ROLE OF INITIATION FACTOR B (F3) IN THE PREFERENTIAL TRANSLATION OF T4 LATE MESSENGER RNA IN T4 INFECTED *E. COLI*

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Received 10 July 1970

1. Introduction

The demonstration of a template specific initiation reaction, by which ribosomes could recognize selectively the proper cistrons of messenger RNA to be translated, would provide a mechanism of gene expression control at the level of translation. Hsu and Weiss [1] and recently Dube and Rudland [2] showed that after infection by phage T4, a protein factor directs *E. coli* ribosomes to select T4 mRNA from host or phage f2 RNA. In our previous work [3, 4], analysis of the function of the three initiation factors in mRNA-ribosomal interaction indicated that the ribosome will recognize specifically natural mRNA from other polynucleotide templates only if initiation factor B (F3) is present. This suggested that initiation factor B (F3) might be responsible for a messenger selection process. In *E. coli*, factor B (F3), indeed, appears to be heterogeneous and we recently fractionated this factor into several species characterized by their difference in activity with T4 and MS2 RNA templates [5]. In the present report, we show that the modification in template specificity after T4 infection, which leads to preferential initiation of late T4 mRNA translation, can also be accounted for by a change in initiation factor B (F3) activity.

2. Methods

Crude extract (S 30), ribosomes, crude initiation factor and supernatant fractions from *E. coli* MRE 600 were prepared as before [3]. Initiation factor C (F2) was purified as reported [3] and factor A (F1) according to Hershey et al. [6]. Purification and fractionation of initiation factor B (F3) was detailed elsewhere [5]. Late T4 mRNA, extracted [7] from an infected *E. coli* culture after 15 min at 37° C, T4 DNA and *E. coli* RNA polymerase [3] and RNA from phage MS2 were obtained and used as before [3, 5]. Conditions for ^{35}S-fMet-tRNA binding to ribosomes [3] under which the reaction is dependent on the presence of each of the three initiation factors were obtained by using non-saturating amounts of the factors. In addition, binding was performed with GMPPCP which reduces markedly the factor dependent fMet-tRNA binding observed in the absence of mRNAs [8].

E. coli MRE 600 was infected with T4 em 74 (lysozyme$^-$) [9], at a multiplicity of 8. Cells were harvested after 13 min at 37°; 14 l of culture yielded 21 g of fresh cells. The crude initiation factor fraction from infected cells was prepared as for uninfected *E.coli* [3].

3. Results and discussion

3.1. *Translation activity of T4 infected E. coli extracts for MS2, T4 early and T4 late mRNAs*

Extracts from *E. coli* infected with T4 for 13 min at 37°, translate MS2 RNA much less efficiently than late T4 mRNA (purified from culture late after infection) (table 1) in agreement with the observation of Hsu and Weiss [1]. In addition, we also detected a marked decrease in the translation of a class of T4 mRNAs, namely, the transcription product of T4 DNA by *E. coli* polymerase, which consists mainly of early T4 mRNA sequences [12]. Table 1 shows that the translation ratio between late and early T4 mRNA is

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Table 1
Translation activity of normal and T4 infected *E. coli* extracts for T4 early, T4 late and MS2 RNA.

Template used	Source of S 30 extract	
	Uninfected *E. coli*	T4 infected *E. coli* (13 min)
T4 DNA transcription product (early T4 mRNA) valine (pmoles)	37.2	10.0
Late T4 mRNA, valine (pmoles)	48.5	65.2
T4 lysozyme mRNA (units)	41.0	35.0
MS2 RNA, valine (pmoles)	25.0	5.5
Late T4 mRNA, MS2 RNA	1.9	11.9
Late T4 mRNA, early T4 mRNA	1.3	6.5
T4 lysozyme, late T4 mRNA	0.84	0.56

Measure of valine-^{14}C (83 μCi/μmole) incorporation in the non-preincubated crude extracts (*E. coli* S 30, 550 μg protein; T4 infected S 30, 950 μg protein) was carried out in 0.125 ml reaction mixture [3] with 120 μg late T4 mRNA [7] or with 2.5 μg T4 DNA and 4 μg RNA polymerase ($MgCl_2$ 14 mM, plus 0.8 mM CTP and UTP), or with 40 μg of MS2 RNA ($MgCl_2$ 10 mM, NH_4Cl, 80 mM). Endogenous incorporation in the absence of template has been subtracted (*E. coli* S 30; 15, 20 and 6 pmoles for early T4; late T4; and MS2 RNA respectively. T4 infected S 30: 25, 9 and 8 pmoles respectively). Lysozyme units as Salser et al. [7].

