ELONGATION FACTOR-DEPENDENT REACTIONS ON RIBOSOMES DEPRIVED OF PROTEINS L7 AND L12

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1. Introduction

In the process of normal translation the elongation factors EF-T_u and EF-G alternately interact with the ribosome and GTP hydrolysis takes place (see, e.g., review [1]). It has been shown that the selective removal of acidic proteins L7 and L12 results in the inhibition of elongation factor-dependent reactions on the ribosome [2–8]. Treatment of ribosomes with antibodies against proteins L7 and L12 gave a similar effect [9]. The use of bifunctional cross-linking agents has shown that proteins L7 and L12 are neighbours of the elongation factors when they interact with the ribosome, both for EF-G [10] and EF-T_u [11]. Thus on the basis of numerous data, a conclusion was made that proteins L7 and L12 play the key role in the functioning of the two elongation factors, EF-T_u and EF-G [12].

This paper reports that the removal of proteins L7 and L12 from the ribosome does not lead to a complete loss of EF-T_u and EF-G interactions with the translating ribosome. Ribosomes without proteins L7 and L12 are found to be still capable, firstly, of forming the complex with EF-G and GTP (GDP); secondly, of carrying out EF-G-dependent hydrolysis of GTP; thirdly, of functioning in the factor-promoted systems of poly(U) translation with the participitation of either EF-G, or EF-T_u, or both.

2. Materials and methods

Ribosomal 30 S and 50 S subparticles were prepared from *Escherichia coli* MRE-600 by sucrose gradient zonal centrifugation in the presence of 0.5 M NH_4Cl with 1 mM $MgCl_2$ [13,14].

The ribosomal proteins L7 and L12 were extracted from the 50 S subparticles with 50% ethanol containing 0.5 M NH_4Cl and 10 mM $MgCl_2$ [3]. Individual proteins L7 and L12 were obtained by chromatography on DEAE-cellulose colums [15]. The extraction was checked by polyacrylamide gel electrophoresis at pH 5 [16] (fig.1).

Individual elongation factors, EF-T_u and EF-G, were prepared according to Kaziro et al. [17,18].

In special experiments 50 S subparticles were treated with antibodies against proteins L7 and L12 (anti-L7/L12 IgG) kindly presented by Dr G. Stöffler, Max-Planck-Institut für Molekulare Genetik, Berlin. The particles were incubated with the anti-L7/L12 IgG at a molar ratio of 1:50, resulting in 50% inhibition of the translation with original ribosomes [9], for 30 min at 25°C.

In experiments on factor-dependent translation the EF-T_u-dependent, the EF-G-dependent and the (EF-T_u + EF-G)-dependent systems [14] were used. The reaction mixtures were prepared in a buffer with 10 mM Tris–HCl, 100 mM KCl or NH_4Cl, 8 mM $MgCl_2$ for the EF-T_u-dependent and the (EF-T_u + EF-G)-dependent translation systems and 14 mM $MgCl_2$ for the EF-G-dependent translation, final pH 7.1 at 37°C. Each 100 μl portion of the mixture contained 7 μg of 30 S subparticles, 14 μg of 50 S subparticles, 10 μg of poly(U) (K^+-salt, Calbiochem), 80 μg of total tRNA aminoacylated with [^{14}C]phenylalanine (900 pmol of [^{14}C]phenylalanyl-tRNA/mg of total tRNA; the initial [^{14}C]phenylalanine was from Amersham, England, 513 mCi/mmol), 12 μg of GTP

