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Conformational Control of the Interaction of Eukaryotic Elongation Factors EF-1 and EF-2 with Ribosomes

(polypeptide chain elongation/eukaryotic ribosomes/*Artemia salina*)

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ABSTRACT **As in the case with prokaryotic systems, *Artemia salina* elongation factors EF-1 and EF-2 interact with a common site or with closely overlapping sites on the *Artemia* ribosome. This feature of ribosomal design must restrict interaction with the ribosome to only one of the factors at alternating steps of chain elongation. In support of this view we find that EF-1, but not EF-2, interacts with the post-translocation ribosome, whereas the reverse is true of the pre-translocation ribosome. Conformational changes probably account for the alternating selectivity of the translating ribosome for each elongation factor.**

The polypeptide chain elongation factors EF-T and EF-G interact with a common site or with closely overlapping sites on the prokaryotic ribosome (2–5). Using *Artemia salina* ribosomes and elongation factors EF-1 and EF-2, we found this to hold also for eukaryotic systems. Similar results were reported for a brain system (6) while our work was in progress.

Binding to a common ribosomal site demands that each factor be excluded from interaction with the ribosome at alternating steps of chain elongation. EF-G (EF-2), but not EF-T (EF-1), should interact with the pre-translocation ribosome while the reverse should be true for the post-translocation ribosome. In this paper we show with eukaryotic ribosomes and elongation factors that whereas both EF-1 and EF-2 interact in a mutually exclusive fashion with the free ribosomes (Fig. 1, *stage 1*), the 80S initiation complex ribosome (Fig. 1, *stage 2*), or the post-translocation ribosome (Fig. 1, *stage 4*), interacts with EF-1 but not with EF-2. On the other hand, the pre-translocation ribosome (Fig. 1, *stage 3*) interacts with EF-2, but it interacts poorly, if at all, with EF-1. In this issue Modolell *et al.* (7) present evidence for similar behavior of prokaryotic ribosomes and elongation factors.

MATERIALS AND METHODS

Ribosome and Ribosomal Subunits. 80S ribosomes and 60S and 40S ribosomal subunits were prepared from *A. salina* embryos as described by Zasloff and Ochoa (8). The 80S ribosomes were washed with 0.5 M KCl.

Factors. Factors were prepared from the pH 5 supernatant of *A. salina* embryos. The supernatant initiation factor EIF-1, prepared as described by Zasloff and Ochoa (9), through the carboxymethylcellulose chromatography step, was kindly provided by Dr. Ralph P. McCroskey of this department. The elongation factor EF-1 was purified to near homogeneity by Sephadex G-200 gel filtration, phosphocellulose chromatography, and repeated gel filtration. The bulk of this preparation consisted of material of molecular weight >200,000 (10, 11). EF-2 was purified to homogeneity by DEAE-cellulose chromatography, phosphocellulose chromatography, and Sephadex G-200 filtration. Its molecular weight of about 90,000, as determined by sodium dodecyl sulfate-gel electrophoresis, is in the range for EF-2 from other eukaryotic sources (12). The EF-1 preparation was free from EF-2 activity and conversely.

Incubations. Unless otherwise stated all incubations were done at all stages in a buffer (buffer A) containing 80 mM Tris·HCl (pH 7.4) (24°), 120 mM KCl, 7 mM $Mg(OAc)_2$, and 2 mM dithiothreitol. For assay of ribosomal binding of Phe-tRNA, samples received 25 μl of a mixture, previously incubated for 5 min at 24°, containing, in buffer A, 0.72 mM GTP, 15–17 pmol of [^{14}C]Phe-tRNA or [^{3}H]Phe-tRNA, and 7–12.5 μg of EF-1. After incubation for 5 min at 24° the bound radioactivity was determined by the Millipore filter assay.

Ribosomal Complexes. These complexes were prepared on the basis of earlier work (13), with *Artemia* ribosomes or ribosomal subunits, using poly(U) as messenger, AcPhe-tRNA for chain initiation, and [^{14}C]Phe-tRNA for chain elongation. The complexes used are shown schematically in Fig. 1.

Stage 1 ribosomes are obtained when 80S ribosomes are incubated in the presence of poly(U). Conditions are described in the legend to Table 1.

Stage 2 ribosomes were prepared as follows: A reaction mixture (0.6 ml) containing 40S ribosomal subunits (2.1 A_{260} units), poly(U) (15 A_{260} units), AcPhe-tRNA (136 pmol), and EIF-1 (33 μg), was incubated for 20 min at 24°, followed by cooling and the addition of 60S subunits (4.8 A_{260} units).

