Intermediate Reactions in the Binding of Aminoacyl-Transfer Ribonucleic Acid to Rat Liver Ribosomes

THE ROLE OF GUANOSINE TRIPHOSPHATE

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1. Transferase I from rat liver binds relatively low quantities of GTP when incubated with this nucleotide in the absence of aminoacyl-tRNA. 2. Transferase I reacts with both aminoacyl-tRNA and GTP to form a relatively stable complex that is retained on cellulose nitrate filters. The ternary complex transferase I–aminoacyl-tRNA–GTP is also formed when the transferase I–aminoacyl-tRNA complex is incubated with GTP or during the incubation of the transferase I–GTP complex with aminoacyl-tRNA. Synthesis of this complex does not require the presence of Mg^{2+} . 3. In the presence of Mg^{2+} the ternary complex becomes readily bound to ribosomes without requirements for any other cofactors. 4. An extensive cleavage of GTP takes place when aminoacyl-tRNA becomes bound to ribosomes. 5. The low interdependence of reactions leading to the formation of transferase I complexes with aminoacyl-tRNA and GTP indicates that the mechanisms of the binding reaction in mammalian systems may be different from those in bacterial cells.

Peptide elongation processes on ribosomes are initiated by the binding of aminoacyl-tRNA to the ribosome, which is a multistep reaction showing a specific requirement for GTP (Gordon, 1969).

In bacterial cells a supernatant fraction called T combines first with GTP (Allende *et al.*, 1967) and this complex subsequently reacts with aminoacyl-tRNA (Lucas-Lenard & Haenni, 1968). Evidence has been presented that this complex II is the key intermediate in the binding reaction (Shorey *et al.*, 1969). The binding of this product to the ribosome is followed by a cleavage of GTP to GDP (Waterson, *et al.*, 1970).

A similar absolute requirement for GTP was also demonstrated for the binding of aminoacyl-tRNA to the ribosome catalysed by the binding enzyme in rabbit reticulocytes (Lin *et al.*, 1969). However, GTP is not necessary for the binding of aminoacyl-tRNA to the smaller subunit of rat liver ribosome in the presence of the transfer enzyme (transferase I) (Rao & Moldave, 1969). Moreover, evidence has been presented in the preceding paper that aminoacyl-tRNA readily combines with transferase I in the absence of GTP (Hradec, 1972). It thus seems that the intermediate reactions involved in the binding of aminoacyl-tRNA to mammalian ribosomes and, in particular, the role of GTP in these reactions may be different from that in bacterial cells.

Evidence is presented in this paper that GTP combines with transferase I and aminoacyl-tRNA to form a ternary complex, which becomes readily

bound to the ribosome. During this latter step most of the GTP is split to GDP.

Experimental

Materials

Biological materials and chemicals. These were the same as described by Hradec (1972).

Radiochemicals. $[8\text{-}^3\text{H}]\text{GTP}$ (11.8 Ci/mmol), $[\text{U-}^{14}\text{C}]\text{GTP}$ (486 mCi/mmol) and $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ (1.7 Ci/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K. Aminoacyl- $[\text{}^{32}\text{P}]\text{tRNA}$ from *Escherichia coli* was purified as described by Hradec (1972). $[\text{}^3\text{H}]\text{Phenylalanyl-tRNA}$ from rat liver was prepared as described by Moldave (1963) from $[\text{}^3\text{H}]\text{phenylalanine}$ (18 Ci/mmol) (The Radiochemical Centre). Individual preparations contained 5.80–6.70 μmol of phenylalanine/mmol of tRNA. $[\text{}^{14}\text{C}]\text{Phenylalanyl-tRNA}$ was prepared by the same technique from $[\text{U-}^{14}\text{C}]\text{phenylalanine}$ (118 mCi/mmol) purchased from the Institute for Research, Production and Uses of Radioisotopes, Prague, Czechoslovakia. Final batches contained 6.00–7.50 μmol of phenylalanine/mmol of tRNA.

