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Amino Acid Transfer Factors from Yeast. III. Relationships between Transfer Factors and Functionally Similar Protein Fractions*

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ABSTRACT: The functions of the factors FI and FII from yeast, complementary in peptide chain elongation, have been further characterized. Factor FI resembles the translocase of bacterial and mammalian systems. Factor FII seems to be a functional analog to the bacterial T factor and to the binding enzyme from mammalian sources. Fusidic acid, as well as diphtheria toxin and nicotinamide–adenine dinucleotide, inhibits the functions of FI but not of FII. Both yeast factors exist in more than one protein fraction. An FI-related form, FI_G, has been isolated; its properties closely resemble those of FI except that FI_G shows a significant ribosome-dependent guanosine triphosphatase activity. Specific treatments selectively eliminate this guanosine triphosphatase activity of FI_G, leaving its transfer activity intact. Besides the binding enzyme FII, two fractions, T_1 and T_2, can be obtained with functions similar to FII.

However, T_1 and T_2 combined with the translocase FI fail to stimulate peptide chain elongation. A relationship between the T fractions and translocase FI can be demonstrated by studying the effect of FI on the guanosine triphosphate–T_1 complex without ribosomes. In the presence of phenylalanyl transfer ribonucleic acid and T_2, factor FI removed the γ-phosphate and the guanine moiety from the guanosine triphosphate–protein complex.

Studies in polypeptide chain elongation so far have led to the finding of separable factors involved in distinct reactions. In microbial systems, two factors (T_u and T_s) have been shown to be involved in the binding of aminoacyl-tRNA to ribosomes (Lucas-Lenard and Lipmann, 1966; Lucas-Lenard and Haenni, 1968; Ravel, 1967), and a third (G) in translocation of peptidyl-tRNA between ribosomal sites (Haenni and Lucas-Lenard, 1968; Erbe and Leder, 1968; Lucas-Lenard and Haenni, 1969). Partial reactions associated with these factors have been (1) the formation of an intermediate aminoacyl-tRNA–T_u–GTP complex (Ravel *et al.*, 1967; Ertel *et al.*, 1968; Gordon, 1968; Skoultchi *et al.*, 1968), and (2) a G factor and ribosome-dependent GTP hydrolysis (Conway and Lipmann, 1964). A GTP hydrolysis has also been associated with transfer of aminoacyl-tRNA from the aminoacyl-tRNA–T_u–GTP complex to ribosomes (Ono *et al.*, 1969; Shorey *et al.*, 1969; Gordon, 1969).

In mammalian systems two factors have been recognized with properties partially analogous to the microbial factors. One catalyzes aminoacyl-tRNA binding to ribosomes (Rao and Moldave, 1967; Arlinghaus *et al.*, 1964; McKeehan *et al.*, 1969) and a concomitant GTP hydrolysis (Arlinghaus *et al.*, 1964; McKeehan *et al.*, 1969), and the other one catalyzes translocation as well as ribosome-dependent GTP cleavage (Skogerson and Moldave, 1968a,b; Felicetti and Lipmann, 1968; Klink *et al.*, 1967).

We have been attempting to find parallels between the yeast system and microbial or mammalian systems. Ayuso and Heredia (1968) described a yeast factor which catalyzed aminoacyl-tRNA binding to ribosomes, and we have isolated two fractions T_1 and T_2 which resemble T_u and T_s from bacteria in that they are complementary in aminoacyl-tRNA-dependent GTP hydrolysis (Richter *et al.*, 1968). They fail to function in chain elongation, however.

