Volume 220, number 1, 126–128 FEB 04975 August 1987

Studies on interaction of 5 S RNA with ribosomal proteins

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Received 12 June 1987

Proteins of the large ribosomal subunit of rat liver (TP 60) were immobilized by diffusion transfer onto nitrocellulose after two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). Incubation of the TP 60 blots with ^{32}P-labeled 5 S RNA under defined ionic conditions (300 mM KCl, 20 mM $MgCl_2$) resulted in specific binding to a limited set of ribosomal proteins consisting of proteins L3, L4, L6, L13/15 and – to a lesser extent – L7 and L19. Under identical conditions, blots with proteins of the small ribosomal subunit (TP 40) did not bind 5 S RNA.

Ribosome; Ribosomal protein; RNA-protein interaction; Protein blotting; 5 S RNA; 5 S RNA-binding protein

1. INTRODUCTION

5 S RNA is a component of the large ribosomal subunit. It interacts with different ribosomal proteins and has been described as contributing to several ribosomal functions such as peptidyltransferase, GTPase and ATPase activities, binding of initiator tRNA and subunit association [1]. However, the question as to which proteins are directly associated with 5 S RNA within the large ribosomal subunit is still under discussion.

In order to obtain information on the proteins interacting with 5 S RNA, studies by affinity chromatography [2–4], UV-cross-linking [5] and nitrocellulose membrane filtration [6] were performed with different results. All the methods used, however, do not exclude protein-protein interactions. Thus proteins could be cross-linked via another one to the RNA, or the very high affinity of one or several proteins to the RNA may suppress the binding of other ribosomal proteins. Such interactions may also restrict or influence the results obtained by affinity chromatography and nitrocellulose membrane filtration of 5 S RNA with ribosomal proteins.

Incubation of proteins blotted on nitrocellulose with rRNA has proved to be a valuable approach for detection of RNA-protein interactions without the mentioned disadvantages.

Therefore we immobilized the separated ribosomal proteins on nitrocellulose and incubated the blots with ^{32}P-labeled 5 S RNA under different ionic conditions, a method which has already been successfully applied for studying interactions of *E. coli* 16 S RNA [7] and yeast 26 S RNA [8,9] with ribosomal proteins.

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Abbreviations: TP 40, total ribosomal protein from small subunits of rat liver ribosomes; TP 60, total protein from large subunits of rat liver ribosomes; 2-D PAGE, two-dimensional polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

Heparin, Tween 80 and toluidene blue were purchased from Serva, Heidelberg; nitrocellulose membrane filters, 0.45 μm, from Sartorius, Göttingen; alkaline phosphatase from Sigma, St.

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00145793/87/$3.50

Louis; [^{32}P]ATP, 1000–2000 Ci/mM from Amersham, Buckinghamshire; and peptone from Difco, Detroit. Polynucleotide kinase and RNA ligase were prepared according to Dolganov et al. [10].

Ribosomal proteins were prepared and separated by 2-D PAGE following the method of Welfle et al. [11] applying 40 μg TP 60 or 30 μg TP 40 per gel. Ribosomal proteins were designated according to the nomenclature of McConkey et al. [12].

5 S RNA was prepared by phenol-SDS treatment of the 7 S RNP complex released from the large ribosomal subunit according to Behlke et al. [13], and 3′- or 5′-end ^{32}P-labeled according to Peattie et al. [14].

Proteins were blotted from the 2-D PAGE gels on nitrocellulose by diffusion following the procedure of Bowen et al. [15] resulting in two replicas from each gel. The transfer efficiency was controlled by staining with Amido black in 45% methanol and 10% acetic acid.

For each assay 2.5 μg ^{32}P-labeled 5 S RNA (about 10^6 cpm) were renatured in 25 μl of 0.02 M Tris-HCl, pH 7.4, containing 1 mM $MgCl_2$ by heating to 60°C for 5 min and cooling down slowly to room temperature [16].

Nitrocellulose blots were incubated with shaking at room temperature for 15 min with 20 ml binding buffer and then blocked for 90 min in binding buffer containing 1% peptone and 0.5% Tween 80. Afterwards the blots were shaken for 60 min in 20 ml of binding buffer, subsequently incubated with the renatured 5 S RNA sample in 20 ml of binding buffer for 120 min and washed twice with 20 ml binding buffer for 30 min each. Then the nitrocellulose sheet was dried at room temperature and exposed for 40 h at −70°C to X-ray film HS-11 (Orwo, Wolfen) using an intensifying screen Perlux (VEB Kali Chemie, Berlin).

