

## Toward an Understanding of the Formylation of Initiator tRNA Methionine in Prokaryotic Protein Synthesis. I. In Vitro Studies of the 30S and 70S Ribosomal-tRNA Complex<sup>†</sup>

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**ABSTRACT:** Formation of the 30S-tRNA initiation complex of *Escherichia coli* with nonformylated initiator tRNA is stimulated by all three initiation factors and is messenger dependent, whereas the complex formation involving the 70S ribosomes is strongly inhibited by initiation factors when the nonformylated species is used. When the 30S-Met-tRNA<sup>Met</sup> complex is first formed and the 50S ribo-

somal subunit added subsequently, there is no significant inhibition by initiation factors and the nonformylated initiator tRNA is puromycin reactive. This leads to the conclusion that the formylation of the methionyl initiator tRNA is only obligatory when polypeptide synthesis is initiated by nondissociated 70S ribosomes.

In prokaryotes, in contrast to what happens in eukaryotes, polypeptide chain initiation proceeds via a mechanism involving a formylated species of initiator tRNA acylated with methionine: fMet-tRNA<sup>Met</sup>. However, several exceptions to this universal rule have been recently described since some prokaryotes can indeed grow under conditions where their initiator tRNA is not formylated (Samuel et al., 1970; Samuel and Rabinowitz, 1974; White and Bayley, 1972). Moreover, it has been shown that *Escherichia coli* can grow to a certain extent, even in media where the level of formylation is strongly depressed (Danchin, 1973; Harvey, 1973); this growth, however, is very slow and does not permit an interpretation of the function of formylation.

Both formylmethionyl-tRNA<sup>Met</sup> and methionyl-tRNA<sup>Met</sup> act as initiators of polypeptide chains in vitro, whereas methionyl-tRNA<sup>mMet</sup> appears to code only for methionine residues in an internal position in a polypeptide (Bretscher and Marcker, 1966; Leder and Bursztyn, 1966a).

These observations suggest that it is the tRNA three-dimensional structure which determines its specificity as polypeptide chain initiator, and not the formyl group, although the latter favors the reaction by a mechanism which is still obscure.

It has been shown that protein factors IF-1, IF-2, and IF-3 are involved in protein synthesis initiation and that they play an active role in the ribosomal site selection process; it has been observed that these factors do not act merely by increasing the affinity of aminoacyl-tRNA and of mRNA for their preexisting attachment regions (Springer and Grunberg-Manago, 1972). The question then arises as to whether formylation of initiator tRNA methionine acts primarily on the recognition of Met-tRNA<sup>Met</sup> by initiation factors, or whether it is involved in another step of the initiation process.

In this paper we show that formation of the 30S-tRNA complex (with nonformylated initiator tRNA) is stimulated by all three initiation factors and is messenger dependent. However, the complex formation involving the 70S ribosomes is strongly inhibited by initiation factors when the nonformylated species is used. When the 30S-Met-tRNA complex is first formed and the 50S ribosomes added subsequently, the inhibition by factors is weaker.

Quantitative data on the behavior of the formylated and nonformylated Met-tRNA<sup>Met</sup> in the presence of 70S ribosomes are presented in the second article of this series (Petersen et al., 1976) in which we suggest a model for the dynamics of the 70S particle.

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## Materials and Methods

L-[methyl-<sup>3</sup>H]Methionine, 7.3 and 8.2 Ci/mmol, were purchased from CEA or from Amersham. Poly(A,U,G), ratio 1:1:1, was a gift from Dr. Thang of this laboratory; trinucleotide ApUpG was a gift from Dr. O. Pongs, Berlin, Germany; ATP (disodium salt) and GTP (trisodium salt) were from Sigma. DNase (RNase free) was from Worthington and puromycin (dihydrochloride) was from Nutritional Biochemical Corp. DEAE-cellulose (DE-52) was from Whatman. tRNA<sub>m</sub><sup>Met</sup> were prepared in our laboratory by Dr. R. Buckingham from the CAJ 64 strain from *E. coli*, by the method of Seno et al. (1968). Pure methionyl-tRNA<sup>Met</sup>-synthetase was a gift from Dr. J. P. Waller and S. Blanquet (Ecole Polytechnique, Palaiseau, France) and was purified by the method of Cassio and Waller (1971). All the other chemicals were reagent grade.

