IN VITRO SYNTHESIS OF YEAST RIBOSOMAL PROTEINS

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1. Introduction

Most yeast messenger RNAs contain a polyA fragment of about 50 nucleotides [1–4]. However, since a substantial amount of the pulse-labelled RNA from yeast polysomes lacks polyA [2,4] the presence of a polyA tract is not a property common to all yeast mRNAs. It is not known whether lack of polyA is limited to mRNA species coding for specific classes of protein or whether it is a reflection of the physiological state (e.g. age) of mRNA species in general. The only eukaryotic messenger RNA known to be devoid of polyA is histone messenger [5]. If indeed only specific mRNA species lack polyA, then messenger RNA for ribosomal protein, because of the physiological analogy between histones and ribosomal protein, would be a likely constituent of the mRNA fraction lacking polyA. Just as histones, ribosomal proteins are synthesized in the cytoplasm [6–8] and then transported into the nucleus [9]. The complex set of nucleocytoplasmic interactions required in each case might be influenced by the absence of polyA from the mRNA involved.

If ribosomal protein messengers could be shown to occur in the fraction lacking polyA, isolation of these messengers and study of the genetic organization of the genes coding for ribosomal protein would be enormously facilitated.

We separated polyA-containing mRNA from mRNA lacking polyA by chromatography on oligodT-cellulose columns and translated both fractions in vitro using a wheat germ system. The products were analyzed using the method recently developed by us for identification of yeast ribosomal protein [10].

The results presented in this paper demonstrate that both mRNA fractions contain messengers coding for yeast ribosomal proteins.

2. Experimental

Saccharomyces carlsbergensis strain S74 was grown on rich medium [10]. Protoplasts, prepared as described previously [11], were conditioned for 2 h at 29°C as described in detail elsewhere [10]. The protoplast suspension (60 ml; density corresponding to $A_{550\,nm}$ = 0.6 as measured in a Kipp colorimeter) was centrifuged at 3000 *g* for 10 min at 4°C, and the pelleted protoplasts were lysed in 4 ml Tris-buffer containing 0.5% Brij 58 at 4°C [10]. The lysate was diluted with an equal volume of 0.1 M NaCl and SDS was added to a final concentration of 0.5%. The suspension was mixed with 8 ml phenol and stirred for 15 min at room temperature. Subsequently, 8 ml chloroform was added and the mixture was stirred for an additional 15 min [12]. The waterplus interphase was re-extracted twice with an equal volume of phenol-chloroform (1:1 v/v) and finally the waterphase was extracted twice with an equal vol of phenol. RNA was precipitated by the addition of 2 vol of alcohol and redissolved in 2 ml of binding buffer (0.01 M Tris–HCl, pH 7.0, containing 0.5 M KCl). The RNA solution was applied to a column (0.5 × 0.5 cm) of oligodT-cellulose (Searle Laboratories). RNA lacking polyA, which does not bind to the column, was recovered by precipitation with 2 vol of alcohol. The column was then washed several times with 2 ml-portions of binding buffer. Subsequently, polyA-containing RNA was eluted with 4 ml distilled water and freeze-dried. About 5 mg RNA lacking

polyA and approx. 50 μg polyA-containing RNA were obtained in this way. Translation was performed in a wheat germ system in the same way as described by others [14]. 20 μg of polyA-containing RNA or 500 μg of RNA lacking polyA were translated in a total volume of 2.0 ml of the system containing 25 μCi/ml L-[^{3}H (U)] lysine (The Radiochemical Centre, 13 Ci/mmol).

3. Results and discussion

As part of our studies on the biosynthesis of ribosomes in yeast we investigated the possibility that messenger RNA coding for yeast ribosomal proteins might lack a polyA-fragment. Localization of the mRNA coding for ribosomal proteins in a specific, easily identifiable, class of mRNAs would enormously facilitate its isolation and, thus, make possible the unravelling of the genetic organization of the yeast ribosomal protein cistrons.

RNA lacking polyA was separated from polyA-containing RNA by chromatography on oligodT-columns. Each fraction was then translated separately in vitro in a system isolated from wheat germ. Fig.1 shows the incorporation of [^{3}H] lysine into protein synthesized on both types of mRNA. The maximal incorporation programmed by 20 μg of polyA-containing mRNA in 1 h (corrected for endogenous incorporation) is about 35 pmol [^{3}H] lysine, and is in good agreement with the results published by other authors [14]. Using 500 μg of RNA lacking polyA an incorporation of 6 pmol was obtained in 1 h. In this case it is difficult to assess the efficiency of translation, because this RNA fraction contains a large amount of other RNA species (e.g. rRNA). However, if we assume the efficiency of translation of polyA-containing mRNA and mRNA lacking polyA to be equal, the data may indicate that the RNA fraction lacking polyA contains approx. 0.7% of mRNA. Since the amount of polyA-containing RNA recovered from the oligodT-cellulose column was only 1% of the amount of RNA lacking polyA (see Experimental), a possible conclusion is that in yeast protoplasts the weight ratio of polyA-containing mRNA to mRNA lacking polyA is 1:0.7. This conclusion is supported by the finding that, after labelling of yeast protoplasts with [^{3}H] uridine for 3 min, about 60% of the isolated, rapidly labelled polysomal RNA appears to bind to an oligodT-cellulose column (unpublished results), indicating a ratio of polyA (+) RNA to polyA (−) RNA of 3:2. In view of the very good agreement between the two sets of observations, our starting assumption, that polyA-containing mRNA and mRNA lacking polyA are translated in vitro with nearly the same efficiency, seems to be correct. Recently, Williamson et al. [15] arrived to a similar conclusion in the case of mouse globin mRNA.

