THE TRANSLATION OF MOUSE GLOBIN mRNA FROM WHICH THE POLYADENYLIC ACID SEQUENCE HAS BEEN REMOVED IN A REINITIATING PROTEIN SYNTHESIS SYSTEM

by

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SUMMARY: The translation of mouse globin messenger RNA from which the polyA sequence had been removed enzymatically was compared with the translation of control globin mRNA in a rabbit reticulocyte lysate cell-free protein synthesis system. Re-initiation of synthesis occurs on average eleven times for mouse β-globin, demonstrating that the polyA sequence is not required for repeated initiation on the same mRNA molecule.

Recent work from this laboratory with mouse globin mRNA (1) and by others using total L cell mRNA (2) has demonstrated that the polyA sequence found on the 3'-terminus of most eukaryotic mRNAs is not required for the initiation of at least a single round of protein synthesis in a heterologous cell-free system. However, neither of these results definitively demonstrated reinitiation of protein synthesis on deadenylated mRNA. The experiment described below shows that reinitiation is possible on an mRNA from which the polyA sequence has been removed. This was done using a heterologous system derived from rabbit reticulocytes, which is known to translate added mRNA many times during a 90 minute period of incubation (3).

MATERIALS AND METHODS

Polysomal mouse globin messenger RNA was prepared using oligo-dT cellulose (Searle Ltd., High Wycombe, Bucks.) and incubated with polynucleotide phosphorylase to remove the polyA sequence as described previously (1). After this treat-

ment, between 60-80% of the mRNA was no longer retained by oligo-dT cellulose. This deadenylated mRNA is free of 3'-terminal polyA sequences as judged by non-retention by oligo-dT cellulose, fingerprint analysis, template ability with oligo-dT primer and reverse transcriptase, and molecular weight reduction on polyacrylamide gel electrophoresis (1). The RNA retained by the column is identical in sequence and behaviour to untreated mRNA and was used as a control in the protein synthesis experiments.

The activity of the mRNA in directing mouse globin synthesis was measured using a rabbit lysate system as described by Palmiter (3). Assays were carried out in a reaction mixture of 250 microlitres containing 2.5 microcuries of ^{3}H-isoleucine (30 Ci/mM, Radiochemical Centre, Amersham). Between 2-6 picomoles of globin mRNA were added to an assay mixture, which is known to be in the linear response range of the system for added mRNA.

After incubation for 6 or 90 minutes at 26°, non-radioactive mouse globin was added as carrier, and total globin extracted and chromatographed on carboxymethyl-cellulose with a 0.01M - 0.05M phosphate gradient as described by Lingrel *et al.* (4).

RESULTS

Figure 1 shows a typical separation of mouse β-globin from mouse α-globin and rabbit globin after 6 minutes incubation. Palmiter has shown that for this system 1.6% of the isoleucine incorporated is radioactive under these conditions (3). Mouse β-globin contains twice as much isoleucine as rabbit globin and it can be calculated that an incorporation of approximately 2,000 dpm represents one picomole of rabbit globin and 0.5 picomole of mouse β-globin. The amounts of mouse β-globin made in each assay is shown in Table 1.

After six minutes the deadenylated mouse mRNA has been translated twice per molecule on average and the control mRNA 2.8 times per molecule on average; after 90 minutes the deadenyl-

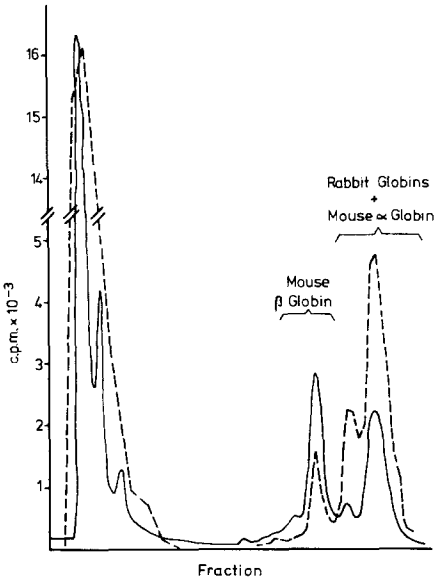

Figure 1. Product analysis of globin chains synthesised in a rabbit reticulocyte lysate cell-free system with added mouse globin mRNA. 2.3 picomoles control mouse globin mRNA were incubated in the system for 6 min at 26°. The mix was cooled to 0° and 40mg unlabelled mouse globin was added. Globin was extracted with acid-acetone and chromatographed on carboxymethylcellulose. The absorbance was monitored at 280 nm (——). Five ml fractions were precipitated onto glass fibre discs, solubilised with NCS (Nuclear Chicago Ltd.) and counted in toluene-based scintillator in a Packard scintillation counter at an efficiency of 48% (– – – –).

ated mRNA has been translated 11.2 times on average and the control mRNA 19.2 times on average. As previously reported, the deadenylated mRNA consistently shows only 60-70% the activity of the control mRNA. However, this figure is similar at 6 and 90 minutes.

DISCUSSION

The amount of mouse globin synthesis obtained with added mouse globin mRNA is that expected for this system, as Palmiter has previously shown a 'transit time' of approximately three minutes for translation of a globin polypeptide chain (3). The somewhat lower globin synthesis

Table 1. The amount of mouse β-globin synthesised at 6 and 90 minutes in a rabbit reticulocyte cell free system.

Added mRNA	Time	pMoles mouse β-globin made	pMoles mRNA added	No. of times each message translated
deadenylated	6 min.	3.81	5.2	1.9
control	6 min.	5.87	4.6	2.8
deadenylated	90 min.	14.01	2.6	11.2
control	90 min.	20.16	2.3	19.2

The picomoles of mRNA added was calculated from the molecular weight of the control (220,000) and deadenylated (185,000) RNAs as determined by gel electrophoresis (1). It was assumed that in both cases 50% of the added mRNA was β-globin message. After 6 minutes each rabbit message had been translated about 10 times, and after 90 minutes about 40 times. The figures in the last column have been corrected for small differences (±10%) in the amount of rabbit globin made in each assay.

directed by the deadenylated as compared to the control mRNA may reflect an inherent effect due to the removal of the polyA sequence, but we feel it is more likely to reflect contamination of the deadenylated mRNA by small fragments of RNA broken by contaminating endonucleases, which contribute to the optical density of the sample but cannot contribute to polypeptide synthesis.

These experiments and others (1,2) demonstrate that the polyA sequence is not obligatory for initiation, reinitiation, translation or termination. However, it is known that the length of the polyA sequence of total HeLa cell cytoplasmic mRNA decreases with time during *in vivo* translation (5). It is therefore likely that polyA is involved in the determination of the lifetime of a messenger RNA in the cytoplasm.

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