Bacterial strains of *E. coli* were used as a source of ribosomes; the wild-type MRE 600 (RNase<sup>-</sup>) was grown according to Danchin (1973) and harvested in the exponential phase at an optical density of 2 at 600 nm.

Ribosomes were prepared as described by Dondon et al. (1974) and washed at high (1.5 M) and twice at low (60 mM) ammonium chloride concentrations. Light scattering measurements showed that almost 100% of these ribosomes were associated at 4 mM magnesium, corresponding to the "tight" couples described by Noll et al. (1973). Of the ribosomes at least 58% were active since 15 pmol of 70S ribosome bound 8.7 pmol of fMet-tRNA<sup>fMet</sup> under the following conditions: ribosomes, 15 pmol; *E. coli* tRNA, 1.9 A<sub>260</sub> units, corresponding to 18 pmol of fMet-tRNA<sup>fMet</sup>; GTP, 1 mM; poly(A,U,G), 0.129 A<sub>260</sub> unit; Mg<sup>2+</sup>, 5 mM; Tris-HCl (pH 7.4), 50 mM; IF-1, 0.15 μg; IF-2, 0.85 μg; IF-3, 0.144 μg. The ribosomes were stored at -90 °C and used over a period of 1 week after defrosting and being kept at 0 °C.

Ribosomal subunits 30S and 50S were prepared by zonal separation. A sucrose gradient (10–30%) was made in the Ti-14 rotor of a Spinco L-65 centrifuge at 3000 rpm. Ribosomes were then added in a 0–4% sucrose gradient and the speed was set to 31 000 rpm for 17 h. The gradient was displaced from the rotor at 3000 rpm with 35% sucrose. The solutions used in the preparation contained 10 mM Tris-HCl (pH 7.5), 60 mM ammonium chloride, 10 mM magnesium acetate, and 0.4 M sodium chloride. The subunits were then suspended in 10 mM Mops buffer (100 mM ammonium chloride, 20 mM magnesium acetate, and 7 mM 2-mercaptoethanol) and activated by heating this solution 15 min at 37 °C.

Crude initiation factors were obtained as the dialyzed supernatant of the 1.5 M ammonium chloride ribosomal wash, centrifuged 90 min at 150 000g; dialysis was against 10 mM Tris-HCl (pH 7.5), 60 mM NH<sub>4</sub>Cl, and 7 mM 2-mercaptoethanol. The crude initiation factors used in some experiments were given by Mr. J. Dondon of this laboratory as were the pure factors. The latter were purified according to Dondon et al. (1974) and appeared homogeneous on the sodium dodecyl sulfate-polyacrylamide gel electrophoretogram.

Aminoacylation of the tRNA<sub>f</sub><sup>Met</sup> and tRNA<sub>m</sub><sup>Met</sup> with methionine was done essentially by the method of Lawrence et al. (1973). We incubated for 15 min at 30 °C the following mixture (total volume of 6 ml in aliquots of 0.5 ml): 6.0 A<sub>260</sub> units of tRNA<sub>f</sub><sup>Met</sup> or tRNA<sub>m</sub><sup>Met</sup>, 0.125 μmol of [<sup>3</sup>H]methionine, 155 pmol of methionyl-tRNA<sup>Met</sup> synthe-

Table I: Effect of Initiation Factors on the Binding of fMet-tRNA and Met-tRNA<sub>f</sub><sup>Met</sup> to 30S Ribosomal Subunits.<sup>a</sup>

