REGULATION OF MESSENGER RNA BY A RIBONUCLEIC FACTOR IN THE PRESENCE OF POLYAMINES

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1. Introduction

Dialyzable components, capable of restoring the activity of crude dialyzed initiation factors have been isolated from the 0.5 KCl wash of rabbit reticulocyte ribosomes [1–4]. Small RNA molecules isolated by this technique are thought to be responsible for this activity in some cases [1–5], while in other cases [6–8], they inhibit specifically the messenger RNA translation.

The purification of dialyzable components produced the evidence of not only one, but two kinds of factors: a ribonucleic component and polyamines. The presence of polyamines, particularly spermine and spermidine in the dialysate of reticulocyte initiation factors, has been demonstrated by Konecki et al. [9]. According to these authors, at low magnesium concentrations, polyamines are able to restore the activity of dialyzed initiation factors. The activity of polyamines in protein synthesis, mainly at the transcriptional level, has also been reported [10,11].

In the present study, we give evidence of two types of factors, and their interaction at the initiation level of protein synthesis. A model to explain the mechanism whereby these factors regulate the translation of messenger RNA is proposed.

2. Materials and methods

Cell-free reticulocyte components were prepared as previously described [2]. The dialysate of crude initiation factors, after lyophilization, was resuspended in water, and purified by Dowex 50 WX 4 chromatography (H^+-form) according to Katz et al. [12]. Samples were eluted stepwise 50 mM HCl, water and 50 mM KOH, and further purified by Bio-Gel P6 chromatography.

The hemoglobin 9 S mRNA was extracted from rabbit reticulocyte ribosomes as described [13].

Cell-free protein synthesis was performed using Miller's method [14] in presence of 2 mM $MgCl_2$ and 65 mM KCl, and 0.1 mg of washed ribosomes, 0.5 mg pH 5-fraction, 0.01 ml supernatant obtained after precipitation of the pH 5-enzyme; initiation factors dialyzed or not and ribonucleic factor, polyamines and messenger RNA as indicated in the figures. After 60 min of incubation at 37°C, the trichloroacetic acid precipitable material was collected and counted, and an aliquot was applied to SDS slab-gels as described by Laemmli et al. [15]. The gel was autoradiographed.

In cell-free translation, two types of ribosomes were used: either salt-wash ribosomes kept at −80°C in pellets and resuspended just before use, for studies with endogenous messenger RNA, or ribosomes in 50% glycerol suspension, kept at −20°C, in which about 50% of messenger RNA is inactivated and able to be stimulated by adding polyamines (personal results), for studies with exogenous messenger RNA.

The experiment carried out for the quantitative measurement of the initiation of protein synthesis was done in the presence of sparsomycin as described by Smith et al. [16].

Phosphorus and ribose contents were estimated by the method of Ames [17] and Trim [18], respectively.

Polyamines were purified and their quantitative

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determination carried out according to Konecki et al. [9].

3. Results

From the dialysate of the protein initiation factors, two kinds of components were isolated: a ribonucleic component and polyamines. These two types of components were obtained after Dowex 50 WX 4 chromatography (fig.1) followed by Bio-Gel P6 chromatography of the A and B peaks.

Following butanol extraction, the B peak was identified as polyamines using Konecki's method [8]. The concentration in polyamines was estimated by titration in presence of fluorescamine and with respect to a spermine solution. There were 60 mol polyamine/ribosome. After dansylation and separation of dansylated compounds, the polyamines of the B peak were identified as spermine and spermidine in the ratio of 1:3.

The ribonucleic component of the A peak was characterized by the ultraviolet spectrum (fig.2), the estimation of phosphorus and ribose, which gave the ratio of 1:1. The determination of the exact structure is in progress by mass spectrometry and the presence of a non-normal base was established.

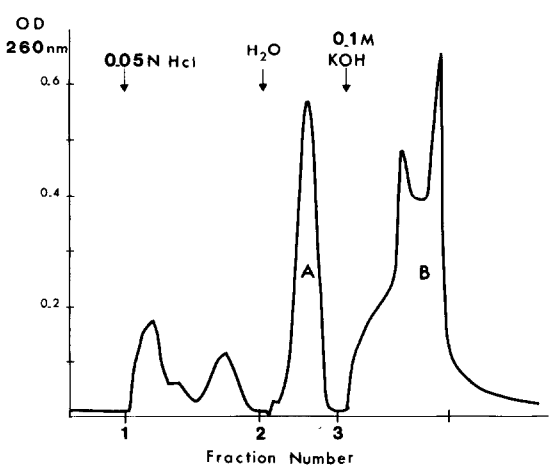

Fig.1. Dowex 50 WX 4 chromatography of dialysate of crude initiation factors. 40 A_{260} were chromatographed on a (1 X 40 cm) Dowex 50 WX (H^+-form) column equilibrated with 50 mM HCl. Step-wise elution was done with 50 mM HCl, water and 0.1 M KOH or 0.1 M Tris, pH 9.

