

*TRANSLATION OF THE GENETIC MESSAGE, II. EFFECT
OF INITIATION FACTORS ON THE BINDING OF
FORMYL-METHIONYL~TRNA TO RIBOSOMES**

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It has been shown¹ that in a cell-free system of protein synthesis from *Escherichia coli*, containing ribosomes purified by *O*-(diethylaminoethyl) cellulose (DEAE-cellulose) chromatography, two factors (F_1 and F_2) usually removed during the purification of the ribosomes are required for the translation of natural messengers such as MS2 phage RNA. Polypeptide synthesis with some synthetic messengers (e.g., poly A) does not require these factors. However, with synthetic oligonucleotides having an AUG codon as the first or second triplet from the 5'-terminus, the incorporation of methionine and other amino acids into acid-insoluble products, as well as the transfer of methionine from met~tRNA_F² into peptide linkage, is markedly stimulated by the factors. On the other hand, methionine from met~tRNA_M is not transferred into peptide linkage, with oligonucleotide messengers having an initial AUG codon, whether in the absence or presence of factors.

Formyl-met~tRNA_F is involved in initiation of polypeptide synthesis in *E. coli*, with synthetic polynucleotides³ and natural RNA messengers,⁴⁻⁷ a reaction mediated by AUG, GUG, or UUG codons.^{3,8} On the other hand, met~tRNA_M, with AUG as codon, effects the insertion of methionine residues into internal positions of the polypeptide chains.³ These and the above observations led to the conclusion that the factors are concerned with initiation of protein synthesis.¹ Independent observations in other laboratories have suggested⁸ or provided evidence⁹⁻¹¹ for the existence of factors that appear to be involved in chain initiation.

Further work in our laboratory has sought answers to the following questions: (a) the specificity of transfer of formylmethionine or methionine residues, from formyl-met~tRNA_F or met~tRNA_M, respectively, with regard to oligonucleotide messengers having an AUG codon at either the 5'-end (as in AUGA_n) or the 3'-end (as in A_nAUG), the latter as a model for an "internal" methionine codon, and the effect of factors thereon; and (b) the mode of action of the factors.

The results, presented in this paper, show: (a) Transfer of methionine into peptide linkage from formyl-met~tRNA_F occurs with AUGA_n messengers and is factor-dependent. There is no transfer with A_nAUG oligonucleotides whether in the absence or presence of factors. Transfer of methionine from met~tRNA_M occurs with A_nAUG, but not with AUGA_n messengers, and factors are not required. (b) The factors accelerate the binding of formyl-met~tRNA_F to purified ribosomes, in the presence of the trinucleotide ApUpG (AUG), but are without effect on the binding of glycyl~tRNA or lysyl~tRNA, in the presence of the trinucleotides GpGpU (GGU) or ApApA (AAA), respectively. This effect, that was missed in previous work,¹ indicates that F_1 and F_2 affect chain initiation, at least in part, through a specific action on the binding of formyl-met~tRNA_F to ribosomes. The effect of the initiation factors is strongly dependent on the concentration of

Mg⁺⁺ in the reaction mixtures. Thus, factor dependence is marked below 10 mM Mg⁺⁺ but becomes smaller at higher Mg⁺⁺ concentrations.

Materials and Methods.—These were as in previous work^{1, 12} unless otherwise noted.

Ribosomes, supernatants, and factors: Ribosomes from *E. coli* Q13 were purified as in previous work¹² except that before chromatography on DEAE-cellulose they were suspended in a buffer containing 0.25 M NH₄Cl, 0.005 M magnesium acetate, and 0.01 M Tris-HCl, pH 7.8. About 4000 A₂₆₀ units of this ribosomal suspension were applied to a column (2 × 25 cm) of DEAE-cellulose (Serva DEAE-SH cellulose, lot B 3999, 0.83 mEq/gm) which was previously equilibrated with the above buffer. The column was washed with 500 ml of the same buffer and the ribosomes were subsequently eluted with 1.0 M NH₄Cl, 0.01 M magnesium acetate, 0.01 M Tris-HCl, pH 7.8. Supernatant fractions were prepared from *Lactobacillus arabinosus*¹² as well as from *E. coli* Q13. The factors were prepared as previously outlined.¹