Addition	pmol	
	fMet-tRNA <sub>f</sub> <sup>Met</sup>	Met-tRNA <sub>f</sub> <sup>Met</sup>
None	0.12	0.18
Crude IF	1.12	0.68
IF-1		0.24
IF-2		0.22
IF-3		0.16
IF-1 + IF-2		0.29
IF-1 + IF-3		0.15
IF-2 + IF-3		0.26
IF-1 + IF-2 + IF-3		0.31

<sup>a</sup> Each assay tube (50 μl) contained: 15 pmol of 30S ribosomal subunits; 0.11 A<sub>260</sub> unit of poly(A,U,G); 50 mM Tris-HCl (pH 7.4); 5 mM magnesium acetate; 50 mM ammonium chloride. Initiation factors, where indicated, were: IF-1, 0.6 μg; IF-2, 1.2 μg; IF-3, 1.0 μg; crude IF, 26 μg; fMet-tRNA<sub>f</sub><sup>Met</sup>, 2.1 pmol (4100 cpm/pmol), or Met-tRNA<sub>f</sub><sup>Met</sup>, 3.0 pmol (4100 cpm/pmol).

tase, 2 mM ATP, 7 mM MgCl<sub>2</sub>, 150 mM potassium chloride, 10 mM 2-mercaptoethanol, 20 mM Tris-HCl (pH 7.5), and 0.1 mM EDTA (pH 7.5).

After incubation, the aliquots were concentrated to 1.8 ml by DEAE column chromatography; we used a layer of 1.0 cm<sup>2</sup> × 1.5 cm DEAE-cellulose previously washed with 5 ml of 2 M NaCl and 0.05 M sodium acetate (pH 5.0) followed by 5 ml of 0.2 M sodium chloride and 0.05 M sodium acetate (pH 5.0). When the reaction mixtures had been applied, 10 ml of 0.2 M sodium chloride and 0.05 M sodium acetate (pH 5.0) was passed through the column which washed out the free [<sup>3</sup>H]methionine. Met-tRNA was eluted with 1.0 M sodium chloride and 0.05 M sodium acetate (pH 5.0). Fractions of 600 μl were collected and approximately 80% of the original amount of tRNA was recovered in 1.8 ml.

To lower the salt concentration, the Met-tRNA was precipitated in 3 vol of ethanol and redissolved in 5 mM sodium acetate (pH 5.0) whereafter it was stored at -20 °C.

By this method, close to 60% of the tRNA was obtained in the aminoacylated form and fractionation on the DEAE column eliminated the eventual contamination by exogenous nucleotides such as GTP.

Formylation of [<sup>3</sup>H]Met-tRNA<sub>f</sub><sup>Met</sup> was obtained chemically in the N position of the methionyl group by the method of Gillam et al. (1968), slightly modified. The formyl ester of N-hydroxysuccinimide (3 mg) was dissolved in dry tetrahydrofuran (500 μl). This solution was mixed with a solution of 1.0 A<sub>260</sub> unit of [<sup>3</sup>H]Met-tRNA<sub>f</sub><sup>Met</sup> in 0.1 M triethanolamine (pH 4.3) and 20 mM magnesium chloride (500 μl). Except for this change in concentration the above-mentioned method was followed.

By this procedure, approximately 90% of the [<sup>3</sup>H]Met-tRNA<sub>f</sub><sup>Met</sup> was formylated, as measured by the degree of formylation of methionine; formylated and unformylated molecules were separated by electrophoresis at pH 3.5.

The binding reaction mixture (50 μl) contained: 50 mM Tris-HCl, 50 mM ammonium chloride, 0.11 A<sub>260</sub> unit of poly(A,U,G), where indicated, 1 mM GTP, where indicated, and ribosomes, magnesium acetate, crude or pure initiation factors, and (formyl-) Met-tRNA<sub>f</sub><sup>Met</sup>, as indicated.