Methods

Isolation of transferase I and ribosomes from rat liver. The methods described by Hradec (1972) were used.

Isolation of transferase I complexes with GTP and aminoacyl-tRNA. For the preparation of transferase I-aminoacyl-tRNA complex the second procedure described by Hradec (1972) was used. For the isolation of the transferase I-aminoacyl-tRNA-GTP complex the same procedure was followed. In this case, the discs after the adsorption of the complex on cellulose DE-20 filters were washed initially with 80mM-KCl in the washing buffer to elute free GTP. The complex was then eluted and stored as described by Hradec (1972). Gel filtration on Sephadex G-100 columns was performed in the same way as described by Hradec (1972).

Incubation procedures and measurements of radioactivity. These were described in the preceding paper (Hradec, 1972).

Results

Formation of a transferase I-GTP complex (complex B)

When transferase I is incubated with GTP (optimum concentration 0.2mM) this nucleotide apparently becomes bound to the enzyme since labelled GTP remains on cellulose nitrate filters through which the incubation mixture has been filtered. Up to a saturation value, the amount of radioactivity retained on filters is linearly proportional to the quantity of transferase I added to the incubation mixture (Fig. 1). However, no important conclusions can be drawn from the saturation values obtained in experiments described in this paper for the reasons discussed by Hradec (1972).

The combination of GTP with the binding enzyme is quite rapid at 37°C and somewhat slower at 0°C (Fig. 2). This complex is highly unstable and becomes completely destroyed even after 30min at 0°C. It is apparently also split during the gel filtration since it was not possible to provide evidence for its existence by using this technique.

The reaction of transferase I with GTP shows an absolute requirement for Mg^{2+} , the optimum concentration being apparently 8–10mM (Fig. 3).

The yields of complex B are rather low. However, the binding of GTP to the enzyme may be severalfold enhanced by the addition of aminoacyl-tRNA (Fig. 4). This suggested that a ternary complex transferase I-GTP-aminoacyl-tRNA may be formed in these conditions.

Formation of a transferase I-aminoacyl-tRNA-GTP complex (complex C)

When transferase I is incubated with labelled aminoacyl-tRNA and GTP and the reaction mixture is filtered through cellulose nitrate membranes, a high proportion of both RNA and GTP is retained on the

Fig. 1. Binding of GTP by transferase I

Reaction mixtures contained in a final volume of 0.1 ml: 50mM-tris-HCl buffer, pH 7.5, 10mM- $MgCl_2$, 50mM-KCl, 0.2mM-GTP (400 000 c.p.m. of $[^3H]$ -GTP and 475 000 c.p.m. of $[\gamma-^{32}P]$ -GTP) and the binding enzyme as indicated. They were incubated at 37°C for 10min, and reaction mixtures after the incubation were filtered through cellulose nitrate filters and the radioactivity retained on filters was assayed as described in the Materials and Methods section. ○, 3H ; ●, ^{32}P .

filter. The radioactivity retained on the membrane is proportional to the amount of enzyme present in the incubation mixture (Fig. 5). This indicated that a ternary complex of the binding enzyme with aminoacyl-tRNA and GTP is formed. Gel filtration of these mixtures demonstrated indeed that radioactivities corresponding to both tRNA and GTP are eluted together with the enzyme protein (Fig. 5). The saturation quantities of the binding enzyme were different for aminoacyl-tRNA and GTP, as shown in Fig. 5.

Binding of GTP to the complex is enhanced by increasing the quantity of aminoacyl-tRNA in the incubation mixture only to a certain value. After this, the quantity of GTP bound to the enzyme rapidly decreases, although the complex is not saturated as yet with respect to aminoacyl-tRNA (Fig. 4). On the other hand, the amount of aminoacyl-tRNA bound to the complex is not affected by increasing the amounts of GTP in the incubation mixtures, at least in the range of 0–0.5mM nucleotide.

