FORMATION OF A TERNARY COMPLEX BETWEEN YEAST AMINOACYL-tRNA BINDING FACTOR, GTP, AND AMINOACYL-tRNA

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SUMMARY: A supernatant factor from yeast that, with GTP, causes binding of aa-tRNA* to ribosomes, forms a ternary complex with GTP and Phe-tRNA which is excluded by Sephadex G-200 and passes through the Millipore filter. The complex of GTP-binding-factor-Phe-tRNA is bound much more rapidly to ribosomes than the separately added components.

It had been shown (1, 2) that the crude soluble fractions, as well as isolated aa-tRNA binding factor and peptidyl-translocase (2, 3) of yeast and liver were interchangeable using liver or yeast ribosomes. Furthermore, yeast ribosomes could be partially complemented by the E. coli soluble fraction, although the reverse reaction did not occur (1). Recently it has been shown (4), using E. coli factors and ribosomes from reticulocytes or tumor cells, that the E. coli aa-tRNA binding factor, T (5), is responsible for this partial complementarity; the reticulocyte binding factor was inactive on E. coli ribosomes. Extending the parallels between eukaryotic and prokaryotic binding factors, it will be shown here that both form a ternary complex with GTP and aa-tRNA.

MATERIALS AND METHODS

The yeast Saccharomyces fragilis was obtained from American Type Culture Collection. ^{3}H-GTP (specific activity 6.05 C/mmole), ^{3}H-GDP (specific activity 1.02 C/mmole), and ^{14}C-phenylalanine (specific activity 325 mC/mmole) were supplied by New England Nuclear Corp. tRNA from E. coli was charged with

* Abbreviations used: aa-tRNA, aminoacyl-tRNA; Phe-tRNA, phenylalanyl-tRNA; GMP-PCP, 5'-guanylyl-methylene diphosphate; DTT, dithiothreitol; PEG, polyethylene glycol; poly U, polyuridylic acid.

^{14}C-Phe by the method of Conway (6) and yielded preparations with 350 μμmoles of Phe/mg of tRNA. N-acetyl-Phe-tRNA and N-acetyl-Val-tRNA were prepared as described (7).

Yeast cells (700 g), harvested in mid-log phase (1), were disrupted in a Manton-Gaulin mill at 9,000 psi; about 70% of the cells were broken. The cells were extracted twice, each time with 500 ml of Buffer 1 (20 mM Tris-HCl, pH 7.4, 10 mM $Mg(CH_3COO)_2$, 1 mM DTT), and were centrifuged first at 15,000 x g for 45 min and then at 105,000 x g for 1 hr. For the isolation of the elongation factors and ribosomes, a slightly modified method of Gordon was used (8). To 100 ml of cell extract were added, with stirring, 30 ml of 30% (w/w) PEG, 10 ml of 20% (w/w) Dextran, and 12 g of KCl. $Mg(CH_3COO)_2$ (10 mM) was present in both the PEG and the Dextran solutions. The phases were separated by centrifugation. With this method, the supernatant factors moved into the PEG phase, and ribosomes and RNA were exclusively found in the Dextran phase. The supernatant factors were isolated from the PEG phase as described (8). The ammonium sulfate fraction obtained at 40-70% saturation was reprecipitated twice at pH 6.6. About 30 mg of this protein solution was passed through a Sephadex G-200 column (2.5 x 90 cm) as described (2, 3). The aa-tRNA binding factor was obtained near the void volume (mol. wt. 220,000), whereas the peptidyl-translocase eluted later (mol. wt. 70,000) (3). Fractions containing the factors were pooled, concentrated with $(NH_4)_2SO_4$ (45-70% saturation), and stored frozen in liquid nitrogen.

After 1:1 dilution with Buffer 1, the ribosomes were isolated from the Dextran phase by centrifugation at 105,000 x g for 4 hr. They were further purified by centrifugation through a sucrose density gradient (9), and were finally dissolved in 20 mM Tris-HCl buffer, pH 7.4, with 10 mM $Mg(CH_3COO)_2$ containing 5 mg/ml protein; they were stored frozen in liquid nitrogen.

Assay Methods.- The Millipore filter technique (10) was used to study the binding of Phe-tRNA to ribosomes and the complex formation of the binding factor. The incubation mixtures were the same as described (8), except that 100 mM NH_4Cl and 60 mM KCl were used and DTT was omitted. Incubation was carried

out as specified in RESULTS. The Millipore filters and buffers used in these assays were pretreated as described (10).

Table 1. Ribosomal Binding of Phe-tRNA with Factors from Yeast and Bacterial Sources.