Stage 3 ribosomes were prepared by supplementing the reaction mixture containing stage 2 ribosomes with 0.4 ml of a solution containing [^{14}C]Phe-tRNA (225 pmol), EF-1 (75 μg), and enough GTP to give a final concentration of 0.18 mM, and incubating this reaction mixture for 30 min at 24°. An identical sample, except for the omission of [^{14}C]Phe-tRNA, served as a stage 2 ribosome control. The samples (each 1.0 ml) were layered on 1 ml of buffer A containing 5% sucrose, in a 2-ml centrifuge tube, and centrifuged at 4° for 120 min at 42,000 rpm in the Ti 50 rotor of the Spinco type L3-50

Abbreviations: Designation of the elongation factors of prokaryotic origin as EF-T (EF-Tu and EF-Ts), EF-G and of the respective factors of eukaryotic cytoplasmic origin as EF-1, EF-2 conforms to currently accepted nomenclature (1). AcPhe-tRNA, *N*-acetylPhe-tRNA; $Mg(OAc)_2$, magnesium acetate; $GMPP(CH_2)P$, 5′-guanylylmethylenediphosphonate.

preparative ultracentrifuge. The pellets were resuspended in a suitable volume of Buffer A.

For Preparation of Stage 4 ribosomes an aliquot of the stage 3 preparation (3.5 A_{260} units) was incubated, in a final volume of 0.46 ml, with EF-2 (33 μg), and enough GTP to give a concentration of 0.23 mM, for 15 min at 24°. This results in translocation of stage 3 to stage 4 ribosomes. The mixture was layered over 1.5 ml of buffer A, containing 5% sucrose, and the ribosomal complex was pelleted and resuspended in buffer A as above.

Stage 2(a) ribosomes were prepared as follows: The reaction mixture (0.7 ml) contained, in buffer A, 80S ribosomes (8 A_{260} units), GTP (0.36 mM), EF-1 (85 μg), and [^{14}C]Phe-tRNA (221 pmol). An identical sample, but without [^{14}C]-Phe-tRNA, served as stage 1 ribosome control. After incubation for 30 min at 24°, the ribosomal complex was pelleted and resuspended in buffer A as above. To the suspension (0.7 ml) was added GTP (0.25 mM) and EF-2 (33 μg), and the mixtures were incubated for 15 min at 24°. This results in translocation of [^{14}C]Phe-tRNA from the A to the P site (see Table 4 of ref. 13). The stage 2(a) ribosomal complex was pelleted and resuspended in buffer A as before.

Stage 2(b) ribosomes were prepared in two ways. In procedure A, stage 2 ribosomes were treated with puromycin. This releases AcPhe-puromycin and, as shown by Modolell

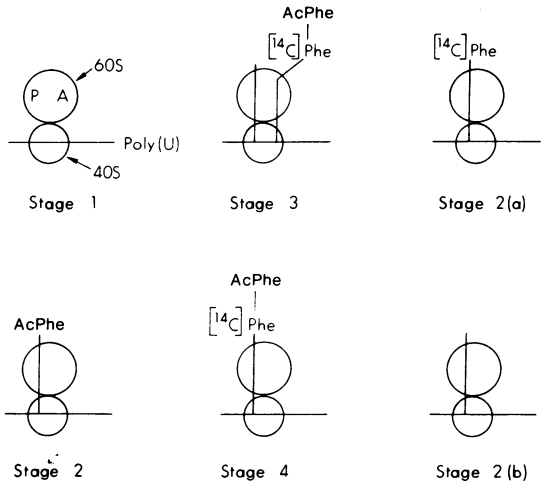

FIG. 1. Diagrammatic representation of the ribosomal complexes used to study interaction with the chain elongation factors EF-1 and EF-2. *Stage 1*, 80S ribosome with bound poly(U). *Stage 2*, 80S initiation complex (80S·poly(U)·AcPhe-tRNA); the ribosome bears nonlabeled AcPhe-tRNA on the P site. *P* is the peptidyl (donor) site; *A*, aminoacyl (acceptor) site. *Stage 3*, first chain elongation step (pre-translocation state); the ribosome bears AcPhe-[^{14}C]Phe-tRNA on the A site and deacylated tRNA^{Phe} on the P site. *Stage 4*, post-translocation state; the ribosome bears AcPhe-[^{14}C]Phe-tRNA on the P site. *Stage 2(a)*; the ribosome bears [^{14}C]Phe-tRNA on the P site. *Stage 2(b)*; the ribosome bears deacylated tRNA^{Phe} on the P site. For the experiments of Table 3 the stage 2 ribosomes carried Ac[^{14}C]Phe-tRNA, rather than nonlabeled AcPhe-tRNA. The stage 3 ribosomes prepared from them, using [^{3}H]Phe-tRNA, carried Ac[^{14}C]Phe-[^{3}H]Phe-tRNA on the A site and deacylated tRNA^{Phe} on the P site. The corresponding stage 4 ribosomes carried Ac[^{14}C]Phe-[^{3}H]Phe-tRNA on the P site.

