THE STOICHIOMETRY OF *E. COLI* 30S RIBOSOMAL PROTEIN S1 ON IN VIVO AND IN VITRO POLYRIBOSOMES

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1. Introduction

We have shown recently [1] that protein S1 of the small ribosomal subunit of *Escherichia coli* is required for the translation of poly U. Ribosomes actively translating this messenger carry S1 at a stoichiometry of close to one copy per particle [1], in line with its suggested role in messenger binding [2–5]. In the same paper we demonstrated that S1 is lost from the ribosomes upon dissociation of the polysomes at low Mg^{2+} concentrations, explaining the low stoichiometry of S1 on purified 30S subunits [6, 7]. It is of obvious interest to know the number of S1 copies on ribosomes translating natural messenger RNA. We have therefore determined the stoichiometry of S1 on polyribosomes isolated directly from the bacterial cell as well as on polysomes generated in vitro by the translation of MS2–RNA. Our data show that the number of S1 copies is approximately the same on all polyribosomes tested and varies from 0.6 to 0.9. This finding suggests that S1 is also required for the translation of natural messengers.

2. Materials and methods

2.1. *Isolation of in vivo polyribosomes and ribosomal particles*

E. coli MRE 600 was grown on minimal salts medium [8] in the presence of 0.5 m Ci [^{3}] amino acid mixture (uniformly labelled, New England Nuclear) per liter. Cells were harvested and lysed as described before [9]. Polyribosomes and 70S particles (these consist for about 70% of free ribosomes and for 30% of monosomes [10]) were recovered by sucrose gradient centrifugation in standard buffer (10 mM Tris–acetate, pH 7.6; 10 mM Mg–acetate; 60 mM NH_4Cl; 6 mM β-mercaptoethanol). Derived subunits were obtained from polysomes after dialysis against standard buffer containing 0.25 mM Mg–acetate instead of 10 mM.

Unwashed ^{3}H-labelled ribosomes, used to prepare in vitro polyribosomes with MS2–RNA were prepared from the same cells by grinding with alumina after slow cooling.

All particles used had a specific activity of about 18 000 counts/min/A_{260} unit.

2.2. *Preparation of polyribosomes in vitro*

20 A_{260} units of ^{3}H-labelled unwashed ribosomes were incubated in a 0.4 ml mixture containing the following components: 225 μg MS2-RNA (gift of Mr. J. M. Gubbens); 60 μl high speed supernatant; 80 μg stripped tRNA; approximately 1 μg unlabelled fMet–tRNA; 36 μg leucovorine; 0.4 μmol ATP; 0.05 μmol GTP; 2 μmol phosphoenolpyruvate; 0.4 μg pyruvate kinase and 4 nmol of the 20 amino acids. Final ionic conditions in the incubation mixture were 40 mM Tris–acetate pH 7.2; 7 mM Mg–acetate; 40 mM NH_4Cl and 6 mM β-mercaptoethanol. After incubation at 35°C for 12 min the mixtures were diluted with ice-cold buffer of the same ionic composition, containing 0.1 mg/ml chloramphenicol, layered on 10–30% sucrose gradients and centrifuged for 6 hr at 25 000 rpm. The optical density tracing is shown in fig. 1.

Tetramers till monomers were cut out and recovered by centrifugation.

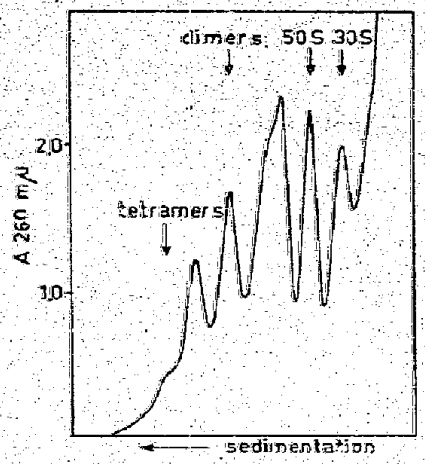

Fig. 1. Sucrose gradient analysis of MS2–RNA induced polysomes. Details of the preparation are described in the Materials and methods section.