Fig. 2. Time-course of complex B formation at (a) 0°C and (b) 37°C

Incubation mixtures contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 10 mM-MgCl₂, 50 mM-KCl, 0.2 mM-GTP (600 000 c.p.m. of [³H]GTP and 750 000 c.p.m. of [γ -³²P]GTP) and 150 μ g of transferase I protein. ○, ³H; ●, ³²P.

Approximately the same yields of complex C are obtained after the incubation of reaction mixtures at 0°C or 37°C. However, the complex C formation proceeds more rapidly at 37°C. At 0°C the binding of aminoacyl-tRNA to the complex is significantly more retarded than that of GTP (Fig. 6). The binding of GTP to the complex is very stable and the amount of the nucleotide bound is not altered even after 60 min of incubation at 37°C. However, the binding of aminoacyl-tRNA to complex C is apparently more labile since at least 30% of aminoacyl-tRNA is released from the complex after 60 min at 37°C. At 0°C the whole complex is stable for at least 60 min (Fig. 6).

Formation of complex C shows no absolute requirement for Mg²⁺ and the complex is obtained in good yields even in the absence of this cation. Low concentrations of Mg²⁺, in particular in the range 1–3 mM, exhibit a strong inhibitory effect on complex C formation, the binding of aminoacyl-tRNA being especially affected. However, Mg²⁺ at a concentration of about 5 mM has some stimulatory effect on

the formation of the ternary complex. Higher concentrations of Mg²⁺ are again inhibitory (Fig. 3).

When complex A, isolated by the ion-exchange procedure described by Hradec (1972), is incubated with increasing amounts of GTP, the nucleotide becomes readily bound to the complex until saturation values are reached characteristic for the formation of complex C from individual components. As expected, the quantity of aminoacyl-tRNA bound to the complex remained unchanged (Fig. 7). GTP is bound to complex C with approximately the same velocity as described for the formation of this ternary complex from individual components. These results suggest that preformed complex A may serve as a substrate for the formation of complex C.

When transferase I is preincubated with GTP, aminoacyl-tRNA added after such preincubation becomes significantly more rapidly bound to the enzyme than when added at the beginning of the incubation (Fig. 7). Moreover, the quantity of GTP bound to the enzyme remains essentially the same from the very beginning of the incubation. The

Fig. 3

Fig. 4

Fig. 3. Mg^{2+} requirement for the formation of (a) complex B and (b) complex C

Mixtures for the assay of complex B formation had the same composition as those described in the legend for Fig. 2 and were incubated at 37°C for 10 min. Incubation mixtures for complex C formation contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 50 mM-KCl, 0.2 mM-GTP (250 000 c.p.m. of [3 H]GTP), 230 pmol of [14 C]phenylalanyl-tRNA and 120 μ g of transferase I protein, and were incubated at 37°C for 15 min. $\circ$, 3 H; $\bullet$, 32 P; Δ , 14 C.

Fig. 4. Enhancement of the binding of GTP to transferase I by aminoacyl-tRNA

Incubation mixtures contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 10 mM-MgCl₂, 50 mM-KCl, 0.2 mM-GTP (100 000 c.p.m. of [3 H]GTP and 200 000 c.p.m. of [γ - 32 P]GTP), 95 μ g of transferase I protein and [14 C]phenylalanyl-tRNA as indicated, and were incubated at 37°C for 10 min. $\circ$, 3 H; $\bullet$, 32 P; Δ , 14 C.

