COMPARATIVE NATURE OF THE REACTIONS CATALYSED BY RETICULOCYTE INITIATION FACTOR IF-M_1 AND *ESCHERICHIA COLI* FACTOR IF_2 ON *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

It is well known that initiation of protein synthesis involves fMet $tRNA_f^{Met}$ in prokaryotes and Met $tRNA_f^{Met}$ in eukaryotes. Although the requirement for the *N*-formyl substitution of the initiator tRNA is absolute with prokaryotic ribosomes, the reverse is not true. The binding of both Met and fMet $tRNA_f^{Met}$ on 80 S ribosomes or on 40 S subunits can be achieved in the presence of the eukaryotic factor IF-M_1 [1,2]. The reaction with the formylated tRNA is however different from a physiological initiation, because of its lack of GTP requirement. The function of factor IF-M_1 thus remains to be elucidated. More precisely, the following questions can be raised. Is IF-M_1 able to function as a true initiation factor in an *E. coli* system and, if so, with what efficiency? What is the degree of similarity between IF-M_1 and the bacterial factor IF_2 which plays a similar role?

In a previous study we have already reported on the degree of interchangeability between bacterial and eukaryotic factors with respect to cognate or heterologous ribosomes. The present paper extends part of this study in showing that the *E. coli* fMet tRNA binding to 70 S ribosomes, directed by IF-M_1, leads to occupancy of the same site as that involved in the normal IF_2 mediated reaction; however, properties of the complex thus formed suggest that the eukaryotic factor cannot be recycled.

2. Materials and methods

2.1. *Materials*

Purified *E. coli* IF_2 was a gift from Dr Jean Thibault.

Crude initiation factors from reticulocytes were obtained by 30 min washing of polysomes with 0.5 M KCl, under conditions previously described [2,3]. Subsequently, IF-M_1 was purified by chromatography on a DEAE-cellulose column (microgranular DE 32) in the following buffer: Tris–HCl 50 mM, pH 7.5, dithiothreitol 10^{-3} M, EDTA 10^{-4} M, KCl 0.2 M. The fractions in the void volume were precipitated with ammonium sulfate (60% saturation) and dialysed overnight against the same buffer.

The other effectors of the *E. coli* and reticulocyte initiation systems were prepared as previously described [2].

2.2. *Methods*

Binding of fMet tRNA to ribosomes was routinely performed by incubating the following mixture for 12 min at 25°C: Tris–HCl 50 mM, pH 7.5, KCl 100 mM, $MgCl_2$ 5 mM, β-mercaptoethanol 7 mM, GTP 1 mM, ribosomes, poly(AUG), initiation factors and radioactive fMet tRNA as indicated in the legends for the figures. The total volume was 50 μl. The fMet tRNA radioactivity bound to the ribosomes was measured by millipore filtration.

The reaction for the transfer of formyl methionine to puromycin was done as previously described [2].

3. Results

3.1. *Interaction between IF-M_1 and 70 S ribosomes*

Preliminary experiments have already indicated that eukaryotic factor IF-M_1 is able to catalyse the binding of *E. coli* fMet tRNA on 70 S ribosomes [2]. Figures 1 and 2 confirm and extend this finding in illustrating the binding of both reticulocyte and *E. coli* fMet tRNA as a function of factor concentration, using either reticulocyte 40 S or *E. coli* 70 S. It is clear that IF-M_1 stimulates these different reactions, a result which emphasizes its ability to interact with the *E. coli* ribosome.

However, one specific point should be mentioned. At the IF-M_1 concentrations used, when working with the reticulocyte 40 S, up to 3.3 μmol of reticulocyte fMet tRNA or 3.8 μmol of *E. coli* fMet tRNA are bound. These two values are quite similar. But when working with the *E. coli* 70 S, a slight difference can be observed: 1.4 μmol of *E. coli* fMet tRNA are bound at IF-M_1 saturation, but only 0.5 μmol of reticulocyte fMet tRNA.

