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Periodicity in the length of 3'-poly(A) tails from native globin mRNA of rabbit

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ABSTRACT

Globin mRNA from rabbit reticulocytes was labelled at the 3'-end with [5'-^{32}P]pCp by T4 RNA ligase. The 3'-poly(A) tail was released by digestion of mRNA with T1 ribonuclease and its size distribution determined by gel electrophoresis and autoradiography. The length of the 3'-poly(A) tails varied from about 15-150 residues, but the size distribution exhibited peaks in the abundance of poly(A) species at intervals of approx. 25 residues. This periodicity appears to reflect the manner in which proteins bind to the 3'-poly(A) tail. The function of such regular interactions may be to control mRNA breakdown in the cytoplasm.

INTRODUCTION

The poly(A) tail which is found at the 3'-end of most eukaryotic mRNA species has been shown to be associated with proteins[1-4] and to undergo size reduction in the cytoplasm in an age-related process[5-7].

In the present study we have labelled native globin mRNA from rabbit reticulocytes at the 3'-end with [5'-^{32}P]pCp under conditions in which the mRNA remains functional in directing protein biosynthesis[10]. Thus the size distribution of 3'-poly(A) tails should reflect their distribution *in vivo*. Analysis of the 3'-poly(A) tails on polyacrylamide gels revealed a size distribution from about 15-150 residues with a sharp cut off at the upper and lower values. Within this range there were peaks in the abundance of poly(A) species of various lengths; the first being at a length of 32-34 residues,followed by further peaks at regular intervals of approx. 25 residues.

This size distribution of the 3'-poly(A) tails of native mRNA, which is the subject of this report, has features in common with the size distribution of 3'-poly(A) tails found after mRNP particles were treated with nuclease T2[9], a procedure which led to a series of fragments differing in size by approx. 27 nucleotides. The periodicity in the sizes of 3'-poly(A) tails observed for both native globin mRNA and for mRNP after T2 ribonuclease

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treatment supports the notion that the proteins associated with the 3'-poly(A) tail may serve to inhibit the action of specific endogenous nucleases and so influence mRNA degradation.

METHODS

Materials. T4 RNA ligase was purchased from PL Biochemicals, T1 ribonuclease was purchased from Calbiochem; [5'-^{32}P]pCp (2000-3000 Ci/mmol) was purchased from Amersham International.

Labelling of mRNA. Highly purified polysomal globin poly(A^+)mRNA was prepared from reticulocytes of New Zealand white rabbits[11]. The mRNA was labelled at the 3'-end in a solution of 50mM Hepes, pH 7.5/3.3mM dithiothreitol/15mM $MgCl_2$/10% dimethylsulphoxide/0.01mg serum albumin/ml at 0°C for 18h. The reaction mixture contained 20µg RNA/800 pmoles ATP/50µC[5'-^{32}P]pCp/20 units T4 ligase in a final volume of 15µl. The reaction was terminated by adding an equal volume of 10M urea/5mM Tris-borate, pH 8.3/0.1mM EDTA-Na_2/0.05% xylene cyanol/0.05% bromophenol blue and heating to 90°C for 30 sec prior to cooling on ice and layering on a gel (4% acrylamide/0.20% N,N^{1}methylenebisacrylamide/7M urea/50mM Tris-borate, pH 8.3/1mM EDTA/0.08% ammonium persulphate). Electrophoresis at 300 v was carried out for 3-4h. Radioactive bands were detected by exposure to Kodak X-omat film for 2 min, after which the RNA was eluted from excised pieces of gel[12] and precipitated with 2.5 volumes of ethanol. This procedure leads to labelling at the 3'-poly(A) tail only and leaves the mRNA intact and functional in protein biosynthesis[10].

Analysis of the 3'-poly(A) tails. 1-5µg of globin mRNA labelled at the 3'-end with [5'-^{32}P]pCp was digested with 5 units of T1 ribonuclease at 37°C for 30 min in a solution of 10mM Tris-HCl, pH 7.5/5mM $MgCl_2$/100mM NaCl (5µl). This procedure releases the 3'-poly(A) tail (also including a cytidine residue at the 5'-end[13,14], see Fig. 2). 10µl of 10M urea/dye (as above) was added and the reaction mixture heated to 90°C for 30 sec, cooled on ice and applied to a 60cm 8% polyacrylamide gel (Fig. 3). Electrophoresis was carried out at 2000 v for 3-8h, depending on the degree of separation required.

The distribution of poly(A) oligonucleotides was visualised by autoradiography. The abundance of a particular length of poly(A) can be calculated from the optical density of the bands on the autoradiograph as measured using a microdensitometer (Joyce-Loebel, Newcastle-upon-Tyne, model E15, mark IIB).