Binding Factors	Binding of ^{14}C-Phe-tRNA to ribosomes	
	yeast	E. coli
	μμmoles	μμmoles
Yeast	6.5	0.3
E. coli	7.8	8.9
Ps. fluorescens	5.7	6.5

The binding of Phe-tRNA to ribosomes was carried out as described in METHODS; 20 μg of the yeast binding factor, 5 μg of the E. coli, and 5 μg of Ps. fluorescens factor were used. The assays with E. coli ribosomes were performed with 50 μg of ribosomal protein; those with yeast ribosomes contained 75 μg of ribosomal protein. Ribosomes and supernatant factors from E. coli (8) and from Ps. fluorescens (5) were gifts from Dr. J. Gordon and Dr. J. Lucas-Lenard, respectively.

RESULTS AND DISCUSSION

Table 1 surveys the binding of Phe-tRNA to yeast and bacterial ribosomes with binding factors of different origin. The yeast factor reacts with yeast ribosomes only, but the factors from Ps. fluorescens and E. coli catalyze the binding of Phe-tRNA to both bacterial and yeast ribosomes. These results are similar to those of Krisko et al.(4) with ascites tumor and reticulocyte ribosomes. In order to extend the parallels between eukaryotes and heterokaryotes further, we examined in detail the partial reactions of the binding factor from yeast.

To study the interaction of yeast aa-tRNA binding factor with GTP and Phe-tRNA, a reaction mixture containing Phe-tRNA, GTP, and the yeast factor was passed through a Sephadex G-200 column. Figure 1 shows that the fraction eluting from the column shortly after the void volume carrying equal amounts of ^{3}H-GTP and ^{14}C-Phe-tRNA bound to the factor. Thus, the yeast binding factor forms a ternary complex just as had been found previously in bacterial systems.

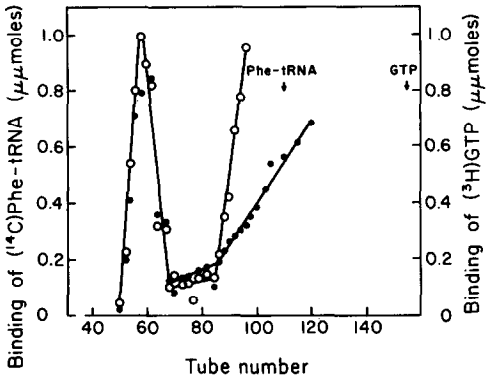

Fig. 1. Gel filtration of the ^{14}C-Phe-tRNA-^{3}H-GTP-enzyme complex on Sephadex G-200. The reaction mixture contained in 300 μl: 20 mM Tris-HCl, pH 7.4; 100 mM NH_4Cl; 60 mM KCl; 10 mM $Mg(CH_3COO)_2$; 300 $\mu\mu$moles of ^{14}C-Phe-tRNA; 800 $\mu\mu$moles of ^{3}H-GTP; and 0.8 mg of the yeast binding factor. Protein was estimated by the method of Warburg and Christian (11). The mixture was incubated at 0° for 5 min, and then was passed through a Sephadex G-200 column (1.2 x 23 cm) equilibrated with Buffer 1. Three drops were collected in each tube and assayed for ^{3}H- and ^{14}C-radioactivity in a liquid scintillation spectrometer. o——o, ^{14}C-Phe-tRNA; ●——●, ^{3}H-GTP. Very little or no ^{3}H-GTP was detectable near the void volume if Phe-tRNA was omitted from the reaction mixture. The arrows indicate the position of the unbound Phe-tRNA and GTP.

Table 2. Interaction of the Preformed Enzyme-^{3}H-GTP-^{14}C-Phe-tRNA Complex with Yeast Ribosomes.

Incubation time	Addition of Complex			
	preformed		not preformed	
	Radioactivity bound to ribosomes			
	^{14}C-Phe-tRNA	^{3}H-GTP	^{14}C-Phe-tRNA	^{3}H-GTP
	$\mu\mu$moles	$\mu\mu$moles	$\mu\mu$moles	$\mu\mu$moles
0	1.2	0.5	0.2	0.4
2	2.5	0.7	0.8	0.6
5	2.9	0.7	1.5	0.6
10	3.2	0.8	2.1	0.7

The complex was preformed as described in Fig. 1. The reaction mixture was passed through a Sephadex G-50 column (1.2 x 44 cm), equilibrated with Buffer 1. The fraction volume was 0.6 ml. The complex appearing in the void volume of the run was immediately used for the following assay: 1 ml contained 20 mM Tris-HCl, pH 7.4, 100 mM NH_4Cl, 60 mM KCl, 10 mM $Mg(CH_3COO)_2$, 100 μg of poly U, 400 μg of ribosomal protein, and 600 μl of the complex containing 3.8 $\mu\mu$moles of ^{14}C-Phe-tRNA and 4.1 $\mu\mu$moles of ^{3}H-GTP. In a second experiment, the complex was not preformed; to the same reaction volume, saturating amounts of ^{14}C-Phe-tRNA (100 μg), ^{3}H-GTP (200 $\mu\mu$moles), and the binding factor (100 μg) were added. The reaction mixtures were incubated at 0° for the times indicated, and then passed through Millipore filters.