TABLE 1. *Inhibition by EF-2 and GMPP(CH$_2$)P of the EF-1- and GTP-dependent binding of Phe-tRNA by stage 1 ribosomes*

Additions to first incubation					[^{14}C]-Phe-tRNA bound (pmol)	Inhibition by EF-2 (%)
Ribosomes	EF-2 (μg)	GMP-P(CH$_2$)P (mM)	GTP (mM)	GDP (mM)		
			Experiment 1			
80S	—	0.28	—	—	3.1	
80S	1.1	0.28	—	—	2.1	32
80S	3.3	0.28	—	—	0.7	77
80S	5.5	0.28	—	—	0.7	77
80S	—	—	0.28	—	3.3	
80S	1.1	—	0.28	—	5.8	0
80S	—	—	—	0.28	3.0	
80S	5.5	—	—	0.28	4.5	0
			Experiment 2			
40S + 60S	—	0.28	—	—	3.0	
40S + 60S	3.3	0.28	—	—	0.9	70
40S	—	0.28	—	—	3.0	
40S	3.3	0.28	—	—	3.0	0
60S	—	0.28	—	—	2.7	
60S	3.3	0.28	—	—	2.2	19

The incubations were conducted in two stages. Samples (35 μl) containing 0.6 A_{260} unit of 80S ribosomes (when present); ribosomal subunits (when present) 40S, 0.15 A_{260} unit, 60S, 0.32 A_{260} unit; poly(U), 1 A_{260} unit; and EF-2, GMPP(CH$_2$)P, GTP, or GDP, as indicated, were incubated for 5 min at 24°. The missing ribosomal subunit (if any) was added and the ribosomal binding of Phe-tRNA measured as described under "*Incubations*" in *Materials and Methods*.

et al. for *Escherichia coli* ribosomes (7), leaves deacylated tRNA^{Phe} on the P site. In procedure B, highly purified *E. coli* tRNA^{Phe} (Phe charging ratio, 1250 pmol/A_{260} unit) was bound nonenzymatically to the P site on 80S ribosomes as described by Zasloff (14). This RNA was a gift of Dr. R. W. Chambers of this department. For procedure A, a reaction mixture (0.4 ml) containing, in buffer A, 40S ribosomal subunits (1.5 A_{260} units), poly(U) (10 A_{260} units), AcPhe-tRNA (90 pmol), and EIF-1 (22 μg), was incubated for 20 min at 25° followed by cooling and addition of 60S subunits (3.2 A_{260} units) and 50 μl of puromycin (15 mg/ml). An identical sample, but with 50 μl of water instead of puromycin, served as a stage 2 ribosome control. The samples were incubated for 15 min at 24° and the ribosomal complexes pelleted and resuspended in buffer A as before. For procedure B, a reaction mixture (25 μl) containing, in buffer A (but with 10 mM rather than 7 mM Mg(OAc)$_2$), 80S ribosomes (0.6 A_{260} unit), poly(U) (1 A_{260} unit), and tRNA^{Phe} (20 pmol), was incubated for 10 min at 24° and cooled. This reaction mixture was immediately used as stage 2(b) ribosomes. An identical sample, but without tRNA^{Phe}, served as stage 1 ribosome control.

