Experimental section) by measuring the S1-nuclease-resistant radioactivity resulting from hybridization with the radiolabelled α - or β -globin-cDNA-specific probes. Thus the S1-nuclease-resistant radioactivity detected in fraction I was 8551 and 7019 c.p.m. after hybridization with α - and β -globin-cDNA transcripts respectively. Experiments with fraction II yielded 6319 and 10925 c.p.m. when hybridization was carried out with α - and β -globin-cDNA transcripts respectively. The α - to β -globin mRNA ratio can be obtained by eqn. (1):

$$\frac{\alpha\text{-mRNA}}{\beta\text{-mRNA}} = \frac{B}{A} \times \frac{NR\alpha}{NR\beta} \quad (1)$$

where $NR\alpha$ and $NR\beta$ represent respectively the S1-nuclease-resistant radioactivity from hybridization experiments performed with α - and β -globin-cDNA transcripts. The correlation between radioactivity into α - and β -globin-cDNA transcripts and their concen-

tration is given by the proportionality factors A and B respectively.

Since the same mRNA concentration was used for hybridization experiments with poly(U)-Sephacrose fraction I and fraction II, it may be assumed that:

$$\alpha\text{-mRNA}_I + \beta\text{-mRNA}_I = \alpha\text{-mRNA}_{II} + \beta\text{-mRNA}_{II} \quad (2)$$

Thus

$$\frac{B}{A} = \frac{NR_{II}^{\alpha} - NR_{II}^{\beta}}{NR_I^{\alpha} - NR_I^{\beta}} \quad (3)$$

where I and II refer to the values obtained with poly(U)-Sephacrose fractions I and II respectively. The B/A ratio obtained in eqn. (3) is 1.7 (consistent with the expected ratio of guanylyl residues incorporated into β - to α -globin-cDNA transcripts). Therefore from eqn. (1) we estimated that the molar ratio of α - to β -globin mRNA was 2:1 and 1:1 for poly(U)-Sephacrose fractions I and II respectively.

Translation in reticulocyte lysates showed that, under optimal ionic conditions for α -globin synthesis, the poly(U)-Sephacrose fraction I contained a higher proportion of translatable α -globin mRNA compared with the β -globin mRNA (Fig. 1) (incorporation of 23308 c.p.m. into α -globin, versus 8090 c.p.m. into β -globin chains). In contrast, mRNA from poly(U)-Sephacrose fraction II directed globin synthesis in the proportions of 51217 c.p.m. for α -chain to 36587 c.p.m. for β -chain (Fig. 1). Fig. 4(a) shows that the mRNA

Fig. 4. Time course of protein synthesis

Reticulocyte lysates were programmed with the mRNA preparation ($10 \mu\text{g/ml}$) from either poly(U)-Sephadex fraction I (Δ) or fraction II ($\bullet$). (a) Radioactivity incorporated into acid-insoluble material ($5 \mu\text{l}$). (b) Samples ($1 \mu\text{l}$) of reaction mixtures were electrophoresed in urea/polyacrylamide gels, and the radioactivity present in the α -globin band was determined.

containing long poly(A) tracts directed protein synthesis with higher rates than did the mRNA containing short poly(A) tracts. Fig. 4(b) shows that the α -globin mRNA present in the poly(U)-Sephadex fraction II directed α -globin synthesis more efficiently than did the α -globin mRNA present in the poly(U)-Sephadex fraction I (it should be noted that the relative concentration of α -globin mRNA in fraction II was lower than that in fraction I). Therefore the low translation rate of the mRNA population from poly(U)-Sephadex fraction I appeared to be directly related to the presence of short poly(A) tracts in mRNA.

