THE ROLE OF *ESCHERICHIA COLI* RIBOSOMAL PROTEINS L7 AND L12 IN PEPTIDE CHAIN PROPAGATION

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1. Introduction

The ribosomal proteins L7 and L12 are involved in a number of factor-dependent partial reactions of initiation [1–3], elongation [4], and termination [5], which in each instance gives rise to the hydrolysis of GTP. It has been suggested that L7 and L12 might constitute a ubiquitous receptor site on the ribosome where the soluble factor·GTP complexes are bound [6–8]. A current model of ribosome functioning envisions a factor·GTP complex binding to the L7/L12 site, hydrolysis of the GTP, dissociation of the factor·GDP complex, and then binding of another factor·GTP complex to the L7/L12 site [9]. Thus the L7/L12 site could be viewed as helping to keep the ribosome in phase, e.g., it could prevent the ribosome from simultaneously binding aminoacyl-tRNA and translocating. The involvement of L7/L12 in the partial reactions of proteins synthesis has been demonstrated in two different types of experiments. Ribosomes are either extracted with NH_4Cl and ethanol to remove L7/L12 or are treated with antibodies against purified L7/L12 [9]. In both cases the ribosomes, after they are treated, are assayed in one of the factor-dependent partial reactions of protein synthesis. A notable exception to the apparent requirement of soluble factors for L7/L12 is the newly discovered elongation factor EF-P, which does not depend on exogenous GTP for its functioning [10–12].

We have examined the ability of ribosomes, from which L7 and L12 have been completely removed, to catalyze the poly(U)-directed synthesis of polyphenylalanyl-tRNA, as a function of time. It was found that ribosomes, lacking both L7 and L12, are after an initial lag, able to support the synthesis of polyphenylalanyl-tRNA, suggesting that EF-T_u and EF-G do not have a stringent requirement for L7/L12 in order to function in peptide chain elongation.

2. Experimental

Ribosomes without L7/L12 were prepared by a modification of the method of Hamel et al. [4]. *E. coli* Q13 ribosomes, 180 mg/ml, in a solution (buffer I) containing 0.5 M NH_4Cl, 10 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.4, and 1 mM DTT were diluted to a final concentration of 4 mg/ml with a solution (buffer II) containing 1.0 M NH_4Cl, 2 mM $MgCl_2$, 10 mM imidazole–HCl, pH 7.4, and 1 mM DTT. Two separate batches of ice-cold ethanol (each being one half the volume of the diluted ribosomes) were added slowly with stirring. Too rapid an addition of ethanol resulted in the incomplete removal of L7/L12. The precipitated ribosomes were pelleted by centrifugation at 30 000 × *g* for 15 min, suspended in buffer II, to a final ribosome concentration of 4 mg/ml and extracted a second time. The final ribosome pellet was dissolved in a

Abbreviations: poly(U), Polyuridylic acid. EF, Elongation factor. DTT, Dithiothreitol.

small volume of buffer I and then dialyzed 3 h against the same buffer.

The extracted protein, from the combined ethanol supernatants, was precipitated with −20°C acetone, pelleted by centrifugation at 30 000 × *g* for 15 min, dissolved in a small amount of buffer I and then dialyzed 3 h against the same buffer.

$EF\text{-}T_u$, $EF\text{-}T_s$, and EF-G were purified by the method of Ravel and Shorey [13]. These elongation factors were free of one another and EF-P.

The polymerization incubation mixture (0.06 ml) contained 6.2 mM Tris–HCl pH 7.4, 100 mM NH_4Cl, 6.3 mM $MgCl_2$, 6.2 mM DTT, 1.2 mM GTP, 40 μg of purified ribosomes, 5 μg of poly(U), 28 pmol [^{14}C]phenylalanyl-tRNA, (522 mCi/mM) 8 μg $EF\text{-}T_u$, 2 μg $EF\text{-}T_s$, and 5 μg EF-G. All incubations were at 35°C and were terminated by the addition of 1–2 ml cold 5% trichloroacetic acid to the reaction mixture, before heating for 15 min at 90°C. The reaction mixtures were then cooled, filtered onto millipore filters, and counted in 5 ml of Brays scintillation fluid. Polyphenylalanine synthesis was completely dependent on addition of elongation factors.