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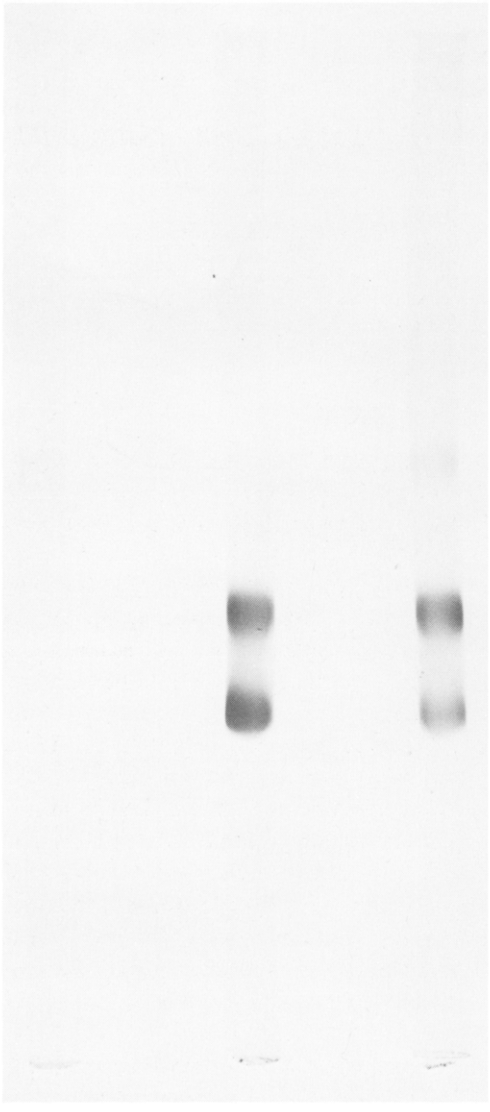

Fig.1. Polyacrylamide gel electrophoresis of proteins L7 and L12 at pH 5. (a) Ethanol/NH_4Cl-extracted 50 S subparticles (250 μg of protein). (b) Protein fraction, extracted by ethanol/NH_4Cl (10 μg of protein). (c) Original 50 S subparticles (250 μg of protein).

(Fluka, Switzerland), 10 μg of dithiothreitol (Calbiochem), 1–2 μg of EF-G in the EF-G-dependent system, 1.2–1.3 μg of EF-T_u in the EF-T_u-dependent system, 1.2 μg of EF-G and 2.8 μg of EF-T_u in the (EF-T_u + EF-G)-dependent system. Incubation was done at 37°C. The radioactivity of hot trichloroacetic acid insoluble polyphenylalanine was determined as described earlier [14].

Experiments on the GTPase reaction were done in a buffer with 20 mM Tris–HCl, 100 mM KCl or NH_4Cl, 14 mM $MgCl_2$, 2 mM dithiothreitol, 0.2 mM ethylenediamine-tetraacetic acid (Na^+-salt), final pH 7.5 at 20°C. Each 100 μg portion of the mixture contained 5.5 μg of 30 S subparticles, 5.5 μg of 50 S subparticles, 100 pmol of [^{14}C]GTP (523 mCi/mmol, Amersham, England), 10 nmol of [^{12}C]GTP, 2 nmol of GDP and 4 μg of EF-G. Incubation was done at 37°C. The amount of hydrolyzed [^{14}C]GTP was determined by the decrease of [^{14}C]GTP and increase of [^{14}C]GDP. The nucleotides were separated by paper electrophoresis at pH 5.0. The spots corresponding to the nucleotides were cut and their radioactivity counted in the standard toluene/PPO/POPOP mixture using the Beckman LS100 liquid scintillation spectrometer.

The technique of Bodley et al. [19] was used in studies of the ribosome·EF-G·GDP complex formation. The reaction mixture was prepared in a buffer containing 50 mM Tris–HCl, 100 mM NH_4Cl or KCl, 14 mM $MgCl_2$, 2 mM dithiothreitol, 1 mM fusidic acid, 0.2 mM ethylenediaminetetraacetic acid (Na^+-salt). 50 μl portions of the reaction mixture contained 9 μg of 30 S subparticles, 18 μg of 50 S subparticles, 7 μg of EF-G, 50 pmol of [^{14}C]GTP (523 mCi/mmol, Amersham, England). Incubation was for 5 min at 37°C. The amount of complexes formed was determined by filtration through nitrocellulose filters [19].

In all the experiments where proteins L7 and L12 were added their molar ratio to the 50 S subparticles was 10:1.

3. Results

3.1. *EF-G-dependent reactions with ribosomes deprived of protein L7 and L12*

Ribosomes deprived of proteins L7 and L12 were tested for their ability to form the ribosome·EF-G·GDP complex in the presence of fusidic acid, to carry out the EF-G-dependent GTPase reaction, and to perform the EF-G-dependent translation of poly(U). Table 1 (columns I, III, V) shows that the removal of proteins L7 and L12 from the ribosome leads to