Fig. 5. Evidence for formation of the ternary complex C

(a) Increased binding of both GTP and aminoacyl-tRNA by increasing amounts of the binding enzyme; (b) analysis of a reaction mixture by gel filtration on Sephadex G-100. Incubation mixtures for the enzyme saturation contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 0.2 mM-GTP (100 000 c.p.m. of [3 H]GTP and 250 000 c.p.m. of [γ - 32 P]GTP) and 200 pmol of [14 C]phenylalanyl-tRNA. The incubation mixture for the gel filtration contained, in a final volume of 1.0 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 0.2 mM-GTP (2 500 000 c.p.m. of [3 H]GTP and 8 000 000 c.p.m. of [γ - 32 P]GTP), 2.18 nmol of [14 C]phenylalanyl-tRNA and 4.5 mg of transferase I protein. The mixture was incubated at 37°C for 15 min, chilled in ice and applied to a column (25 mm $\times$ 360 mm) of Sephadex G-100 equilibrated with 50 mM-tris-HCl buffer, pH 7.5, containing 6 mM-MgCl₂ and 50 mM-KCl. The column was eluted with the same buffer and fractions (2.0 ml) were collected. The separation was performed at 2°C. 3 H and 32 P radioactivities in fraction nos. 50–70 were multiplied by 0.1. $\circ$, 3 H; $\bullet$, 32 P; Δ , 14 C.

significant enhancement of GTP binding to the complex by aminoacyl-tRNA, typical for the formation of complex C from individual components (Fig.

4), is much less pronounced if aminoacyl-tRNA is added after the preincubation of transferase I with GTP. These results indicate that complex B formed

Fig. 6. Formation of complex C at (a) 0°C and (b) 37°C

Incubation mixtures contained, in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 0.2 mM-GTP (100 000 c.p.m. of [³H]GTP and 175 000 c.p.m. of [γ -³²P]GTP), 250 pmol of [¹⁴C]-phenylalanyl-tRNA and 125 μ g of transferase I protein. $\circ$, ³H; $\bullet$, ³²P; Δ , ¹⁴C.

Fig. 7

Fig. 8. Binding of (a) aminoacyl-tRNA and GTP to ribosomes in the presence of the binding enzyme and (b) complex B and aminoacyl-tRNA to ribosomes

Incubation mixtures for the binding of individual components contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 50 μg of poly(U), 0.2 mM-GTP (100 000 c.p.m. of ^3H]GTP and 150 000 c.p.m. of $[\gamma\text{-}^{32}\text{P}]\text{GTP}$), 200 pmol of ^{14}C]phenylalanyl-tRNA, 125 μg of transferase I protein and 350 μg of ribosomes, and were incubated at 37°C for the time indicated. Mixtures for the binding of complex B to ribosome contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 50 μg of poly(U), 0.2 mM-GTP (100 000 c.p.m. of ^3H]GTP and 150 000 c.p.m. of $[\gamma\text{-}^{32}\text{P}]\text{GTP}$) and 125 μg of transferase I protein, and were preincubated at 37°C for 10 min. After this, the tubes were chilled in ice and 200 pmol of ^{14}C]phenylalanyl-tRNA and 350 μg of ribosomes were added to each tube. These complete mixtures were then incubated at 0°C for the time indicated. $\circ$, ^3H ; $\bullet$, ^{32}P ; Δ , ^{14}C .

during the preincubation of the binding enzyme with GTP may be utilized as a direct substrate for the synthesis of the ternary complex C.

Experiments on the formation of complex C from deacylated tRNA showed that less than 10% of this

tRNA becomes bound to the binding enzyme compared with aminoacyl-tRNA. Surprisingly, the amount of GTP bound to the complex is essentially the same as in the presence of aminoacyl-tRNA. Moreover, deacylated tRNA enhances the binding

Fig. 7. Formation of complex C from (a) complex B and (b) complex A

Incubation mixtures for the synthesis from complex B contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 0.2 mM-GTP (500 000 c.p.m. of ^3H]GTP and 750 000 c.p.m. of $[\gamma\text{-}^{32}\text{P}]\text{GTP}$) and 125 μg of transferase I protein. They were incubated at 37°C for 10 min; after this preincubation 250 pmol of ^{14}C]phenylalanyl-tRNA was added and the incubation was continued for the time indicated. Mixtures for the assay of complex C from complex A formation contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 0.2 mM-GTP (100 000 c.p.m. of ^3H]GTP and 150 000 c.p.m. of $[\gamma\text{-}^{32}\text{P}]\text{GTP}$) and complex A (125 μg of enzyme protein containing 220 pmol of ^{14}C]phenylalanyl-tRNA). $\circ$, ^3H ; $\bullet$, ^{32}P ; Δ , ^{14}C .