Incubation was performed for 20 min at 37 °C, or as indicated, and the reaction was stopped by dilution with 3 ml

Table II: Effect of Different Messengers on the Binding of fMet-tRNA<sup>Met</sup>, Met-tRNA<sup>Met</sup>, and Met-tRNA<sup>Met</sup> to 30S Ribosomal Subunits in the Absence and in the Presence of Initiation Factors.<sup>a</sup>

Messenger	Crude IF	pmol		
		fMet-tRNA <sup>Met</sup>	Met-tRNA <sup>Met</sup>	Met-tRNA <sup>Met</sup>
No	-	0.01	0.03	0.02
	+	0.04	0.02	0.01
ApUpG	-	0.03	0.02	0.02
	+	0.27	0.07	0.03
Poly(A,U,G)	-	0.21	0.20	0.03
	+	1.83	0.67	0.08
R17 RNA	-	0.01	0.06	0.01
	+	0.74	0.13	0.01

<sup>a</sup> Same conditions as in Table I except where indicated: Met-tRNA<sup>Met</sup>, 4.4 pmol (4100 cpm/pmol); ApUpG, 0.30 A<sub>260</sub> unit; R17 RNA, 1.3 A<sub>260</sub> units; and in the assays containing fMet-tRNA, 1 mM GTP is present.

Table III: Effect of GTP and Magnesium Concentration on the Binding of fMet-tRNA and Met-tRNA<sup>Met</sup> to 30S Ribosomal Subunits in the Absence and the Presence of Initiation Factors.<sup>a</sup>

	[Mg] (mM)		pmol	
			fMet-tRNA <sup>Met</sup>	Met-tRNA <sup>Met</sup>
+GTP	5	-IF	0.12	0.18
		+IF	1.12	0.68
	5	-IF	0.21	0.17
		+IF	1.83	0.35
	15	-IF	0.46	0.45
		+IF	1.27	1.07
35	-IF	0.49	0.52	
	+IF	1.17	0.95	

<sup>a</sup> Same conditions as for Table I, except that crude initiation factors were used, and for the magnesium acetate concentration which is as indicated. In the series where GTP is present the concentration of GTP was 1 mM.

of ice-cold 50 mM Tris-HCl (pH 7.4), 5 mM magnesium acetate, and 50 mM ammonium chloride, followed by filtration on nitrocellulose filters (Millipore). After washing the filters twice with 3 ml of the cold dilution buffer they were dried at 85 °C and radioactivity was measured in 5 ml of standard toluene-2,5-diphenyloxazole-1,4-bis[2-(5-phenyloxazolyl)]benzene scintillation liquid in an Intertrichine scintillation counter.

**Puromycin Reaction.** The binding incubation mixture was the same as above. After 20 min incubation (except where otherwise stated) 5 μl of a puromycin aqueous solution (5 μg/μl) was added to the reaction mixture and incubation was continued at 37 °C for the indicated times. The reaction was stopped by dilution with 1 ml of ice-cold 0.1 M sodium phosphate (pH 8.0) and the (formyl-)methionylpuromycin was extracted with ethyl acetate, as described by Leder and Burszty (1966b). At about pH 5, only fMet-puromycin is extracted, whereas at pH 8 both Met- and fMet-puromycin, but not methionine, are extracted.

## Results

**I. Formation of the 30S Initiation Complex.** Table I shows that binding of Met-tRNA<sup>Met</sup> to 30S ribosomal units is stimulated in the presence of poly(A,U,G) by IF-1 and IF-2, but not by IF-3 alone. Maximal binding stimulation, however, requires all three initiation factors. The stimulation by purified initiation factors eliminates the possibility of a contaminating transformylase formylating the Met-tRNA<sup>Met</sup>. Stimulation by crude initiation factors is somewhat higher than by all three pure factors (at saturating conditions). We therefore tried to find an additional stimulating factor in DEAE-cellulose fractionated crude initiation factors, but could not, however, detect such an activity. The higher activity of crude initiation factors could probably be explained by a contamination of the extracts by ribosomal proteins restoring the activity of some ribosomes deprived of their protein content, as has been previously observed by Noll and Noll (1974).