Evidence for the formation of stage 2 and 3 ribosomes was given previously (13). Proof for formation of stage 4 ribosomes was obtained as follows: Stage 3 ribosomes were prepared with Ac[^{14}C]Phe-tRNA and [^{3}H]Phe-tRNA and repelleted. They carried 3.7 pmol of AcPhe and 4.9 pmol of Phe per A_{260} unit. Upon incubation (20 min, 24°) of these ribosomes with EF-1, [^{3}H]Phe-tRNA, and GTP there was no change, but incubation with EF-1, EF-2, [^{3}H]Phe-tRNA, and GTP resulted in

TABLE 2. *No inhibition by EF-2 and GMPP(CH$_2$)P of the EF-1- and GTP-dependent binding of Phe-tRNA by stage 2 ribosomes*

Additions to first incubation	Ribosomes at stage	[^{14}C]Phe-tRNA bound (pmol) EF-2 absent	EF-2 present	Inhibition by EF-2 (%)
EIF-1	1	3.5	1.0	68
AcPhe-tRNA	1	3.6	1.1	65
EIF-1, AcPhe-tRNA	2	3.1	3.1	0

The incubations were conducted in three stages: (*a*) Samples (40 μl) containing 0.15 A_{260} unit of 40S ribosomal subunits, 1 A_{260} unit of poly(U), 2.2 μg of EIF-1 (when present), and 10.5 pmol of AcPhe-tRNA (when present) were incubated for 5 min at 24°. (*b*) The samples were supplemented with 0.48 A_{260} unit of 60S ribosomal subunits, 3.3 μg of EF-2 (when present), and 0.16 mM GMPP(CH$_2$)P (total volume now 55 μl), and incubated for 5 min at 24°. (*c*) For assay of Phe-tRNA binding the samples were then processed as described under "*Incubations*" in *Materials and Methods*.

chain elongation The acid-insoluble product contained 3.4 pmol of Ac[^{14}C]Phe and 60 pmol of [^{3}H]Phe. The ribosomes prepared from an aliquot of the stage 3 ribosomes by translocation (incubation with GTP and EF-2) followed by repelleting, contained 4.0 and 4.7 pmol, respectively, of Ac[^{14}C]-Phe and [^{3}H]Phe per A_{260} unit; consistent with stage 4 ribosomes.

Other Preparations. Nonlabeled Phe-tRNA, AcPhe-tRNA, [^{14}C]Phe-tRNA, Ac[^{14}C]Phe-tRNA, and [^{3}H]Phe-tRNA were prepared by amino-acylation of crude *E. coli* W tRNA (Schwarz BioResearch) as described (8). The charging ratio of the tRNA was 31–38 pmol of Phe per A_{260} unit. The specific radioactivities (cpm/pmol) were: [^{14}C]Phe-tRNA, 410 (all experiments except those described in Table 5) and 800 (Table 5); Ac[^{14}C]Phe-tRNA, 800; [^{3}H]Phe-tRNA, 2450. The preparation of Ac[^{14}C]Phe-tRNA according to Haenni and Chapeville (15), has been described (13). Poly(U) was from the Miles Laboratories and [γ-^{32}P]GTP from ICN, Irvine, Calif. For use, the labeled GTP was diluted with unlabeled GTP to a specific radioactivity of 638 cpm/pmol. The source of other materials was as in previous work (8, 9, 13).

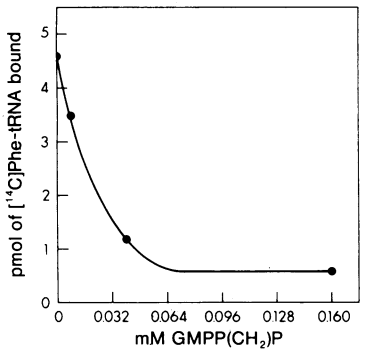

FIG. 2. Inhibition by EF-2 and GMPP(CH$_2$)P of the EF-1- and GTP-dependent binding of [^{14}C]Phe-tRNA by stage 1 ribosomes as a function of the GMPP(CH$_2$)P concentration. Conditions as in Table 1 with 60S + 40S ribosomal subunits, 3.3 μg of EF-2, and GMPP(CH$_2$)P as indicated.

Assays. Ribosomal binding of Ac[^{14}C]Phe-tRNA, [^{14}C]Phe-tRNA, or [^{3}H]Phe-tRNA was determined by the Millipore filter procedure (16). Filters were dried under an infrared lamp and the retained radioactivity was measured in Omnifluor (New England Nuclear Corp.) in a Packard Tri-Carb liquid scintillation spectrometer. ^{14}C and ^{3}H were counted at 90% and 35% efficiency, respectively. Hydrolysis of [γ-^{32}P]GTP was determined by counting an aliquot of a trichloroacetic acid supernatant after removing unreacted triphosphate by adsorption on charcoal (17). Protein was determined by the Lowry procedure (18) with bovine-serum albumin as standard.

