FRACTIONATION OF TRANSLATION INITIATION FACTOR B (F3) INTO CISTRON-SPECIFIC SPECIES

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1. Introduction

Previous studies on the specificity of messenger RNA-ribosome interaction have shown that *E.coli* ribosomes recognize and bind natural mRNA preferentially to AUG-containing polynucleotides [1]. Attachment of ribosomes to mRNA completely depends on the presence of the initiation factors [2, 3] but, while factor C (F2) promotes 30 S mRNA binding without apparent specificity for the template, initiation factor B (F3) determines the specific recognition of natural mRNA [1, 4].

Ribosomes do not recognize identically all cistrons of mRNA. Thus, Lodish [5] by comparing bacterial species, demonstrated that ribosomes differentiate between the three cistrons of f2 RNA. Further, Hsu and Weiss reported [6] that after infection with phage T4, *E. coli* ribosomes translate T4 mRNA much more efficiently than f2 RNA. These experiments, therefore, suggest that in mRNA there is for each, or each group of cistrons, a specific signal which is recognized by some element of the translation machinery.

If initiation factor B (F3) plays a role in the recognition of this signal, it should be possible to demonstrate the existence of cistron specific fractions of this factor. We report here, the fractionation and purification from *E. coli* of several species of factor B characterized by their differences in activity for the translation of T4 mRNA and MS2 RNA.

2. Materials and methods

Ribosomes and supernatant from *E. coli* MRE 600 were prepared as described previously [2]. Late T4 mRNA was purified from T4 infected *E. coli*, as by Salser et al. [7], and was used to measure lysozyme synthesis as before [4]. RNA from phage MS2 (a gift from Dr. E.Ron) was purified and used as in previous studies [2]. ^{35}S-Formylmethionyl-tRNA (100 μCi/μmole) was prepared from *E. coli* B tRNA (Schwartz) and used for binding and incorporation studies as before [1]. ^{14}C-Valine (50 μCi/μmole) and ^{3}H-histidine (1100 μCi/μmole) were from Schwartz.

Initiation factor C (F2) was purified according to our published procedure [8] and factor A (F1) was obtained after purification through phosphocellulose [9]. Initiation factor B was fractionated and purified as follows: 0.7 kg *E. coli* MRE-600 were suspended in 700 ml of tris-HCl (pH 7.8) 0.02 M, $MgCl_2$ 0.01 M and β-mercaptoethanol (EtSH) 7 mM and homogenized twice through a continuous flow French Press at 7000 Psi. The extract was treated with DNAase (1 μg/ml), and ribosomes were sedimented at 250,000 *g* for 2.5 hr, suspended overnight in NH_4Cl 2 M, tris-HCl (pH 7.5) 0.03 M, $MgCl_2$ 0.01 M, EtSH 12 mM and recentrifuged at 250,000 *g* for 2.5 hr. From the ribosomal wash fluid obtained, proteins were precipitated between 45 and 75% ammonium sulfate and adsorbed on a DEAE cellulose column (Serva, 0.65 meq/g; 6 X 22 cm) equilibrated in K phosphate buffer pH 7.2, 0.02 M, $MgCl_2$ 0.2 mM, EtSH 7 mM, glycerol 5%. (The non-adsorbed material containing factor A (F1) was recovered.) Fractions were eluted with a linear gradient of 0.02–0.3 M K phosphate buffer and each fraction assayed for factor B activity as in fig. 1. Active fractions (100–170 of fig. 1) were concentrated by vacuum dialysis and chromatographed through a 2.8 X 72 cm column of Sephadex G-100 (in 0.1 M K phosphate buffer as above), in which factor B is in-