The requirement for messenger is shown in Table II. Unformylated Met-tRNA<sup>Met</sup> binding is stimulated by crude IF. There is a stimulation not only in the presence of poly(A,U,G), but also when phage R 17 RNA is used as messenger; the stimulation, about three times, is less than what

we find using the formylated species. As a comparison, binding of Met-tRNA<sup>Met</sup> is shown. The small enhancement found can be explained by the contamination of tRNA<sup>Met</sup> by tRNA<sup>fMet</sup> (which is about 5%).

The effect of magnesium concentration is shown in Table III. One observes that the stimulation by initiation factors occurs at Mg<sup>2+</sup> concentrations as high as 35 mM. Here too, formylated and unformylated initiator tRNA behave similarly although fMet-tRNA binding is somewhat better at 5 mM Mg<sup>2+</sup>, whereas at 15 mM the two species are barely distinguishable. The difference between the two species is better seen in the presence of GTP. We find that GTP inhibits the binding of Met-tRNA<sup>Met</sup> in the presence of initiation factors (Table III). Contrariwise, GTP enhances fMet-tRNA<sup>Met</sup> binding. This shows that the role of GTP in the binding of the initiator tRNA to the ribosome is complex; it is likely that a ternary complex GTP-fMet-tRNA-IF-2 is formed on the 30S subunit and that such a complex is sensitive to formylation of the methionine of the initiator tRNA.

**II. Complex Formation on 30S + 50S Ribosomes.** In contrast to what is found with the 30S subunits, where it does not have a drastic influence, formylation plays an important role when both 30S and 50S subunits are present. At 5 mM magnesium the unformylated Met-tRNA<sup>Met</sup> binds very well to ribosomes in the presence of poly(A,U,G) without initiation factors, whereas under these conditions fMet-tRNA<sup>Met</sup> binds to a very small extent (Figure 1A,B). Initiation factors show an opposite effect on the two species the binding of Met-tRNA<sup>Met</sup> being highly inhibited whereas the binding of fMet-tRNA<sup>Met</sup> shows the well-known stimulation. The same phenomenon had been previously observed by Giègé et al. (1973) using mischarged Val-tRNA<sup>Met</sup> and Phe-tRNA<sup>Met</sup> in formylated and unformylated forms.

As can be seen in Table IV the inhibition is mostly due to IF-2 and IF-3; maximal inhibition occurs when these two factors are present and addition of IF-1 does not increase the inhibition. Since we had observed an effect of GTP on 30S subunits we investigated its influence on a preformed mixture of 30S + 50S subunits. Table V shows that the inhibition by initiation factors is not dependent on GTP.

Comparing the results of Figures 1A and 1B it can be seen that in the absence of initiation factors, changes in