RESULTS

Stage 1 Ribosomes. Phe-tRNA binds readily to stage 1 ribosomes at 7 mM Mg^{2+} and, as shown previously (13), the

TABLE 3. *No inhibition by EF-2 and GMPP(CH$_2$)P of the EF-1 dependent binding of Phe-tRNA to stage 2 or stage 4 ribosomes*

	Aminoacyl-tRNA bound to ribosomes (pmol/A_{260} unit)					
	Present before incubation		Present after incubation			Inhibition by EF-2 (%)
Fusidic acid (pmol)	Ac-[^{14}C]-Phe-tRNA	[^{3}H]-Phe-tRNA	Ac-[^{14}C]-Phe-tRNA	[^{3}H]Phe-tRNA EF-2 absent	[^{3}H]Phe-tRNA EF-2 present	
			Ribosome Stage 2			
0	5.5	0	4.5	2.9	4.7	0
100	5.5	0	4.5	2.9	2.7	7
			Ribosome Stage 4			
0	4.3	4.0	3.7	7.6(3.6)	8.7(4.7)	0
100	4.3	4.0	3.6	7.4(3.4)	7.0(3.0)	12

Stage 2 and 3 ribosomes were prepared as described in *Materials and Methods* except that [^{14}C]Phe-labeled AcPhe-tRNA and [^{3}H]Phe-labeled Phe-tRNA were used and that stage 4 ribosomes were treated with *N*-ethylmaleimide to inactivate adsorbed EF-2. A mixture (0.2 ml) of stage 3 ribosomes (5 A_{260} units), 33 μg of EF-2, and 0.28 mM GTP in buffer A was incubated for 15 min at 24° to promote translocation to stage 4. *N*-Ethylmaleimide in buffer A was added to 20 mM concentration and, after further incubation for 25 min at 24°, unreacted *N*-ethylmaleimide was neutralized with dithiothreitol (40 mM). After repelleting, 2.3 A_{260} units of the above ribosomes (final volume, 40 μl in buffer A) were reincubated with *N*-ethylmaleimide (25 mM) as before and unreacted *N*-ethylmaleimide was neutralized with dithiothreitol. The resulting (stage 4) ribosomal suspension was used for the experiments given in this table. When these ribosomes are supplemented with EF-1, GTP, and Phe-tRNA there is no chain elongation beyond the tripeptide stage unless EF-2 is also added. The experimental samples (30 μl) containing 0.5 A_{260} unit of poly(U), 0.34 mM GMPP(CH$_2$)P, 0.35 A_{260} unit of stage 2 ribosomes or 0.4 A_{260} unit of stage 4 ribosomes, and, when present, 3.3 μg of EF-2, were incubated for 5 min at 24°. They were then processed for assay of [^{3}H]Phe-tRNA binding as described under "*Incubations*" in *Materials and Methods*, in the absence or presence of fusidic acid as indicated. Values in parentheses give the amount of extra [^{3}H]Phe-tRNA bound during this assay. The simultaneous counting efficiency was 50% for ^{14}C and 25% for ^{3}H.

reaction is GTP- and EF-1-dependent. Prior binding of EF-2 by formation of the stable EF-2·ribosome·GMPP(CH$_2$)P complex strongly inhibits Phe-tRNA binding (Table 1). GTP or GDP cannot replace GMPP(CH$_2$)P (Exp. 1) because no stable EF-2·ribosome complex is formed if fusidic acid is not present. When fusidic acid and GTP were substituted for GMPP(CH$_2$)P (not shown) there was also inhibition. Maximal inhibition (80% or greater) was obtained with about 0.06 mM GMPP(CH$_2$)P (Fig. 2) and 3 μg of EF-2 per A_{260} unit of 80S ribosome per 35 μl.

When the preincubation with EF-2 and GMPP(CH$_2$)P is carried out with 40S or 60S subunits, and the 80S ribosomes are formed by adding the missing subunit just prior to assaying for Phe-tRNA binding (Exp. 2), there is little or no inhibition by EF-2*. These results show: (*a*) that in *Artemia*, as in brain (6) or *E. coli* (2–5), the elongation factors EF-1 (EF-Tu) and EF-2 (EF-G) compete for a common ribosomal site or interact with closely overlapping sites, and (*b*) that such site(s) is (are) formed by association of the two ribosomal subunits.