To verify further that the variations in translational efficiency of globin mRNA observed above were due to differences in poly(A) length, we performed experiments with de-adenylated mRNAs. For this, the mRNA populations eluted from both poly(U)-Sephadex fractions were digested with RNAase H. It should be noted that control mRNA was also incubated with RNAase H, but oligo(dT) was omitted, in order to avoid translational differences between de-adenylated and

control mRNA owing to non-specific nuclease digestion. When saturation curves for mRNA from poly(U)-Sephadex fraction II were performed, lower amounts of α - and β -globin synthesis were obtained with the de-adenylated mRNA, as compared with the control mRNA. These differences were observed up to an mRNA concentration of $20 \mu\text{g/ml}$ (Fig. 5). When the translational machinery was saturated with mRNA, similar amounts of globin synthesis were obtained with control and RNAase-H-digested mRNAs, indicating that translational differences can only be observed in the mRNA-dose-response linear range. In addition, these results suggested that the poly(A)-deficient mRNAs interacted with the translational machinery less efficiently than did mRNAs with long poly(A) tracts, but were able, however, to direct similar amounts of protein synthesis when their concentration increased. This observation correlates well with the model proposed by Palatnik *et al.* (1984), explaining radical changes in the pattern of protein synthesis without major destruction of pre-

Fig. 5. Relationship between de-adenylation of globin mRNAs and translational capacity

Increasing concentrations of the mRNAs from poly(U)-Sephadex fraction II were translated in reticulocyte lysates: control ($\bullet$) and RNAase-H-digested ($\circ$) mRNAs. (a) Synthesis of α -globin; (b) synthesis of β -globin; (c) α - to β -globin synthesis ratio.

Fig. 6. Effect of K^+ concentration on translation of globin mRNAs with different poly(A) contents

(a) Synthesis of α - and β -globin chains directed by the mRNA preparations ($10 \mu\text{g}/\text{ml}$) from either poly(U)-Sephacrose fraction I or fraction II and their de-adenylated forms (RNAase-H-digested) in the presence of added KCl concentrations of $70 \mu\text{M}$ ($\square$) and 100 mM ($\blacksquare$). (b) The ratio of α - to β -globin synthesis is indicated in each case for 70 mM - ($\triangle$) and 100 mM - ($\blacktriangle$) added KCl.

existing mRNAs during *Dictyostelium* development. This model proposes that transcriptional and translational controls can be coupled by altering the state of adenylation of mRNAs. In addition, we suggest that poly(A)-deficient mRNAs may direct high extents of protein synthesis if their concentration is increased by novel transcription.

The de-adenylation of the mRNA containing long poly(A) tracts [poly(U)-Sephacrose fraction II] induced a preferential decrease in the relative synthesis of β -globin as compared with α -globin (i.e. increase of the α - to β -globin synthesis ratio in the mRNA-dose-response linear range; Fig. 5c). However, in accordance with a lower translational competitive ability of the α -globin mRNA versus β -globin mRNA, the α - to β -globin synthesis ratio must decrease when total mRNA

concentration increases (Lodish, 1974; McKeehan, 1974; Kabat & Chappell, 1977; Di Segni *et al.*, 1979). Indeed, when translation was programmed with the RNAase-H-digested mRNA from poly(U)-Sephacrose fraction II, the α - to β -globin ratio decreased as a function of the mRNA concentration (Fig. 5c). Therefore, the high translational efficiency of the β -globin mRNA may be greatly linked to the presence of long poly(A) tails, but other structural features must play an important role, as discussed below.

