UNPROTECTED 5′-SEGMENTS OF POLY(A)$^+$-mRNA IN *SACCHAROMYCES CEREVISIAE* POLYSOMES

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1. Introduction

Recent studies on some specific mRNAs from eukaryotes show that 30–50% of their sequences are non-coding [1–7]. In the case of mRNA for α- and β-globin chains part of these non-coding sequences are located at their 5′-ends [1,8]. The role of these non-coding sequences in eukaryotic mRNA is not clarified, but it is likely that they may be important in the control mechanisms of gene expression.

Previous studies on total yeast poly(A)$^+$-mRNA showed that its size is markedly larger than expected from a population of monocistronic mRNA [9,10]. In the present work we studied the degradation of short-term labelled, polysomal poly(A)$^+$-mRNA from an osmotic-sensitive *Saccharomyces cerevisiae* mutant [11]. The results show that large 5′-segments of total poly(A)$^+$-mRNA from polysomes are unprotected by ribosomes against ribonuclease attack. These results suggest that the presence of non-coding 5′-end segments is characteristic for the bulk of poly(A)$^+$-mRNA molecules in yeast polysomes.

2. Methods and materials

The osmotic-sensitive *S. cerevisiae* mutant VY 1160 cells [11] were grown to a culture density of 4–5 × 10^6 cells/ml in YM-5 medium [12] supplemented with 10% sorbitol. The cells were labelled with 2 μCi [^{3}H]uridine/ml (for analysing the effect of incubation time on the content of poly(A)$^+$-mRNA in total RNA) or with 100 μCi [^{3}H]uridine/ml (for isolation of polysomal poly(A)$^+$-mRNA). At the end of the labelling period 100 μg/ml cycloheximide were added and the cultures were poured over an equal volume of ice-crushed 10% sorbitol, containing 100 μg/ml cycloheximide. The cells were centrifuged and washed with cold 10% sorbitol, containing 100 μg/ml cycloheximide. They were lysed by suspending in 1/10th culture volume of low ionic strength buffer (0.01 M Tris–hydrochloride, pH 7.6; 0.05 M NaCl; 0.01 M $MgCl_2$). Unlysed cells and cell debris were removed by centrifugation. Total RNA was isolated by shaking portions of the lysate with an equal volume of phenol–chloroform mixture (3:1), containing 1% sodium dodecylsulfate for 10 min at 60°C and precipitated with 3 vol. 96% ethanol, containing 1% potassium acetate at −20°C overnight.

Poly(A)$^+$-RNA was separated from total RNA by the use of oligo(dT)cellulose [13]. The samples (0.5 ml) were counted in a mixture of 2 parts toluene/PPO(2,5-diphenyloxazole)/dimethyl-POPOP(1,4-bis-(5-phenyloxazolyl)benzene) phosphor and 1 part Triton X-100 in a Packard Tri-Carb 3320 scintillation spectrometer. For preparative purposes poly(A)$^+$-mRNA was precipitated with 10 vol. 96% ethanol, containing 1% potassium acetate at −20°C.

Polyacrylamide-formamide gel electrophoresis was carried out in 3% polyacrylamide gels in 98% formamide as in [14]. Before electrophoresis the RNA samples, dissolved in 98% formamide, were heated for 3 min at 65°C in order to destroy any aggregates and secondary structure [15]. The gels were cut in 1 mm slices, digested with 10% NH_4OH and counted as above.

For isolation of polysomes the lysate was centrifuged in a 10–40% linear sucrose gradient for 90 min at 40 000 rev./min at 4°C in the SW 40 rotor of a Beckman L5-50 ultracentrifuge. The gradients were collected in 40 fractions, precipitated with 5% trichloroacetic acid and counted. For preparative purposes the tubes with a specified region of the gradient were pooled, mixed with an equal volume of phenol–chloroform (3:1), containing 1% sodium dodecyl sulfate and total RNA was isolated as above.

Nutritional media components and sorbitol were from Difco Laboratories, Detroit, MI; oligo(dT)cellulose was from Collaborative Research Inc., Waltham, MA; cycloheximide, from Koch-Light Lab, England; [^{3}H]uridine (24.7 Ci/mmol) was obtained from Institut für Forschung, Herstellung und Anwendung der Radioisotope, Prague. All other reagents were analytical grade.

3. Results and discussion

Saccharomyces cerevisiae VY 1160 cells were labelled for 15 min with [^{3}H]uridine. After addition of cycloheximide, the cells were lysed with low ionic strength buffer. The lysate was incubated at 20°C. Control experiments showed that incorporation of [^{3}H]uridine into RNA is blocked after lysis of the cells. At different time intervals aliquots were taken, total RNA was isolated and the % of labelled poly(A)$^+$-mRNA was determined. The results (fig.1) clearly show that the relative amount of labelled poly(A)$^+$-mRNA decreases in the initial 20–25 min to reach a plateau level at longer incubation times. These results revealed that a fraction of poly(A)$^+$-mRNA (~60% of the initial amount) in yeast lysates is relatively resistant to nuclease attack.