Fig.1. Blot of TP 60 after separation by 2-D PAGE, subsequent diffusion transfer to nitrocellulose and staining with Amido black 10 B.

Detection of ribosomal proteins on blocked RNA-treated nitrocellulose sheets was done, according to [17], by incubation with heparin and subsequent staining of the protein-bound heparin with toluidene blue and superposition of the stained sheet with the corresponding developed X-ray film.

3. RESULTS AND DISCUSSION

The proteins of the large ribosomal subunit (TP 60) were separated by 2-D PAGE and subsequently transferred to nitrocellulose by diffusion. In contrast to rapid blotting by electrotransfer, this method allows the proteins to renature and recover their nucleic acid binding ability [15]. The pattern

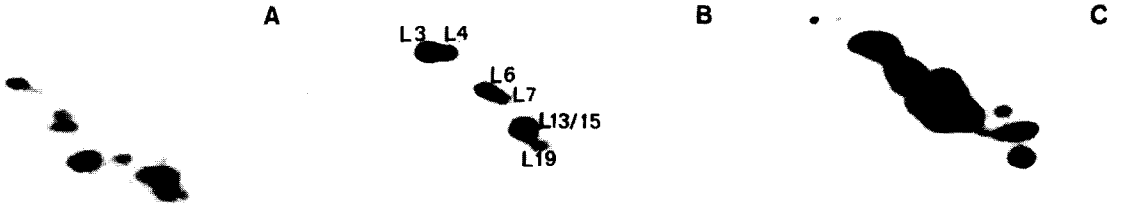

Fig.2. Autoradiographs of TP 60 blots after incubation with ^{32}P-labeled 5 S RNA under different ionic conditions. (A) 100 mM KCl, 20 mM $MgCl_2$; (B) 300 mM KCl, 20 mM $MgCl_2$; (C) 300 mM KCl, 1 mM $MgCl_2$.

of transferred proteins is nearly complete after 48 h with the exception of proteins L3 and L4 which – due to slow diffusion – are only visible as small spots on nitrocellulose sheets (fig.1) while parts of them remain within the gels as enlarged faint spots (not shown). For the 5 S RNA binding experiments incubation conditions of low RNA to protein ratios and short incubation periods were chosen which allowed preferential occupation of the high-affinity binding sites of the immobilized ribosomal proteins. Initial tests on the reliability of the method showed the necessity to block the TP 60 nitrocellulose blots with 1% peptone and 0.5% Tween 80 prior to the incubation with ^{32}P-labeled 5 S RNA. Thereafter the distribution of radioactivity was detected by exposure of the dried blots to X-ray films. As is obvious from fig.2, under different ionic conditions varying labeling patterns were obtained. At 100 mM KCl and 20 mM $MgCl_2$ (fig.2A) or at 300 mM KCl and 1 mM $MgCl_2$ (fig.2C), 5 S RNA bound unspecifically to almost all individual proteins of TP 60. However, under conditions favouring specific protein-nucleic acid interactions, at 300 mM KCl and 20 mM $MgCl_2$ [2–4,6], only proteins L3, L4, L6, L13/15, and to a lesser extent L7 and L19 bound 5 S RNA (fig.2B) whereas proteins of the small ribosomal subunit blotted to nitrocellulose did not bind 5 S RNA under these conditions.

For *E. coli* ribosomes it was shown by different approaches that ribosomal proteins L5, L18 and L25 specifically interact with 5 S RNA. These proteins were released from ribosomes upon EDTA treatment in complex with 5 S RNA [18] and determined to be the most strongly binding proteins to 5 S RNA-Sepharose [19]. In eukaryotic ribosomes, however, the situation is somewhat different. The complex released by comparable EDTA concentrations only consists of protein L5 and 5 S RNA [20,21] and, in addition, in binding studies by affinity chromatography proteins L6, L7 and L19 [2,3] were shown to be 5 S RNA binding proteins. The results obtained here reflecting the binding of 5 S RNA to immobilized single ribosomal proteins confirm and, with regard to proteins L3, L4 and L13/15, extend the results of affinity chromatography.

Furthermore, immobilization of ribosomal protein also seems to be a useful approach to study the binding properties of 18 S RNA and 28 S RNA, or defined fragments of both moieties. Information of this kind could also contribute to accomplishing the reconstitution of eukaryotic ribosomal subunits from their constituents.

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