Table 1
EF-G-dependent reactions with ribosomes deprived of proteins L7 and L12

Ribosomal and protein components of the mixture	Formation of ribosome·EF-G·[^{14}C]GTP complex in presence of fusidic acid. Bound [^{14}C]-GDP (pmol)		EF-G-dependent GTPase reaction. [^{14}C]-GTP hydrolyzed (nmol)[a]		EF-G-dependent translation of poly(U) (in presence of GTP). [^{14}C] Phe polymerized (pmol)[b]	
	I	II	III	IV	V	VI
	KCl	NH_4Cl	KCl	NH_4Cl	KCl	NH_4Cl
Original 30 S + 50 S	0.1	0.1	0	0	0.4	0.3
30 S + 50 S[-L7/L12]	0.1	0.1	0	0	0.3	0.4
Original 30 S + 50 S + EF-G	5.7	5.8	1.5	2.1	5.9	5.9
30 S + 50 S[-L7/L12] + EF-G	2.0	3.0	0.1	0.3	1.6	4.1
30 S + 50 S[-L7/L12] + EF-G + L7/L12	4.8	5.2	1.5	2.0	5.0	5.9

[a]After 5 min incubation at 37°C
[b]After 20 min incubation at 37°C

the inhibition of each of these ribosomal functions. The addition of proteins L7 and L12 restores the EF-G-dependent functions. However, the partial retention of the ribosome ability to carry out the EF-G-dependent reactions despite the removal of proteins L7 and L12 deserves special attention. This particularly concerns the EF-G-dependent translation and the formation of the ribosome·EF-G·GDP complex in the presence of fusidic acid.

It was found that the substitution of ammonium ions for potassium ions in the incubation medium (table 1, columns II, IV, VI) stimulates the ability of the ribosome without proteins L7 and L12 to form the ribosome·EF-G·GDP complex (1.5 times), the GTPase reaction (2 times) and the EF-G-dependent translation (2.5–3 times). At the same time, such a substitution does not noticeably change the capabilities of the original particles in these reactions. Thus, in the presence of NH_4^+, the difference in the ability to carry out EF-G-dependent reactions between the original ribosomes and the ribosomes without L7/L12 decreases.

The ability of ribosomes deprived of proteins L7 and L12 to hydrolyze GTP and to synthesize polyphenylalanine in the presence of EF-G is especially well revealed from kinetic studies of these reactions (figs.2 and 3). As seen in fig.2, the polyphenylalanine synthesis rate with the original (30 S + 50 S) and the

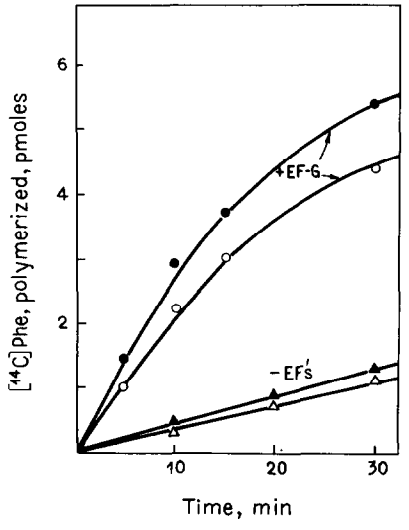

Fig.2. Kinetics of poly(U)-directed [^{14}C]polyphenylalanine synthesis in the EF-G-dependent translation system in the presence of NH_4Cl at 37°C (as compared with factor-free translation). (●) Original ribosomes in the EF-G-dependent system with GTP. (○) Ribosomes, deprived of proteins L7 and L12, in the EF-G-dependent system with GTP. (▲) Original ribosomes in the factor-free system. (△) Ribosomes, deprived of proteins L7 and L12, in the factor-free system.

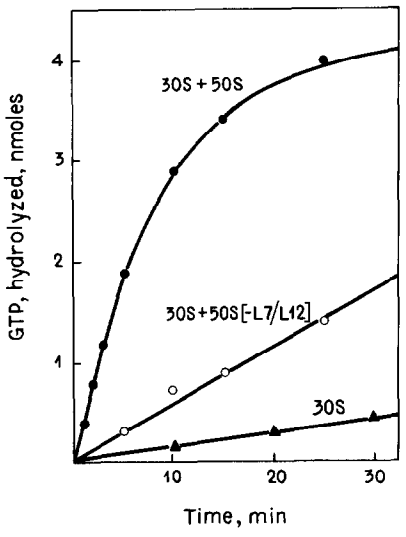

Fig.3. Kinetics of EF-G-dependent GTP hydrolysis in the presence of NH_4Cl at 37°C. (•) Original ribosomes. (○) Ribosomes, deprived of proteins L7 and L12. (▲) isolated 30 S subparticles (control).

extracted (30 S + 50 S[-L7/L12]) ribosomes in the EF-G-dependent translation system [14] in the presence of ammonium ions differs little. (As expected, the removal of proteins L7 and L12 practically does not affect factor-free translation; fig.2, lower curves.)