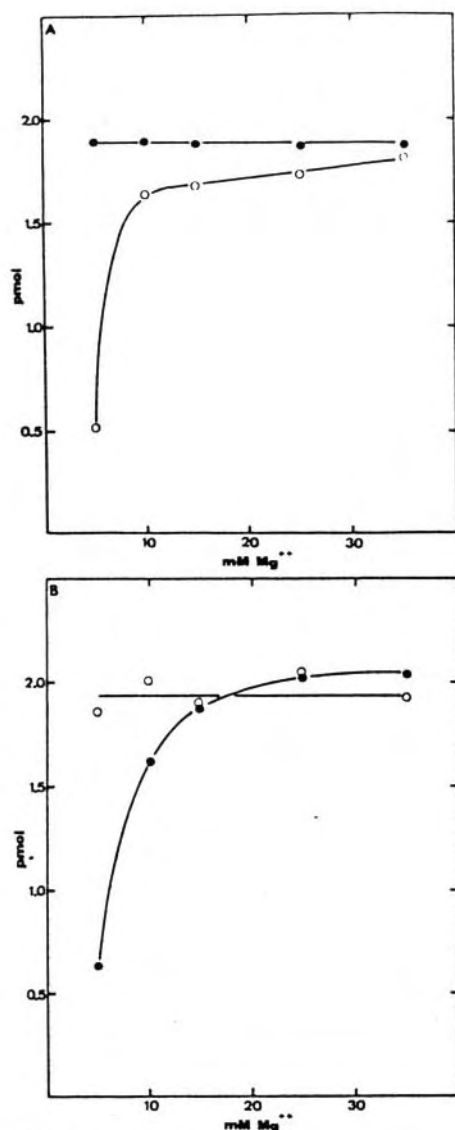


FIGURE 1: (A) Effect of magnesium concentration on the binding of fMet-tRNA<sup>Met</sup> to 70S ribosomes. Incubation mixtures (50  $\mu$ l) contained: 15 pmol of 70S ribosomes; 2.8 pmol of fMet-tRNA<sup>Met</sup> (4100 cpm/pmol); 0.11  $A_{260}$  unit of poly(A,U,G); 1 mM GTP; 50 mM Tris-HCl buffer (pH 7.4); 50 mM ammonium chloride; magnesium acetate, as indicated; and, where indicated, 50  $\mu$ g of crude initiation factors. (B) Effect of magnesium concentration on the binding of Met-tRNA<sup>Met</sup> to 70S ribosomes. Incubation mixtures are the same as described in A except that no GTP was present and 3.2 pmol of Met-tRNA<sup>Met</sup> (4100 cpm/pmol) was used instead of fMet-tRNA<sup>Met</sup>: (○) in the absence of initiation factors; (●) in the presence of initiation factors.

magnesium concentration have no effect on the binding of Met-tRNA<sup>Met</sup>, whereas a considerable increase in the binding of the formylated species is found, maximal binding of fMet-tRNA<sup>Met</sup> being reached at 10 mM magnesium.

Although initiation factors inhibit the binding of Met-tRNA<sup>Met</sup> at magnesium concentrations up to 15 mM, the inhibition is decreased at higher Mg<sup>2+</sup> concentrations.

We then bound initiator tRNA, formylated or not, first to 30S ribosomal subunits and subsequently added 50S subunits after 10-min incubation. The effect of initiation factors in this case is seen in Figures 2A and 2B: addition of 50S subunits yields no significant stimulation of binding of fMet-tRNA<sup>Met</sup> in the presence of initiation factors, whereas additional binding can be seen in the absence of factors.

With Met-tRNA<sup>Met</sup> a marked effect is found when 50S

Table IV: Effect of Purified Initiation Factors on Binding of Met-tRNA<sup>Met</sup> to 70S Ribosomes.<sup>a</sup>

Additions	Met-tRNA <sup>Met</sup> Bound (pmol)
None	0.48
IF-1	0.45
IF-2	0.09
IF-3	0.14
IF-1 + IF-3	0.13
IF-2 + IF-3	0.08
IF-1 + IF-2 + IF-3	0.08

<sup>a</sup> The incubation mixture (50  $\mu$ l) contained: 70S ribosomes, 15 pmol; poly(A,U,G), 0.11  $A_{260}$  unit; Tris-HCl (pH 7.4), 50 mM; magnesium acetate, 5 mM; ammonium chloride, 50 mM; initiation factors (where indicated), 0.25  $\mu$ g of IF-1, 0.44  $\mu$ g of IF-2, 0.5  $\mu$ g of IF-3; and Met-tRNA<sup>Met</sup>, 3.0 pmol (4100 cpm/pmol). In the absence of poly(A,U,G) the amount of Met-tRNA<sup>Met</sup> bound was 0.10 pmol.