Transfer RNA: The two methionine-accepting species of tRNA were obtained from *E. coli* B as previously described.¹ The tRNA_M fraction, which was contaminated with about 5% of tRNA_F, was acylated¹³ with C¹⁴-methionine (specific radioactivity, 190 μc/μmole). For the preparation of formyl-met~tRNA_F, the tRNA_F fraction was acylated with either C¹⁴-methionine (specific radioactivity, 224 μc/μmole) or S³⁵-methionine (specific radioactivity, 175 μc/μmole) in the presence of N¹⁰-formyltetrahydrofolic acid.⁴ The product was shown by electrophoresis¹⁴ to be at least 95% formylated. We are indebted to Dr. J. C. Rabinowitz, University of California, Berkeley, for the formyltetrahydrofolate synthetase. An enzyme fraction obtained by precipitation of *L. arabinosus* supernatant with ammonium sulfate at 75% saturation, dissolving the precipitate in 0.02 M Tris-HCl, pH 7.8, 0.01 M magnesium acetate, and 0.01 M 2-mercaptoethanol, and dialyzing the resulting solution for 16 hr at 4° against the same buffer, served as a source of aminoacyl-tRNA synthetases and of the enzyme³ transferring the formyl group from N¹⁰-tetrahydrofolic acid to met~tRNA_F.

For the preparation of lysine-labeled lys~tRNA, unfractionated tRNA from *E. coli* B was acylated with H³-lysine (specific radioactivity, 480 μc/μmole) and then treated with a 20-fold excess of periodate.¹⁵ This treatment eliminated 90% of the methionine-accepting species. Glycyl~tRNA was prepared, using unfractionated tRNA from *E. coli* W or *E. coli* Q13 supernatant as a source of aminoacyl-tRNA synthetases, with C¹⁴-glycine (specific radioactivity, 116 μc/μmole).

Polynucleotides: Oligonucleotides of the type A_nAUG((Ap)_nApUpG), with the methionine codon AUG at the 3'-end, were prepared as models of messengers having a single "internal" methionine codon. For this purpose (Ap)_nU (ApApAp ApApU) was prepared as previously described¹² and used as primer for the addition of a single guanylic acid residue (from H³-labeled guanosine 5'-diphosphate (pyro)) at the 3'-terminus. The reaction was carried out with polynucleotide phosphorylase, in the presence of ribonuclease T₁, to yield ApApAp ApUpG*p. The resulting polymers were chromatographed on a column of DEAE-cellulose, equilibrated with 0.01 M Tris-HCl, 8.0 M urea, pH 7.8, with an exponential gradient of NaCl.¹² Fractions were dialyzed exhaustively against distilled water and chain lengths estimated from the ratio of H³-radioactivity to A₂₆₀. Removal of the 3'-terminal phosphate with bacterial alkaline phosphatase (Worthington), followed by phenol extraction and dialysis, yielded ApApAp ApUpG*.

The preparation of other oligonucleotides, including AUGA_n, A₃UGA_n, A₄UGA_n, and the trinucleotides ApUpG (AUG) and ApApA (AAA), has been described.^{1, 12, 16, 17} GpGpU (GGU), isolated from pancreatic ribonuclease digests of *E. coli* ribosomal RNA, was provided by Dr. W. M. Stanley, Jr. We are indebted to Dr. Marshall W. Nirenberg, National Institutes of Health, Bethesda, Maryland, for a gift of GpUpG (GUG) and UpUpG (UUG).

Amino acid incorporation: Samples contained the following components in a final volume of 0.14 ml: Tris-HCl buffer, pH 7.8, 60 mM; NH₄Cl, 50 mM; 2-mercaptoethanol, 16 mM; magnesium acetate, either 14 or 18 mM; ATP, 1.2 mM; GTP, 0.3 mM; phosphocreatine, 18 mM; creatine kinase, 9 μg; *E. coli* tRNA, 1.1 mg; purified *E. coli* Q13 ribosomes, 9–12 A₂₆₀ units; *L. arabinosus* supernatant with 0.2 mg of protein; 20 amino acids (one of them with radioactive label), each 0.2 mM; RNA or polynucleotide messenger, 20–30 μg; without or with addition of both factors F₁ and F₂. The specific radioactivity of the labeled amino acids and the amount of each factor used are given in Table 1. After incubation for 40 min at 37° the reaction was terminated by addition of KOH (final concentration, 0.5 M). Mixtures were kept at 37° for 3 hr,

followed by neutralization, and the acid-insoluble radioactivity was determined as previously described.¹

Transfer of methionine from formyl-met~tRNA_F or met~tRNA_M into peptide linkage: The composition and treatment of the samples (final volume, 0.14 ml) was as in the amino acid incorporation experiments except for the omission of uncharged tRNA, the omission of amino acids other than nonlabeled methionine and lysine, and the addition of lys~tRNA labeled with H³-lysine and methionine-labeled formyl-met~tRNA_F or met~tRNA_M in the amounts given in Table 2. The incubation was for 10 or 20 min at 37°.