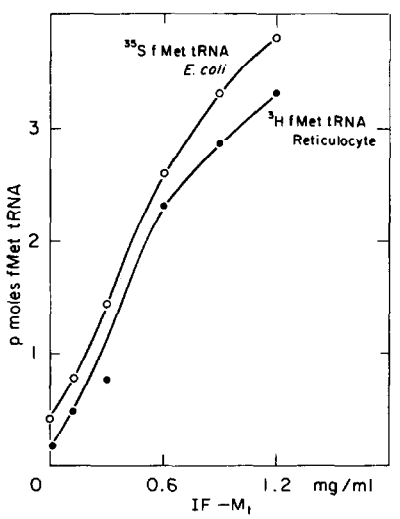

Fig.1. Stimulation by IF-M_1 of the binding reaction of *E. coli* and reticulocyte fMet tRNAs on the reticulocyte 40 S. The incubation was done as described in Materials and methods. Each sample contained 0.6 A_{260} unit of 40 S, 0.12 A_{260} unit of poly(AUG), 15 μmol reticulocyte ^{3}H-labeled fMet tRNA (900 mCi/mmol) or 15 μmol *E. coli* ^{35}S-labeled fMet tRNA (300 mCi/mmol). Factor IF-M_1 was added at the concentrations indicated on the figure.

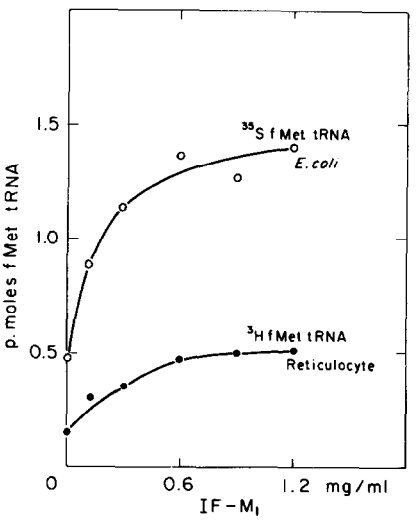

Fig.2. Stimulation by IF-M_1 of the binding reaction of *E. coli* and reticulocyte fMet tRNAs on the *E. coli* 70 S. The incubation was done as described in Materials and methods. Each sample contained: 1 A_{260} unit of 70 S, 0.12 A_{260} unit of poly(AUG), 15 μmol reticulocyte ^{3}H-labeled fMet tRNA (900 mCi/mmol) or 15 μmoles of *E. coli* ^{35}S-labeled fMet tRNA (300 mCi/mmol). Factor IF-M_1 was added at the concentrations indicated on the figures.

Thus, in the case of the interaction between IF-M_1 and the 70 S, we can emphasize three points:

(1) The binding is three times more efficient with the bacterial fMet tRNA than with the eukaryotic one.

(2) The plateau is lower than that obtained with the 40 S.

(3) Saturation is reached more rapidly.

Nevertheless, this reaction does occur and proves that the 70 S and IF-M_1 recognize each other. Whether this recognition is of the same type as that which has been described [4] between the 70 S and the homologous factor IF_2 deserves further investigation.

3.2. *Similarities between IF_2 and IF-M_1*

In the *E. coli* initiation system, the factor which catalyses the binding of fMet tRNA on the ribosome is IF_2. Its activity is stimulated by factor IF_1, and requires GTP. It also catalyses the reaction for the

transfer of the formyl methionine to puromycin.

As reported in a preceding paper [2], although IF-M_1 catalyses the same binding reaction of fMet tRNA to 70 S, its mechanism of action exhibits three differences with that of IF_2: First, no complementation with IF_1 has been observed. Second, it does not require GTP. Third, it does not stimulate any transfer to puromycin.

The lack of complementation between IF_1 and IF-M_1 leads us to a tentative hypothesis. It is known that IF_1 permits IF_2 to function catalytically, by favouring its release after each initiation round. Thus it can be thought that IF-M_1, once the initiation complex has been formed, remains bound on the ribosome instead of being released. This would explain equally the insufficiency of IF-M_1 in the reaction of transfer to puromycin which requires this release.

If our model is correct, once the binding reaction is done in the presence of IF-M_1, further addition of IF_2 should not stimulate more binding of the fMet tRNA. That is, once the receptory site for IF_2 is occupied with IF-M_1, IF_2 is unable to act. The simplest way to verify this assertion consists in doing the binding reaction in the presence of IF-M_1 and at the end of the incubation, adding IF_2 and allowing the reaction to proceed during another incubation period. The results (table 1) show a clear inhibition of IF_2 action, by the preliminary addition of IF-M_1.

