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

BY MARGARITA SALAS,† MERRILL B. HILLE,‡ JEROLD A. LAST,§ ALBERT J. WAHBA, AND SEVERO OCHOA

DEPARTMENT OF BIOCHEMISTRY, NEW YORK UNIVERSITY SCHOOL OF MEDICINE, NEW YORK

Communicated December 1, 1966

It has been shown[1] 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~$tRNA_F$[2] 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 polynucleotides[3] 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 suggested[8] or provided evidence[9–11] 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 work[12] except that before chromatography on DEAE-cellulose they were suspended in a buffer containing 0.25 *M* NH_4Cl, 0.005 *M* magnesium acetate, and 0.01 *M* Tris-HCl, pH 7.8. About 4000 A_{260} 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* NH_4Cl, 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 acylated[13] with C^{14}-methionine (specific radioactivity, 190 $\mu c/\mu mole$). For the preparation of formyl-met~$tRNA_F$, the $tRNA_F$ fraction was acylated with either C^{14}-methionine (specific radioactivity, 224 $\mu c/\mu mole$) or S^{35}-methionine (specific radioactivity, 175 $\mu c/\mu mole$) in the presence of N^{10}-formyltetrahydrofolic acid.[4] The product was shown by electrophoresis[14] 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 enzyme[3] transferring the formyl group from N^{10}-tetrahydrofolic acid to met~$tRNA_F$.

For the preparation of lysine-labeled lys~tRNA, unfractionated tRNA from *E. coli* B was acylated with H^3-lysine (specific radioactivity, 480 $\mu c/\mu mole$) 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 C^{14}-glycine (specific radioactivity, 116 $\mu c/\mu mole$).

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 described[12] and used as primer for the addition of a single guanylic acid residue (from H^3-labeled guanosine 5′-diphosphate (pyro)) at the 3′-terminus. The reaction was carried out with polynucleotide phosphorylase, in the presence of ribonuclease T_1, 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 H^3-radioactivity to A_{260}. 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 $AUGA_n$, A_3UGA_n, A_4UGA_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; NH_4Cl, 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 A_{260} 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 F_1 and F_2. 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 H^3-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; NH_4Cl, 50 mM; magnesium acetate, 5–20 mM; purified ribosomes, 3.0 A_{260} units; labeled aminoacyl~tRNA, 11–16 $\mu\mu$moles; without or with trinucleotide, 0.2 A_{260} 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 $A_3UGA_{\overline{24}}$, 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 view[8] 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 $A_{\overline{21}}AUG$.

TABLE 1

POLYPEPTIDE SYNTHESIS WITH $AUGA_n$, A_3UGA_n, AND A_nAUG OLIGONUCLEOTIDE MESSENGERS*

Expt. no.	Messenger	Mg^{++} concentration (mM)	Factor additions	Amino Acid Incorporation† Lysine	Methionine
1	$AUGA_{\overline{13}}$ (20 μg)	14	None	387	37
			$F_1 + F_2$	1696	341
	$A_3UGA_{\overline{24}}$ (25 μg)	14	None	669	19
			$F_1 + F_2$	3047	430
2	$A_{\overline{21}}AUG$ (32 μg)	14	None	111	0
			$F_1 + F_2$	67	4
	$AUGA_{\overline{13}}$ (20 μg)	18	None	2145	85
			$F_1 + F_2$	2363	147
	$A_{\overline{21}}AUG$ (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 A_{260} units/sample. The specific radioactivity of the labeled amino acids (in μc/μmole) was C^{14}-lysine, 2; S^{35}-methionine, 30. F_1 and F_2 with 9 and 26 μg of protein, respectively. In expt. 2, the amount of ribosomes was 12 A_{260} units/sample. The specific activity of the labeled amino acid was C^{14}-lysine, 2; C^{14}-methionine, 50. F_1 and F_2 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)
	$A_{\overline{21}}AUG$ (19 μg)	None	0	0.81
		$F_1 + F_2$	0	0.48
2	None	None	(0.04)	(0.18)
		$F_1 + F_2$	(0.12)	(0.18)
	$AUGA_{\overline{13}}$ (20 μg)	None	0.08	0.01
		$F_1 + F_2$	3.94	0.42
	$A_4UGA_{\overline{21}}$ (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 H^3-labeled lysine, 62 $\mu\mu$moles, and either C^{14}-methionine-labeled formyl-met~tRNA$_F$, 15 $\mu\mu$moles, or met~tRNA$_M$, 15 $\mu\mu$moles. 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°. F_1 and F_2 with 7 and 24 μg of protein, respectively.

