FACTOR REQUIREMENT OF FORMYLMETHIONYL-tRNA BINDING TO *E. COLI* RIBOSOMES PROGRAMMED WITH A PLANT VIRAL RNA OR A PHAGE RNA

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1. Introduction

Three ribosomal proteins, required for the initation of polypeptide synthesis in cell-free systems of *E. coli* have recently been isolated [1-5] and purified [6-7]. One of them (designated F_3 by Iwasaki et al. [1] has been reported to be essential for the binding of natural messengers like phage RNA to 30 S ribosomes, while the others (F_1 and F_2 in addition to GTP) are required for the binding of F-Met-tRNA to the mRNA-ribosome complex [2,4].

So far contradictory data are available [4,8] with regard to the question whether all natural messengers, including plant viral RNA, depend on all three factors for translation in the cell-free system of *E. coli*. In this paper we show that F_1, F_2 and F_3 are required for the binding of F-Met-tRNA to *E. coli* ribosomes in the presence of the plant viral messenger AMV-RNA* as they are in the presence of phage RNA.

2. Materials and methods

RNA derived from the top component *a* of AMV was labeled with ^{32}P and MS_2-RNA with ^{3}H as described previously [9]. *N*-formyl-^{35}S-methionyl-tRNA and *N*-formyl-^{3}H-methionyl-tRNA were kindly provided by Dr. H.O.Voorma.

For the isolation and purification of ribosomes (washing with high salt and chromatography on DEAE-cellulose), the isolation of initiation factors and their

* AMV, alfalfa mosaic virus.

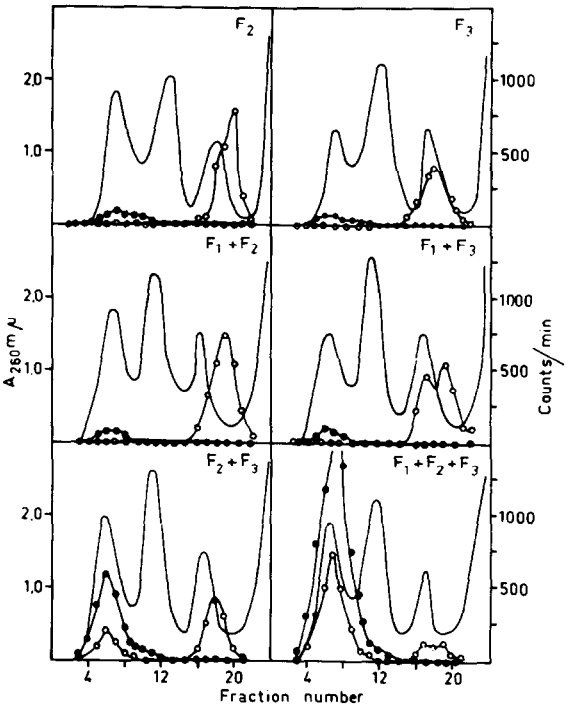

Fig. 1. Factor requirement of the binding of both ^{3}H-MS_2-RNA and ^{35}S-Met-tRNA to ribosomes. The incubation mixtures (0.5 ml) containing 20 μμmole ^{3}H-MS_2-RNA, 200 μμmole F-^{35}S-Met-tRNA, 0.1 μmole GTP, 600 μg of purified ribosomes (see Materials and Methods), 40 μg of each initiation factor (as indicated), 37 μmole NH_4Cl, 3.4 μmole Mg acetate, 3 μmole β-mercaptoethanol and 25 μmole tris-HCl (pH 7.1) were incubated at 37°C for 10 min. They were submitted to centrifugation in a gradient of 15-30% sucrose in the same buffer in a SW25.3 rotor at 20,000 rpm for 15.5 hr. Absorbancy at 260 mμ was monitored continuously with a Gilford spectrophotometer. Radioactivity was assayed as described in the text. o——o Binding of ^{3}H-MS_2-RNA; •——• Binding of F-^{35}S-Met-tRNA; —— A_{260} mμ.

fractionation (separation of F_1, F_2 and F_3) essentially the procedure of Iwasaki et al. [1] was followed (compare also Albrecht et al. [9].

3. Results

3.1. *Binding of ^{3}H-MS$_2$-RNA and F-^{35}S-Met-tRNA*

The effectiveness of the separation of the factors F_1, F_2 and F_3 is shown in the dual-label experiment of fig. 1. *E. coli* ribosomes, washed with high salt and purified by DEAE-chromatography (see Materials and Methods) were incubated with both ^{3}H-MS$_2$-RNA and F-^{35}S-Met-tRNA in the presence and absence of factors. Subsequently the reaction mixtures were centrifuged in a 15–30% sucrose gradient. All gradient fractions were passed through Millipore filters and the radioactivities trapped on the filters were measured. Binding of both phage messenger and F-Met-tRNA required the presence of all three factors, as was originally found by Iwasaki et al. [1]. Binding of F-Met-tRNA in the absence of MS$_2$-RNA was much lower (table 1, columns 2 and 3). The trapping of ^{3}H-MS$_2$ RNA in the zone between the meniscus and the 30 S ribosomes is under study and will be discussed elsewhere.