The gels (10% acrylamide) for polyacrylamide gel electrophoresis, were run at pH 4.5 and stained with Coomassie Brilliant Blue as described by Gesteland and Staehlin [14].

3. Results and discussion

If the 50 S ribosomal proteins L7 and L12 constitute a common ribosomal site [6,7] through which the soluble factors mediate the intrinsic activity of the ribosome [15,16] then L7 and L12 should be indispensable for protein synthesis. Evidence that this expectation is fulfilled comes from several studies where ribosomes have been treated with anti-

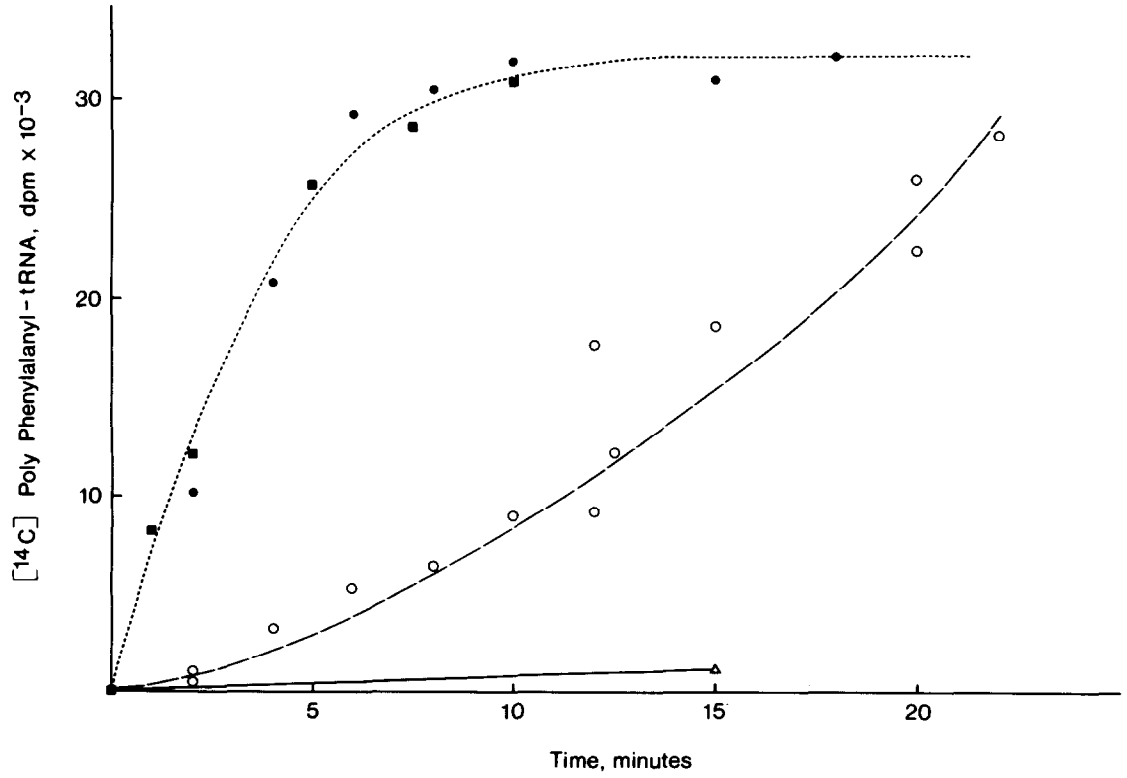

Fig. 1. The effect of ribosomal proteins L7 and L12 on peptide chain propagation. All reaction mixtures contained $EF\text{-}T_u$, $EF\text{-}T_s$, and EF-G and were incubated at 35°C for the time indicated. Symbols: (■) Native 70 S ribosomes. (○) 70 S ribosomes extracted to remove L7/L12. (●) Extracted 70 S ribosomes to which L7/L12 was added back at zero time (in the ratio of approximately 1 μg of L7/L12 to 3 μg of ribosomes). (△) Native 70 S ribosomes in the absence of added poly(U).