Stage 2 and 4 Ribosomes. Phe-tRNA is also readily bound by stage 2 ribosomes. However, as shown in Tables 2 and 3, preincubation with EF-2·GMPP(CH$_2$)P fails to inhibit this binding. Controls (Table 2) show that if stage 2 ribosomes are not formed, e.g., by omitting AcPhe-tRNA or the initiation factor EIF-1 from the incubation with 40S subunits (8), there is good inhibition. Stage 4 ribosomes (Table 3) also bind Phe-tRNA easily and, here again, this binding is insensitive to EF-2·GMPP(CH$_2$)P. Note that stage 4 ribosomes bind roughly one extra equivalent of Phe-tRNA in the presence of fusidic acid. Without fusidic acid, which inhibits translocation, there is some more elongation. These results suggest that the initiating or post-translocation ribosome, bearing an aminoacyl or peptidyl group, respectively, on the P site, does not interact with EF-2. Otherwise EF-2·GMPP(CH$_2$)P would inhibit Phe-tRNA binding.

Stage 3 Ribosomes. The stage 3 ribosome, in the pre-translocation state, interacts readily with EF-2 as shown by translocation to stage 4 upon addition of EF-2 and GTP. This ribosome cannot bind additional Phe-tRNA, as both the A and P sites are occupied, and has a sharply reduced reactivity with EF-1. This is best shown (Table 4) by comparing the EF-1- and Phe-tRNA-dependent (coupled) GTPase activity (19) of stage 2 and stage 3 ribosomes. Under the conditions used (4 mM Mg^{2+}), stage 3 ribosomes show close to 80% reduction of EF-1-dependent, coupled GTPase activity as compared to stage 2 control ribosomes.

Stage 2(a) and 2(b) Ribosomes. Since the stage 2 ribosome bears an *N*-blocked aminoacyl-tRNA on the P site, the question arises whether its EF-2 reactivity is affected or not by the blocking group. This question is important, for the aminoacyl residue of the eukaryotic initiator, Met-tRNA$_f$, is thought not to be acylated (for review, see ref. 20). Table 5 (Exps. *1* and *1a*) shows that the EF-1-dependent binding of Phe-tRNA to stage 2(a) ribosomes is much less sensitive to EF-2·GMPP(CH$_2$)P than the binding to stage 1 control ribosomes. Thus, the EF-2 reactivity of ribosomes bearing aminoacyl-tRNA on the P site is low regardless of whether the α-amino group is blocked or not.

Modolell *et al.* (7) showed that puromycin releases AcPhe as AcPhe-puromycin from *E. coli* stage 2 ribosomes but leaves the deacylated tRNA^{Phe} on the P site [Fig. 1, stage 2(b)]. Moreover, the EF-Tu-GTP-dependent binding of Phe-tRNA is now inhibited by preincubation with EF-G·GTP, in the presence of fusidic acid, indicating that EF-G interacts with these ribosomes (7). We have confirmed these results with *Artemia* stage 2(b) ribosomes prepared by two methods: (*a*) puromycin treatment of stage 2 ribosomes (Table 5, Exp. *2*); (*b*) nonenzymatic binding of tRNA^{Phe} to the P site of stage 1 ribosomes (Table 5, Exp. *3*). In both cases stage 2(b) ribosomes behave like stage 1, but unlike stage 2, ribosomes in being sensitive to preincubation with EF-2·GMPP(CH$_2$)P.

TABLE 4. *Decreased interaction of EF-1 with stage 3 ribosomes*

Ribosome stage	[γ-^{32}P]GTP hydrolysis (pmol of $^{32}P_i$)				
	Ribosomes	EF-1	Ribosomes + EF-1 + Phe-tRNA	Coupled hydrolysis	Reactivity with EF-1 (%)
2 (control)	9.6	7.4	28.8	11.8	100
3	11.2	7.4	21.2	2.6	22

Interaction of ribosomes with EF-1 was determined indirectly as the EF-1- and Phe-tRNA-dependent (coupled) hydrolysis of [γ-^{32}P]GTP. The samples contained, in a volume of 50 μl, 80 mM Tris·HCl buffer (pH 7.4), 120 mM KCl, 4 mM Mg(OAc)$_2$, 2 mM dithiothreitol, 0.57 A_{260} unit of stage 2 (control) or stage 3 ribosomes, 18 pmol of nonlabeled Phe-tRNA, 7.5 μg of EF-1, and 10 μM [γ-^{32}P]GTP (320 × 10^3 cpm). After incubation for 10 min at 24°, the reaction was stopped by addition of 1 ml of a solution containing 0.3 mM KH_2PO_4, 65 mM potassium acetate, 2% trichloroacetic acid, and 40 mg of acid-washed activated charcoal. The samples were centrifuged and 0.5 ml of the supernatant was taken for counting of $^{32}P_i$.

