*TRANSLATION OF THE GENETIC MESSAGE, VI. THE ROLE OF RIBOSOMAL SUBUNITS IN BINDING OF FORMYLMETHIONYL~TRNA AND ITS REACTION WITH PUROMYCIN**

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It was previously shown[1, 2] that the chain initiation factors F_1 and F_2, derived from *E. coli* ribosomes, are involved in the binding of formylmet~$tRNA_F$ to the ribosomes. These experiments had been carried out with purified, unfractionated ribosomes containing both 30*S* and 50*S* subunits. Further investigation has shown that formylmet~$tRNA_F$ binds only to the 30*S* ribosomal subunits. However, both the 30*S* and 50*S* subunits are required for the synthesis of formylmet-puromycin. As with unfractionated ribosomes both messenger and initiation factors are needed for maximal binding by the 30*S* subunits. We have used the trinucleoside diphosphate ApUpG (AUG) as messenger. We have confirmed recent reports[3–6] that the initiation binding reaction is guanosine 5′-triphosphate-dependent (GTP-dependent). While this work was in progress, we learned that Nomura and Lowry[7] also found that 30*S* ribosomal subunits bind formylmet~$tRNA_F$ in the presence of natural (f2 phage RNA) and synthetic messengers.

Materials and Methods.—These were as in previous work[1, 2] unless otherwise stated.

Initiation factors: Initiation factors were purified from *E. coli* Q13 ribosomes as previously described.[2]

Ribosomes and ribosomal subunits: Ribosomes from *E. coli* Q13 were purified by *O*-(diethylaminoethyl) cellulose (DEAE-cellulose) chromatography,[1] and were stored at 4° in a buffer containing 0.5 *M* NH_4Cl, 0.002 *M* magnesium acetate, and 0.02 *M* Tris-HCl, pH 8.1 (buffer A). These ribosomes (DEAE-ribosomes) were shown by sucrose density gradient centrifugation to consist of 30*S* and 50*S* subunits.

Fractionation of ribosomes into subunits was carried out with linear 5–20% sucrose gradients (28 ml) in buffer A. Gradients were equilibrated for 5 hr at 4°, loaded with 0.5 ml of sample containing 180 A_{260} units of DEAE-ribosomes, and run at 23,000 rpm in the SW25.1 Spinco rotor for 10 hr at 5°. To obtain 50*S* subunits, contaminated with less than 15% of 30*S* particles, only fractions comprising the faster sedimenting 10% of the 50*S* peak were pooled.[8] The slower moving fractions of the 30*S* peak (66%) were pooled, resulting in less than 6% contamination with 50*S* particles. The 50*S* and 30*S* fractions from each gradient were dialyzed against buffer A for 6 hr at 4°, then concentrated by centrifugation in the Spinco 40 rotor at 38,000 rpm for 15 hr at 5°. Pellets were suspended in buffer A and stored at 4°. Each subunit preparation was further characterized by sucrose density gradient centrifugation.

An aliquot of 60 A_{260} units of DEAE-ribosomes was suspended in 10 ml 20% sucrose in buffer A for 10 hr at 4°, then dialyzed and concentrated as above. These ribosomes were used as controls and are referred to as unfractionated. Their activity is approximately one fifth that of untreated DEAE-ribosomes. All binding assays and puromycin reactions were completed within 24 hr of the subunit isolation.

Transfer RNA: E. coli W tRNA was purchased from Schwarz BioResearch, Inc. Formylmethionine~tRNA was prepared by acylation of *E. coli* W tRNA with C^{14}-methionine in the presence of N^{10}-formyltetrahydrofolic acid and *L. arabinosus* supernatant,[1] and was passed through a Sephadex G-25 column to remove contaminating nucleotides.[9]

Binding of aminoacyl~tRNA to ribosomes and ribosomal subunits: Samples contained in a volume of 0.05 ml, Tris-HCl buffer, pH 7.2, 50 mM; NH_4Cl, 130 mM (Table 1) or 160 mM

(Fig. 1); magnesium acetate, 5–20 mM; dithiothreitol (DTT), 10 mM; ribosomes or ribosomal subunits, as specified in the legends; labeled formylmet~tRNA, as specified in the legends; with or without the trinucleoside diphosphate ApUpG (AUG), 0.18 A_{260} units; with or without F_1 (4 μg) and F_2 (8 μg); with or without GTP, 0.2 mM. Incubations were for either 2 or 5 min at 25°. Binding was determined as described by Nirenberg and Leder.[10] The Millipore filters were dried, inserted into scintillation vials, and the ribosome-bound radioactivity was measured in a Tri-Carb liquid scintillation spectrometer. The scintillation fluid contained 4 gm of 2,5-diphenyloxazole (PPO) and 50 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (POPOP) per liter of toluene.