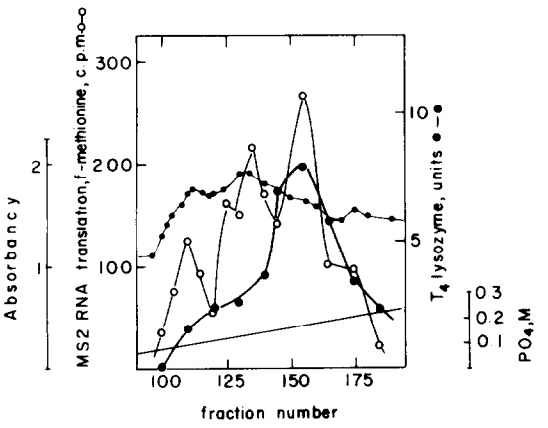

Fig. 1. Elution pattern of factor B activity from DEAE cellulose. Conditions as in Methods. Assay of 25 μl from each fraction as in table 2. Lysozyme expressed in units as Salser et al. [7]. Factor A (F1) is not adsorbed on the column and A activity is clearly separated from B fractions shown in the fig.

cluded. Fractions were then adsorbed on a phosphocellulose (Whatman P11, 7.4 meq/g) column (1 X 5 cm) and washed with 0.05 M K phosphate buffer pH 7.9, EtSH 7 mM, glycerol 5%. Factor B was eluted with a linear gradient of K phosphate (0.1–0.8 M). Finally, the active fractions were adsorbed on a 1 X 5 cm hydroxylapatite (BioRad) column in 0.02 M K phosphate buffer as above. Stepwise elution was carried out and factor B activity came out at 0.18 and 0.22 M K phosphate buffer. Fractions were concentrated and stored in liquid nitrogen. Before assay, all fractions were dialyzed against 0.02 M K phosphate buffer as above. Results of the purification are shown in table 1.

3. Results

3.1. *Fractionation and purification of initiation factor B (F3)*

Initiation factor B activity can be assayed by its effect on the translation of natural messenger RNA in the presence of purified initiation factors C (F2) and A (F1) [2]. In the present work, we compared the effect of factor B on the translation of two different mRNAs used by the *E. coli* system: T4 mRNA and in particular the lysozyme cistron [7], and RNA from phage MS2. During the purification procedure outlined in table 1, considerable purification of the activity of both mRNAs was observed. However, the ratio between activities changed very markedly during the purification. The most purified fraction (BH1) is markedly enriched in MS2 translation activity (MS2/T4 lysozyme ratio is 6 times higher than that of the DEAE-cellulose fraction B4). In contrast, another fraction (BH2) from the hydroxylapatite column is enriched in T4 lysozyme activity (T4 lysozyme/MS2

Table 1
Fractionation and purification of initiation factor B (F3).

Fraction	T4 lysozyme mRNA translation		MS2 RNA translation		MS2
	Lysozyme activity (units X 10^{-2} per μg protein)	Purification factor	Valine incorporation (pmoles X 10^{-2} per μg proteins)	Purification factor	T4 lysozyme
I) Ribosomal wash fluid	4.4	1	6.6	1	1.52
II) DEAE-cellulose (fraction B4)	15.8	3.6	15.0	2.3	0.98
III) Sephadex G-100	76	17	174	26	2.32
IV) Phosphocellulose	224	51	950	145	4.35
V) Hydroxylapatite					
0.18 M fraction (BH1)	912	208	5164	790	5.90
0.22 M fraction (BH2)	688	156	556	84	0.81

Purification procedure is described in Methods. Assays as in table 2.

ratio 7 times higher than for BH1). The decrease of T4 lysozyme translation relative to MS2 seen in BH1 can therefore be ascribed to the separation of the fraction specific for T4 lysozyme translation during the purification procedure. Fraction BH1 is not yet pure; analysis by polyacrylamide gel electrophoresis still showed two bands at pH 4.5.

From the purification data, it thus appears that the factor B activities responsible for the translation of two different mRNAs are not due to the same protein. This is further shown by the difference in the elution pattern of factor B activities from DEAE-cellulose (fig. 1): T4 lysozyme activity gives a major peak at fraction 155 (B4) while the activity for MS2 RNA shows three peaks at fraction 110 (B2), 135 (B3) and 155 (B4).