Binding of aminoacyl~tRNA to ribosomes: Samples contained, in a volume of 0.05 ml, Tris-HCl buffer, pH 7.2, 100 mM; NH₄Cl, 50 mM; magnesium acetate, 5–20 mM; purified ribosomes, 3.0 A₂₆₀ units; labeled aminoacyl~tRNA, 11–16 μmoles; without or with trinucleotide, 0.2 A₂₆₀ units; and without or with factors in the amounts specified in the legends. The course of the binding reaction was followed by incubating samples for various time intervals from 1 to 10 min. The incubation temperature was 16°, 24°, or 25°, depending on the experiment. Binding was determined as described by Nirenberg and Leder,¹⁸ washing in each case with the same buffer in which the incubation had been conducted. The filters were dried, inserted into scintillation vials, and the ribosome-bound radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. The scintillation fluid contained 4 gm of PPO and 50 mg of POPOP per liter of toluene.

Results.—Translation of oligonucleotides with an AUG codon in different positions of the chain: Previously it was shown that the effect of the factors can be correlated with an AUG codon, when present as the initial or the second triplet at the 5'-terminus of synthetic oligonucleotides.¹ In Table 1 (expt. 1) it may be seen that the translation of a polymer with the AUG triplet near the 5'-end but out of phase, as in A₃UGA₂₄, is markedly enhanced by the factors. This is in line with recent results of Thach *et al.*⁸ showing that AUG codes for formylmethionine, whether at the 5'-end or in the immediate vicinity, even when out of phase. In contrast, shifts in reading frame rarely occurred with oligonucleotides having codons other than AUG near the 5'-terminus.¹⁶ This supports the view⁸ that in the vicinity of the 5'-end of a polynucleotide, AUG acts as a phase-selector codon. Clearly, AUG triplets near the 5'-terminus do not behave as internal methionine codons. Oligonucleotides of the type A_nAUG were therefore synthesized and tested. As shown in Table 1 (expt. 2) the factors do not affect the translation of A₂₁AUG.

TABLE 1
POLYPEPTIDE SYNTHESIS WITH AUGA_n, A₃UGA_n, AND A_nAUG OLIGONUCLEOTIDE
MESSENGERS*

Expt. no.	Messenger	Mg ⁺⁺ concentration (mM)	Factor additions	Amino Acid Incorporation†	
				Lysine	Methionine
1	AUGA ₁₃ (20 μg)	14	None	387	37
	A ₃ UGA ₂₄ (25 μg)	14	F ₁ + F ₂	1696	341
2			None	669	19
			F ₁ + F ₂	3047	430
	A ₂₁ AUG (32 μg)	14	None	111	0
			F ₁ + F ₂	67	4
	AUGA ₁₃ (20 μg)	18	None	2145	85
			F ₁ + F ₂	2363	147
	A ₂₁ AUG (32 μg)	18	None	975	60
			F ₁ + F ₂	835	55

* Conditions as described under "Amino acid incorporation." In expt. 1, the amount of ribosomes was 9 A₂₆₀ units/sample. The specific radioactivity of the labeled amino acids (in μμmole) was C¹⁴-lysine, 2; S³⁵-methionine, 30. F₁ and F₂ with 9 and 28 μg of protein, respectively. In expt. 2, the amount of ribosomes was 12 A₂₆₀ units/sample. The specific activity of the labeled amino acid was C¹⁴-lysine, 2; C¹⁴-methionine, 50. F₁ and F₂ with 6 and 21 μg of protein, respectively.

† Net values (blanks without polynucleotide subtracted from values with polynucleotide). All values are averages of duplicate runs. The blanks (virtually the same without or with the additions of factors) averaged 58 and 23 for lysine and methionine, respectively, in expt. 1, and 149 and 71 in expt. 2.