2.3. *Gel electrophoresis and counting*

Approximately 30 000 counts/min of each of the ribosomal preparation were analyzed by electrophoresis on 10% polyacrylamide gels in SDS according to Weber and Osborn [11]. The gels were stained with Coomassie brilliant blue as described previously [12] and sliced after destaining. One mm slices were incubated overnight at 50°C in 0.7 ml Soluene (Packard) containing 10% water, then supplemented with toluene–Triton X100 scintillation cocktail and counted.

3. Results

Because of its high molecular weight (65 000 daltons) [13, 14] S1 can easily be separated from all other ribosomal proteins by electrophoresis in polyacrylamide gels in SDS (fig. 2). The mass fraction of S1 in a mixture of radioactive 30S ribosomal proteins is equivalent to the fraction of radioactivity present in S1 [6, 7]. By using known data for the molecular weight of S1 and the molar protein content of a 30S particle (260 000 daltons) [14] its stoichiometry can be computed. The value we obtained for the stoichiometry of S1 on derived 30S subunits (figure not shown) varied from 0.15 to 0.25 with an average of 0.21 obtained from 3 independent measurements (table 1). These values are well within the range of published data for this protein [6, 7]. Further evidence for the reliability of the method employed here was derived from stoichiometry measurements of the 50S protein L2. Except for S1 this is the only other protein (31 500 daltons) [13] which gives a single band after SDS electrophoresis of total 70S ri-

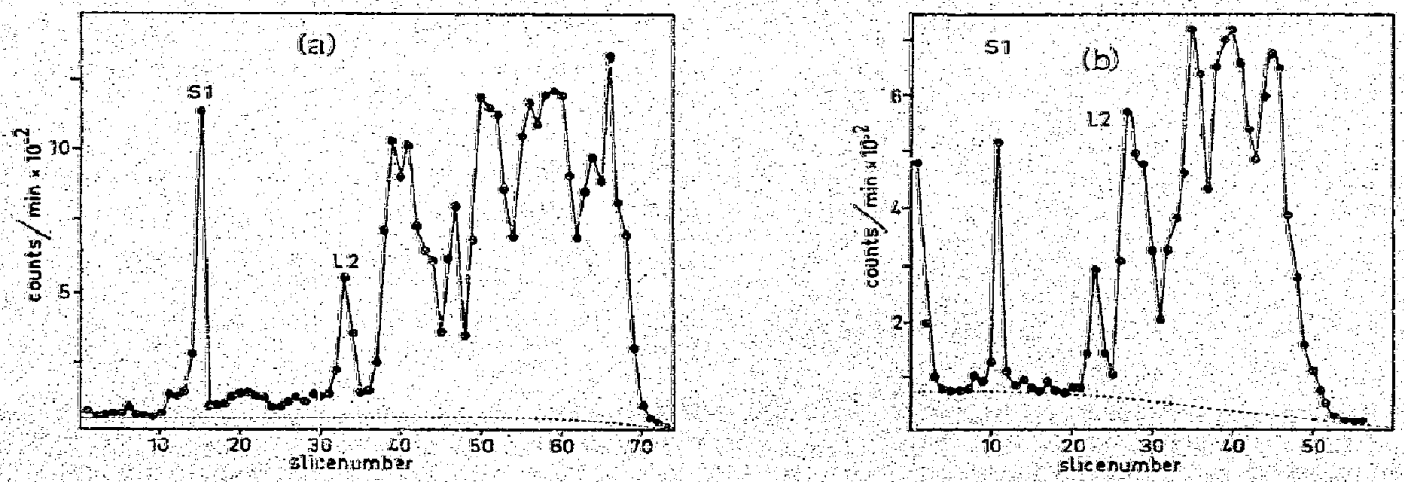

Fig. 2(A). Gel electrophoresis analysis of free 70S ribosomes from a cell lysate. For stoichiometry calculations the radioactivity of each slice was corrected for background values as indicated by the dotted line. Details of sample preparation and electrophoresis are mentioned in the Materials and methods section. (B). Gel electrophoresis analysis of trimer fraction of MS2–RNA induced polysomes. For stoichiometry calculations the radioactivity of each slice was corrected as described in the legend to 2A. Details of the calculation and sample preparation are described in the Materials and methods section.