Here, we described two factors FI and FII which have been

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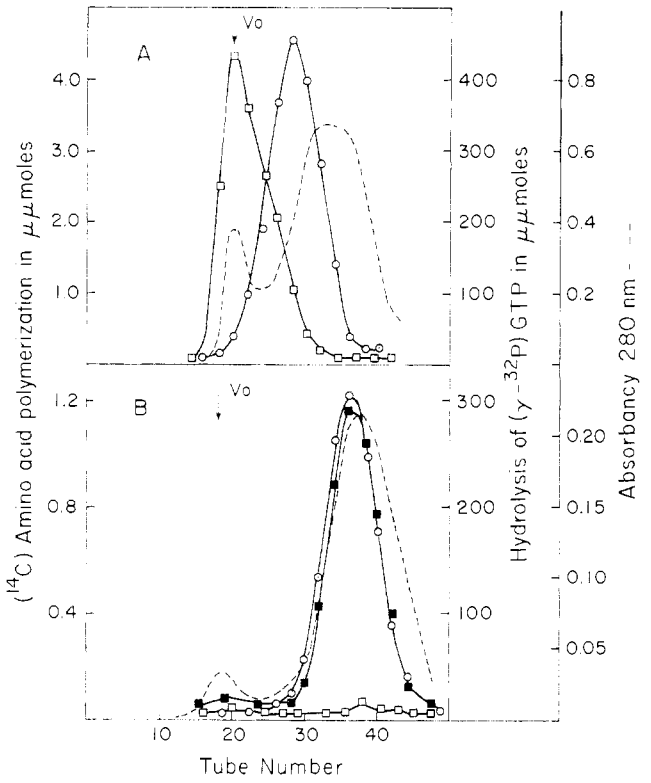
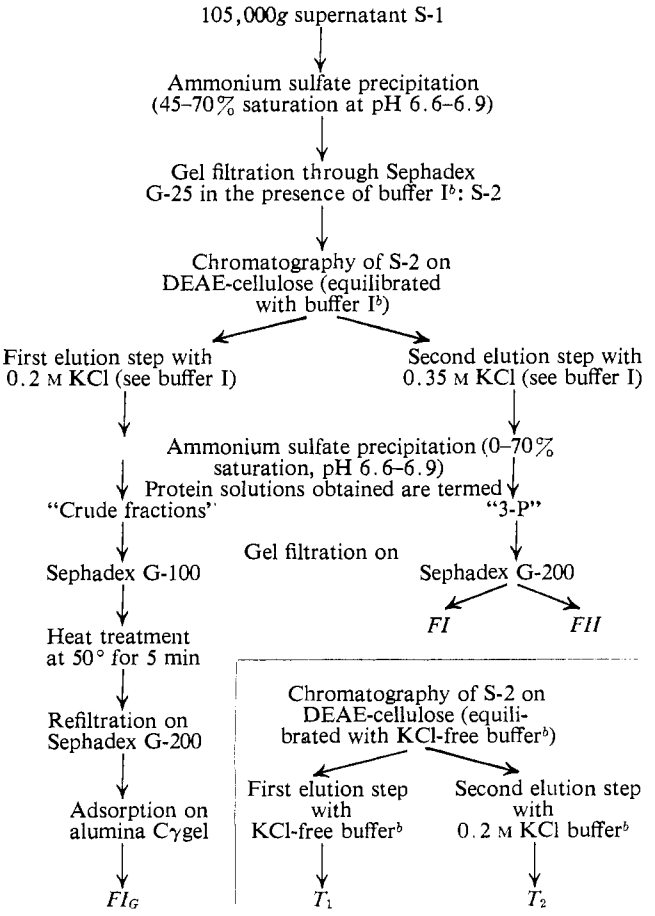


FIGURE 1: Isolation of a ribosome-dependent GTPase by gel filtration on Sephadex G-100 (A) and G-200 (B), respectively. For the conditions of chromatography, GTP splitting and amino acid polymerization see Methods. GTP hydrolysis by: (○—○) 50 μl of the eluate fractions. Polymerization activity by: (□—□) 50 μl of the eluate fractions; (■—■) 50 μl of the eluate fractions in combination with 50 μl of FII.

SCHEME I[a]



[a] The isolation procedures of the various steps were described previously (Richter and Klink, 1967b; Richter *et al.*, 1968). [b] Buffer I: 0.02 M Tris-HCl (pH 7.4), 0.001 M $Mg(CH_3COO)_2$, 0.001 M dithiothreitol, 0.2 M KCl, or KCl concentrations as indicated.

isolated on the basis of complementation in chain elongation (Richter and Klink, 1967a). These were studied in order to find out whether they resemble mammalian or microbial factors. We found that an FI-related protein, FI_G, appeared to resemble the G factor of *E. coli* in possessing a high ribosome-dependent GTPase activity; this activity could be selectively removed by various treatments. In addition, we attempted to relate our functions T_1 and T_2 to chain elongation by looking for functional interactions with FI and FII. We found that a T_1–GTP complex is specifically hydrolyzed in the presence of T_2, FI, and tRNA.

Materials and Methods

Assay for [^{14}C]Phenylalanyl-tRNA Binding to Ribosomes. The binding reaction was carried out with poly U dependent 80S ribosomes from liver and [^{14}C]phenylalanyl-tRNA. The isolation of the phenylalanyl-tRNA bound to ribosomes was performed by the assay of Nirenberg and Leder (1964). The incubation mixture contained, in 0.5 ml, 100 μl of ribosome solution (containing 2 mg/ml of protein), 40–60 μg of [^{14}C]-phenylalanyl-tRNA (3000–4000 cpm), 50 mμmoles of GTP, 15 μmoles of cysteamine, 50 μmoles of KCl, 30 μmoles of NH_4Cl, 5 μmoles of $Mg(CH_3COO)_2$, 100 μg of poly U, 25 μmoles of Tris-HCl (pH 7.6), and the protein fractions as indicated. The reaction was started at 30° by addition of GTP and [^{14}C]phenylalanyl-tRNA. After incubation for 20 min the reaction mixture was diluted with 5 ml of ice-cold buffer I (0.05 M Tris-HCl (pH 7.6), 0.1 M KCl, 0.06 M NH_4Cl, 0.01 M $Mg(CH_3COO)_2$, and 0.03 M cysteamine) and passed through a Millipore filter. The filter was washed three times with 5 ml of buffer I, dried, and counted in a liquid scintillation spectrometer.

Assay for GTPase Activity. GTP hydrolysis was measured by determination of [^{32}P]P_i liberated from γ-phosphate-labeled GTP. The reaction mixture and the conditions were the same as described previously (Richter *et al.*, 1968). If not otherwise indicated the ribosome-dependent GTPase was calculated by subtraction of the ribosomal blank, of the blank caused by the factors, and of the [^{32}P]P_i blank in the [γ-^{32}P]GTP solution from the overall reaction.

