DIFFERENCE IN DEGRADATION MODES OF CAPPED AND DECAPPED mRNAs IN VARIOUS EUKARYOTIC CELLS

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1. Introduction

Most eukaryotic mRNAs contain a cap structure $m^7GpppX(m)$ at their 5′-termini. Many attempts have been made to elucidate the biological significance of this modified structure. The cap-carrying mRNA shows higher translation efficiency in protein synthesis than the cap-deleted mRNA [1–3]. Capped mRNA binds to ribosomes more efficiently than the decapped one.

The cap structure stabilizes eukaryotic mRNA against degradation by 5′-exonucleases. The capped mRNA is more stable than the unblocked one when mRNA is injected into *Xenopus laevis* oocytes or incubated with extracts of wheat germ, mouse L cell and *Saccharomyces cerevisiae* [1,3,4]. These results suggest that the unblocked mRNA is degraded from the 5′-terminus exonucleolytically, while the capped mRNA is not degraded by exonuclease. 5′-Exonuclease has been obtained from the supernatant fraction of wheat germ extract [5]. The enzyme digests an unblocked mRNA from the 5′-terminus to release 5′-mononucleotides, but it is not able to hydrolyze both the 5′-capped mRNA and the 5′-OH mRNA without free phosphates at the 5′-terminus. A 5′-exonuclease has been purified also from *Saccharomyces cerevisiae* [4]. However, stability of mRNA may be different in various kinds of cell, depending on the relative contents of the enzymes digesting mRNA. Such difference in mRNA stability is especially expected between a highly differentiated cell and a cell carrying a possibility of further differentiation, as sometimes the former has lost a nucleus or its function including transcriptional machinery during differentiation.

[^{3}H]Uracil-labeled mRNA of cytoplasmic polyhedrosis virus (CPV) was incubated with the extracts prepared from various kinds of cell under the condition of protein synthesis. Depending on the kind of cell, there are two types of degradation of an mRNA. In the extract of highly differentiated cells such as reticulocyte and muscle, the 5′-capped mRNA is degraded at similar rate as the cap-deleted mRNA. In the extract of a cell carrying a possibility of further differentiation such as erythroblast, BHK cell (cultured cell), and wheat germ, the decapped mRNA is degraded more rapidly than the 5′-capped one. This difference seems to be mainly dependent on the activity of 5′-exonuclease in a cell.

2. Materials and methods

The radioisotopically labeled CPV mRNA was prepared in vitro as in [1,6]. To delete the 5′-capped structure without degradation of RNA chain, mRNA was hydrolyzed with tobacco phosphodiesterase as in [7].

Bone marrow cells were prepared from New Zealand White rabbits made anemic by subcutaneous injection of 5 mg acetylphenylhydrazine/kg. The erythroblasts were separated from the other nucleated cells as in [8] by centrifugation 3 times through solution of bovine serum albumin (fraction V) at different densities. Of the prepared cells >90% were found to be erythroblasts in the various stages of maturation by Wright-Giemsa staining [8]. Reticulocytes were collected from the peripheral blood of these anemic rabbits. Lysates of reticulocyte and erythroblast were prepared as in [9]. Wheat germ extract was prepared as in [1].

To assay the rate of degradation of mRNA, CPV

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mRNA labeled with [^{3}H]UTP (5×10^8 cpm) was added to the cell lysate which was adjusted to the condition used for in vitro protein synthesis. After the mixture was incubated at 30°C for the indicated time, trichloroacetic acid solution was added to 5% finally in the ice cold bath. Radioactivity in the resulting precipitate was measured as in [3]. Degradation of the 5′-terminal labeled CPV mRNA after incubation in erythroblast lysate was analyzed by centrifugation in glycerol density gradient as in [1]. Muscle extract was prepared as in [10], liver extract as in [11] and BHK cell extract as in [12].

Degradation products of the unblocked mRNA by incubation in erythroblast lysate were analyzed by DEAE-cellulose chromatography with 7 M urea containing salt solution as in [13].