Table 1
Number of copies of S1 and L2 in various ribosomal preparations

Source of ribosomes	Protein S1		Protein L2
	Method of calculation		Method of calculation
	Recovery	L2 standard	Recovery
Endogenous *E. coli* polysomes	0.68 (2)	0.72 (3)	1.08 (2)
Free 70S ribosomes from cell lysates	0.71 (2)	0.69 (3)	1.06 (2)
MS2–RNA polysomes			
Tetramers	0.91 (1)		
Trimers	0.74 (1)	0.77 (1)	0.96 (1)
Dimers	0.65 (1)	0.73 (1)	0.90 (1)
Monomers + free 70S	0.70 (1)		
30S Derived from endogenous polysomes by low Mg^{2+} dissociation	0.21 (3)		
50S Derived from endogenous polysomes by low Mg^{2+} dissociation			0.93 (2)

The isolation of the various ribosomal preparations is described in Materials and methods. Formula used in recovery method:

$$\text{stoichiometry } N_i = \frac{\text{counts in protein i}}{\text{total counts}} \times \frac{\text{daltons of protein in particle}}{\text{M.W.}_i}$$

Formula used in L2 standard method:

$$N_i = \frac{\text{counts in protein i}}{\text{counts in L2}} \times \frac{\text{M.W. L2}}{\text{M.W.}_i}$$

Numbers between brackets represent numbers of independent determinations.

bosomal proteins (fig. 2). Taking 550 000 [13] and 885 000 daltons [15] for the molar protein content of 50S and 70S ribosomes respectively, we found the number of L2 copies listed in table 1. These data are in excellent agreement with those of Weber [7] and show that L2 is a unit protein. We can now use two procedures to determine the number of S1 copies on polysomes and free 70S ribosomes:

1) by the recovery procedure as described above and using 885 000 daltons for the protein content of a 70S particle;
2) by simply relating the counts in the S1 peak to those in the L2 peak and assuming a stoichiometry of 1.00 for L2. This method is independent of the molar protein content of the ribosome under study.

Both procedures yield essentially the same value for the S1 stoichiometry (table 1). The number of S1 copies on both in vivo and in vitro prepared polysomes (and also on free 70S particles) varies from 0.6 to 0.9. Similar results were found when T4–mRNA was used as messenger instead of MS2–RNA (data not shown).

4. Discussion

The existing evidence concerning the possible function of S1 in protein synthesis points to a direct role of the protein in messenger RNA binding [1–5]. This evidence is mainly based on experiments using poly U

as template. Although there is no direct proof that S1 is also required for the translation of natural mRNA, the results presented above show that the majority of ribosomes translating these messengers carry S1. The fact that the stoichiometry of S1 on 'natural' polysomes is somewhat less than the value we obtained for poly U generated polysomes [1], might be due to several reasons. Except for possible experimental errors it might be that phage RNA induced polysomes are heterogeneous indeed. It is conceivable that a minor fraction of ribosomes gets stuck at a stage that does not require S1 (for instance a step in initiation). It is of interest that Bollen et al. [16] found that S1 is absent from a 30S initiation complex with poly AUG as messenger. Apparently mRNA binding does not require S1 in this stage of the reaction.

Recent reports have claimed the identity of the translational interference factor i [17, 18] as well as of subunit I of the Qβ replicase with protein S1 [19, 20]. Kamen et al. [21] had already shown that the i-factor and subunit I are the same protein. It is reported that the interference factor inhibits the translation of native MS2–RNA by selectively blocking initiation at the coat protein initiation site [17, 18, 22] At first glance this property seems at variance with the now established presence of S1 on MS2–RNA generated polysomes. However, it is by no means excluded that the same protein is involved in messenger RNA binding to ribosomes during elongation and in cistron selection during initiation. In this connection we may point out that both S1 [4, 23] and i-factor show a strong affinity for RNA in vitro. We could postulate that, depending on the way in which the activity of the protein is assayed, it either behaves as an interference factor or as a factor required for elongation. Further experiments will be necessary to test this assumption.

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