BINDING OF THE *IN VITRO* SYNTHESIZED RNA TO RAT LIVER 40 S RIBOSOMAL SUBUNIT

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1. Introduction

Studies with microbial systems have shown that mRNA binds to the small ribosomal subunit [1, 2], but the way a messenger RNA binds to mammalian ribosomes has not yet been clearly explained. This is due to the inability to obtain mammalian mRNA and mammalian ribosomal subunits in appropriate quantity to study this process. However, a recently developed method for the separation of mammalian ribosomes (80 S ribosome) into the subunits (60 S and 40 S ribosomal subunits) by high KCl concentration [3], offers the possibility to study much more precisely the processes of the interaction of mammalian RNA with the mammalian 80 S ribosomes and their subunits. In this communication data will be presented indicating that labelled mRNA synthesized *in vitro* on the DNA template from rat liver cells can be attached to the 40 S ribosomal subunit obtained by high KCl concentration from 80 S ribosomes of rat liver cells. This process seems to be stimulated by transferase I and tRNA.

2. Materials and methods

Male albino rats weighing approximately 250 g were used and ribosomes from rat liver were prepared according to Rendi and Hultin [4]. The ribosomes were dissociated into subunits following the method of Martin and Wool [3] (for details see fig. 2). All chemicals were purchased from Calbiochem. ^{3}H-CTP was purchased from Schwarz BioResearch, specific activity 13 Ci/mmole.

The binding of *in vitro* synthesized labelled RNA with ribosomal 40 S subunit was usually followed in 0.2 ml of reaction mixture consisting of the components indicated in fig. 3, the Millipore filtration technique of Nirenberg and Leder [5]. The incubation was effected at 24° during 30 min.

Protein was determined by the method of Lowry et al. [6]. Transferase I was prepared by the method of Schneir and Moldave [7]. As a template for the synthesis of labelled mRNA, we used DNA prepared from rat liver cells by the method of Zamenhoff et al. [8]. RNA polymerase was prepared from *E. coli* MRE 600 by the method of Chamberlin and Berg [9].

The labelled ^{3}H-mRNA was synthesized *in vitro* on the DNA from the rat liver cells in the presence of RNA polymerase and appropriate four nucleoside triphosphates, CTP was labelled with ^{3}H, by the method already described [10].

3. Results

Sedimentation profile of the labelled mRNA synthesized *in vitro* on the DNA template from rat liver cells is presented in the fig. 1. The ^{3}H-labelled mRNA shows a profile close to that of the 4 S tRNA. In fig. 2 are presented the profiles of the ribosomal sununits obtained after the treatment of the 80 S ribosomes with high KCl concentration. The fractions corresponding to the 40 S subunits were taken and their binding capacity was tested. The results obtained in the study of the formation of complex between the 40 S subunit and labelled RNA depending on the Mg^{2+} concentration are presented on fig. 3. As can be seen from the figure, significant binding of ^{3}H-labelled

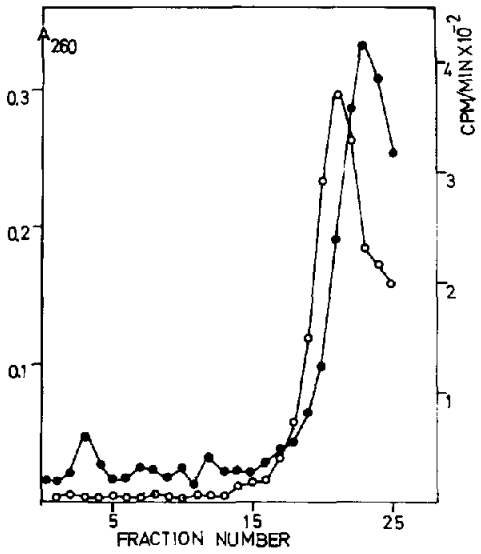

Fig. 1. Sedimentation profile of labelled mRNA synthesized *in vitro* on the DNA template. DNA was extracted from rat liver cells. 600 μg DNA, 20 units of RNA polymerase separated from *E. coli* MRE_{600} (obtained from Prof. François Gros, Institut de Biologie Moléculaire, Paris, France), tris-HCl 0.04 M, pH 8.0, $MnCl_2$ 0.001 M, $MgCl_2$ 0.008 M, four nucleoside triphosphates 0.008 M each (only ^{3}H-CTP, specific activity of 13 Ci/mmole, Schwarz BioResearch) and mercaptoethanol 0.005 M in 10 ml were incubated for 40 min at 37°. The mixture was treated with DNase (10 μg per ml) for another 15 min. Synthesized ^{3}H-mRNA was extracted with phenol saturated with 1XSSC in the presence of 0.5% sodium dodesyl sulfate. After extraction the labelled mRNA was extensively dialysed against 0.01% SSC and used for *in vitro* binding. o—o—o ^{3}H-labelled mRNA; o—o—o tRNA from rat liver cells.