3. Results and discussion

In wheat germ extract CPV mRNA with capped 5′-terminus is more stable than decapped mRNA, which is prepared by treatment with the pyrophosphatase purified from cultured tobacco cells (fig.1a). The cap structure protects mRNA from 5′-exonuclease attack and consequently stabilizes it in wheat germ or in *Xenopus* oocyte [1,3]. However, we could not detect any significant difference in the stability between the capped and the decapped mRNAs in reticulocyte lysate (fig.1b). Similar results were obtained in [3]. We next compared results obtained from reticulocytes with those from erythroblasts, which are in the younger stage of erythroid cell maturation. Erythroblast-rich fraction was isolated from rabbit bone marrow by the same procedure as used for isolation of reticulocytes and the stability of mRNA was examined. We call the lysate of the fraction the 'erythroblast lysate'. In the erythroblast lysate, CPV mRNA with the capped 5′-terminus was clearly more stable than the decapped one (fig.1c). This result suggests that the unblocked mRNA is degraded by exonuclease attack from the 5′-terminus.

Degradation process of CPV mRNA in erythroblast lysate was then analyzed by sedimentation in a glycerol density gradient. CPV mRNA labeled at its 5′-terminus by [*methyl*-^{3}H]SAM was incubated with erythroblast lysate under the conditions, in which the in vitro protein synthesis is carried out. After 30 s incubation at 30°C, degradation products were analyzed with centrifugation in a glycerol density gradient (fig.2). A large proportion of the 5′-capped CPV mRNA did not change in size, whereas the decapped CPV mRNA labeled at its 5′-terminus with [γ-^{32}P]-ATP was immediately degraded during incubation in the lysate. The degradation product was analyzed by DEAE-cellulose/7 M urea chromatography to determine the product sizes. The radioactivity of the degradation product was detected only in the mononucleotide fraction, but not in oligonucleotide fractions (fig.3). These results suggest the presence of

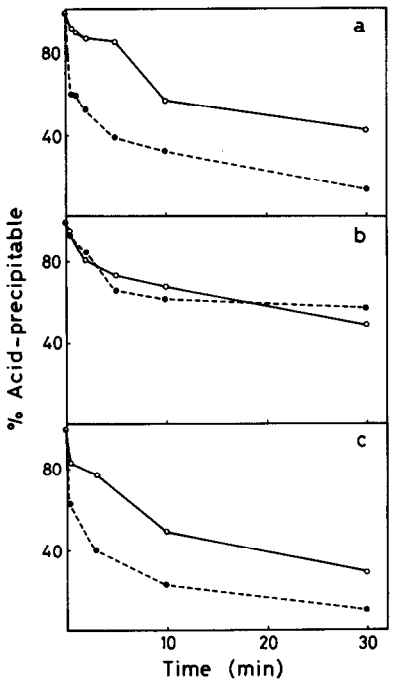

Fig.1. Degradation of CPV mRNA in wheat germ extract, reticulocyte lysate and erythroblast lysate. CPV mRNA labeled internally with [^{3}H]UTP (1.5×10^3 cpm/μg, 5×10^3 cpm) was incubated at 30°C in a reaction mixture used for in vitro protein synthesis. (a) Wheat germ extract: the reaction mixture (50 μl) contained 20 mM Hepes buffer (pH 7.6), 2.5 mM magnesium acetate, 100 mM KCl, 4 mM dithiothreitol, 1 mM ATP, 20 μM GTP, 8 mM creatine phosphate, 2 μg creatine phosphate kinase and 3 μM each of 20 amino acids. (b) Reticulocyte lysate: the mixture contained 12 mM Hepes buffer (pH 7.5), 7.5 mM KCl, 1.5 mM magnesium acetate, 0.8 mM ATP, 0.16 mM GTP, 10 mM creatine phosphate, 11 μg creatine phosphate kinase, 5 mM dithiothreitol, 0.1 mM each of 20 amino acids and 20 μg/ml haemin. (c) Erythroblast lysate: same conditions as in (b). After incubation, radioactive polynucleotides precipitated in 5% trichloroacetic acid were collected on a nitro-cellulose filter, and radioactivity was counted in toluene-based scintillant: (o——o) capped mRNA; (•– – –•) decapped mRNA.