In the kinetic studies of the GTPase reaction it was found that the initial rate (up to 5 min) is 8–10 times faster with the original ribosomes compared to extracted ones. Nonetheless, it is seen that in the absence of proteins L7 and L12, ribosomes with EF-G are still capable of hydrolyzing GTP, though at a slower rate.

To exclude the possibility of the partial presence of the proteins L7 and L12 in some small active fraction of the extracted ribosomes, a special experiment was done in which the ribosomes were treated with antibodies against proteins L7 and L12. A 50-fold molar excess of the antibodies over the ribosomes was used which induces, according to Highland et al., a 50% inhibition of the translating acitivty of normal ribosomes [9]. The results of the EF-G-dependent poly(U) translation in the system where the 50 S subparticles were treated with antibodies against proteins L7 and L12, and when the antibodies continued to be present in the reaction mixture, are given in table 2. It is seen that treatment of the ribosomes deprived of proteins L7 and L12 with the antibodies does not affect their ability to carry out EF-G-dependent translation. At the same time treatment of the original ribosomes by antibodies against L7 and L12 leads to a two-fold inhibition of EF-G-dependent translation, as expected. These data directly indicate that the preparations of extracted ribosomes are not contaminated with proteins L7 and L12, or, in any case, that the observed activity in the EF-G-dependent translation is not due to the contamination.

3.2. *EF-T_u-dependent poly(U) translation by ribosomes deprived of proteins L7 and L12*

The ability of ribosomes deprived of proteins L7 and L12 to interact with EF-T_u was tested in the EF-T_u-dependent poly(U) translation system [14]. The kinetics of polyphenylalanine synthesis is represented in fig.4. It is seen here that the removal of proteins L7 and L12 from the ribosome slows down the rate of synthesis 2.5 times. However, just as with the EF-G-dependent translation system, ribosomes without

Table 2
Effect of antibodies against proteins L7 and L12 on the EF-G-dependent poly(U) translation (in the presence of GTP)

Ribosomal and protein components of the mixture	Polyphenylalanine precipitated by trichloroacetic acid [^{14}C]-Phe(pmol)[a]
Original 30 S + 50 S + EF-G	7.2
Original 30 S + 50 S + anti L7/L12 IgG + EF-G	3.6
30 S + 50 S[-L7/L12] + EF-G	7.0
30 S + 50 S[-L7/L12] + anti L7/L12 IgG + EF-G	6.9

[a]Incubation time 50 min at 37°C

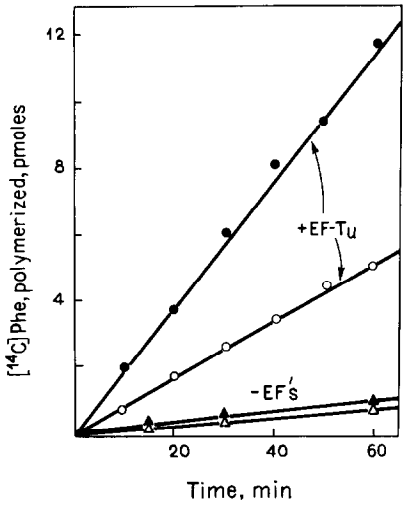

Fig.4. Kinetics of poly(U)-directed [^{14}C]polyphenylalanine synthesis in the EF-T_u-depended translation system in the presence of NH_4Cl at 37°C. Designations as in fig.2.

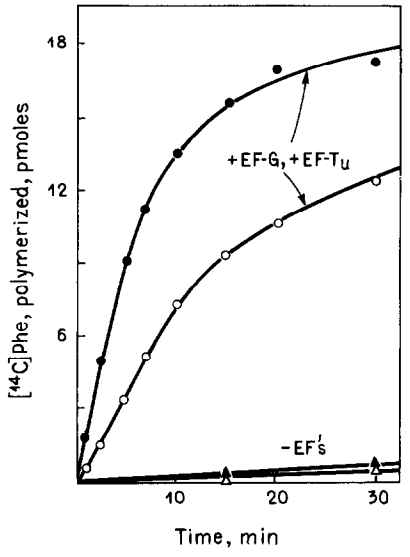

Fig.5. Kinetics of poly(U)-directed polyphenylalanine synthesis in the two-factor (EF-T_u + EF-G)-dependent translation system in the presence of NH_4Cl at 37°C. Designations as in fig.2.

proteins L7 and L12 are still able to perform EF-T_u-dependent polyphenylalanine synthesis and thus interact with EF-T_u in the process of translation.