TABLE 2
TRANSFER OF METHIONINE FROM FORMYLMETHIONYL~tRNA_F OR METHIONYL~tRNA_M
INTO PEPTIDE LINKAGE*

Expt. no.	Messenger	Factor additions	Methionine Transferred from†		
			Formyl-met~tRNA _F	Met~tRNA _M	
1	None	None	(0.20)	(0.16)	
		F ₁ + F ₂	(0.33)	(0.25)	
		A ₂₇ AUG (19 μg)	None	0	0.81
2	None	F ₁ + F ₂	0	0.48	
		None	(0.04)	(0.18)	
		F ₁ + F ₂	(0.12)	(0.18)	
		AUGA ₁₃ (20 μg)	None	0.08	0.01
		F ₁ + F ₂	3.94	0.42	
		A ₄ UGA ₂₁ (20 μg)	None	0.08	0.06
		F ₁ + F ₂	3.78	0.39	

* Conditions as described under the same heading in *Materials and Methods*. Samples contained lys~tRNA with H³-labeled lysine, 62 μmoles, and either C¹⁴-methionine-labeled formyl-met~tRNA_F, 15 μmoles, or met~tRNA_M, 15 μmoles. The specific radioactivity of the amino acids used to charge the various tRNA species is given under "Transfer RNA" in *Materials and Methods*. In expt. 1, the samples had 18 mM Mg⁺⁺ and were incubated for 20 min; in expt. 2, they had 14 mM Mg⁺⁺ and were incubated for 10 min. Incubation temperature, 37°. F₁ and F₂ with 7 and 24 μg of protein, respectively.

† All values are averages of duplicate runs and are given in μmoles/sample. The blank values in the samples with no added messenger (given in parentheses) were subtracted from those obtained with added messenger to give the net transfer in the presence of messenger oligonucleotides.

At 14 mM Mg⁺⁺, a concentration at which there is a clear stimulation of methionine incorporation by the factors with AUGA₁₃, the incorporation of lysine and methionine with A₂₇AUG is small and is not affected by the factors. A higher magnesium concentration (18 mM) is required for an efficient reading of this polymer, and no effect of factors is observed. The factor requirement of methionine incorporation directed by AUGA₁₃ is smaller at 18 than at 14 mM Mg⁺⁺, but the effect of the factors is still apparent.

Transfer of methionine from met~tRNA's into peptide linkage: The results of the previous section, showing that, in the presence of A_nAUG oligonucleotides, incorporation of methionine into acid-insoluble products occurs independently of the absence or presence of initiation factors, indicated that polymers with an AUG codon at the 3'-end can be used as models of messengers containing a single, non-initiation ("internal") methionine codon. Experiments were therefore carried out to study the effect of AUGA_n, A₄UGA_n, and A_nAUG oligonucleotides on the transfer into peptide linkage of methionine from either formyl-met~tRNA_F or met~tRNA_M, in the presence of lys~tRNA. The source of lys~tRNA was an unfractionated tRNA preparation, charged with H³-labeled lysine, in which the bulk of the remaining, uncharged tRNA's had been functionally destroyed by periodate oxidation. Since methionine incorporation with oligo A_nAUG is low at 14 mM Mg⁺⁺, experiments with this polymer were done at 18 mM Mg⁺⁺. Experiments with the other two polymers were conducted at 14 mM Mg⁺⁺.

The results, shown in Table 2, may be summarized as follows: (a) With A₂₇AUG messenger, methionine is transferred from met~tRNA_M, but not from formyl-met~tRNA_F, whether in the absence or presence of factors (Table 2, expt. 1). (b) With AUGA₁₃ or A₄UGA₂₁ messenger, there is methionine transfer from formyl-met~tRNA_F and this transfer is factor-dependent (Table 1, expt. 2). The small, factor-dependent transfer observed with met~tRNA_M is to be attributed to the contamination (about 5 per cent) of the tRNA_M fraction with tRNA_F. It may be concluded that with these polymers, there is no transfer from met~tRNA_M whether the factors are present or not. A previously published result, indicating