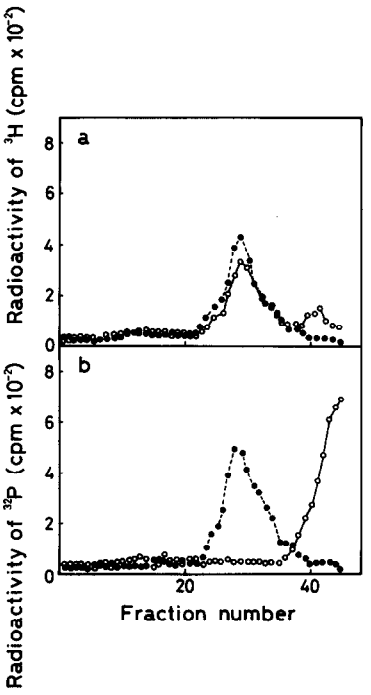

Fig.2. Stability of CPV mRNA during incubation with the erythroblast lysate. CPV mRNA labeled with [*methyl*-^{3}H]-SAM (a) and unblocked CPV mRNA labeled at the 5'-terminus with [γ-^{32}P]ATP (b) were incubated with the S-30 fraction obtained from erythroblasts in 100 μl of a reaction mixture in erythroblasts at 30°C for 30 s. After incubation, 100 μl water-saturated phenol was added and mixture was shaken immediately. The aqueous phase was applied on a glycerol density gradient (10–30%) in 20 mM EDTA/50 mM sodium acetate (pH 5.5) and centrifuged at 38 000 rev./min for 15 h in a SW40 swinging bucket rotor in Beckman-Spinco ultracentrifuge. Fractions (0.28 ml) were collected from the bottom of the tube, and the radioactivity was counted in Kinard's scintillation fluid: (●---●) no incubation; (○——○) incubation for 30 s.

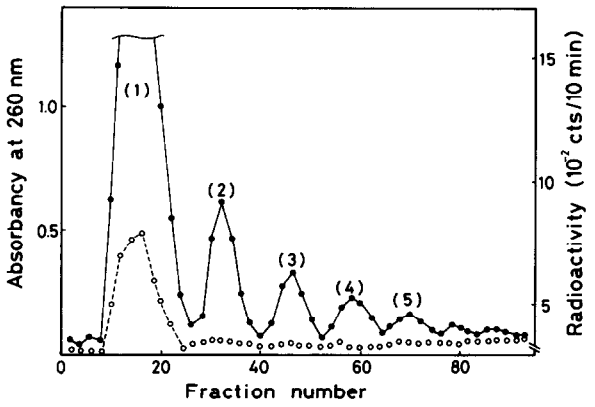

Fig.3. DEAE–urea column chromatography of degradation products from the incubation of the unblocked mRNA (fraction 43–46, fig.2b) in the erythroblast extract. The sample solution was applied to a column (diam. 0.6 cm × length 40 cm) of DEAE-cellulose (Whatman DE-1). Elution was carried out with a linear gradient from 0–0.25 M NaCl (100 ml) in 7 M urea, 0.02 M Tris–HCl buffer (pH 7.6): (○---○) sample; (●——●) pancreatic RNase A digest of ribosomal RNA as a size marker.