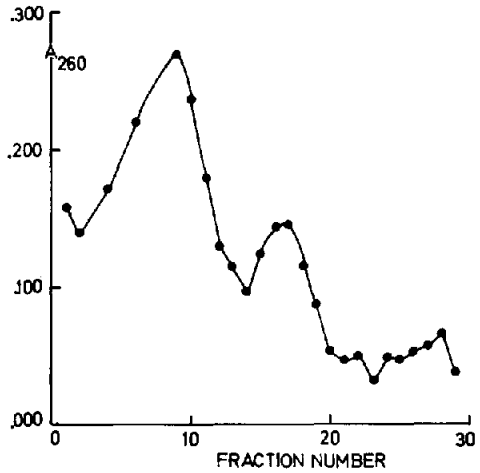

Fig. 2. Separation of ribosomal subunits by high KCl concentration. In order to obtain the ribosomal subunits, 2.5 M KCl in 0.01 M $MgCl_2$ was added to ribosomal suspension containing 0.25 M sucrose, 0.01 M $MgCl_2$, 0.025 M KCl, 0.01 M mercaptoethanol and 0.05 M tris-HCl pH 7.6 to a final concentration of 1.3 M. The suspension was incubated at 37° for 1 hr and layered on 4.6 ml of a 10–30% linear sucrose gradient containing 0.850 M KCl, 0.010 M $MgCl_2$, 0.010 M mercaptoethanol and 0.05 M tris-HCl pH 7.6 centrifuged in rotor SW 50 (Spinco) at 39,000 rpm for 2.5 hr at 24–28°. Fractions were collected and a portion was used to measure the absorbancy at 260 nm. The fractions 17 to 20 were used as a 40 S subunit.

RNA to 40 S subunit was observed in the presence of transferase I and tRNA. This suggests that the complex, formed in the presence of unpurified transferase I, could possibly be similar to that observed with a bacterial system by Revel et al. [11], and that unpurified transferase I contained possibly some materials responsible for the interaction between mRNA and 40 S subunit. Formation of this complex was augmented by an increase of magnesium concentration up to 10 mM, but with higher concentrations of this ion the formation of the complex decreases. Moreover, the results presented on the same figure show that the formation of the complex is much more stimulated in the presence of both transferase I and tRNA, suggesting that tRNA participates in an unknown manner in the formation of the complex between the 40 S subunits and labelled RNA used.

4. Discussion

The results presented show that labelled RNA synthesized on the DNA extracted from rat liver cells can bind to the 40 S ribosomal subunit obtained after treatment of the 80 S ribosomes by a high KCl concentration and that this binding is stimulated by the transferase I and tRNA. The effect of the transferase I on the attachment of labelled RNA to the 40 S ribosomal subunit suggests that this complex might be similar to that formed in bacterial systems, i.e. that this complex could be the initiation complex [11]. The finding that tRNA stimulates the formation of this complex suggests that either tRNA participates

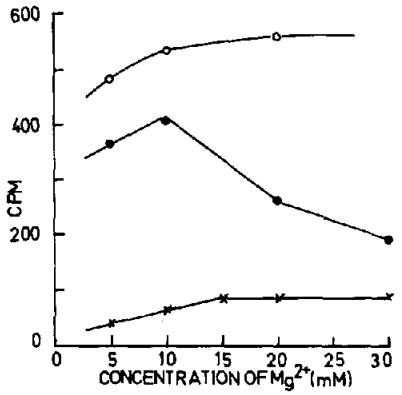

Fig. 3. Binding of *in vitro* synthesized labelled mRNA with ribosomal 40 S subunits depending on the $MgCl_2$ concentration, was followed in a 0.2 ml reaction mixture consisting of the following components: 40 S ribosomal subunit 15 μg, labelled ^{3}H-mRNA 1.5 μg (11,000 cpm), transferase I 40 μg of protein, tris-HCl 0.010 M pH 7.6, KCl 0.005 M and $MgCl_2$ as is indicated in the fig. o—o—o Binding in the presence of transferase I only; o—o—o binding in the presence of transferase I and tRNA from rat liver; x—x—x binding in the absence of transferase I and tRNA. cpm per A_{260} units.

as an active component in the interaction between 40 S subunit and mRNA or that tRNA contains some quantity of fMet-tRNA, as it was recently shown by Smith and Marcker [12] that eucaryotic cells contain two types of Met-tRNA, and that fMet-tRNA in the presence of transferase I in an unknown manner stabilizes the formation of the complex between 40 S subunit and mRNA *in vitro*. Thus, it can be assumed that under our experimental conditions the formation of initiation complex can occur.

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