To characterize these fractions, we compared the effect of B2, B4 and BH1 on the translation of T4 and MS2 RNA.

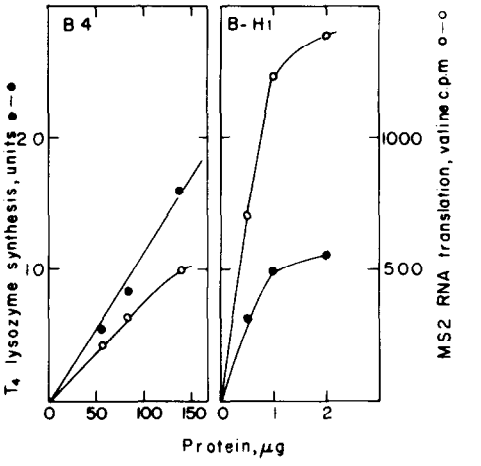

Fig. 2. Factor B (F3) dependent translation T4 and MS2 RNA. Conditions as in table 2. Background with A + C alone was subtracted (T4 lysozyme 2.4 units; MS2 dependent valine incorporation 150 cpm).

3.2. *Effect of the different factor B (F3) fractions on T4 and MS2 RNA translation*

Fig. 2 shows the differential effects of fractions B4

Table 2
Differential initiation of T4 and MS2 RNA translation.

Experiment	Factor B added	Late* T4 mRNA		MS2 RNA	$\frac{\text{T4 (total)}^{**}}{\text{MS2}}$	$\frac{\text{T4 lysozyme}}{\text{MS2}}$	$\frac{\text{T4 lysozyme}}{\text{T4 (total)}^{**}} \times 10^{-1}$
		Lysozyme synthesis units	Valine incorporation (pmoles)	Valine incorporation (pmoles)			
1) Translation	B2	1.1	26.9	11.2	2.4	0.10	0.4
	B4	27.3	74.8	26.0	2.9	1.05	3.7
	BH1	6.2	42.9	38.7	1.1	0.16	1.4
		fMet-tRNA bound (pmoles)		fMet-tRNA bound (pmoles)			
2) fMet-tRNA binding	B4	1.6		0.6	2.7		
	BH1	2.8		4.0	0.7		

Late* T4 mRNA (100 μg) and MS2 RNA (30 μg) translation were performed in 0.125 ml reaction mixture [2] containing 150 μg ribosomes, 18 μg factor A, 14 μg factor C and 15 μl of high-speed supernatant, 140 μg B4 or 120 μg B2 or 1.2 μg BH1. Lysozyme units as Salser et al. [7]. Background with factor A + C alone was subtracted (lysozyme 3.0, T4 mRNA 11.2, MS2 RNA 3.0). Binding of ^{35}S-fMet-tRNA was carried out in a 0.05 ml reaction mixture [4] with MS2 RNA (8 μg) or T4 mRNA (40 μg), 100 μg ribosomes, 5 μg factor A, 5 μg factor C, 30 μg factor B4 or 0.3 μg BH1 and analyzed by zone sedimentation as before [4]. Background with factor A + C alone (0.2) was subtracted.

* Late T4 mRNA was extracted from infected culture after 15 min at 37°.

** T4 (total) refers to the overall valine incorporation observed with T4 mRNA.

and BH1 on T4 lysozyme and MS2 RNA translation, under conditions where synthesis is linearly dependent on the amount of factor B added. The difference is not due to inhibition: mixing B4 and BH1 shows that their effects are additive. Addition of more mRNA does not change the result. Table 2 summarizes the differences observed between the templates: T4 lysozyme translation differs from that of MS2 RNA and even from that of the bulk of T4 mRNAs. Thus table 2 shows that with factor B2, almost no T4 lysozyme was synthesized, although this factor stimulates overall amino acid incorporation with the T4 template.