Assays for polymerization and for binding of GTP by the protein fractions as well as the preparation of the radioactive-labeled components, see Richter *et al.* (1968). Preparation of the cell-free extract from the yeast *Saccharomyces cerevisiae*, strain Kaneka, was carried out as described (Richter *et al.*, 1968).

Isolation of the Ribosomes. In the experiment described we used polysomes or poly U dependent ribosomes (80S ribo-

TABLE I: Comparison of the Elongation Factors from Yeast in Various Functions.

Conditions	Properties of the Elongation Factors	
	FI	FII
Peptide synthesis in combination with	FII	FI
GTP hydrolysis[a]	8.0	22.5
Binding of GTP[b]	0.11	7.2
Binding of Phe-tRNA to ribosomes[c]	0.14	3.28
Inhibition (%)		
By 1.2 mM fusidic acid	80	2.0
By diphtheria toxin and NAD[d]	91	1.2
Heating at 50° for 5 min[e] (%)	97	56
Molecular weight[f]	70,000	220,000

[a] The GTPase activity of the fractions was calculated for $\mu\mu$moles of GTP hydrolyzed/100 μg of protein. GTP hydrolysis was measured in the presence of poly U, phenylalanyl-tRNA, and 80S ribosomes. [b] Binding of GTP by the fraction was calculated for $\mu\mu$moles of GTP bound/100 μg of protein. [c] Binding of the phenylalanyl-tRNA to ribosomes was calculated in the same way. [d] The factors were preincubated with toxin and NAD as described in Methods. [e] The per cent values indicate the polymerization activities of the factors after heating; the transfer activity of the unheated control = 100%. [f] The molecular weights of the factors were estimated by gel filtration on Sephadex columns.

TABLE II: GTP Splitting by the Elongation Factors.

Conditions	[γ-^{32}P]GTP Hydrolysis[a] by the Factors (in $\mu\mu$moles)	
	FI	FII
Addition of		
80S ribosomes	7.3	10.0
80 S + poly U	7.0	11.7
80 S + Phe-tRNA	7.5	19.0
80 S + poly U + Phe-tRNA	7.0	22.5
Complete[b] + 1.2 mM fusidic acid	0.9	21.4
Complete + diphtheria toxin–NAD[c]	1.0	20.1

[a] GTP hydrolysis was calculated as described in Methods. [b] The complete system contained 200 μg of 80S ribosomes, 50 μg of phenylalanyl-tRNA, and 100 μg of poly U. For other conditions, see Methods. [c] The factors were preincubated with diphtheria toxin and NAD as indicated and as described in the Methods.

somes) from liver (Kramer and Klink, 1967). These preparations were practically free of GTPase activity.

Isolation and Purification of the Polymerization Enzymes. For a typical preparation of the elongation factors 120 g of lyophilized yeast cells was disrupted and the cell sap was isolated as reported (Richter *et al.*, 1968). The high-speed supernatant fluid was fractionated by addition of ammonium sulfate, applied to a DEAE-cellulose column, and eluted as shown in Scheme I. By this procedure we obtained two protein fractions termed "crude fraction" and 3-P which were both by themselves still highly active in amino acid polymerization. Further purification of these fractions was carried out by gel filtration on the dextran gels Sephadex G-100 and G-200 using an ascending technique with Pharmacia columns (2.5 × 45 cm); 0.8 ml of each protein material (about 2–10 mg/ml of protein) was applied to the column by a peristaltic pump. A flow rate of 15 ml/hr was used and 3-ml fractions were collected. Gel fitration of the protein material 3-P yielded FI and FII which were complementary in amino acid polymerization (Richter and Klink, 1967a,b). For isolation of a ribosome-dependent GTPase (recently termed stable GTPase, Richter *et al.*, 1968), the crude fraction (10–25 mg of protein) was filtered through Sephadex G-100. A typical elution diagram is shown in Figure 1A. The first peak had high transfer activity by itself (refiltration of this peak on Sephadex G-200 yielded FI and FII; K. G. Petersen and D. Richter, unpublished data); the second peak contained a ribosome-linked GTPase activity. Fractions 24–36 were pooled and heated at 50° for 5 min under stirring. The denatured protein was removed by centrifugation and the clear supernatant (about 22 ml) was concentrated by Aquacide I to about 1 ml. The concentrate (4.0–6.0 mg) was refiltered on Sephadex G-200. Gel filtration was carried out as described above. The eluted fractions were assayed for peptide synthesis and for ribosome-dependent GTPase activity (see Figure 1B). Fractions 30–44 were active in GTP hydrolysis when combined with ribosomes; the same fractions were able to catalyze amino acid polymerization in combination with the elongation factor FII.

By adsorption chromatography on alumina Cγgel the ribosome-linked GTPase from yeast was further purified (Richter *et al.*, 1968). The purified ribosome-dependent GTPase was called FI_G.

Isolation of T_1 and T_2. The T fractions were prepared by stepwise chromatography of the high-speed supernatant fraction (S-2) on DEAE-cellulose. In contrast to the isolation procedure of the elongation factors, the elution was started with KCl-free buffer, followed by 0.2 M KCl (see Scheme I). The crude protein fractions were further purified by salt fractionation, adsorption on alumina, and gel filtration on Sephadex G-200 (Richter *et al.*, 1968).