5 times greater in the infected cell free system than in uninfected extracts. Furthermore, the data indicate that not all the mRNAs cistrons present in late T4 mRNA are preferentially translated after infection: thus, the translation of the T4 lysozyme cistron is even relatively decreased in the infected extracts, although much less than the true early mRNAs. These changes were not apparent in extracts of cultures 3 min after T4 infection. The modification in translation specificity observed late after infection might indicate that the "turn off" of early enzymes synthesis, late after infection, [10] results from a decreased translation of early genes messengers.

3.2. *Modification of template specificity is due to initiation factor B (F3) activity*

Although some modification of the ribosome fraction after T4 infection cannot be excluded, the change in template specificity can clearly be ascribed to the initiation factor fraction [1, 2]. To determine which of the three initiation factor activities is responsible for the messenger selection process, we compared the effect of each of the *E. coli* factors with that of the T4 infected factors on T4 and MS2 RNA dependent fMet-tRNA binding to ribosomes.

The crude initiation factor fraction prepared from T4 infected cells was used to replace in turn each one of the three purified factors. If template specificity is determined by one of the three initiation factors, the change in specificity should be most apparent when the T4 infected fraction is used as the only source of this factor. Table 2 shows that the change in ratio T4/MS2 RNA activity depends very much on which of the initiation factors is replaced by the T4 infected factors. When the T4 infected fraction replaces factor B (line 9) it produces a large stimulation in T4 mRNA dependent fMet-tRNA binding but none in the MS2 RNA dependent reaction. In all combinations when T4 infected factors replace initiator protein A (F1) or C (F2), stimulation is observed with both, although a small increase in T4/MS2 RNA ratio is seen. As a control, addition of a crude fraction from uninfected *E. coli* cells (line 4,7,10), gives in all combinations a T4/MS2 RNA ratio similar to that observed with the purified proteins.

The data in table 2 thus indicate that the T4 infected factors do exhibit factor C (F2) and A (F1) activity for both MS2 and T4 mRNA, but factor B (F3) activity only for T4 mRNA and not for the MS2 template. The absence of activity toward the

Table 2
Effect of the three initiation factors on template specificity.

Line	Initiation factor added	F-Met-tRNA binding to ribosomes			
		Late T4 mRNA dependent (pmoles)	MS2 RNA dependent (pmoles)	T4/MS2	ApUpG dependent (pmoles)
1	A (F1) + B (F3) + C (F2)	6.70	2.65	2.5	9.05
2	A + B	0.28	0		
3	A + B + crude T4	7.08	1.43	4.9	
4	A + B + crude Ec	4.92	1.94	2.5	
5	B + C	3.55	1.4		
6	B + C + crude T4	8.02	2.23	3.5	
7	B + C + crude Ec	6.12	2.91	2.1	
8	A + C	1.01	0.51		8.54
9	A + C + crude T4	4.50	0.31	14.5	8.91
10	A + C + crude Ec	3.79	1.66	2.2	9.62
11	A + B + C + crude T4	7.24	1.56	4.6	
12	A + B + C + crude Ec	5.48	3.02	1.8	

^{35}S-F-Met-tRNA (24,000 cpm; 2000 cpm/pmole; free of unformylated species) was incubated at 32° for 10 min in a 0.05 ml reaction mixture containing tris-HCl, pH 7.5, 110 mM, NH_4Cl 90 mM, $MgCl_2$ 7.0 mM, GMP PCP (Miles) 1 mM, DTT 8 mM, glycerol 2.5% with 100 μg *E. coli* ribosomes, 10 μg MS2 RNA, 14 μg late T4 mRNA or 3 μg ApUpG; factor A (F1) after phosphocellulose [6] 5 μg, factor B (F3) step IV [5] 2 μg and factor C (F2) step IV [3] 1.6 μg were used in combinations with crude *E. coli* factor* 39 μg or crude T4 infected factor*, 45 μg. Binding measured on Selectron (Schleicher and Schull) filter membranes. Values in the absence of mRNA were subtracted (line 1 to 12 respectively: 1.9; 0.1; 1.18; 0.96; 0.69; 0.70; 1.01; 0.71; 1.34; 0.73; 1.35; 1.65 pmoles).