In order to determine whether this nuclease-resistant poly(A)$^+$-mRNA is in polysomes we analysed the polysome profiles in yeast lysates taken immediately after lysis or upon 50 min incubation at 20°C (fig.2). The results demonstrate that the distribution of ^{3}H-labelled RNA in the two polysomal profiles is identical. The observed stability of polysomes upon incubation of the lysate was unexpected. This could be correlated with the established low activity of ribonucleases in lysates from the osmotic-sensitive yeast mutant studied here [10]. Analysis of the %

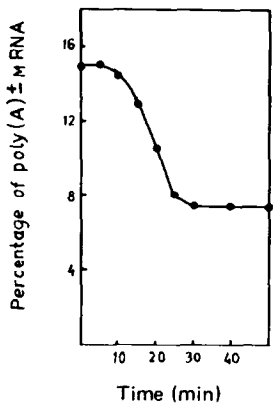

Fig.1. Effect of incubation time of the yeast cell lysate at 20°C on the relative content of poly(A)$^+$-mRNA in total RNA, isolated from the lysate. *S. cerevisiae* VY 1160 cells growing exponentially were labelled with 2 μCi/ml [^{3}H]uridine for 15 min. The cells were lysed and the lysate incubated at 20°C. Samples of the lysate were taken off at different time intervals, total RNA was isolated and the content of poly(A)$^+$-RNA was determined as in section 2.

of poly(A)$^+$-mRNA from the polysome region showed that incubation of the lysate (50 min at 20°C) results in a reduction of labelled polysomal poly(A)$^+$-mRNA to ~60% of the level in polysomes obtained from

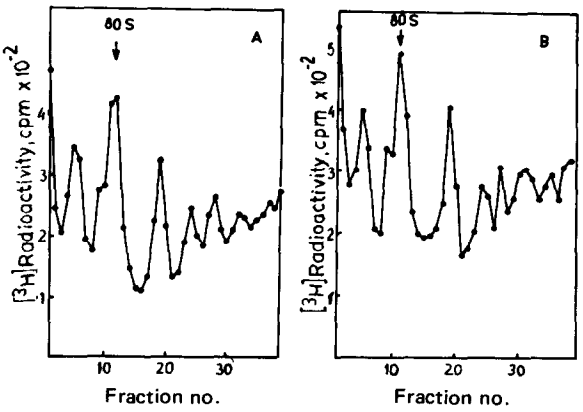

Fig.2. Sedimentation analysis of cell lysates. *S. cerevisiae* VY 1160 cells, growing exponentially were labelled with 100 μCi/ml [^{3}H]uridine for 15 min. Cell lysates were obtained and polysomes were fractionated by sucrose density gradient centrifugation as in section 2. (A) Non-incubated lysate; (B) lysate, incubated for 50 min at 20°C.

control lysates. These observations indicate that the presence of stable and unstable components is inherent to polysomal poly(A)$^+$-mRNA, rather than to the interference by free mRNA–protein particles, known to cosediment with the free ribosomal particles [16]. The fact that labelled polysomal poly(A)$^+$-mRNA is decreased by ~40% without appreciable alteration of the polysomal profile shows that most likely the degraded segments of poly(A)$^+$-mRNA are not protected by ribosomes. Since we analyse only poly(A)-containing mRNA molecules it is evident that the unprotected segments of polysomal poly(A)$^+$-mRNA are located at the 5′-end of the molecule.

In order to verify this possibility we isolated poly(A)$^+$-mRNA of polysomes, obtained from non-incubated lysates and lysates, incubated for 50 min at 20°C. The size of poly(A)$^+$-mRNA was determined by electrophoresis in polyacrylamide–formamide gels [10,14] under conditions where formation of aggregates and secondary structure interactions are abolished [15]. The results (fig.3) show that polysomal poly(A)$^+$-mRNA molecules, obtained from incubated lysates are shorter than their counterparts isolated from control lysates. These results show also that the presence of unprotected 5′-end segments is characteristic for the bulk of polysomal poly(A)$^+$-mRNA of different size classes. Estimates of the average molecular weight of labelled polysomal poly(A)$^+$-mRNA showed that 0.75×10^6 in controls was decreased to 0.44×10^6 after incubation. Thus, it may be concluded that the unprotected 5′-end segments constitute about 40% of the length of labelled polysomal poly(A)$^+$-mRNA.

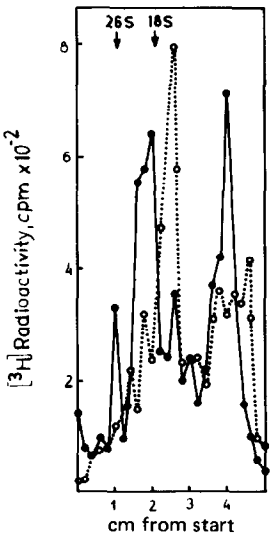

Fig.3. Polyacrylamide–formamide gel electrophoresis of polysomal poly(A)$^+$-mRNA. Poly(A)$^+$-mRNA was isolated from the polysome region of the gradients (fig.2) as in section 2. (——) Poly(A)$^+$-mRNA, isolated from non-incubated lysate; (– – –) poly(A)$^+$mRNA, isolated from lysate, incubated at 20°C for 50 min.

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