Preincubation of the Elongation Factors with Diphtheria Toxin and NAD. If not otherwise indicated the preincubation mixture contained, in 1 ml, 500 μl of the elongation factor (200–300 μg/ml of protein), 40 μmoles of cysteamine, 100 μmoles of NAD, 50 μl of diphtheria toxin (11 flocculation units/ml), 100 μmoles of KCl, 8 μmoles of $Mg(CH_3COO)_2$, and 50 μmoles of Tris-HCl (pH 7.6). After incubation at 30°

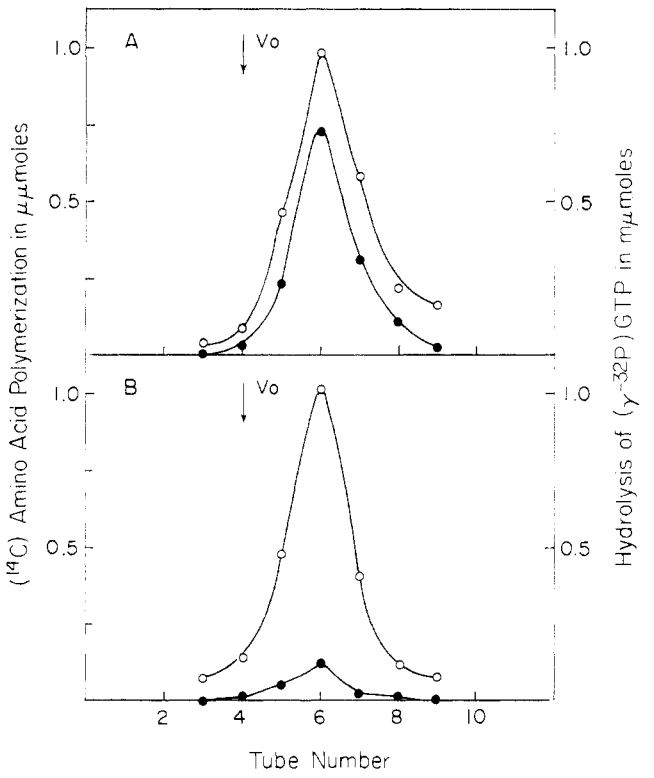


FIGURE 2: Gel filtration of factor FI_G in urea; 25 μl of 8 M urea was added to 0.5 ml of factor FI_G (500 μg/ml of protein), dialyzed against 2 M urea (pH 8.0), and applied to a Sephadex G-100 column (1.0 × 15 cm). The column was equilibrated and washed with 0.8 M urea (pH 8.0). Fractions (1.5 ml) were collected, dialyzed against 0.02 M Tris-HCl buffer (pH 7.4), 0.008 M $Mg(CH_3OOO)_2$, and 0.1 M KCl, and tested for polymerization and GTP hydrolysis. (A) Untreated FI_G was filtered in the absence of urea. (B) Treated FI_G was filtered in the presence of 0.8 M urea. Amino acid polymerization by: (○—○) eluted fractions combined with FII. No polymerization was measured by the fractions alone. GTP hydrolysis by: (●—●) eluted fractions in combination with polysomes. For assay for GTP hydrolysis and polymerization, see Methods.

for 40 min the reaction was stopped by the addition of 20 μl of antiserum (140 IU/ml). This preincubated mixture (100 μl) was used for polymerization or for GTP hydrolysis.

Materials. Fusidic acid was a gift of the Pharmaceutical Products (Copenhagen, Denmark); diphtheria toxin and antiserum were a gift of the Behring Werke (Marburg, Germany).

Results

Comparison of the Properties of FI and FII from Yeast. Factors FI and FII were first compared with respect to various catalytic properties which are summarized in Table I. Peptide chain elongation was dependent on both factors, FI and FII. Factor FII was active in the formation of a GTP–protein complex and in the GTP-dependent binding of phenylalanyl-tRNA to ribosomes. Both functions of FII were affected neither by fusidic acid nor by diphtheria toxin and NAD. Moreover, hydrolysis of GTP was catalyzed by a combination of FII with ribosomes and phenylalanyl-tRNA (Table II). A slight GTPase activity was obtained by combination of FI with ribosomes. Poly U and/or phenylalanyl-tRNA did not

TABLE III: Inhibition of the Ribosome-Dependent GTPase and of the Peptide Synthesis by Fusidic Acid and Diphtheria Toxin and NAD.

Conditions	Hydrolysis of [γ-32P]-GTP (μμ-moles)[a]	Amino Acid Polymeriza-tion (μμ-moles)[b]
FI	6.0	0.21
FI_G	294.0	0.08
FI_G + 1.2 mM fusidic acid	60.8	0.10
FI_G (diphtheria toxin–NAD[c])	71.9	0.12
FI + FII		2.65
FI_G + FII		1.74
FI + FII + 1.2 mM fusidic acid		0.52
FI_G + FII + 1.2 mM fusidic acid		0.36
FI (toxin–NAD)[c] + FII		0.45
FI_G (toxin–NAD)[c] + FII		0.31
FII (toxin–NAD)[c] + FI		2.70

[a] Hydrolysis of GTP was calculated as described in Methods. [b] GTP hydrolysis as well as polymerization were carried out with polysomes as described in Methods. The blank of factor FII alone in the polymerization test was 0.35 μμmole of amino acids polymerized. [c] Preincubation of the factors with diphtheria toxin and NAD was carried out as described in Methods. No inhibition was obtained if FI or FI_G was preincubated with toxin or NAD alone.

stimulate the GTPase activity of FI. In contrast to FII the function of FI was inhibited by fusidic acid as well as by diphtheria toxin and NAD (Tables I and II). Factor FI, though showing some functional analogies to the bacterial and mammalian translocase, failed to stimulate the ribosome-dependent GTP hydrolysis significantly.