Table V: Effect of GTP on the Binding of Met-tRNA<sup>Met</sup> to 70S Ribosomes in the Absence and in the Presence of Initiation Factors.<sup>a</sup>

Addition	Crude IF	pmol	
		Met-tRNA <sup>Met</sup>	fMet-tRNA <sup>Met</sup>
None	-	1.89	0.15
GTP	-	1.64	0.5
None	+	0.07	0.24
GTP	+	0.04	1.85

<sup>a</sup> Incubation mixtures contained: 3.8 pmol of Met-tRNA<sup>Met</sup> (4600 cpm/pmol) or 2.8 pmol of fMet-tRNA<sup>Met</sup> (4100 cpm/pmol); 15 pmol of 70S ribosomes and, where indicated, 1 mM GTP and 49  $\mu$ g of crude IF. Magnesium acetate, Tris-HCl buffer, ammonium chloride, and poly(A,U,G) were in the amounts described in Table I.

subunits are added. In the absence of initiation factors, a very high stimulation is observed so that a level is reached which equals that which was obtained using preformed 70S ribosomes. In the presence of factors, the addition of 50S subunits results in a stimulation of Met-tRNA<sup>Met</sup> binding, but this stimulation is much less than that formed in the absence of initiation factors. However, it is clear that initiation factors do not inhibit further binding when 50S subunits are added, nor do they destabilize the Met-tRNA previously bound, which is very different from that found with 70S ribosomes.

The Met-tRNA<sup>Met</sup> bound under these conditions (at low Mg<sup>2+</sup>) is, at least partly, able to react with puromycin but, as was observed previously (Bretscher and Marcker, 1966), the reaction with the antibiotic is slower than with the formylated species; however, the extent of the reaction is much higher than when preformed 70S ribosomes are used.

#### Discussion

The experiments reported here show that, in contrast to what was believed, formylation of the methionyl-tRNA<sup>Met</sup> does not appear to be absolutely required for the initiation of protein synthesis in the usually accepted mechanism, namely formation of a 30S initiation complex followed by an association of the 50S ribosomal subunit. Under such conditions (binding of messenger RNA and initiator tRNA on the 30S particles), unformylated Met-tRNA<sup>Met</sup> is recognized by initiation factors and is bound as well as fMet-