Puromycin reaction: Samples contained in a volume of 0.05 ml the same components as in the binding assay except for the concentrations of NH_4Cl, 130 mM, and magnesium acetate, 5 mM. Puromycin dihydrochloride, 1 mM, was added last, and the mixtures were incubated for 5 min at 25°. The formation of formylmet-puromycin was determined[11] after adding 1.0 ml of sodium phosphate, pH 8.0, and 1.5 ml of ethylacetate. The mixtures were stirred vigorously with a Vortex mixer, and the radioactivity of the ethylacetate phase was measured by scintillation counting in Bray's solution.[12]

Results and Discussion.—Table 1 (expt. 1) shows that formylmet~tRNA binds predominantly to the 30*S* subunits. As expected, the binding was dependent on the presence of AUG, GTP, and initiation factors. Binding to the 50*S* subunit can be accounted for by the 15 per cent contamination with 30*S* subunits. Addition of 50*S* to the 30*S* particles appeared to give slightly increased binding in one experiment but did not do so in another. Probably there is little, if any, additional effect of the 50*S* subunits in this binding. Study of the synthesis of formylmet-puromycin (Table 1, expt. 2) showed that both the 30*S* and 50*S* ribosomal subunits are required. This reaction exhibited a pronounced dependence on AUG, GTP, and factors. The lower and more variable dependence of the binding reaction on these components probably reflects the occurrence of some nonspecific binding.[6] Moreover, peptide bond synthesis has an additional requirement for GTP.[5] The puromycin reaction is linear up to 15 minutes, while the binding of formylmet~tRNA is complete within 2 minutes.

The dependence of the binding of formylmet~tRNA to purified, unfractionated

TABLE 1

ROLE OF RIBOSOMAL SUBUNITS IN THE BINDING OF FORMYLMET~TRNA AND FORMYLMET-PUROMYCIN SYNTHESIS*

Expt. no.	Additions: AUG	GTP	Factors	Formylmet~tRNA Bound to Ribosomal Particles†: 30S	50S	30S + 50S	Unfractionated ribosomes
1	−	+	+	0.42	0.14	0.44	0.32
	+	−	+	0.94	0.22	0.61	0.48
	+	+	−	0.36 (0.24)	0.05 (0.58)	0.33 (0.36)	0.19
	+	+	+	1.80 (1.35)	0.48 (0.76)	2.60 (1.37)	1.80
	Additions: AUG	GTP	Factors	Formylmet-Puromycin Synthesis with Ribisomal Particles‡: 30S	50S	30S + 50S	Unfractionated ribosomes
2	−	+	+	0.17	0	0.75	0.51
	+	−	+	0.03	0	0.15	0
	+	+	−	0	0	0	0.15
	+	+	+	0.69 (0.70)	1.10 (1.20)	12.0 (6.05)	8.90

* Conditions as described under *Binding of aminoacyl~tRNA to ribosomes and ribosomal subunits* and *Puromycin reaction*. Ribosome additions of 30*S*, 50*S*, and unfractionated ribosomes were 0.42, 0.78, and 0.99 A_{260} units, respectively. C^{14}-methionine-labeled formylmet~tRNA (specific radioactivity, 199 μc/μmole); expt. 1, 32 $\mu\mu$moles; expt. 2, 64 $\mu\mu$moles. All samples contained 5 mM magnesium acetate and were incubated for 5 min at 25°. All values are the average of duplicate runs. The values given in parentheses are results of another experiment carried out with a different preparation of ribosomal subunits.

† Net values in $\mu\mu$moles/sample. The blanks (samples without ribosomes) averaged 0.02.

‡ Net values (blanks without puromycin, subtracted from values with puromycin) in $\mu\mu$moles/sample. The blanks (essentially the same without or with the addition of AUG, GTP, or ribosomes) were 0.62 and 0.23 in the presence and absence of factors, respectively.

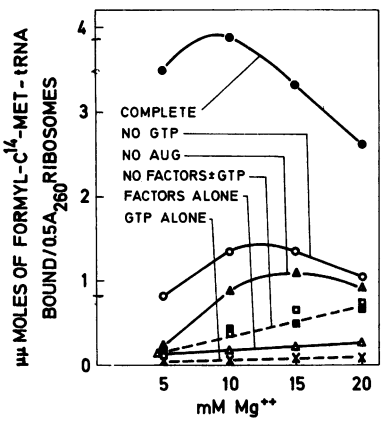

FIG. 1.—GTP and factor dependence of formylmet ~ tRNA binding to ribosomes as a function of the Mg^{++} concentration. Conditions as described under *Binding of aminoacyl ~ tRNA to ribosomes and ribosomal subunits.* All samples contained 18 μμmoles of C^{14}-methionine-labeled formylmet ~ tRNA (specific radioactivity, 187 μc/μmole) and 0.5 A_{260} units of DEAE-ribosomes. Incubation was for 2 min at 25°.

ribosomes on AUG, GTP, and initiation factors, at different Mg^{++} concentrations, is shown in Figure 1. Good GTP and factor dependence was observed throughout the 5–20 mM Mg^{++} concentration range, although, as already pointed out in the case of the initiation factors,[1] it becomes less pronounced as the Mg^{++} concentration increases.

The requirement for both ribosomal subunits in the synthesis of formylmet-puromycin, i.e., in peptide bond synthesis, is in line with Monro's[13] results suggesting that the 50*S* ribosomal particle contains peptidyl transferase and with the finding by Nomura and Lowry[7] that both the 30*S* and 50*S* ribosomal subunits are required for binding of aminoacyl~tRNA's other than formylmet~tRNA. Our results, together with those of Nomura and Lowry,[7] indicate the existence of a specific chain initiation site on the 30*S* subunit.

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