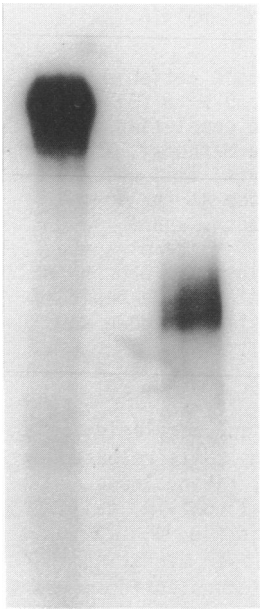

Fig. 1. Autoradiograph of globin mRNA, labelled at the 3'-end with [5'-^{32}P]pCp, after polyacrylamide gel electrophoresis.

mRNA was isolated, labelled and applied to a 4% polyacrylamide gel as described in the Methods section. Electrophoresis at 300v proceeded for 2h (left) and 6h (right). The longer separation enabled two distinct bands to be resolved.

RESULTS

When globin mRNA, labelled with [5'-^{32}P]pCp at the 3'-end, was isolated and purified on polyacrylamide gels (2.5%-5%), autoradiography revealed two distinct major components (Fig. 1) which were not always resolved. The mRNA was eluted from the gel[12] either as one component representing total mRNA or as two distinct fractions and digested to completion with T1 ribonuclease to release the 3'-end labelled poly(A) tails (Fig. 2). The size distribution of the poly(A) tails was analysed by resolving the components of the digest mixture on long (60cm) 8% polyacrylamide gels (Fig. 3).

Size distribution of 3'-poly(A) tails in total globin mRNA

Autoradiography revealed that the size of the 3'-poly(A) tails of total globin mRNA from rabbit reticulocytes varies from about 15-150 nucleotides and that there is a sharp fall off in the abundance of poly(A) species outside this range. The size profile of the 3'-poly(A) tails was analysed by scrutinising the optical density of the bands on the autoradiograph using a microdensitometer. The abundance of a particular length of poly(A) is proportional to the radioactivity of the corresponding band on the gel, which is, within limits, proportional to the darkening caused by the radioactivity on the X-ray film. Therefore, by obtaining the optical density of each of the poly(A) bands on the autoradiograph, it is possible to

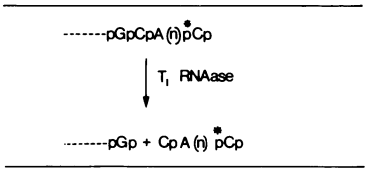

Fig. 2. Release of 3'-poly(A) tails from mRNA.

Globin mRNA from rabbit reticulocytes, labelled at the 3'-end with [5'-^{32}P] pCp, was digested to completion with T1 ribonuclease (see Methods). This releases the 3'-poly(A) tail which has a cytidine residue at the 5'-end in the case of both α and β globin mRNA [13,14]. (*) denotes the position of the radioactive phosphate (^{32}P). n, the no. of adenine residues in the 3'-poly(A) tail was shown to vary between about 15-150.

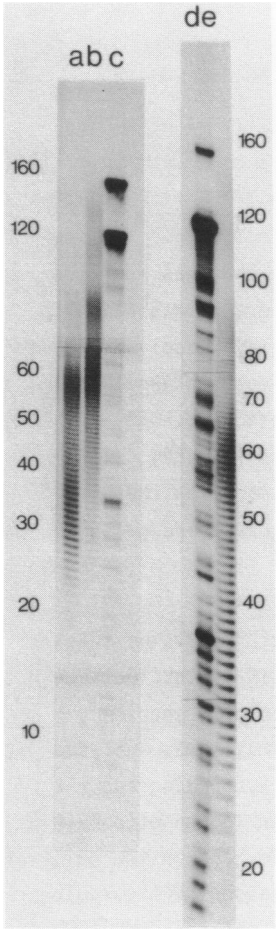

Fig. 3. Autoradiographs of 8% polyacrylamide gels after fractionation of 3'-poly(A) tails released by digestion of globin mRNA with T1 ribonuclease. The tracks are as follows: (a) 3'-poly(A) tails from the faster migrating mRNA band in Fig. 1; (b) 3'-poly(A) tails from the slower migrating mRNA band in Fig. 1; (c) and (d) oligonucleotide markers, comprising N. crassa 5S-rRNA (118 nucleotides), N. crassa 5.8S-rRNA (157 nucleotides) and products of N. crassa 5S-rRNA obtained by chemical cleavage using the A>G sequencing reaction of Peattie (1979); (e) 3'-poly(A) tails from total globin mRNA.

determine the relative abundance of each poly(A) oligonucleotide fraction (Fig. 4).