In contrast with the β -globin mRNA, the translational capacity of the α -globin mRNA in mammalian cell-free systems has been shown to be severely decreased by increasing the K^+ concentration (Di Segni *et al.*, 1979; Parets Soler *et al.*, 1981). The K^+ concentration optimum for translation of mRNAs arises because of the effect of this cation on the initiation-factor-mediated interaction of mRNAs with components of the translational apparatus (Weber *et al.*, 1977; Brendler *et al.*, 1981; Rosen *et al.*, 1982; Edery *et al.*, 1984; Sarkar *et al.*, 1984). In order to analyse a possible relationship between poly(A) content and ionic requirements for mRNA translation, control and RNAase-H-digested mRNA populations from both poly(U)-Sephacrose fractions I and II were translated in the reticulocyte lysate in the presence of added KCl concentrations of 70 or 100 mM . The results obtained with 70 mM -KCl confirmed that the de-adenylation of mRNA induced a decrease in globin synthesis (Fig. 6a), and showed again that β -globin mRNA translation was more affected than that of the α -globin mRNA, since the α - to β -globin synthesis ratios increased (Fig. 6b). On the other hand, an increase in the added KCl concentration (to 100 mM) decreased the synthesis of α -globin, whereas β -globin synthesis was little affected (Fig. 6a) and the α - to β -globin ratio decreased (Fig. 6b). Therefore these results suggested a lack of correlation between the size of the poly(A) tail in mRNA and the optimal K^+ requirement for translation.

In conclusion, the length of the poly(A) tracts affects the translational efficiency of the α -globin mRNA, and in particular that of the β -globin mRNA. However, β -globin mRNA must possess other features permitting it to interact efficiently with the translational machinery, as is revealed by the low susceptibility to high K^+ concentrations and the competitive abilities of the de-adenylated β -globin mRNA under saturating mRNA concentrations. The translational efficiency of the mRNAs may be the result of a diversity of parameters associated with different structural features of the mRNAs. The parameters associated with the 5'-terminal structure of the mRNA molecule relate to the affinity of the mRNA for translational initiation factors (Godefroy-Colburn & Thach, 1981; Sonenberg *et al.*, 1981; Rosen *et al.*, 1982; Ray *et al.*, 1983; Parets Soler *et al.*, 1984). Our observations agree with the idea that the 5'- and 3'-terminal structural domains may modulate translation independently. Nevertheless, a close association of poly(A) with other regions of mRNAs, as part of a mechanism for modulating mRNA translation, might take place (Jeffery & Brawerman, 1975). Our results, together with those reported by other authors (Doel & Carey, 1976; Deshpande *et al.*, 1979; Jacobson & Favreau, 1983; Rosenthal *et al.*, 1983; Palatnik *et al.*, 1984; Muschel *et al.*, 1986), suggest that the poly(A) tail of the 3'-terminal structure confers additional com-

petitive abilities to the mRNA in order to initiate translation.

We thank Dr. C. López-Otín (Centro de Biología Molecular, Universidad Autónoma, Madrid) for the preparation of the oligodeoxyribonucleotides. This work was supported by grants from the U.S.-Spain Joint Committee for Scientific and Technological Cooperation (CCB8402068).