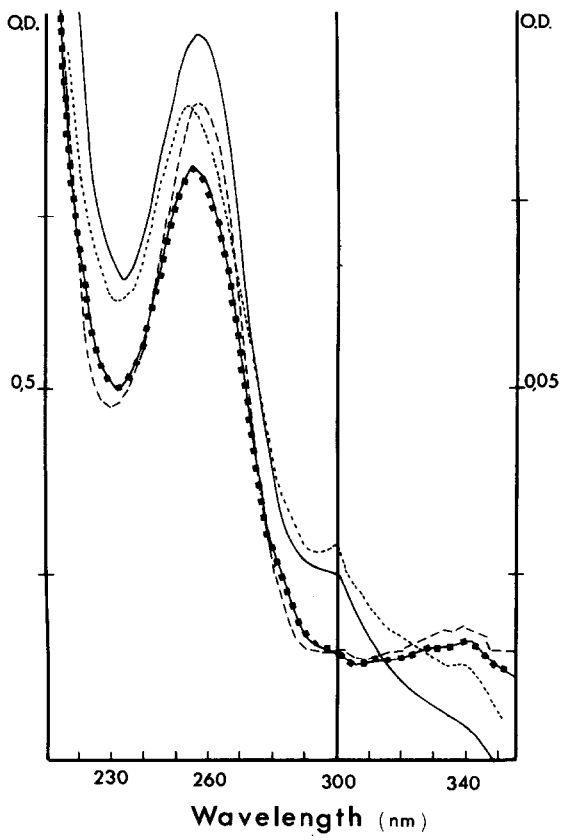

Fig.2. Ultraviolet spectrum of the ribonucleic factor. The ribonucleic factor was eluted from Dowex 50 WX 4 chromatography with water, purified by Bio-Gel P_6 chromatography after which the ultraviolet spectrum was done at different pH-values: (——) 12.5, (-·-) 5.5, (■——■) 2.5, (. . . .) 1.0.

The effects of polyamines on cell-free translation of synthetic polynucleotides as well as natural messenger RNA are well-documentated [19,20]. In the case of reticulocyte system, the ribonucleic compound enhances the polyamines effect. The biosynthesis of hemoglobin was demonstrated by total incorporation, checked by electrophoresis on polyacrylamide gel.

In a cell-free system, and in the presence of dialyzed initiation factors, the following results were obtained (table 1):

(i) In the best conditions, only a 1.5-fold of protein synthesis was obtained by addition of the ribonucleic component to the cell-free system.

Table 1
The effect of the ribonucleic factor on hemoglobin synthesis

Experiments	Ribonucleic factor				
	0 μg	0.35 μg	0.75 μg	1.06 μg	1.23 μg
	(pmol Leu incorporation)				
Dialyzed initiation factors	7.6	9	7.5	13	10
Dialyzed initiation factors					
+ spermine 0.205 mM	107	109	155	188	96
0.102 mM	76	67	112	115	92
+ spermidine 0.56 mM	67	75	74	68	68
0.28 mM	21.4	26.3	47	48	48

Incubations were performed as described in Materials and methods, in the presence of salt-wash ribosomes kept at −80°C in pellets and resuspended just before use. In every case, a control incubation was done with non-dialyzed initiation factors yielding 25–30 pmol leucine incorporation due to the polyamines and ribonucleic factor contents of the initiation factors.

(ii) Using polyamines alone, protein synthesis was 9–14-fold stimulated, with optimal concentrations of 0.205 mM spermine and 0.56 mM of spermidine, respectively.

(iii) Using polyamines together with the ribonucleic component, protein synthesis was greatly increased and this, depending on the concentrations of those two types of factors. As shown in table 1, there was a 1.75-fold increase of protein synthesis by spermine and a 2.23-fold increase with spermidine, but only at low concentration of spermidine. These increases relate to polyamines alone.

(iv) The influence of magnesium was also studied because it is known that Mg^{2+} can partly substitute for polyamines. At 3.5 mM Mg^{2+}, the ribonucleic factor was not able to stimulate synthesis. Therefore, the protein synthesis activity of the ribonucleic factor, in the presence of polyamines, is not due to the polycationic nature of the polyamines.