Correlation between a Ribosome-Dependent GTPase and Factor FI. We found that one of the fractions from DEAE-cellulose (see Scheme I), FI_G, resembled FI, but contained a high ribosome-dependent GTPase activity. As shown in Figure 1B, the GTPase peak coincides exactly with the polymerization peak obtained by combination of the eluted fractions with FII. The approximate molecular weights of FI and FI_G were calculated to be 70,000 (Auricchio and Bruni, 1964). To shed more light on the correlation between FI_G and FI, fraction FI_G was treated with urea. Figure 2A,B shows a comparison between fractions FI_G before and after treatment with urea. Urea did not remove the ability of FI_G to complement FII in peptide synthesis but did eliminate the ribosome-linked GTPase to a large extent. A corresponding result was obtained if FI_G was rechromatographed stepwise with KCl-free buffer, 0.2 M KCl, and 0.35 M KCl on DEAE-cellulose. At 0.2 and 0.35 M KCl a protein fraction was obtained which, like FI, was complementary with FII in polymerization, but was unable to hydrolyze GTP in the presence of ribosomes. Starting the chromatography with 0.2 M KCl buffer, in contrast, yielded a protein fraction completely resembling FI_G in

TABLE IV: Comparison of the Functions of T_1 and T_2.[a]

Fractions	Binding of [^{3}H]GTP ($\mu\mu$moles)	Binding of [^{14}C]Phe-tRNA to Ribosomes ($\mu\mu$moles) −GTP	Binding of [^{14}C]Phe-tRNA to Ribosomes ($\mu\mu$moles) +GTP	Hydrolysis of [γ-^{32}P]GTP ($\mu\mu$moles) −Phe-tRNA	Hydrolysis of [γ-^{32}P]GTP ($\mu\mu$moles) +Phe-tRNA
T_1	19.4	0.3	0.8	29.5	31.0
T_2	3.1	0.5	2.5	14.1	15.0
$T_1 + T_2$	26.5	0.5	3.7	36.4	125.0
$T_1 + T_2$ + 5 mM fusidic acid	24.5	0.5	3.5	35.1	120.1
FII	7.1	0.4	2.4	9.3	23.1

[a] GTP hydrolysis was calculated as described in Methods. Hydrolysis of GTP and binding of phenylalanyl-tRNA were carried out with poly U dependent ribosomes.

polymerization and GTPase activity. Obviously specific treatments selectively inactivated the GTPase activity of FI_G, leaving its transfer activity uneffected. This suggests that the GTPase is unrelated to polypeptide synthesis function. However, the following experiments show that agents which specifically react with the translocase in other systems also inhibited the GTPase here (Tanaka *et al.*, 1968; Malkin and Lipmann, 1969; Honjo *et al.*, 1968; Haenni and Lucas-Lenard, 1968). A concentration of 1.2 mM fusidic acid caused 80% inhibition of the ribosome-dependent GTPase and transfer activity (Table III). Similarly inhibition of peptide chain elongation occurred when FI_G or FI was treated with diphtheria toxin and NAD. Inhibition of the FI_G-linked GTPase activity by toxin–NAD occurred only in the presence of higher NAD concentrations (U. Albrecht and D. Richter, unpublished data).

Interaction of the T_1 Fractions with GTP, Phenylalanyl-tRNA, and Ribosomes. Recently evidence has been presented that the binding enzyme FII may also exist in more than one protein fraction (Richter and Klink, 1967a,b). Although no direct proof has been obtained, these fractions T_1 and T_2 probably represent subfractions of FII. To get more details concerning T_1 and T_2 from yeast we examined their role in the formation of a GTP–protein complex, in the enzymatic binding of phenylalanyl-tRNA to ribosomes, and in GTP breakdown. As shown in Table IV, T_1 bound GTP in a larger extent than the more labile T_2. Combination of T_1 with T_2 resulted in a slight stimulation of the formation of a GTP–protein complex.

TABLE V: Polymerization of Phenylalanyl-tRNA by the T Fractions and the Elongation Factors.[a]

Fractions	[^{14}C]Phenylalanine Polymerized ($\mu\mu$moles)
T_1	0.04
T_2	0.08
$T_1 + T_2$	0.14
$T_1 + T_2$ + FI	0.36
$T_1 + T_2 + FI_G$	0.38
$T_1 + T_2$ + FII	0.56
FII	0.29
FI + FII	2.85

[a] See Methods for assay for polymerization.

TABLE VI: FI-Dependent GTP Hydrolysis.