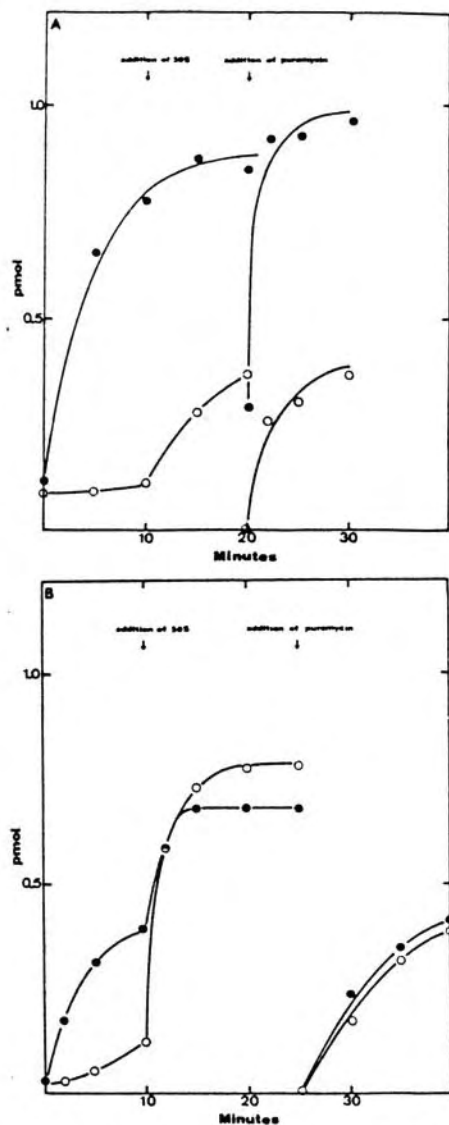


FIGURE 2: (A) Binding of fMet-tRNA<sup>Met</sup> to 30S ribosomal subunits, effect of 50S subunits, and puromycin sensitivity. Incubation mixtures contained, at time 0, 30S ribosomal subunits, buffer, magnesium acetate, ammonium chloride, and fMet-tRNA<sup>Met</sup>, and, where indicated, crude IF as described under Table I plus 1 mM GTP. After 10-min incubation at 37 °C 15 pmol of 50S ribosomal subunits was added. After an additional 10-min incubation 5  $\mu$ l of puromycin (5  $\mu$ g/ $\mu$ l) was added. The amount of fMet-tRNA<sup>Met</sup> bound and reaction with puromycin are analyzed as described under Materials and Methods. (B) Binding of Met-tRNA<sup>Met</sup> to 30S ribosomal subunits, effect of 50S subunits, and puromycin sensitivity: (O) in the absence of initiation factors; (●) in the presence of initiation factors. Same incubation mixtures as in A except Met-tRNA<sup>Met</sup> was used instead of fMet-tRNA<sup>Met</sup> and there was no GTP. Incubation periods are as indicated (the arrows correspond to the time of the various additions).

tRNA<sup>Met</sup> at a site which is reactive toward puromycin. This applies to complex formation directed by poly(A,U,G). Poly(A,U,G) can be considered as an analogue of natural messenger RNA since it not only provides a large number of ApUpG initiator codons but also exhibits the purine-rich sequence located near the initiator codon and which, as suggested by Shine and Dolgarno (1974), can pair with the sequence located at the 3'-OH end of 16S ribosomal RNA. With phage R17 mRNA there was also some stimulation by initiation factors of the binding of nonformylated initiator tRNA, but in this case the binding was much lower. With the triplet AUG the binding of Met-tRNA<sup>Met</sup> was

too small to be interpretable, which is consistent with the results previously reported (Grunberg-Manago et al., 1969). In the formation of the 30S initiation complex, both formylated and nonformylated initiator tRNAs are active; this is found in response to poly(A,U,G), even without initiation factors. Thus, it is the structure of the initiator tRNA that is recognized directly by the ribosome, and not the presence or absence of formylation.

It has been shown previously that the specificity for initiation does not reside in the methionine moiety since Met-tRNA<sup>m</sup>Met is not recognized by initiation factors (whereas it is recognized by elongation factor EF-T<sub>u</sub>, in contrast to Met-tRNA<sup>r</sup>Met), and since mischarged tRNA<sup>r</sup>Met (Val-tRNA<sup>r</sup>Met and fPhe-tRNA<sup>r</sup>Met; Giègé et al., 1973) appear to be as good initiators as fMet-tRNA<sup>r</sup>Met. We observe now that formylation is not absolutely indispensable; this may explain how *E. coli* can grow on trimethoprim, with a low level of formylation, when the one-carbon pool metabolites are present (Danchin, 1973; Harvey, 1973).

What is, therefore, formylation necessary for? A striking difference between the formylated and nonformylated initiator tRNA is observed when one measures their binding in the presence of both ribosomal subunits (i.e., when 70S ribosomes are present during a large portion of the time). Under these conditions, the binding of the unformylated species is strongly inhibited by initiation factors, whereas that of formyl-Met-tRNA<sup>r</sup>Met is stimulated; thus, initiation using 70S ribosomes directly requires absolutely that the charged initiator tRNA be formylated.

This indicates that the formyl group is necessary only when initiation of polypeptide synthesis takes place on whole 70S ribosomes. We have therefore undertaken a study on the effect of formylation of the methionine of initiator tRNA in the presence of 70S ribosomes, which is discussed in the second article of this series (Petersen et al., 1976).

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