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A special repressor/activator system controls distribution of mRNA between translationally active and inactive mRNPs in rabbit reticulocytes

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Translation of free mRNPs and polyribosomal mRNPs from rabbit reticulocytes was studied in a rabbit reticulocyte and wheat germ cell-free systems It has been shown that the translation efficiency of polyribosomal mRNPs and the mRNA isolated from the particles is nearly the same in both systems At the same time, free mRNPs' translatability, which is high in the homologous cell-free system, is very low in the system from wheat germs Translation efficiency of free mRNPs in the wheat germ system can be restored by addition of 0 5 M KCl-wash of rabbit reticulocyte ribosomes These results testify to the existence of some special repressor/activator system which controls the distribution of mRNA between free mRNPs and polyribosomes in rabbit reticulocytes

Ribonucleoprotein complex, mRNA, Repressor, Activator, Translation

1. INTRODUCTION

All messenger mRNA is present in eukaryotic cells in the form of ribonucleoprotein complex (mRNPs or informosomes) [1]. Two classes of cytoplasmatic mRNPs can be distinguished, polyribosomal mRNPs and free mRNPs. Polyribosomal mRNPs are a part of polyribosomal complexes and their mRNAs are translated. Free mRNPs can represent: (1) newly sythesized mRNAs in transit from the nucleus to polyribosomes; (2) mRNA which is in equilibrium with mRNA in polyribosomes; or (3) masked mRNA which is capable of translating only under specific physiological conditions [2]. In the literature there is a large body of data on mRNPs containing masked mRNA [2–6]. This paper is devoted to a study of free mRNPs containing mRNA in equilibrium with the mRNA of polyribosomes. We have shown that the state of this equilibrium in rabbit reticulocytes is controlled by a special repressor/activator system.

2. MATERIALS AND METHODS

Polyribosomal and mRNPs were isolated from rabbit reticulocytes by chromatography on oligo(dT)-cellulose as in [7], except that binding of mRNPs with resin was done at 150 mM NaCl [8] mRNA was extracted from mRNPs by SDS/chloroform/phenol deproteinization [8]. The concentration of free mRNA and mRNA within mRNPs was determined spectrophotometrically [8]. The rabbit reticulocyte cell-free translation system treated with micrococcal nuclease was prepared as described previously [9] The cell-free translation system from wheat germs was prepared as in [10]. The fraction of the 0 5 M KCl-wash of rabbit reticulocyte ribosomes was obtained as described previously [11] SDS gel electrophoresis was done according to Laemmli [12].

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3. RESULTS AND DISCUSSION

Polyribosomal and free mRNPs isolated from rabbit reticulocytes were translated in two different cell-free systems of protein biosynthesis: (1) in a homologous rabbit reticulocyte system; (2) in a heterologous wheat germ system. The translation of mRNPs was compared with that of mRNA isolated from the same particles. It is shown (fig.1a,b) that polyribosomal and free mRNPs, as well as deproteinized mRNA, are translated in rabbit reticulocyte lysates with a high efficiency. It is worthwhile mentioning the significant difference in translation of mRNA and mRNPs depending on their concentration. Some reasons for this phenomenon have been discussed in detail previously [8]. The translatability of free mRNPs (fig.1b) as well as the similarity of the products of translation of free mRNPs and their mRNA (fig.2a,b) show that free mRNPs from rabbit reticulocytes do not seem to contain masked mRNA. These particles therefore contain the mRNA which is in equilibrium with the mRNA of polyribosomes. The similarity of products of translation of free and polyribosomal mRNPs (fig.2a,c) also confirms this conclusion.

mRNA, isolated from polyribosomal and free mRNPs, as well as polyribosomal mRNPs, are effectively translated in the cell-free system from wheat germs, while the translation of free mRNPs in this system proceeds far less efficiently (a 10–25% level of mRNA translation) (fig.1c,d). On the other hand, free