Conditions	[γ-^{32}P]GTP Hydrolyzed ($\mu\mu$moles) Without 80S Ribosomes[a] −Phe-tRNA	Without 80S Ribosomes[a] +Phe-tRNA	With 80S Ribosomes[a] −Phe-tRNA	With 80S Ribosomes[a] +Phe-tRNA
$T_1 + T_2$	105	210	244	802
T_1 + FI	87	105	110	125
T_2 + FI	60	85	95	100
$T_1 + T_2$ + FI	135	850	615	1735
$T_1 + T_2$ + FI				2125
T_1[b] + T_2 + FI				1808
$T_1 + T_2$[b] + FI				425
$T_1 + T_2$ + FI[b]				1957
$T_1 + T_2$				715
$T_1 + T_2$ + 5 mM fusidic acid				635
$T_1 + T_2$ + 5 mM fusidic acid				778

[a] The ribosome-dependent GTP hydrolysis was tested in the presence of 100 μg of poly U. [b] The various fractions were heated at 50° for 5 min.

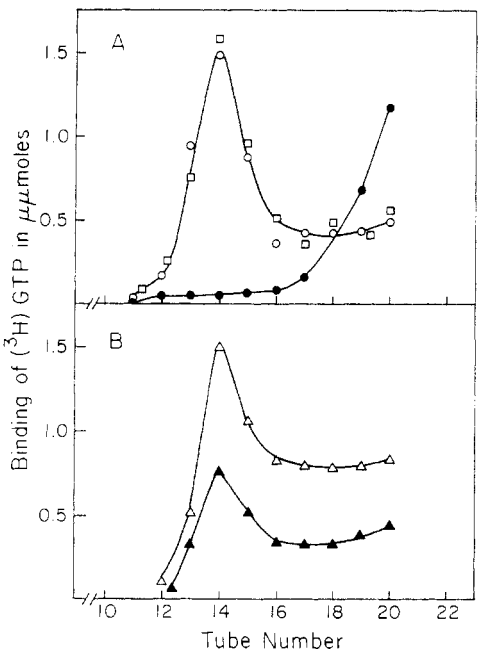


FIGURE 3: Influence of FI, T_2, and [^{12}C]phenylalanyl-tRNA on the [^{3}H]GTP–protein complex. Formation and isolation of the complex: 2 ml of T_1 (6 mg/ml of protein) was incubated at 0° for 10 min in the presence of 20 mμmoles of [^{3}H]GTP, and filtered on Sephadex G-25 (column size 1.8 × 40 cm). The fractions containing the complex were pooled. Aliquots of 300 μl containing 20 μμmoles of bound [^{3}H]-GTP were immediately used for the experiments. A total volume of 600 μl was incubated in the presence of the enzyme fractions as indicated (at 30° for 10 min) and refiltered on Sephadex G-25 column (1.5 × 15 cm). Fractions of 20 μl were collected and assayed for bound [^{3}H]GTP. (A) Bound [^{3}H]GTP by (○—○) refiltered T_1 and (●—●) refiltered T_1 combined with FI, T_2, and [^{12}C]phenylalanyl-tRNA, (□—□) refiltered T_1 combined with FI and T_2. (B) Bound [^{3}H]-GTP by (▲—▲) refiltered T_1 combined with T_2 and [^{12}C]phenylalanyl-tRNA, (△—△) refiltered T_1 combined with FI and [^{12}C]phenylalanyl-tRNA. For the experiments 50 μl of [^{14}C]phenylalanyl-tRNA (60 μg), 50 μl of T_2, and 50 μl of FI were used as indicated.

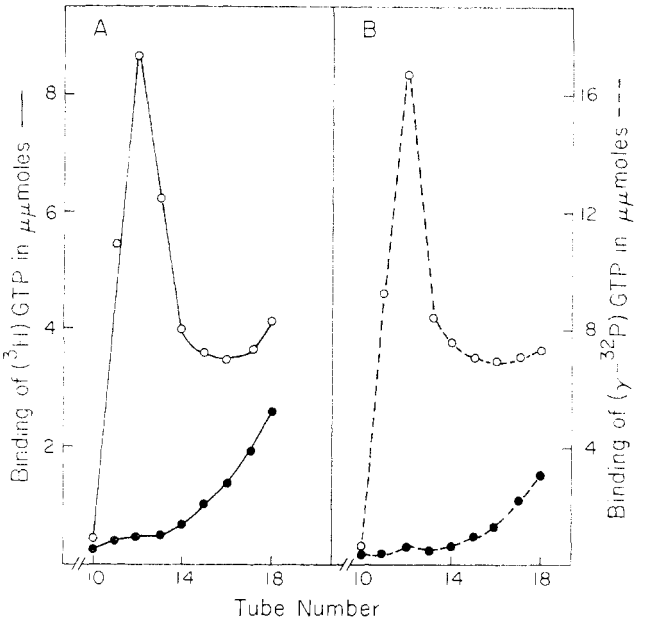


FIGURE 4: The dissociation of the GTP–protein complex in the presence of fusidic acid. The complete reaction mixture contained in a total volume of 0.5 ml: 150 μl of T_1, 50 μl of T_2, 50 μl of FI, 50 μl of [^{12}C]phenylalanyl-tRNA (60 μg), 5 mμmoles of [^{3}H]GTP, and 12 mμmoles of [γ-^{32}P]GTP. The reaction mixtures were incubated at 30° for 10 min and filtered on Sephadex G-25 columns (1.5 × 15 cm). Fractions of 20 μl were collected and assayed for [^{3}H]GTP and for [γ-^{32}P]GTP. (A) Bound [^{3}H]GTP by: (●—●) complete reaction mixture, (○—○) complete reaction mixture in the presence of 5 mM fusidic acid. The dotted lines in part B indicate the amount of [γ-^{32}P]GTP bound by the reaction mixtures as described above.