DISCUSSION

The present work discloses the existence of a fine mechanism of control of ribosomal reactivity with the chain elongation factors during growth of the polypeptide chain. The occurrence of such a mechanism was likely since both elongation factors interact with a common site or with closely overlapping sites on the ribosomal surface. Thus, whereas free ribosomes can interact with either elongation factors EF-T (EF-1) or EF-G (EF-2) in prokaryotes and eukaryotes (2–6, this paper), the interaction with elongation factors of *Artemia* ribosomes bearing aminoacyl- or peptidyl-tRNA on the P site (initiation or post-translocation) is restricted to EF-1, whereas that of ribosomes bearing peptidyl-tRNA on the A site and deacylated tRNA on the P site (pre-translocation) is largely restricted to EF-2. Similar observations have been made (7) with *E. coli* ribosomes and factors. The feasibility of preparing radioactively labeled *Artemia* factors for closer study of these interactions is being explored. The observed changes in ribosome reactivity are consistent with, but not mandatory

* The small inhibition observed on preincubation with 60S subunits may be largely due to the small contamination (<5%, ref. 8) with 40S subunits.

TABLE 5. *Elongation factor interaction with stage 2(a) and 2(b) ribosomes*

Ribosomes at stage	Fusidic acid (pmol)	[^{14}C]Phe-tRNA (or [^{3}H]-Phe-tRNA) bound by ribosomes (pmol)			Inhibition by EF-2 (%)
		Present before incubation	Bound after incubation		
			EF-2 absent	EF-2 present	
Experiment 1					
1	0	0	3.6	1.3	64
1	100	0	2.2	0.6	73
2(a)	0	4.3	5.2	5.5	0
2(a)	100	4.3	5.1	3.8	26
Experiment 1a					
2(a)	0	3.2	2.2	2.4	0
2(a)	100	3.2	1.9	1.6	16
Experiment 2					
2	0	0	4.3	4.7	0
2	100	0	3.9	3.6	8
2(b)	0	0	3.4	2.1	38
2(b)	100	0	3.7	1.8	51
Experiment 3					
1	0	0	3.2	1.2	63
2(b)	0	0	4.2	1.8	57

Stage 2(a) and 2(b) ribosomes (Fig. 1), with appropriate controls, were used to determine the effect of prior incubation with EF-2 and GMPP(CH$_2$)P on the EF-1- and GTP-dependent binding of Phe-tRNA. The incubations were conducted in two stages. All samples (30 μl) contained 1 A_{260} unit of poly(U), 0.285 mM GMPP(CH$_2$)P, and, when present, 3.3 μg of EF-2. In addition, in experiment *1*, the samples contained stage 2(a) ribosomes, 0.53 A_{260} unit, or stage 1 control ribosomes, 0.61 A_{260} unit. In experiment *1a* the composition of the samples was as in experiment *1;* the stage 2(a) ribosomes carried [^{14}C]Phe-tRNA but the EF-1- and GTP-dependent binding of Phe-tRNA was done with [^{3}H]Phe-tRNA. Before pelleting these ribosomes were treated with *N*-ethylmaleimide as described for stage 4 ribosomes in the legend to Table 3. In experiment *2*, the samples contained stage 2(b) ribosomes, prepared by procedure A (see *Materials and Methods*), 0.58 A_{260} unit, or stage 2 control ribosomes, 0.55 A_{260} unit. In experiment *3*, the samples contained stage 2(b) ribosomes, prepared by procedure B (*Materials and Methods*), 0.6 A_{260} unit, or the same amount of stage 1 control ribosomes. After incubation for 5 min at 24°, some samples received fusidic acid, as indicated, and all were processed for Phe-tRNA binding as described under "*Incubations*" in *Materials and Methods.*

for, the known catalytic activity of the elongation factors†, which must involve their repeated uptake and ejection by the functioning ribosome.

The alternating interaction of the ribosome with each elongation factor is probably due to conformation changes brought about by the alternative presence of peptidyl-tRNA on the ribosomal A or P site. The subtle nature of the mechanisms involved is emphasized by the fact (ref. 7 and this paper) that whereas occupancy of the P site by aminoacyl-tRNA inhibits ribosomal interaction with EF-2, occupancy of the same site by deacylated tRNA has little or no effect on this interaction.