3.3. *(EF-T_u + EF-G)-dependent poly(U) translation by ribosomes deprived of proteins L7 and L12*

The interaction of the two elongation factors, EF-T_u and EF-G, with ribosomes deprived of proteins L7 and L12 was tested in the two-factor-dependent poly(U) translation system [14]. Figure 5 shows the kinetics of polyphenylalanine synthesis by the original and extracted ribosomes in the presence of both EF-T_u and EF-G with GTP. It is seen that removal of proteins L7 and L12 leads to a two-fold decrease in the rate of the polyphenylalanine synthesis. The important fact is, however, that ribosomes without proteins L7 and L12 are, nonetheless, still able to perform a quite active polypeptide synthesis in the cellfree system promoted by both the elongation factors with GTP.

4. Discussion

The data obtained permit one to assert that ribosomes deprived of proteins L7 and L12 retain, to a considerable degree, the EF-G- and EF-T_u-dependent functions, i.e., the ability to form the ribosome·EF-G·GDP complex in the presence of fusidic acid, to carry out EF-G-dependent hydrolysis of GTP and finally to perform polyphenylalanine synthesis in the one-factor (EF-G-promoted or EF-T_u-promoted) and the two-factor (EF-G + EF-T_u) promoted poly(U) translation systems.

It should noted that the experimental data of all the earlier published reports on the functions of ribosomes deprived of proteins L7 and L12 [2–8] do not contradict factually the above results. In all the cited reports the greatest difference between the original and ethanol/NH_4Cl-extracted ribosomes was observed in the level of EF-G-dependent GTPase; but even in the case of this function, which is the most sensitive to the removal of proteins L7 and L12, a noticeable EF-G-dependent GTPase activity in extracted ribosomes is always observed and is usually from 3–15% of the original ribosomal activity, according to different authors [2–6]. Further, the activity of extracted ribosomes in binding guanyl nucleotides and EF-G in the presence of fusidic acid was found to be from 5–50% of the original ribosomal activity [3–5,8]. The EF-G-dependent translocation in the

extracted ribosomes proceeded at a rate of no less than 10%, and, as a rule was about 50%, of the translocation rate with the original ribosomes [3,4]. The capabilities for both EF-T_u-dependent aminoacyl-tRNA binding and EF-T_u-dependent GTPase were retained to no less than 15–30% after the removal of proteins L7 and L12 [3,4,6,8]. Finally, an analysis of the published experimental data shows that the ability of ribosomes without proteins L7 and L12 to perform complete elongation-factor-dependent poly(U) translation was not equal to zero, though it did not exceed 10–20% of that of the original ribosomes in conditions used by the authors [3,6].

This paper shows that in other conditions the effect of protein L7 and L12 removal on the factor-dependent functions of the ribosome can be even less. It seems very important that the elongation factors, EF-G with GTP and EF-T_u with GTP, can effectively promote elongation in a system with ribosomes deprived of proteins L7 and L12. Consequently, though the proteins L7 and L12 are undoubtedly in close proximity to the binding site of both the elongation factors [2,9–11] and undoubtedly facilitate the interaction of the factors with the ribosome [2–8], they cannot be regarded as the settling site itself for the elongation factors on the ribosome. Their presence is not a strict requirement for factor binding, for factor-dependent binding of aminoacyl-tRNA and translocation and for factor-dependent GTP hydrolysis. Thus, it can hardly be asserted that proteins L7 and L12 play the 'key role' in the binding and functioning of EF-T_u and EF-G, the more so that the interaction between the ribosomes and the elongation factors occurs on, and is limited to, proteins L7 and L12.

At the same time, if antibodies against any other ribosomal proteins except L7 and L12 do not affect the binding of EF-G with the ribosome [9], then there is nothing left but to assume that ribosomal RNA could play the key role in the interaction of the elongation factors with the ribosome.

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