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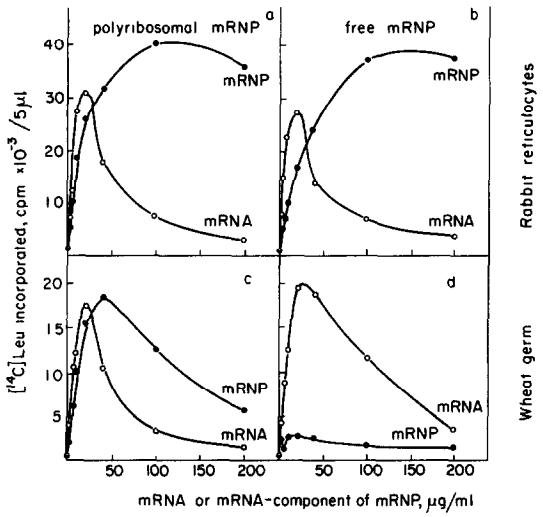

Fig 1 Translation of polyribosomal (a,c) and free (b,d) mRNPs from rabbit reticulocytes in cell-free systems of protein biosynthesis from the lysate of rabbit reticulocytes (a,b) and wheat germs (c,d) (●—●) mRNPs, (○—○) mRNA, isolated from the corresponding classes of mRNPs Cell-free translation systems were incubated 1 h

mRNPs do not inhibit the translation of free mRNA in this system (table 1). The formation of polyribosomes can be detected during mRNA translation in a wheat germ cell-free system. At the same time polyribosomes are not formed at incubation of free mRNPs (fig.3). This testifies that translation of free mRNPs in a wheat germ cell-free system is inhibited at the initiation stage.

Our data indicate that free mRNPs from rabbit reticulocytes contain a component preventing the initiation of free mRNP translation in a wheat germ cell-free system. This component is absent in preparations of

Table 1

Effect of free mRNPs from rabbit reticulocytes on mRNA translation in the cell-free system of protein biosynthesis from wheat germs

RNA concentration (μg/ml)		[14C]Leucine incorporated, (cpm/ 5 μl)
mRNA	mRNP*	
10	–	15 026
–	10	2 956
10	10	16 372
20	–	23 952
–	20	3 748
–	–	647

* The concentration of the mRNA-component of mRNP is indicated

polyribosomal mRNPs and isolated mRNA. We suppose that the indicated component is a repressor, preventing the process of initiation of mRNA translation not only in what germs but also in reticulocytes. This assumption is supported by the localization of the repressor component only within free mRNPs and its absence within translating polyribosome-bound mRNPs. In this case the translatability of free mRNPs in rabbit reticulocyte lysates can be explained by the presence in reticulocytes (in contrast to wheat germs) of a special activator component overcoming the effect of the repressor. If this proves to be the case, the addition of some fraction from the reticulocyte lysates into the wheat germ cell-free system must stimulate the translation of free mRNPs in this system. Indeed, the addition of a 0.5 M KCl-wash of rabbit reticulocyte ribosomes into the wheat germ cell-free system considerably stimulates translation of free mRNPs (fig.4). The translation of deproteinized mRNA in these conditions was not stimulated but decreased (fig.4). Hence, ac-

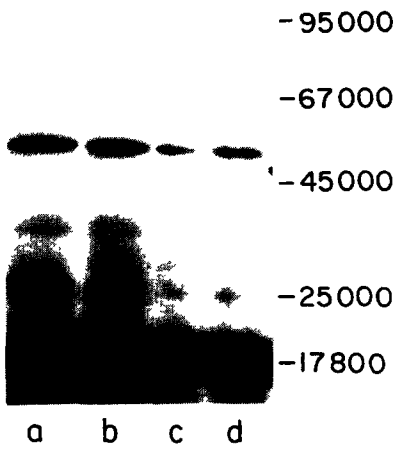

Fig 2. Fluorogram of SDS gel electrophoresis of proteins synthesized in the cell-free translation system from rabbit reticulocytes after translation of free mRNPs (a), of mRNA extracted from free mRNPs (b), polyribosomal mRNPs (c), mRNA extracted from polyribosomal mRNPs (d) Each line contains 100 000 cpm [35S]methionine-incorporated material