The ternary complex was examined for its capacity to transfer the Phe-tRNA to ribosomes. As shown in Table 2, the rate of Phe-tRNA binding to ribosomes was considerably faster from the preformed complex as compared with the separate addition of equivalent amounts of the individual components.

In the bacterial system, the ternary complex was found to pass through Millipore filters, whereas the complex between binding factor and GTP or GDP was retained (12, 13). Furthermore, the bacterial binding factor preferentially bound GDP rather than GTP in the absence of aa-tRNA (10). A comparison of the binding factors from yeast and E. coli shows that in both cases GTP binding was lowered in the presence of GDP (Fig. 2). Addition of GMP-PCP to the reaction mixture similarly inhibited the binding of ^{3}H-GTP to the filter, but other nucleoside phosphates had no effect. As shown in Fig. 3, the yeast binding factor had a higher affinity for GDP than for GTP, but the preference for GDP was not as strong as in the E. coli system. Only with GTP, however, was there a

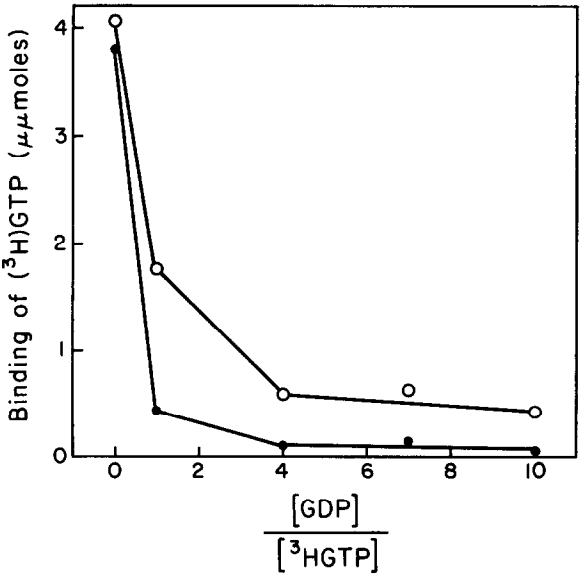

Fig. 2. Binding of ^{3}H-GTP by the binding factors from yeast and E. coli. ^{3}H-GTP binding to the factors was carried out in the presence of varying amounts of unlabeled GDP and 150 μg of the binding factor from yeast, o——o, or with 20 μg of the E. coli T factor, ●——●. The incubation was performed at 0° for 1 min, and the complex formed was isolated on Millipore filters.

release of GTP from Millipore filters when Phe-tRNA was present. Uncharged tRNA did not decrease the retention of the ^{3}H-GTP protein complex, nor did N-acetyl-Phe-tRNA or N-acetyl-Val-tRNA.

The results reported here indicate that the function of the binding factor from both sources is similar, and that from this point of view, both factors are equivalent and quite comparable.

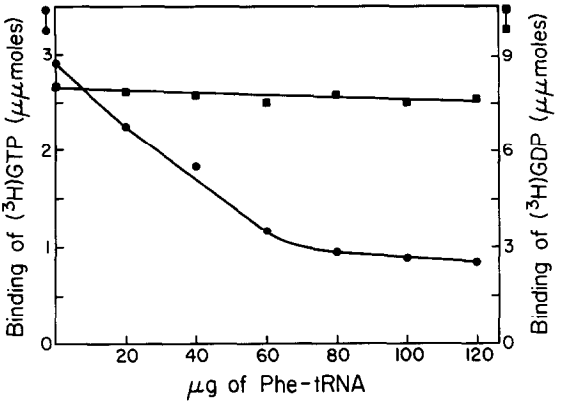

Fig. 3. Influence of Phe-tRNA on the binding of ^{3}H-GTP or ^{3}H-GDP to the yeast binding factor. The conditions for formation of the complex were the same as in Fig. 2, except that 500 $\mu\mu$moles of ^{3}H-GTP or ^{3}H-GDP were used. The complex was isolated on Millipore filters.

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