However, an absolute requirement for both fractions in GTP binding was never observed. On the other hand, the enzymatic binding of phenylalanyl-tRNA to ribosomes was stimulated by T_2 to a higher extent than by T_1 and was almost dependent on the presence of GTP. A combination of T_1 and T_2 was as effective as T_2 alone. The requirement of only one fraction for this reaction may be explained by the impurity of T_2. Table IV indicates further that the hydrolysis of GTP significantly depended on both T fractions, ribosomes, and phenylalanyl-tRNA. T_1 and T_2 were not inhibited by fusidic acid. As so far examined, the functions of the T fractions are closely similar to those of the binding enzyme FII, supporting the assumption that the T fractions derive from FII. Although T_1 and T_2 are involved in partial steps of peptide synthesis they are unable to catalyze the overall reaction in the presence of FI or FI_G (Table V).

Interaction of FI on the T_1–GTP Complex. In an attempt to get evidence for the participation of the T fractions in the peptide-synthesizing system, we investigated a secondary reaction which may suggest that the translocase reacts with the GTP–T_1 complex even in the absence of ribosomes. As shown in Table VI, the phenylalanyl-tRNA-dependent GTP breakdown caused by T_1 and T_2 was considerably increased in the presence of FI. Without ribosomes the GTP hydrolysis was almost completely dependent on phenylalanyl-tRNA. Uncharged tRNA was as effective as charged tRNA (K. Prenzel and D. Richter, unpublished data). It should be noted that the FI-dependent GTPase activity required higher temperatures (30°) than the complex formation which rapidly occurred in the cold (Richter *et al.*, 1968). The dependence of GTP breakdown on both T fractions was shown by heat inactivation of T_2 (Table VI). GTP hydrolysis was reduced to 80% if T_2 was heated at 50° for 5 min, whereas T_1 as well as FI remained stable under these conditions. It is of particular interest that fusidic acid did not influence the phenylalanyl-tRNA- and ribosome-dependent GTPase activity of the T fractions alone. However, in the presence of FI GTP hydrolysis was blocked by the antibiotic, indicating that this GTPase activity was linked with the translocase. The FI-linked GTP breakdown indicated that an interrelationship between the T fractions and one of the elongation factors may exist. To examine the fate of the GTP–protein complex when treated with T_2, FI, and phenylalanyl-tRNA, the isolated T_1–GTP complex was used as a substrate. After incubation of T_1–GTP with FI, T_2, and phenylalanyl-tRNA, the reaction mixture was refiltered on Sephadex G-25. Figure 3 demonstrates that FI not only caused GTP hydrolysis but also the removal of the guanine moiety from the complex. The profiles show that phenylalanyl-tRNA as well as T_2 were required for the dissociation of the complex. Fusidic acid, known to inhibit the translocase,

prevented a dissociation of the complex (Figure 4), supporting the contention that FI was the factor which reacted with the GTP–protein complex.

Discussion

The results presented here indicate that the yeast factors bear some similarities to both mammalian and microbial systems. However, they have features which have not been observed with the other systems. First, the translocase from yeast exists in two forms, FI and FI_G. The latter contains the ribosome-dependent GTPase activity. Specific treatments of FI_G selectively eliminate the ribosome-dependent GTPase from the FI_G without removing the transfer activity. This conversion of FI_G to a fraction quite similar to FI may be explained in several ways. The first possibility is that factor FI_G is nothing more than FI contaminated with an enzyme of similar molecular weight unrelated to peptide synthesis and possessing a GTPase activity. However, the dependence on ribosomes of FI_G-dependent GTPase activity, as well as the fusidic acid inhibition of that activity, argue that the observed GTPase is related to the elongation enzyme. Therefore, a more likely explanation of the selective elimination of the GTPase activity of FI_G might be that the FI_G fraction represents two modifications of one enzyme with the same molecular weight. One form could be active in translocation and GTP hydrolysis only in combination with the complete protein-synthesizing system. The other form, somewhat damaged, might split GTP with ribosomes in an uncoupled reaction. Following this supposition, FI would represent one form of the FI_G fraction.