It is apparent from Fig. 4 that particular lengths of 3'-poly(A) tails are more favoured than others. The peaks in the relative abundance of the 3'-poly(A) tails appear at regular intervals, the first is at a length of 32-34 residues and thereafter approx. every 25 residues. No peaks could be detected at a length greater than 140 residues.

The absence of mRNA with very short 3'-poly(A) tails (less than about 15 adenine residues) was also noted when 3'-poly(A) tails were labelled with [5'-^{32}P]pCp using mRNP as the substrate for T4 RNA ligase. The mRNP particles were isolated[15] without using an oligo(dT) cellulose column and the profile of the abundance of 3'-poly(A) tails as a function of size was similar to that found for mRNA isolated by means of oligo(dT)-cellulose[11]. The rarity of 3'-poly(A) tails of fewer than 15 residues appears to be an intrinsic property of globin mRNA.

Size distribution of 3'-poly(A) tails in two different mRNA fractions

Rabbit globin mRNA was occasionally resolved into two major bands on polyacrylamide gel electrophoresis (see Fig. 1). The components were found to differ in the size distribution of their 3'-poly(A) tails (Fig.3). These differences in 3'-poly(A) tail length are sufficient to account for the different mobilities of the two components in electrophoresis. There is also a difference in the sizes of α-globin and β-globin mRNA of 38 nucleotides that could make a contribution affecting the separation of

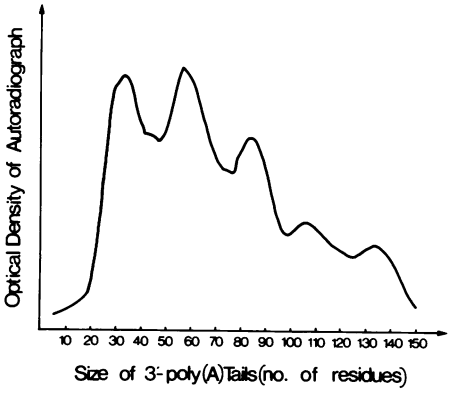

Fig. 4. Size distribution of 3'-poly(A) tails of globin mRNA from rabbit reticulocytes. The optical density of bands on the autoradiographs were measured by a microdensitometer and plotted on a linear scale against the length of the 3'-poly(A) tails. The relative abundance of each particular poly(A) oligonucleotide is proportional to the optical density of the relevant band. Peaks in abundance occur at 32-34 residues, 57-59 residues, 82-84 residues, 105-110 residues and 130-135 residues. These results were verified in several independent experiments. As an illustration of the results, data for total mRNA (see Fig. 3e) are presented.

the two components. However, the dominating factor is the size of the 3'-poly(A) tails.

DISCUSSION

The occurrence of a poly(A) component at the 3'-end of mRNA is almost universal in eukaryotes, suggesting that it plays an important role in mRNA function. The proposal has been made that the 3'-poly(A) tail has a role in the mechanism of mRNA ageing since it has been observed that there is a decrease in the size of the 3'-poly(A) tail after the appearance of mRNA in the cytoplasm[5-7]. This gradual loss of the 3'-poly(A) tail, it has been suggested, would ultimately leave the mRNA sequence accessible to digestion by 3'-exonucleases[8]. It has been demonstrated that proteins which bind to the 3'-poly(A) tail protect it from digestion with snake venom diesterase, a 3'-exonuclease[8]. These protein interactions could serve to provide the cell with a means of controlling mRNA degradation.

In this study we have examined the size distribution of the native rabbit reticulocyte globin mRNA 3'-poly(A) tail that reflects the distribution in vivo. The findings indicate that there is a mechanism within the cytoplasm which leads to some 3'-poly(A) tail lengths being more abundant than others. The favoured lengths of the 3'-poly(A) tail occur at regular intervals of approx. 25 residues over the range 15-150. These results complement a recent report that T2 ribonuclease treatment of RNP particles yields a pattern of fragments in regular multiples of 27 nucleotides[9]. It is therefore feasible to conclude that the periodicity found in the 3'-poly(A) tail length arises from the manner in which poly(A) binding proteins interact with the 3'-poly(A) tail conferring some resistance to the action of endogenous nucleases.

The proportion of mRNA molecules detected with 3'-poly(A) tails less than 15 residues in length was small. It is possible that below this critical length,the poly(A) binding proteins are unable to interact, thereby leaving the 3'-poly(A) tail vulnerable to endogenous nucleases leading rapidly to the degradation of the whole mRNA.

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