bodies directed against purified L7/L12 (e.g., ref. [7]). However, both antibody molecules and their Fab fragments are much larger than ribosomal proteins and as a result the level of resolution of this technique is low. It is conceivable, for example, that regions adjacent to the putative L7/L12 site would be directly affected by bound immunoglobin. Thus, antibody inactivation, in this instance, may not be directly affecting the presumed binding site. Since the proteins which, together with RNA, comprise the ribosome are highly interdependent it may be misleading to assign function to any ribosomal protein solely on the basis that antibodies to that protein inhibit a particular function.

Another piece of evidence that L7/L12 is important for protein synthesis comes from studies where ribosomes are extracted to remove L7/L12. The data shown in fig.1 indicate that ribosomes from which L7/L12 have been removed by extraction with ethanol and NH_4Cl display a significantly decreased initial rate of poly(U)-directed polyphenylalanine synthesis, as compared to native ribosomes. This lesion in protein synthesis is alleviated upon adding back L7/L12 at zero time. With native 70 S ribosomes, the poly(U)-directed synthesis of polyphenylalanine which occurs is complete after a 10 min incubation at 35°C whereas the synthesis which occurs on extracted 70 S ribosomes (i.e., ribosomes lacking L7/L12) has a very slow initial rate which, after a short lag, increases steadily for at least 20 min, at which point most of the added Phe-tRNA can be recovered as polyphenylalanyl-tRNA. The poly(U)-directed synthesis of polyphenylalanyl-tRNA which occurs on native 70 S ribosomes is a pseudo-first-order process with a rate constant of 0.31 min^{-1} (determined from a first-order plot of the data of fig.1). The synthesis which occurs on extracted 70 S ribosomes is initially characterized by a rate constant of approximately