Second, the formation of a GTP–protein complex and the transfer of phenylalanyl-tRNA to ribosomes is catalyzed by the binding enzyme, FII, as well as by T_1 and T_2, respectively. Although complete dependence for these reactions on both T fractions was not observed, we suggest that T_1 and T_2 represent two different subfractions. A complementarity of T_1 and T_2 in the phenylalanyl-tRNA-linked GTP-splitting reaction strengthen this contention. The inability of the T fractions to catalyze the overall reaction in the presence of the translocase may be clarified by preliminary results suggesting that one of the T fractions is contaminated by a protein fraction which inhibits polymerization (K. Prenzel and D. Richter, unpublished data). Although no direct evidence has been obtained for the relationship between the T fractions and FII, the similarity in their functions makes this a reasonable possibility. The lower molecular weights of T_1 and T_2 (about 65,000, U. Abrecht and D. Richter, unpublished data) also might indicate that these are derived from the native larger fraction FII (about 220,000). Recently, Schneir and Moldave (1968) reported on a binding enzyme from liver which occurs in three protein fractions with different molecular weights and relative specific activities. These different forms were complementary to the translocase of liver. The only case in which the T fractions of yeast were complementary with the yeast translocase FI was the effect of FI on the GTP–T_1 complex. The latter phenylalanyl-tRNA- and T_2-dependent reaction resulted in the removal of the γ-phosphate and of the guanine moiety of GTP from the complex. That FI is the factor acting on the GTP–T_1 complex is supported by the inhibition of the FI-linked GTP breakdown by fusidic acid; the antibiotic does not block the functions of T_1 or T_2. Should the complementarity of the T fractions and of the translocase FI apply also to peptide chain elongation, the FI-dependent release of the γ-phosphate and of the nucleotide moiety from the GTP–protein complex may be regarded as circumstantial evidence that the energy required for cyclic chain elongation derives from the GTP originally utilized in the binding of aminoacyl-tRNA to ribosomes.

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Comparative Study of Polyribonucleotides in Aqueous and Glycol Solutions*

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ABSTRACT: In an effort to investigate the nature of the ordered but anomalous structure assumed by deoxyribonucleic acid dissolved in ethylene glycol, we have carried out a systematic study of polyribonucleotides, transfer ribonucleic acid, and stoichiometric equivalents of mononucleotides in aqueous and glycolic solutions. Determination of optical rotatory dispersion, circular dichroism, and absorption spectra, as well as of temperature–absorbance profiles, were the techniques used. The rotatory spectra suggest that deoxyribonucleic acid in glycol must have a structure that is distinctly non-Watson–Crick–Wilkins in character, yet, on the basis of its hypochromicity and the sharpness of its thermal transition, must be a highly ordered one. Glycolic solutions of the polynucleotides and of transfer ribonucleic acid, on the other hand, show rotatory spectra which do not exhibit the anomaly characteristic of deoxyribonucleic acid.

We have recently reported (Green and Mahler, 1968) an apparently anomalous and nonconservative (Bush and Brahms, 1967; Tinoco, 1968) rotatory behavior of DNA in ethylene glycol. In this context, "nonconservative" defines a circular dichroic spectrum of an oligo- or polynucleotide, characterized by a single band *centered* on the wavelength of an absorption maximum, in contrast to "conservative," which contains both a positive and a negative band of equal magnitude with a *crossover* at the wavelength of the absorption maximum: $(rC)_n$[1] is an example of a polymer producing the former, $(rA)_n$ one producing the latter type.

Ethylene glycol, due to its high polarizability and the decreased magnitude of resultant solvophobic forces, exerts profound effects on the transition of a polynucleotide between its ordered (helix) and disordered (coil) forms. Among them are the following (all statements relative to water): to increase the contributions to helix stability due to hydrogen bonding (both between complementary bases and those due to sugar hydroxyls) and to electrostatic interactions, and to diminish all contributions due to solvophobic interactions between stacked bases (Singer, 1962; Sage and Singer, 1962; Sinanoglu and Abdulnur, 1964, 1965; Hanlon and Major, 1968). From studies of the behavior of polynucleotides in such solvents, many authors have implicated hydrophobic interactions as a major contribution to the stability of nucleic acids and polynucleotide structures in water (Ts'o *et al.*, 1961, 1962; Gordon and Jencks, 1963; Levine *et al.*, 1963; Marmur *et al.*, 1963; Brahms *et al.*, 1964). Our own results (Green and Mahler, 1968) raised serious doubts whether DNA dissolved in glycol retained a Watson–Crick–Wilkins (WCW) B helix as suggested by Luzzati *et al.* (1964, 1967), but, we did not venture to suggest an acceptable alternative model.

In an attempt to lay the groundwork for such a model, we have now made a systematic study of the behavior of various synthetic polynucleotides and of mixtures of their component mononucleotides, in both solvents. This present work can also be regarded as an extension of the systematic studies by Ts'o and Helmkamp (Ts'o and Helmkamp, 1961; Helmkamp and Ts'o, 1961, 1962; Ts'o *et al.*, 1962), who previously studied the optical rotatory properties of polynucleotides, as well as their mononucleotide components, in both aqueous and nonaqueous solvents and provided strong evidence for the importance of noncovalent interactions to optical rotatory

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[1] We use here the system of abbreviations for nucleotides and their polymers suggested in the Revised Tentative Rules (1965) of the IUPAC–IUB Combined Commission on Biochemical Nomenclature. In this system, polyriboadenylic acid (3′–5′ linked) becomes $(rA)_n$ or poly rA and its duplex with polyribouridylic acid is $(rA)_n \cdot (rU)_n$. Other abbreviations used are: ϵ_{max}, molar extinction coefficient at wavelength of maximum absorption on a mononucleotide basis; λ_{max}, wavelength of maximum absorption; λ_c wavelength of crossover.