The template specificity of B4 and BH1 is clearly in the initiation reaction, as shown by fMet-tRNA binding studies. For this experiment, the amount of ^{35}S-fMet-tRNA bound to the 70 S region after zone sedimentation was analyzed. With factor A and C alone, very little fMet-tRNA binding was obtained [3]. Table 2 shows the effect of B4 and BH1 on fMet-tRNA binding with T4 and MS2 RNA: a 4 fold difference between the relative activities was found, in agreement with the translation experiments.

When ribosomes are challenged by two different mRNAs, the ratio of competition between them should be determined by the initiation factor B added. We investigated the effect of MS2 RNA addition on the translation of the T4 lysozyme cistron, under conditions where synthesis depends linearly on the amount of factor B added (as in fig. 2). With B4, which has a higher activity with T4 than MS2, addition of the latter did not decrease the synthesis of lysozyme With BH1, however, which preferentially directs the ribosomes to translate MS2 RNA, addition of this RNA produced a 65% decrease in the amount of lysozyme produced per total protein. It thus seems possible to determine the ratio of translation between two mRNAs according to which initiation factor B is used.

The DEAE cellulose elution profile of factor B activity for MS2 RNA translation showed the existence of multiple fractions (fig. 1). To distinguish between the coat protein cistron and the two other cistrons, incorporation of valine and histidine were compared [10]. Table 3 shows that fraction B2 directs a much lower incorporation of histidine than valine when compared to fraction B4. In constrast, BH1 gives even a higher histidine/valine ratio than B4. For the same amount of total MS2 proteins, B2 therefore directs the synthesis of over 3 times more coat protein, containing no histidine, than does BH1. This would indicate the existence of factor B species which differentiate between the cistrons of this phage RNA.

4. Discussion

The differences between the fractions of factor B in their relative activities with various templates are about 5 fold, but even the most purified fraction BH1 still has activity for both T4 and MS2 RNAs. This fraction could, of course, still be heterogeneous and contain more than one factor B species. Alternatively, differences between mRNAs may not be absolute, and one factor could recognize different mRNA signals, although with varying affinities. Finally, it is possible that there are some common signals between T4 and MS2 RNA. Little is known about what is recognized by initiation factor in mRNA. Previous studies, based on template competition [1], showed that the presumed initiation signal must be more than the codon AUG. The mechanism of action of initiation

Table 3
Comparison of histidine and valine incorporation with MS2 RNA.

Factor B2 added	Valine incorporation (pmoles)	Histidine incorporation (pmoles)	$\frac{\text{Histidine}}{\text{Valine}} \times 10^2$
B2	16.8	0.6	3.6
B4	22.7	2.0	8.8
BH1	47.7	5.3	11.1

Condition as in table 2. Background with A + C alone (^{14}C-valine, 1.4; ^{3}H-histidine, 0.5) was subtracted.

factor B is also unclear. It is required to form the stable 70 S mRNA–fMet-tRNA complex [3, 4] while a 30 S mRNA complex can be formed in its absence [2, 4]. Under certain conditions (limiting amounts of factor C or of template) it stimulates AUG-dependent fMet-tRNA binding to ribosomes [11, 12]. Template competition studies showed, however, that factor B (F3) promotes the recognition of some structure which exists only in natural mRNA [1] and the present work demonstrates directly its specificity of action towards mRNA.

Recently, Lodish showed that difference in cistron-specificity between two bacterial species resides in the 30 S ribosomal subunit and not in the initiation factor fraction [15]. In *E. coli*, ribosomal heterogeneity might exist in addition to initiation factor heterogeneity. After T4 phage infection, however, a change in template specificity occurs in some protein factor [6, 13] and we have been able to demonstrate as reported elsewhere [14] that this change is due to a specific initiation factor B activity which directs ribosomes from uninfected cells to initiate selectively translation of late T4 mRNA. The existence of different factor B species for different mRNAs explains how these changes can occur; it will now be of great interest to study the role played by translation control in bacteria as well as in differentiating eukaryotic cells.

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