† All values are averages of duplicate runs and are given in $\mu\mu$moles/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 $AUGA_{\overline{13}}$, the incorporation of lysine and methionine with $A_{\overline{21}}AUG$ 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 $AUGA_{\overline{13}}$ 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, noninitiation ("internal") methionine codon. Experiments were therefore carried out to study the effect of $AUGA_n$, A_4UGA_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 H^3-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 $A_{\overline{21}}AUG$ 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 $AUGA_{\overline{13}}$ or $A_4UGA_{\overline{21}}$ 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

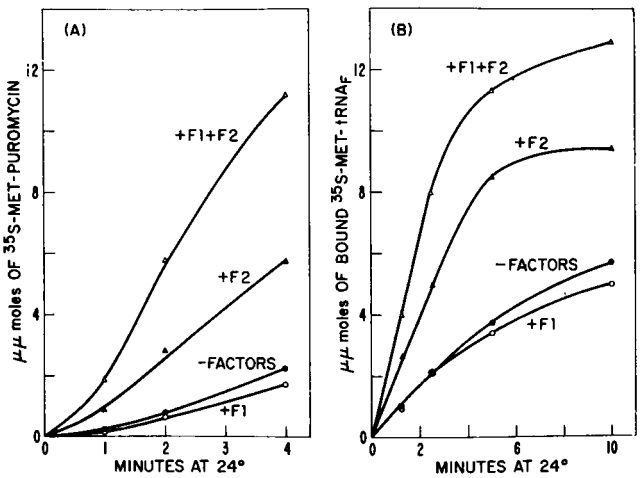

FIG. 1.—Effect of factors on the binding of formyl-met ~ $tRNA_F$ to ribosomes at 10 mM Mg^{++}. Conditions as described under "Binding of aminoacyl ~ tRNA to ribosomes." Samples contained formyl-met ~ $tRNA_F$ (with S^{35}-methionine label), 16 μμ-moles, and magnesium acetate, 10 mM. The trinucleotide was ApUpG (AUG). F_1 and F_2 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-pyromycin was determined[20] 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's[21] 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, F_1, F_2, and $F_1 + F_2$, respectively.

the occurrence of a factor-independent transfer from met~$tRNA_M$ in the presence of $A_4UGA_{\bar{10}}$ (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 suggested[1] 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~$tRNA_F$ and puromycin[10] 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~$tRNA_F$, 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 1*A* are obtained. The formation of formyl-methionyl-puromycin is slow when no factors are added or with F_1 alone. Addition of F_2 stimulates the reaction and both factors together are still more effective. These observations suggested that some other step in the reaction between formyl-met~$tRNA_F$ and puromycin was rate-limiting. When the kinetics of binding of formyl-met~$tRNA_F$ to ribosomes in the presence of AUG is studied (Fig. 1*B*), 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~$tRNA_F$ 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~$tRNA_F$ or whether they are also involved in the binding of other aminoacyl ~tRNA's, the binding of glycyl~tRNA and lysyl~tRNA to ribosomes was studied. A kinetic analysis of the binding of glycyl~tRNA to ribosomes in the presence of

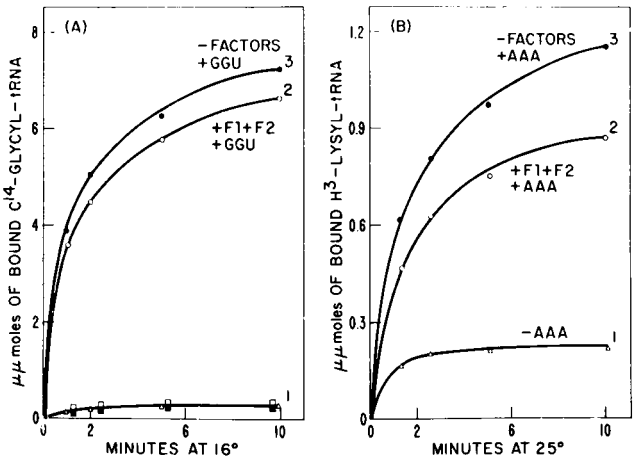

FIG. 2.—Binding of glycyl ~ tRNA or lysyl ~ tRNA to ribosomes. Conditions of Fig. 1*B* except as noted. (*A*) Glycyl ~ tRNA (with C^{14}-glycine label), 14 μμmoles; the trinucleotide was GpGpU (GGU). Incubation at 16°. (*B*) Lysyl ~ tRNA (with H^3-lysine label), 11 μμmoles; the trinucleotide was ApApA (AAA). Incubation at 25°. In both cases, F_1 and F_2 with 3 and 5 μg of protein, respectively. The Mg^{++} concentration was 10 mM in all but the experiments of curve 1 (*A*), which were as follows: -△-△-△-, 5 or 10 mM Mg^{++}, no GGU, with or without factors; -□-□-□-, 5 mM Mg^{++}, GGU, no factors; -■-■-■-, 5 mM Mg^{++}, GGU, $F_1 + F_2$.