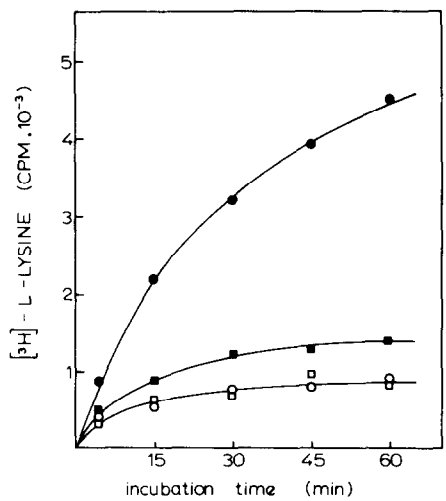

Fig.1. In vitro translation of yeast mRNA in a wheat germ system. Translation was carried out as described in the Experimental section. At the times indicated 10 μl fractions were pipetted into 0.1 N KOH and incubated for 10 min to hydrolyse amino acyl-tRNA. Protein was precipitated with cold 5% trichloroacetic acid in the presence of 500 μg/ml bovine serum albumin, and counted in toluene-NCS. (○) blank; (□) ribosomal RNA (500 μg); (●) polyA-containing RNA (20 μg); (■) polyA-lacking RNA (500 μg).

In order to determine the presence of ribosomal proteins among the translation products, we used the method recently devised by us [10]. Protein was extracted from the incubation mixture, dissolved in 8 M urea and loaded on 15% polyacrylamide gels. Electrophoresis was performed at pH 8.6 with the anode at the top. Under these conditions only ribosomal proteins will migrate towards the cathode and no other cellular proteins are found in the gel [10]. Fig.2 shows the results when this technique is applied to the proteins synthesized in vitro on mRNA lacking polyA-containing mRNA, respectively. While

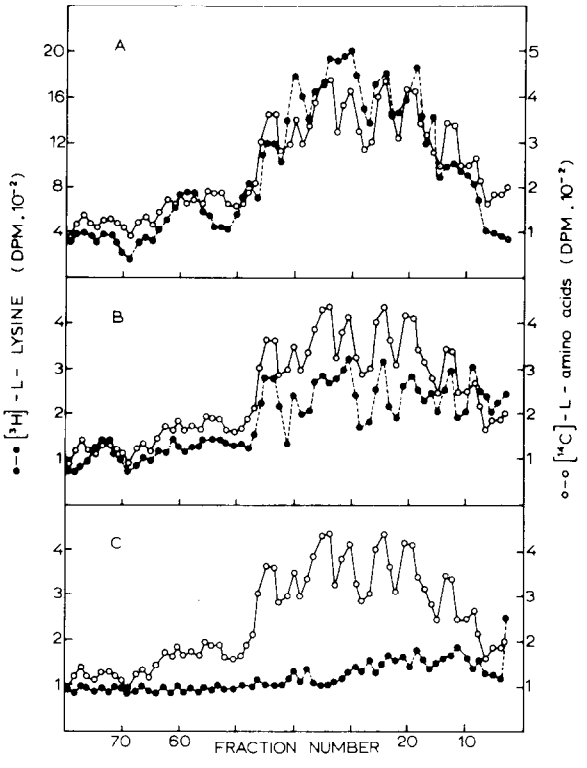

Fig.2. Analysis of protein synthesized in vitro on isolated yeast mRNA in the presence of [^{3}H]lysine. Protein was extracted from the incubation mixture as described previously [10]. Electrophoresis was performed on 15% polyacrylamide gels at pH 8.6 with the anode at the top. Direction of electrophoresis was from right to left. [^{14}C] uniformly labelled ribosomal protein was added as marker. (A) Protein synthesized in vitro on polyA-containing mRNA; (B) Protein synthesized in vitro on RNA lacking polyA; (C) Protein synthesized in vitro when no exogenous mRNA was added. Similar results were obtained when ribosomal RNA was added.

hardly any label can be detected in the control gels, yeast ribosomal protein peaks are present in the other two gels. We must conclude that ribosomal proteins have been synthesized on both polyA-lacking and polyA-containing mRNA. Moreover, no significant differences between the products synthesized on both types of mRNA can be observed.

Though we can not exclude the possibility that there occurs a specific loss of polyA-tracts during the isolation procedure, the results are in agreement with the hypothesis that the presence of polyA-fragments is a general property of mRNA species and that, during the lifetime of the messenger, polyA is split off [16].

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