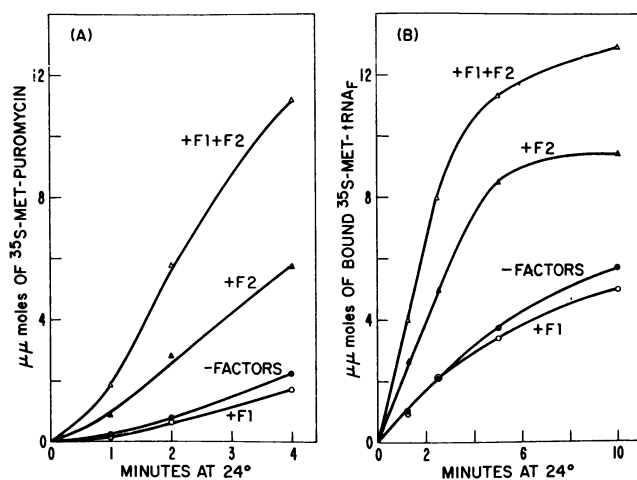


FIG. 1.—Effect of factors on the binding of formyl-met \sim tRNA_F to ribosomes at 10 mM Mg⁺⁺. Conditions as described under "Binding of aminoacyl \sim tRNA to ribosomes." Samples contained formyl-met \sim tRNA_F (with S³⁵-methionine label), 16 μμ-moles, and magnesium acetate, 10 mM. The trinucleotide was ApUpG (AUG). F₁ and F₂ with 3 and 5 μg of protein, respectively. The specific radioactivity of the amino acids used to charge the tRNA's used in the experiments of this and the remaining figures is given under "Transfer RNA" in *Materials and Methods*. (A) Puromycin dihydrochloride followed by ribosomes was added and the mixtures were incubated

for various time intervals at 24°. The formation of formyl-met-puromycin was determined²⁰ after adding 1.0 ml of sodium phosphate, pH 8.0, by extraction with ethylacetate and measurement of the radioactivity of the ethylacetate phase by scintillation counting in Bray's²¹ scintillation mixture. (B) Binding was measured after incubation at 24° for various time intervals. Blanks without trinucleotide (not shown on the figure) were run simultaneously. The 10-min blank binding values were (in μμmoles/sample), 0.28, 0.30, 0.37, and 1.31, with no factors, F₁, F₂, and F₁ + F₂, respectively.

the occurrence of a factor-independent transfer from met \sim tRNA_M in the presence of A₄UGA₁₀ (ref. 1, Table 4, expt. 2), must be ascribed to experimental error.

Effect of factors on the binding of formyl-methionyl-tRNA_F to ribosomes: It was previously suggested¹ that the factors might be involved in the formation of the first peptide bond between formyl-methionyl-tRNA_F and the next amino acid. In order to elucidate this point, the reaction between formyl-met \sim tRNA_F and puromycin¹⁰ was studied. The formation of formyl-methionyl-puromycin is not dependent on factors when, prior to the addition of puromycin, the ribosomes are preincubated (at 10 mM Mg⁺⁺) with the trinucleotide ApUpG (AUG) and formyl-met \sim tRNA_F, according to Leder and Bursztyn.²⁰ However, when the kinetics of formyl-methionyl-puromycin formation is followed under the same conditions, but without preincubation, the results shown in Figure 1A are obtained. The formation of formyl-methionyl-puromycin is slow when no factors are added or with F₁ alone. Addition of F₂ stimulates the reaction and both factors together are still more effective. These observations suggested that some other step in the reaction between formyl-met \sim tRNA_F and puromycin was rate-limiting. When the kinetics of binding of formyl-met \sim tRNA_F to ribosomes in the presence of AUG is studied (Fig. 1B), the effect of the factors is found to be similar to that observed in the reaction with puromycin. These results indicate that the factors are involved in the binding of formyl-met \sim tRNA_F to ribosomes and not in the formation of the peptide bond between formylmethionine and puromycin.

In order to ascertain whether the factors affect specifically the binding of formyl-met \sim tRNA_F or whether they are also involved in the binding of other aminoacyl \sim tRNA's, the binding of glycyl \sim tRNA and lysyl \sim tRNA to ribosomes was studied. A kinetic analysis of the binding of glycyl \sim tRNA to ribosomes in the presence of