To summarize, under optimal conditions, the stimulation of protein synthesis was 1.5-fold with the ribonucleic factor, 9–14-fold with polyamines and 16–30-fold with polyamines and ribonucleic factor together. By polyacrylamide gel autoradiographic analysis of the cell-free incubation products, all the radioactive synthesized material was located in the hemoglobin area.

In the presence of 0.07 mM sparsomycine which blocks elongation [16], the cell-free incubations were done as in table 1, using [^{35}S]methionine as labelled amino acid. The initiation dipeptides containing methionine (Met–Val) were isolated and purified by electrophoresis, and we obtained a 1.7-fold increase of dipeptides Met–Val by addition of the ribonucleic factor to a system containing polyamines. We can conclude that this factor has some role in the initiation process.

We further studied the stimulation of the protein synthesis by ribonucleic factors and polyamines in the presence of added messenger RNA. Ribosomes partially stripped of messenger RNA were used (suspension of 50% glycerol salt-wash ribosomes). In this case, the ribonucleic factor stimulated very weakly the protein synthesis in the presence of exogenous messenger RNA. Polyamines stimulated exogenous messenger RNA translation 1.5–2.5-fold compared to the stimulation obtained without added messenger RNA (table 2).

Moreover in the presence of polyamines and messenger RNA, the stimulation of protein synthesis by the ribonucleic factor was under optimal conditions, 2.2-fold as compared to stimulation with polyamines and messenger RNA. Table 2 shows:

(i) At a high concentration of spermine, 0.205 mM and saturating or non-saturating concentration of messenger RNA, the ribonucleic factor stimulates protein synthesis 1.5-fold. At low concentration of spermine 0.105 mM, stimulation was only obtained

Table 2
Stimulation of globin messenger RNA by the ribonucleic factor in the presence of polyamines

Experiments	9 S							
	0.9 μg				2.25 μg			
	Ribonucleic factor							
	0 μg	0.8 μg	1.18 μg	1.5 μg	0 μg	0.8 μg	1.18 μg	1.5 μg
	(pmol Leu incorporation)							
Dialyzed initiation factors	5	6.9	7.5	7.5	4.7	10.3	9	7
Dialyzed initiation factors								
+ Spermine 0.205 mM	200	202	230	167	181	258	320	259
0.102 mM	76.2	172	176	203	143	168	174	185
+ Spermidine 0.84 mM	116	162	138	120	202	156	227	168
0.56 mM	115.2	140	123	97	187	180	192	284

Incubations were performed as described in Materials and methods in the presence of salt-wash ribosomes kept in 50% glycerol suspension at −20°C.

with a non-saturating concentration of messenger RNA.

(ii) In the case of spermidine the results were different. Stimulation of protein synthesis was observed only with a low concentration of spermidine and the optimal dose of ribonucleic factor was lower with a non-saturating concentration of messenger RNA than with an excess of messenger RNA.

The results of those experiments are summarized in table 2. In each case, 4–6 experiments were performed; the nature of the protein (hemoglobin) was always checked by polyacrylamide gel electrophoresis as control. The differences observed between pmol leucine, incorporated in a cell-free system containing protein initiation factors and polyamines, were due to different preparations of initiation factors; but the stimulation of the protein synthesis by added ribonucleic factor and messenger RNA gave always the same ratio.

4. Discussion

Suggestions have been made that the translational control of protein synthesis in eukaryotes can be achieved by different kinds of factors [5,21,22]. Several lines of evidence point out to the importance of small ribonucleic factors [1–8].

We have been able to isolate from the dialysate of crude protein initiation factors a biologically active ribonucleic factor and in the mean time polyamine molecules.

It is known that polyamines bind to nucleic acids with the stabilization of the two combined molecules [23]. We obtained a two-fold stimulation of the protein synthesis by addition of the ribonucleic factor to a system containing polyamines and messenger RNA. This stimulation was entirely dependent on the concentration of the ribonucleic factor, polyamines and messenger RNA. This complex increases the affinity between messenger RNA and ribosomes, in the initiation process resulting in a positive control. Heywood et al. [7,8] suggest a translational control by the small RNAs (called tcRNAs), which are able to pair with a specific sequence of a messenger RNA, making it inactive to protein synthesis. In the case of a positive control, it is possible that the ribonucleic factor, in the presence of polyamines, react with the messenger RNA, making it more accessible in the initiation process. The following hypothesis can be made: messenger RNA forms a binary complex with polyamines which interact with the ribonucleic factor, resulting in a transitory complex, very active in the stimulation of the protein synthesis:

[Messenger RNA – polyamines – ribonucleic factor],

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