A similar experiment was made for the puromycin reaction. If our model is correct and knowing that IF-M_1 alone does not catalyse this reaction, addition of IF_2 after the incubation period should not stimulate any transfer to puromycin. The results (table 2) clearly confirm this hypothesis.

Table 1
Inhibition by reticulocyte factor IF-M_1 of *E. coli* factor IF_2 action in binding

	fMet tRNA (μmoles)	
	E. coli	Reticulocyte
−factor	<0.01	<0.01
+ IF-M_1	1.0	0.4
+ IF_2	2.4	1.2
+ IF-M_1 + IF_2	1.2	0.6

Complete system contains 60 μg IF-M_1, 4 μg IF_2 and one A_{260} unit of 70 S. Other effectors as usual. In the tubes containing both factors, IF_2 is added after 12 min incubation and the reaction continued for 12 min. In other cases incubation time is 24 min.

Table 2
Inhibition by reticulocyte factor IF-M_1 of *E. coli* factor IF_2 action in transfer to puromycin on 70 S

	fMet tRNA (μmoles)	
	E. coli	Reticulocyte
−factor	<0.01	<0.01
+ IF-M_1	<0.01	<0.01
+ IF_2	5.0	1.2
+ IF-M_1 + IF_2	0.5	0.3

The conditions of incubation are the same as in table 1, puromycin being added at zero time, to give a final concentration of 10^{-3} M.

Also, it can be pointed out that when using either *E. coli* or reticulocyte fMet tRNA, IF-M_1 is approximately two-fold less efficient in the binding reaction than IF_2 (table 1). This suggests once more that the interaction between the bacterial ribosome and the eukaryotic factor is not identical to that obtained with the bacterial factor, although it allows the same binding reaction to take place.

4. Discussion

According to the preceding results, an effective similarity between reticulocyte IF-M_1 and *E. coli* IF_2 can be exhibited. However, three differences do exist:

(1) In fact, IF-M_1 catalyses the binding of fMet tRNA to the 70 S ribosome, but it does not require the hydrolysis of GTP.

(2) IF-M_1 does not catalyse the transfer of formyl methionine to puromycin.

(3) IF-M_1 is not stimulated by IF_1. Moreover, when it is preincubated with the 70 S and fMet tRNA in the presence of the initiation effectors (except *E. coli* factors), it inhibits the further action of IF_2.

We suggest the following interpretation: IF-M_1 recognizes the same site as IF_2 on the 70 S ribosome, favouring the binding of fMet tRNA. But unlike IF_2, it occupies this site irreversibly. Consequently the following steps of initiation which require its release from the ribosome (GTP hydrolysis, formation of the first peptide bond) cannot take place.

Thus, the biological function of IF-M_1 remains

unclear. Although the reaction it catalyses differs from the physiological initiation one (mainly by using a formylated Met tRNA as a substrate), it is considered by Anderson et al. [4–6] as a real initiation factor. In fact, in a reticulocyte initiation system, IF-M_1 enhances the level of binding obtained when using IF-M_{2A+B} alone. On the contrary, Schreier and Staehelin [7] do not find any factor with the M_1 properties. Nevertheless, they have reported another factor, IF-E_2, which (like IF-MP of Anderson et al. [8] stimulates the binding of Met tRNA on the ribosome, but in the presence of a natural mRNA. But both IF-E_2 and IF-MP are structurally quite different from IF-M_1.

Concluding we mention that in different eukaryotic organisms there exist factors which, like IF-M_1, exhibit analogies with the *E. coli* factor IF_2. For instance, the *Artemia salina* factor EIF-1, described by Ochoa et al. [9,10], which catalyses the GTP-independant binding of *E. coli* fMet tRNA on the *Artemia* 40 S subunit. An initiation factor has also been isolated in the Krebs ascites tumor cell [11] exhibiting properties very similar to that of *Artemia salina* EIF-1. However, these factors are different from IF-M_1 since they catalyse the reaction of transfer to puromycin, contrary to IF-M_1. Moreover, their molecular weight (150 000) is greater than that of IF-M_1 (63 000). Thus the real physiological function of IF-M_1 remains an open problem.

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