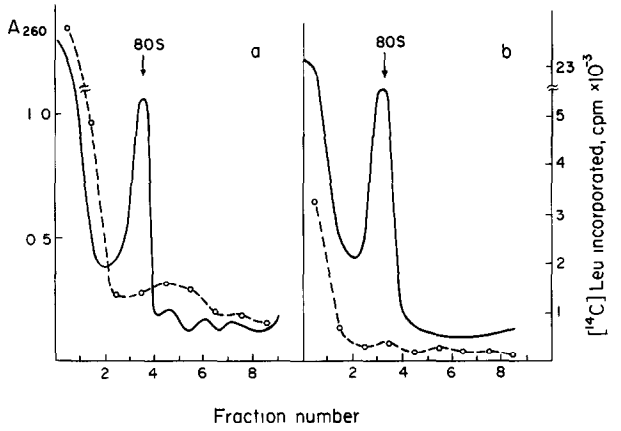

Fig 3 Sucrose gradient analysis of wheat germ extracts incubated with mRNA (a) and free mRNPs (b) (——) adsorption at 260 nm, ((○---○) distribution of a [14]leucine-incorporated material, cpm per fraction The extracts containing 20 μg/ml free mRNA or mRNA within mRNPs were centrifuged in the linear 15–33% sucrose gradient in a SW-55 rotor, at 4°C, 35 000 rpm for 1 5 h Sedimentation was from left to right

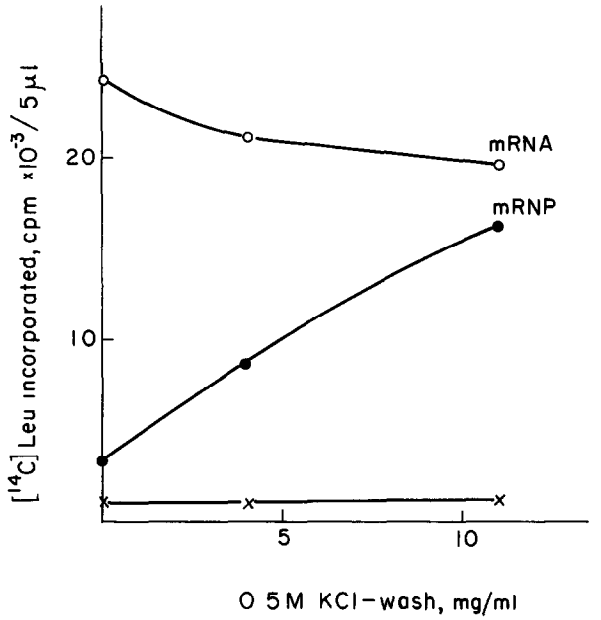

Fig 4 Effect of various concentrations of 0 5 M KCl-wash of rabbit reticulocyte ribosomes on translation of free mRNPs (●—●) and their mRNAs (○—○) in the cell-free system from wheat germs, control without exogenous templates (×—×). Cell-free systems without exogenous mRNA as well as those containing 20 µg/ml mRNA or mRNA within mRNPs were incubated for 1 h at 25°C

tivator component is present in the 0.5 M KCl-wash of ribosomes. It is absent in the fraction of the postribosomal (ribosome-free) supernatant (data not shown).

The results presented in this work demonstrate that the process of initiation of translation occurs differently in the case of free mRNPs and in that of polyribosomal mRNPs. This testifies to the existence of some special system which controls the distribution of mRNA between free mRNPs and polyribosomes in rabbit reticulocytes. This system includes such components as: (1) a translation repressor associated with mRNAs within free mRNPs preventing the initiation of mRNA translation; and (2) a translation activator associated with ribosomes overcoming the effect of the repressor. Such an activator of translation of free mRNPs from rabbit reticulocyte is absent in wheat germs.

Earlier Walden and Thach based on different translation competition of mRNA and mRNPs from mouse fibroblasts suggested the existence of a special translational induction/repression system in eukaryotes [13].

Our work on isolation of pure preparations of repressor and activator from rabbit reticulocytes is now in progress.

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