† The turnover number of freshly prepared *A. salina* EF-2 in our standard assay (poly(U) translation with [^{14}C]Phe-tRNA as substrate), assuming a molecular weight of 90,000, is 290 (mol of Phe incorporated per mol of EF-2 per min) at 24°.

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1. Caskey, T., Leder, P., Moldave, K. & Schlessinger, D. (1972) "Translation: Its mechanism and control," *Science* **176**, 195–197.
2. Richman, N. & Bodley, J. W. (1972) "Ribosomes cannot interact simultaneously with elongation factors EF Tu and EF G," *Proc. Nat. Acad. Sci. USA* **69**, 686–689.
3. Cabrer, B., Vázquez, D. & Modolell, J. (1972) "Inhibition by elongation factor EF G of aminoacyl-tRNA binding to ribosomes," *Proc. Nat. Acad. Sci. USA* **69**, 733–736.
4. Miller, D. L. (1972) "Elongation factors EF Tu and EFG interact at related sites on ribosomes," *Proc. Nat. Acad. Sci. USA* **69**, 752–755.
5. Richter, D. (1972) "Inability of *E. coli* ribosomes to interact simultaneously with bacterial elongation factors EFTu and EFG," *Biochem. Biophys. Res. Commun.* **46**, 1850–1856.
6. Richter, D. (1973) "Competition between the elongation factors 1 and 2 and phenylalanyl transfer ribonucleic acid for the ribosomal binding sites in a polypeptide synthesizing system from brain," *J. Biol. Chem.* **248**, 2853–2857.
7. Modolell, J., Cabrer, B. & Vázquez, D. (1973) "The interaction of elongation factor G with *N*-acetylphenylalanyl transfer ribonucleic acid-ribosome complexes," *Proc. Nat. Acad. Sci. USA* **70**, in press.
8. Zasloff, M. & Ochoa, S. (1971) "A supernatant factor involved in initiation complex formation with eukaryotic ribosomes," *Proc. Nat. Acad. Sci. USA* **68**, 3059–3063.
9. Zasloff, M. & Ochoa, S. (1973) "Polypeptide chain initiation in eukaryotes IV. Purification and properties of supernatant initiation factor from *Artemia salina* embryos," *J. Mol. Biol.* **73**, 65–76.
10. McKeehan, W. L. & Hardesty, B. (1969) "Purification and partial characterization of the aminoacyl transfer ribonucleic acid binding enzyme from rabbit reticulocytes," *J. Biol. Chem.* **244**, 4330–4339.
11. Weissbach, H., Redfield, B. & Moon, H.-M. (1973) "Further studies on the interaction of elongation factor 1 from animal tissues," *Arch. Biochem. Biophys.* **156**, 267–275.
12. Collins, J. F., Raeburn, S. & Maxwell, E. S. (1971) "Aminoacyltransferase II from rat liver II. Some physical and chemical properties of the purified enzyme and its adenosinediphosphate ribose derivative," *J. Biol. Chem.* **246**, 1049–1054.
13. McCroskey, R. P., Zasloff, M. & Ochoa, S. (1972) "Polypeptide chain initiation and stepwise elongation with *Artemia* ribosomes and factors," *Proc. Nat. Acad. Sci. USA* **69**, 2451–2455.
14. Zasloff, M. (1973) "Non-enzymic binding of formylmethionyl-transfer RNA to *Artemia salina* ribosomes," *J. Mol. Biol.* **76**, 445–453.
15. Haenni, A. L. & Chapeville, F. (1966) "The behaviour of acetylphenylalanyl soluble ribonucleic acid in polyphenylalanine synthesis," *Biochim. Biophys. Acta* **114**, 135–148.
16. Nirenberg, M. & Leder, P. (1964) "RNA codewords and protein synthesis: The effect of trinucleotides upon the binding of sRNA to ribosomes," *Science* **145**, 1399–1407.
17. Richter, D. & Klink, F. (1971) "Isolation of peptide chain elongation factors from yeast," *Methods in Enzymology, 20C*, 349–359.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) "Protein measurement with the Folin phenol reagent," *J. Biol. Chem.* **193**, 265–275.
19. Lin, S-Y., McKeehan, W. L., Culp, W. & Hardesty, B. (1969) "Partial characterization of the enzymatic properties of the aminoacyl transfer ribonucleic acid binding enzyme," *J. Biol. Chem.* **244**, 4340–4350.
20. Lucas-Lenard, J. & Lipmann, F. (1971) "Protein biosynthesis," *Annu. Rev. Biochem.* **40**, 409–448.