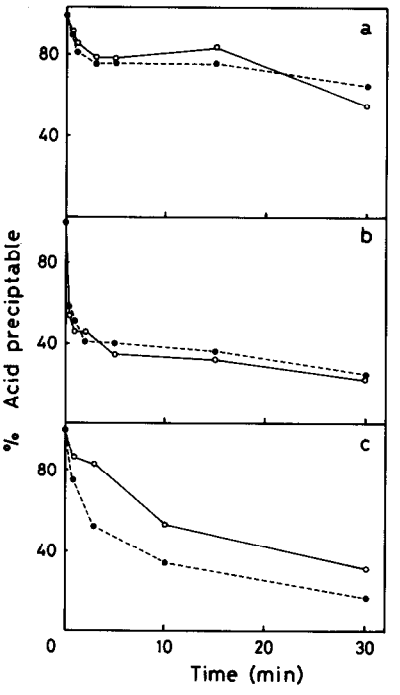

Fig.4. Degradation of CPV mRNA in extracts from muscle, liver and BHK cell. The experimental conditions were as in fig.1 except for the reaction mixture. (a) Muscle extract: the reaction mixture (50 μl) contained 0.15 M KCl, 0.01 M $MgCl_2$, 6 mM β-mercaptoethanol, 0.01 M Tris–HCl buffer (pH 7.4). 2 mM ATP, 0.5 mM GTP, 10 mM phosphoenol pyruvate, 0.5 μM each of 20 amino acids and 2.5 μg pyruvate kinase. (b) Liver extract: the reaction mixture (50 μl) contained 35 mM KCl, 3.5 mM $MgCl_2$, 1 mM dithiothreitol, 35 mM Tris–HCl (pH 7.5), 0.17 mM GTP, 0.7 mM ATP, 7 mM creatine phosphate, 0.035 mM each of 20 amino acids and 2 μg creatine phosphate kinase. (c) BHK cell extract: the reaction mixture (50 μl) contained 120 mM KCl, 5 mM magnesium acetate, 20 mM Hepes–KOH (pH 7.6), 6 mM β-mercaptoethanol, 1 mM ATP, 0.2 mM GTP, 0.1 mM each of 20 amino acids, 10 mM creatine phosphate and 10 μg creatine phospho kinase: (○——○) capped mRNA; (●---●) decapped mRNA.

exonuclease activity in the erythroblast-rich fraction. It seems that this exonuclease cleaves the decapped mRNA progressively in the direction from 5′–3′-terminus as the 5′-exonuclease activity found in wheat germ extract [5]. In rabbit reticulocyte lysate, the exonuclease activity may be overcome by endonuclease activity or be repressed, or the exonuclease may not be contained.

As a comparison, the stability of mRNAs, either with or without the 5′-cap structure, was also assayed in cell lysates prepared from various kinds of tissues. When CPV mRNA was added to the cell-free system obtained from muscle, the mode of degradation of mRNA carrying the cap was the same as the decapped one (fig.4a). As degradation of either mRNA with or without a cap proceeds very slowly, nuclease activity in muscle tissue may be low. The mRNAs added to the cell-free system from the liver were degraded rapidly in both the cases of capped and decapped mRNAs as shown in fig.4b. Although the amounts of proteins in muscle extract and liver extract (fig.4) were made equal, mRNA degradation in liver extract was rapid compared with the case of muscle extract. Nuclease activity is very strong in liver cells. In an in vitro system of BHK cell, which is a cell line established from baby hamster kidney, the mode of degradation of the decapped mRNA was different from the capped one (fig.4c) as in erythroblasts.

Summarizing these results, degradation modes of mRNA are classified into two types:

(1) Cap-carrying mRNA is degraded at the same rate as cap-deleted mRNA, e.g., in the extracts of muscle, liver, and reticulocytes;

(2) Cap-deleted mRNA is degraded rapidly, while cap-carrying mRNA slowly, e.g., in the extracts of wheat germ, BHK cells, and erythroblasts.

In (2), activity of the 5′-exonuclease in a cell is strong compared with endonuclease activity. Presence of the 5′-cap structure must protect mRNA from exonucleolytic degradation. In the highly differentiated cells such as reticulocyte and muscle (1), the 5′-exonuclease seems to be repressed or lost during differentiation.

Acknowledgements

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