REFERENCES

- Beaucage, S. L. & Caruthers, M. H. (1981) *Tetrahedron Lett.* **22**, 1859–1862
- Bird, R. C., Jacobs, F. A., Stein, G., Stein, J. & Sells, B. H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6760–6764
- Blake, K. R., Murakami, A. & Miller, P. S. (1985) *Biochemistry* **24**, 6132–6138
- Brendler, T., Godefroy-Colburn, T., Yu, S. & Thach, R. E. (1981) *J. Biol. Chem.* **256**, 11755–11761
- Bynum, J. W. & Volkin, E. (1980) *Anal. Biochem.* **107**, 406–416
- Deshpande, A. K., Chatterjee, B. & Roy, A. K. (1979) *J. Biol. Chem.* **254**, 8937–8942
- Di Segni, G., Rosen, H. & Kaempfer, R. (1979) *Biochemistry* **18**, 2847–2854
- Doel, M. T. & Carey, N. H. (1976) *Cell* **8**, 51–58
- Edery, I., Lee, K. A. W. & Sonenberg, N. (1984) *Biochemistry* **23**, 2456–2462
- Efstratiadis, A., Kafatos, F. C. & Maniatis, T. (1977) *Cell* **10**, 571–585
- Godefroy-Colburn, T. & Thach, R. E. (1981) *J. Biol. Chem.* **256**, 11762–11773
- Heindell, H. D., Liu, A., Paddock, G. V., Studnicka, G. M. & Salser, W. A. (1978) *Cell* **15**, 43–54
- Huez, G., Marbaix, G., Burny, A., Hubert, E., Leclercq, M., Cleuter, Y., Chantrenne, H., Soreq, H. & Littauer, U. Z. (1977) *Nature (London)* **266**, 473–474
- Jacobson, A. & Favreau, M. (1983) *Nucleic Acids Res.* **11**, 6353–6368
- Jeffery, W. R. & Brawerman, G. (1975) *Biochemistry* **14**, 3445–3451
- Kabat, D. & Chappell, M. R. (1977) *J. Biol. Chem.* **252**, 2684–2690
- Lodish, H. F. (1974) *Nature (London)* **251**, 385–388
- Mateucci, M. D. & Caruthers, M. H. (1981) *J. Am. Chem. Soc.* **103**, 3185–3191
- McKeehan, W. L. (1974) *J. Biol. Chem.* **249**, 6517–6526
- Muschel, R., Khoury, G. & Reid, L. M. (1986) *Mol. Cell. Biol.* **6**, 337–341
- Nudel, U., Soreq, H., Littauer, U. Z., Marbaix, G., Huez, G., Leclercq, M., Hubert, E. & Chantrenne, H. (1976) *Eur. J. Biochem.* **64**, 115–121
- Palatnik, C. M., Storti, R. V. & Jacobson, A. (1979) *J. Mol. Biol.* **128**, 371–395
- Palatnik, C. M., Storti, R. V., Capone, A. K. & Jacobson, A. (1980) *J. Mol. Biol.* **141**, 99–118
- Palatnik, C. M., Wilkins, C. & Jacobson, A. (1984) *Cell* **36**, 1017–1025
- Parets Soler, A., Reibel, L. & Schapira, G. (1981) *Biochem. Biophys. Res. Commun.* **99**, 1427–1432
- Parets Soler, A., Reibel, L. & Schapira, G. (1984) *Biochem. Biophys. Res. Commun.* **120**, 804–811
- Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256
- Ray, B. K., Brendler, T. G., Adya, S., Daniels-McQueen, S., Miller, J. K., Hershey, J. W. B., Grifo, J. A., Merrick, W. C. & Thach, R. E. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 663–667
- Rosbash, M. & Ford, P. J. (1974) *J. Mol. Biol.* **85**, 87–101
- Rosen, H., Di Segni, G. & Kaempfer, R. (1982) *J. Biol. Chem.* **257**, 946–952
- Rosenthal, E. T., Tansey, T. R. & Ruderman, J. V. (1983) *J. Mol. Biol.* **166**, 309–327
- Rovera, G., Magarian, C. & Borun, T. W. (1978) *Anal. Biochem.* **85**, 506–518
- Sarkar, G., Edery, I., Gallo, R. & Sonenberg, N. (1984) *Biochim. Biophys. Acta* **783**, 122–129
- Schönfelder, M., Horsch, A. & Schmid, H.-P. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6884–6888
- Schreier, M. H., Erni, B. & Staehelin, T. (1977) *J. Mol. Biol.* **116**, 727–753
- Sippel, A. E., Stavrianopoulos, J. G., Schutz, G. & Feigelson, P. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4635–4639
- Sonenberg, N., Guertin, D., Cleveland, D. & Trachsel, H. (1981) *Cell* **27**, 563–572
- Soreq, H., Nudel, U., Salomon, R., Revel, M. & Littauer, U. Z. (1974) *J. Mol. Biol.* **88**, 233–243
- Soreq, H., Sagar, A. D. & Sehgal, P. B. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1741–1745
- Weber, L. A., Hickey, E. D., Nuss, D. L. & Baglioni, C. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3254–3258
- Williamson, R., Crossley, J. & Humphries, S. (1974) *Biochemistry* **13**, 703–707

Received 23 February 1987/7 April 1987; accepted 27 May 1987