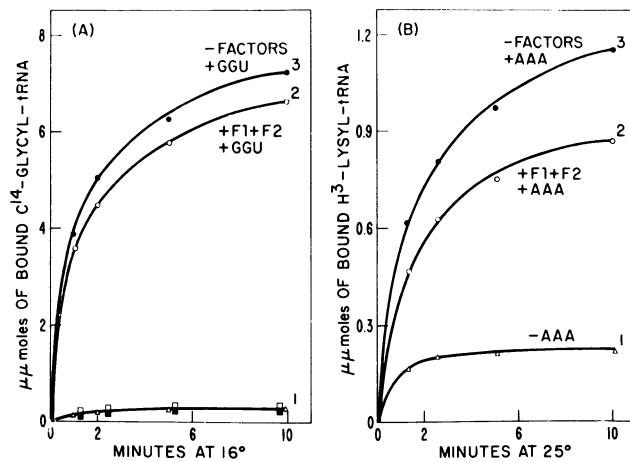


FIG. 2.—Binding of glycyl \sim tRNA or lysyl \sim tRNA to ribosomes. Conditions of Fig. 1B except as noted. (A) Glycyl \sim tRNA (with C¹⁴-glycine label), 14 $\mu\mu$ moles; the trinucleotide was GpGpU (GGU). Incubation at 16°. (B) Lysyl \sim tRNA (with H³-lysine label), 11 $\mu\mu$ moles; the trinucleotide was ApApA (AAA). Incubation at 25°. In both cases, F₁ and F₂ with 3 and 5 μ g of protein, respectively. The Mg⁺⁺ concentration was 10 mM in all but the experiments of curve 1 (A), which were as follows: Δ - Δ - Δ , 5 or 10 mM Mg⁺⁺, no GGU, with or without factors; \square - \square - \square , 5 mM Mg⁺⁺, GGU, no factors; \blacksquare - \blacksquare - \blacksquare , 5 mM Mg⁺⁺, GGU, F₁ + F₂.

the trinucleotide GpGpU (GGU) is presented in Figure 2A. It is evident that at 10 mM Mg⁺⁺, glycyl-tRNA binds very efficiently in the absence of factors and that the factors play no role in this reaction. As further shown in Figure 2A, there was no significant binding of glycyl-tRNA, at 5 mM Mg⁺⁺, whether in the absence or presence of factors. Similar results for the binding of lysyl-tRNA to ribosomes at 10 mM Mg⁺⁺, in the presence of the trinucleotide ApApA (AAA), are shown in Figure 2B.

Complete dependence on factors of the ribosomal binding of formyl-met-tRNA_F mediated by AUG is obtained at 5 mM Mg⁺⁺ as shown in Figure 3. In the absence of both factors, or with F₁ alone, there is practically no binding. Although F₂ alone causes a clear stimulation, a maximal effect is only obtained with F₁ + F₂.

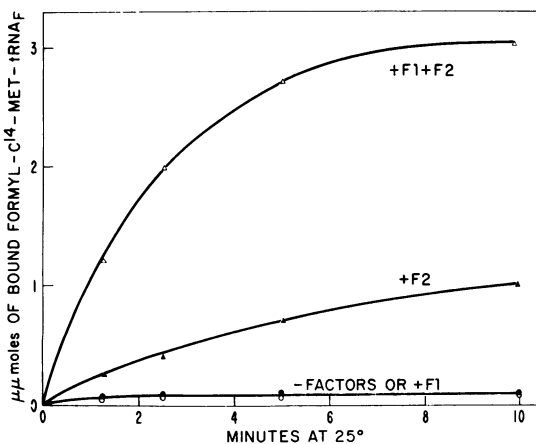


FIG. 3.—Effect of factors on the binding of formyl-met \sim tRNA_F to ribosomes at 5 mM Mg⁺⁺. Conditions of Fig. 1B except as noted. Mg⁺⁺, 5 mM; formyl-met \sim tRNA_F (with C¹⁴-methionine label), 11 $\mu\mu$ moles; the trinucleotide was ApUpG(AUG). F₁ and F₂ with 4 and 5 μ g of protein, respectively. Blanks without trinucleotide (not shown on the figure) were run simultaneously. The 10-min blank binding values were (in $\mu\mu$ moles/sample), 0.05, 0.08, 0.15, and 0.38, with no factors, F₁, F₂, and F₁ + F₂, respectively.