* Crude Ec and crude T4, respectively, are the proteins obtained from the 2 M NH_4Cl wash of the ribosomes from uninfected and T4 infected *E. coli*.

MS2 RNA can be reversed by adding purified factor from uninfected *E. coli* (line 11). The ratio T4/MS2 obtained is still higher than the value observed in the uninfected system and probably results from the combined effects of normal and infected factor B. Competition between the factor activities is seen also in all other cases where *E. coli* and T4 infected fractions are added together (line 3 and 6 compared to line 9).

The observation that purified factor B from *E. coli* stimulates MS2 RNA activity in the presence of the T4 infected fraction shows that the decreased activity of the T4 infected fraction for MS2 RNA results from some change in factor B activity. This change could result from either of two mechanisms: (1) the normal factor B activity for MS2 is destroyed after T4 infection [11]; (2) a new factor B activity having affinity only for T4 mRNA appears and determines the ribosome to recognize selectively T4 mRNA and not MS2 RNA. We cannot yet distinguish unambiguously between these two possibilities*. Both mechanisms, however, imply that factor B activities for T4 and MS2 RNA are due to different proteins. Factor B from uninfected *E. coli* can indeed be fractionated into several species with different specificities for T4 and MS2 RNAs [5]. As shown in table 3, one of the fractions (factor B4) has a higher T4/MS2 translation ratio than the crude *E. coli* factor, while another (fac-

* The decrease in translation activity after T4 infection is not restricted to MS2 RNA but is seen also with bulk *E. coli* mRNA [2], with early T4 mRNAs and to a certain extent even in the lysozyme cistron. If all these changes were due to inactivation of the host factor B, heterogeneity in the factor B would require that this inactivation affects several proteins. We therefore favor the hypothesis that the effect observed is due to the synthesis after T4 infection of some new species of factor B.

Table 3
Effect of initiation factor on the T4/MS2 RNA translation ratio.

Factor added	Late T4 mRNA translation		MS2 RNA translation	T4 (total)**	T4 lysozyme
	Valine incorporation (pmoles)	Lysozyme synthesis (units)	Valine incorporation (pmoles)	MS2	MS2
Crude T4	58.4	31.5	7.1	8.2	4.4
Crude Ec	32.4	34	10	3.2	3.4
Fraction B*	49.5	63	5.9	8.4	10.6
Fraction BH1*	37.7	24.4	14.9	2.5	1.6
Crude T4 + BH1	51.6	30.8	11.1	4.6	2.7
Crude T4 + B4	74.0	78	9.6	7.7	8.0
Crude Ec + BH1	28.3	14.4	12.0	2.3	1.2
Crude Ec + B4	56	47.6	13.2	4.2	3.6

* These fractions were purified from crude *E. coli* factor (see [5]). Fraction B4 is obtained from DEAE cellulose column, fraction BH1 after purification through hydroxylapatite.
** T4 (total) refers to the overall valine incorporation observed with T4 mRNA.

Valine-^{14}C (83 μCi/μmoles) incorporation was measured in 0.125 ml reaction mixture [3] containing 200 μg *E. coli* ribosomes, 60 μg late T4 mRNA, 15 μg MS2 RNA; high speed supernatant 300 μg; when factor B4 [5], 140 μg, or BH1 [5] 0.5 μg, were used purified factor A (F1) 10 μg and factor C (F2) 14 μg were added; crude *E. coli* factor (crude Ec) 80 μg and T4 infected factor (crude T4) 90 μg were used in combination with factor B fractions. Endogenous incorporation (3.5 pmoles) was subtracted.

tor BH1) is characterized by a ratio lower than that of the crude factor. Existence of different factor B species for T4 mRNA and MS2 RNA would explain how changes in relative activity of factor B for different templates are possible. As an example, in line with hypothesis 2 above, the template specificity of the T4 infected crude factor could be understood by assuming than after infection the extract is enriched for a new factor B species specific for late T4 mRNA. Thus, as illustrated by table 3, the translation activity of the cell free system for different templates varies according to which factor B is added, suggesting that, *in vivo*, variations in the amount of the different factor B (F3) species could control the relative translation of different messenger RNAs.

Acknowledgements

The expert technical assistance of Mrs. P.Federman is gratefully acknowledged. Work supported in part by a grant from Service d'Aide à la Recherche et au Development Français.

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