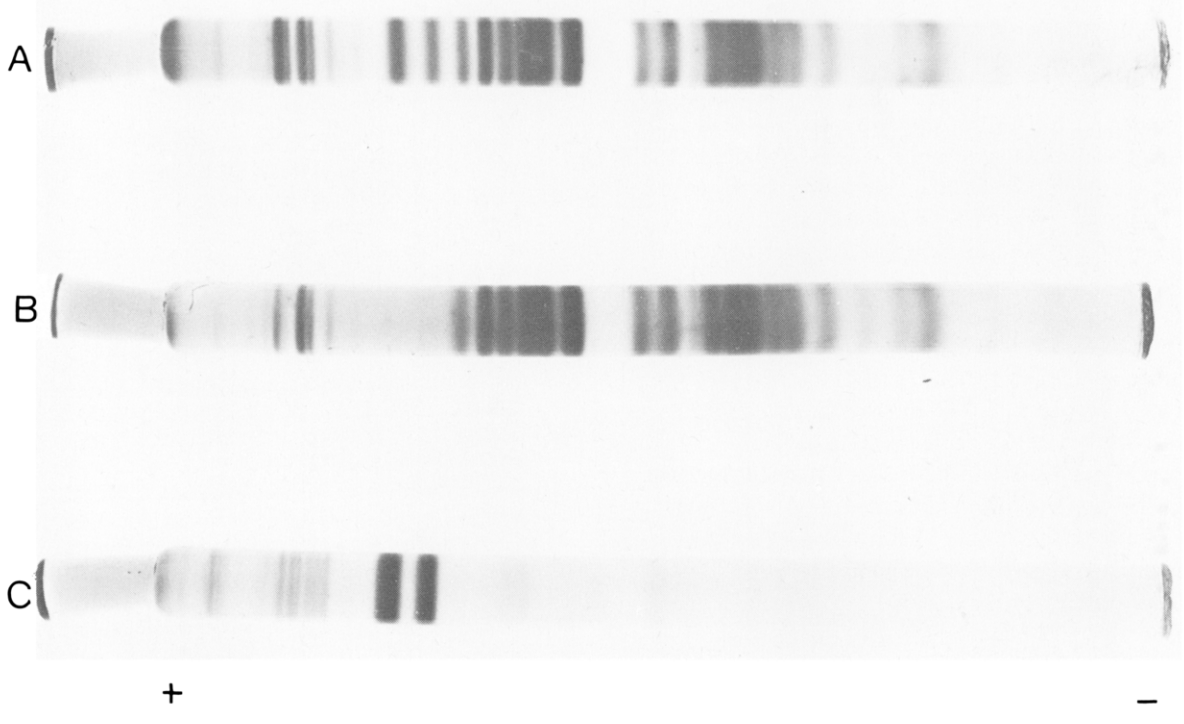

Fig. 2. One-dimensional polyacrylamide gel electrophoresis of 70 S ribosomal proteins and extracts. Migration was towards the cathode. Gel A shows protein from native 70 S ribosomes (180 μg of protein were loaded on the gel). Gel B shows protein from extracted 70 S ribosomes (150 μg of protein were loaded on the gel). Gel C shows proteins removed from 70 S ribosomes by the NH_4Cl–ethanol extraction (50 μg of protein were loaded on the gel). The minor, acidic, bands which appear in all three gels are non-ribosomal protein contaminants.

0.027 min^{-1}. The rate of synthesis with extracted ribosomes gradually increases until it equals the rate of synthesis with native ribosomes.

Figure 2 shows a one-dimensional polyacrylamide gel analysis of proteins (from (A) native 70 S ribosome, (B) extracted 70 S ribosomes and (C) the proteins extracted from 70 S ribosomes. This figure indicates that: (1) the extracted ribosomes used in this study are free of L7/L12 and (2) L7 and L12 are the only ribosomal proteins removed in the extraction. A two-dimensional polyacrylamide gel indicated that the NH_4Cl–ethanol extraction also removes protein L8 (data not shown), which has recently been reported to be a complex of L7/L12 and L10 [17].

Hamel et al. [4] observed that the major effect of removing L7/L12 from ribosomes, on the binding of Phe-tRNA to extracted ribosomes was a ten-fold reduction in the initial rate of this binding. In addition, while both the EF-G-dependent and EF-T_u-dependent GTPase activities of extracted ribosomes are practically nil, these ribosomes are only 50% impaired in their ability to translocate [4].

Unfortunately, the role in protein synthesis of the factor-dependent GTPase activity, which is impaired by removal of L7/L12, is not clear. Most (>90%) of the factor-dependent ribosomal GTPase activity is uncoupled from peptide chain elongation [18]. In fact, ribosomes may have the intrinsic ability to synthesize some peptides without the intervention of extrinsic factors or GTP [15,16,19].

If, as has been suggested [20–22], the primary function of factor coupled GTP hydrolysis is to facilitate removal of the factor from the ribosome, then an impairment in GTP hydrolysis would be expected to result in a decreased rate of factor removal. Thus a lesion affecting GTP hydrolysis could affect the rate and not the extent of both substrate binding and translocation. In this way a serious impairment in GTP hydrolysis need not be lethal. An auxiliary rather than an essential role in protein synthesis for L7/L12 may explain why the *E. coli* mutant ts 9, which has a mutation in the gene coding for L7/L12, has an identical growth rate, at several different temperatures, to its parental strain [23,24]. This conclusion is consistent with the demonstration by Hamel and Nakamoto [25] that extracted ribosomes assayed in the presence of 20% methanol did not require the presence of L7/L12 for factor-dependent GTPase activity.

L7/L12 while unquestionably involved in the factor-dependent GTPase activity of the ribosome cannot constitute the entire binding site for the soluble factors and probably plays an auxiliary rather than an essential role in mediating the effects of the soluble factors.

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