3.2. *Binding of ^{32}P-AMV-RNA and F-^{35}S-Met-tRNA*

Fig. 2 shows the effect of factors on binding to ribosomes of ^{32}P-AMV-RNA (in the presence of non-

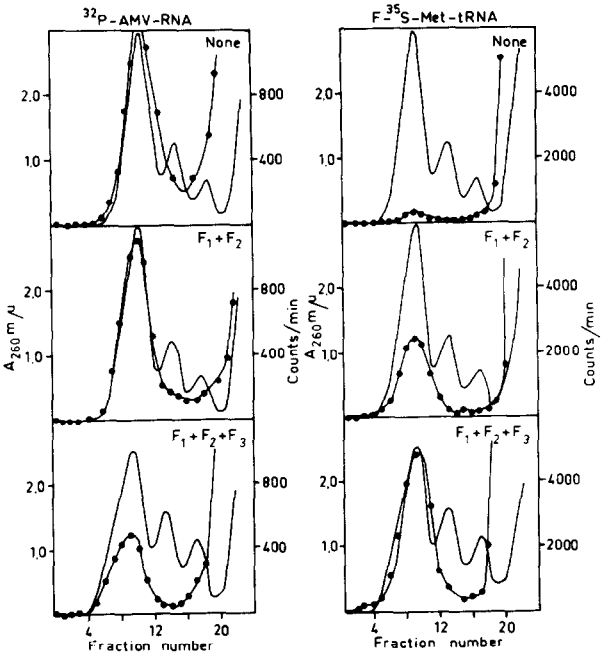

Fig. 2. Factor requirement of the binding of both ^{32}P-AMV-RNA and F-^{35}S-Met-tRNA to ribosomes. For experimental details see legend of fig. 1, except that 10 μg of ^{32}P-AMV-RNA were incubated with 200 μμmole unlabeled F-Met-tRNA in three experiments and 10 μg of unlabeled AMV-RNA with 200 μμmole of F-^{35}S-Met-tRNA in the other three. ●—● counts/min; — A_{260} μμ.

Table 1
Factor requirement of F-Met-tRNA binding to *E. coli* ribosomes in the presence and absence of MS$_2$-RNA and AMV-RNA, respectively.

Additions	F-^{3}H-Met-tRNA associated with 70S ribosomes		F-^{35}S-Met-tRNA associated with 70S ribosomes	
	+MS$_2$-RNA (counts/min)	-MS$_2$-RNA (counts/min)	+AMV-RNA (counts/min)	-AMV-RNA (counts/min)
None	–	–	1430	400
F_1	100	100		
F_2	3550	2520		
F_3	1720	–		
$F_1 + F_2$	5050	–	11150	4610
$F_2 + F_3$	6500	3200		
$F_1 + F_2 + F_3$	24700	6450	18300	4710

For experimental details compare legends of fig. 1 and 2 except that non-labeled viral messengers were used. The radioactivities of the sucrose fractions, containing 70S-ribosomes, were enumerated.

labeled F-Met-tRNA) and of F-^{35}S-Met-tRNA (in the presence of non-labeled AMV-RNA). Evidently the formation of complexes between 70 S ribosomes, AMV-RNA and F-^{35}S-Met-tRNA also required the combined action of F_1, F_2 and F_3. Association of the plant viral messenger, however, was found to occur in the complete absence of factors (compare also Albrecht et al. [9]). In fact addition of factors caused a substantial decrease in AMV-RNA binding to 70 S ribosomes. (Note that gradient fractions were not passed through Millipore.) Binding of F-^{35}S-Met-tRNA dropped considerably when AMV-RNA was omitted (table 1, columns 4 and 5).

4. Discussion and conclusion

The conclusion that three separate ribosomal factors (F_1, F_2 and F_3) are essential for the binding of F-Met-tRNA to *E. coli* ribosomes in the presence of a phage messenger ([1–5], fig. 1 and table 1) can now be extented to another natural messenger: RNA derived from the plant virus AMV. Whether F_3 is also a requisite for the association of the plant viral messenger with the ribosomes, as seems to be the case with the homologous phage RNA, remains to be seen. AMV-RNA can bind to *E. coli* ribosomes in the complete absence of initiation factors (Albrecht et al. [9] and fig. 2). Such a binding does not require binding of F-Met-tRNA either [9], and addition of factors even lowers the binding of plant viral messenger. Various explanations for the latter phenomenon are possible and further studies are underway to clarify this point.

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