the trinucleotide GpGpU (GGU) is presented in Figure 2*A*. 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 2*A*, 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 2*B*.

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 F_1 alone, there is practically no binding. Although F_2 alone causes a clear stimulation, a maximal effect is only obtained with $F_1 + F_2$.

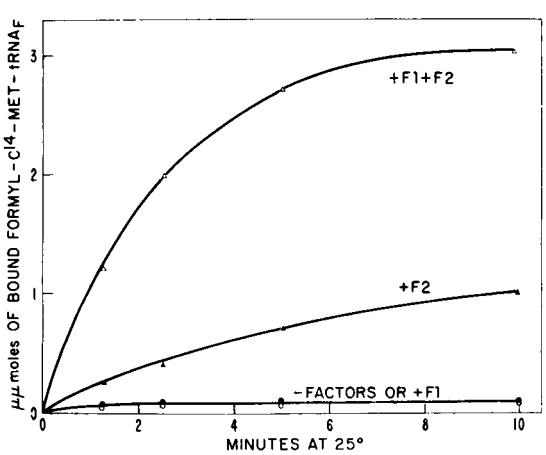

FIG. 3.—Effect of factors on the binding of formyl-met ~ $tRNA_F$ to ribosomes at 5 mM Mg^{++}. Conditions of Fig. 1*B* except as noted. Mg^{++}, 5 mM; formyl-met ~ $tRNA_F$ (with C^{14}-methionine label), 11 μμmoles; the trinucleotide was ApUpG(AUG). F_1 and F_2 with 4 and 5 μg of protein, respectively. Incubation at 25°. Blanks without trinucleotide (not shown on the figure) were run simultaneously. The 10-min blank binding values were (in μμmoles/sample), 0.05, 0.08, 0.15, and 0.38, with no factors, F_1, F_2, and $F_1 + F_2$, respectively.

TABLE 3

RIBOSOMAL BINDING OF AMINOACYL tRNA'S*

No.	Mg^{++}	Spermidine	Factors	Aminoacyl tRNA Bound ($\mu\mu$ moles) — Formyl-met~$tRNA_F$: No nucleotide	AUG	GUG	UUG	Glycyl~tRNA: 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." C^{14}-methionine-labeled formyl-met~$tRNA_F$, 11 $\mu\mu$moles; C^{14}-glycine-labeled glycyl~tRNA, 14 $\mu\mu$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 Marcker[3] 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 $F_1 + F_2$ 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 F_1 and F_2 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 paper[1] 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 not[3]) 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 F_1 and F_2 are needed for maximal stimulation of binding. The fact that F_2 alone has some effect may be due to contamination of this factor with F_1, to the presence of some F_1 on the purified ribosomes, or both. The actual role of each factor remains to be determined.

We are indebted to Miss Martha J. Miller for her assistance in the preparation of ribosomal and supernatant fractions, to Mr. Horace Lozina for growth of bacterial cells, and to Miss Maria Piney for technical assistance in some of this work.

* Aided by grants AM-01845, AM-08953, and FR-05399 from the National Institutes of Health, U.S. Public Health Service, the Jane Coffin Childs Fund for Medical Research, and E. I. du Pont de Nemours and Company, Inc.

† International postdoctoral fellow of the Jane Coffin Childs Fund for Medical Research. Permanent address: Instituto Marañón, Centro de Investigaciones Biológicas, C.S.I.C., Madrid, Spain.

‡ Postdoctoral fellow of the National Institutes of Health, U.S. Public Health Service.

§ Postdoctoral fellow of the American Cancer Society.

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[2] $tRNA_F$ (formerly $tRNA_2$) refers to the methionine-tRNA species that can be formylated; $tRNA_M$ (formerly $tRNA_1$) to the one that does not undergo formylation. Other abbreviations are as in previous work (ref. 1).

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