TABLE 3
RIBOSOMAL BINDING OF AMINOACYL tRNA'S*

No.	Mg ⁺⁺	Spermi- dine	Factors	Aminoacyl tRNA Bound (μ moles)					
				Formyl-met~tRNA _F			Glycyl~tRNA		
				No nucleotide	AUG	GUG	UUG	No nucleotide	GGU
1	20	0	None	0.23	4.54	1.52	1.18	—	—
2	5	0	None	0.05	0.09	0.06	0.09	0.07	0.10
3	5	5	None	0.07	2.08	—	—	0.10	1.83
4	5	0	F ₁ + F ₂	0.44	3.10	1.35	0.45	0.07	0.10
5	5	5	F ₁ + F ₂	0.36	3.66	—	—	0.16	1.10

* Conditions as described under "Binding of aminoacyl tRNA to ribosomes." C¹⁴-methionine-labeled formyl-met~tRNA_F, 11 μ moles; C¹⁴-glycine-labeled glycyl~tRNA, 14 μ moles. Incubation, 5 min at 25°. The specific radioactivity of the amino acids used to charge the various tRNA's is given under "Transfer RNA" in *Materials and Methods*.

Clark and Marcker³ first reported that methionine from formyl-met (or met)~tRNA_F is incorporated into amino-terminal positions of polypeptide chains in *E. coli* systems, in the presence of either poly AUG or poly UG. They further observed ribosomal binding of this aminoacyl~tRNA with the following trinucleotides: ApUpG > GpUpG > UpUpG. At high Mg⁺⁺ concentrations (20 mM) we find binding of formyl-met~tRNA_F, without added factors, with each of the trinucleotides mentioned and in the above order of effectiveness (Table 3, line 1). At 5 mM Mg⁺⁺ there is no binding of formyl-met~tRNA_F with any of the above oligonucleotides (AUG, GUG, UUG) or of glycyl~tRNA with GGU, in the absence of factors (Table 3, line 2). Addition of F₁ + F₂ promotes the binding of formyl-met~tRNA_F with AUG and GUG, but not with UUG (Table 3, line 4). Also, as already noted, at 5 mM Mg⁺⁺ the factors have no effect on the binding of glycyl~tRNA with GGU.

Since polyamines reportedly stimulate polypeptide synthesis directed by synthetic polynucleotides,²² the effect of spermidine on the binding of formyl-met~tRNA_F (with AUG) and glycyl~tRNA (with GGU), at 5 mM Mg⁺⁺ and in the absence or presence of factors, was investigated. As shown in Table 3 (lines 2 through 5), 5 mM spermidine stimulates the AUG-dependent binding of formyl-met~tRNA_F and the GGU-dependent binding of glycyl~tRNA, in the absence or presence of factors. However, since F₁ and F₂ promote the binding of formyl-met~tRNA_F, but not that of glycyl~tRNA, the spermidine effect, like that of Mg⁺⁺, is nonspecific and therefore differs from that of the initiation factors.

Discussion.—The results presented in this and in the preceding paper¹ show that two factors recovered from *E. coli* ribosomes are involved in the initiation of translation at least in part through a specific effect on the binding of formyl-met~tRNA_F to ribosomes mediated by the AUG codon. Independent evidence for the occurrence of factors required for the translation of natural messenger RNA's, and thus probably concerned with initiation, has been recently presented by Brawerman and Eisenstadt^{9, 10} and by Revel and Gros.¹¹

Dependence of the binding reaction and polypeptide synthesis on the initiation factors is pronounced at low Mg⁺⁺ concentrations (5–10 mM) and decreases at higher concentrations of this cation. Nevertheless, the factors specifically affect the binding of formyl-met~tRNA_F for, even at low Mg⁺⁺ concentrations, they have no effect on the binding of other aminoacyl~tRNAs, e.g., the GGU-mediated binding of glycyl~tRNA. Spermidine can partially substitute for Mg⁺⁺, but its effect on binding like that of Mg⁺⁺ is nonspecific.

The initiation factors probably facilitate the interaction of met~tRNA_F (whether formylated or not³) with the peptidyl (donor) binding site of the ribosome, for there are indications^{19, 23} that met~tRNA_F binds to this site, whereas met~tRNA_M binds to a different one, probably the amino-acyl (acceptor) site.

As previously shown for peptide synthesis,¹ both F₁ and F₂ are needed for maximal stimulation of binding. The fact that F₂ alone has some effect may be due to contamination of this factor with F₁, to the presence of some F₁ on the purified ribosomes, or both. The actual role of each factor remains to be determined.

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² tRNA_F (formerly tRNA₂) refers to the methionine-tRNA species that can be formylated; tRNA_M (formerly tRNA₁) to the one that does not undergo formylation. Other abbreviations are as in previous work (ref. 1).

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