Fig. 9. Binding of (a) complex C and (b) complex A to ribosomes

Incubation mixtures for the binding of complex C contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, complex C (16.8 μ g of protein with 0.28 pmol of aminoacyl-[³²P]-tRNA and 20 pmol of [³H]GTP bound, isolated as described in the Materials and Methods section) and 180 μ g of ribosomes. Mixtures for the binding of complex A contained in a final volume of 0.1 ml: 50 mM-tris-HCl buffer, pH 7.5, 6 mM-MgCl₂, 50 mM-KCl, 20 μ M-GTP, complex A (12.4 μ g of protein with 0.28 pmol of aminoacyl-[³²P]tRNA) and 180 μ g of ribosomes. Both mixtures were incubated at 37°C for the time indicated. $\circ$, ³H; $\bullet$, ³²P.

of GTP to the complex to the same extent as for the aminoacylated species. These results indicate that the charging of tRNA is not essential for the combination of GTP with the binding enzyme.

No significant splitting of GTP to GDP could be observed during the formation of complex C (Figs. 5–7).

Binding of aminoacyl-tRNA to ribosomes in the free form and from its complexes with the binding enzyme

When the complete system (i.e. transferase I, aminoacyl-tRNA, GTP and ribosomes) is incubated at 37°C, the quantity of aminoacyl-tRNA bound to ribosomes increases linearly for 20 min, after which a saturation value is reached. However, the binding of GTP under these conditions proceeds more quickly and the amount of this nucleotide bound to ribosomes decreases significantly to the end of the incubation period (30 min). A high proportion of GTP is cleaved to GDP, in particular at the later periods of incubation (Fig. 8).

The binding of aminoacyl-tRNA from complex

A to ribosomes proceeds much more rapidly and the time required for the maximal bindings of both aminoacyl-tRNA and GTP to ribosomes is apparently the same. In this case again, GTP is rather rapidly released from the ribosome and a high proportion of it is split to GDP (Fig. 9). When ribosomes are incubated with complex C, essentially all the aminoacyl-tRNA and GTP present in the presynthesized complex are bound to ribosomes, even at 0°C, in a few seconds. Already after 2 min of incubation at 37°C a significant release of GTP could be demonstrated together with the cleavage of this nucleotide (Fig. 9).

No cofactors are required for the binding of both aminoacyl-tRNA and GTP to the ribosomes except in the presence of Mg²⁺. Whereas the binding of aminoacyl-tRNA to ribosomes shows a rather broad optimum Mg²⁺ concentration in the range 5–20 mM, the optimum requirement for the binding of GTP is very sharp (6 mM) and higher concentrations of Mg²⁺ exhibit an inhibitory effect.

When transferase I was preincubated with GTP before the addition of both aminoacyl-tRNA and ribosomes, the binding of both aminoacyl-tRNA and

Fig. 10. Evidence for the binding of aminoacyl-tRNA and GTP to ribosomes

The incubation mixture had the same composition as that described in the legend for Fig. 5 and contained, in addition, 4.5 mg of ribosomes. It was incubated at 37°C for 30 min, chilled in ice and separated by gel filtration as described in the legend for Fig. 5. ^3H and ^{32}P radioactivities in fraction nos. 50–70 were multiplied by 0.1. $\circ$, ^3H ; $\bullet$, ^{32}P ; Δ , ^{14}C .

GTP to ribosomes is rather rapid even at 0°C (Fig. 8). Again a cleavage of GTP was demonstrated at later periods of the incubation.

When the binding enzyme is incubated with deacylated tRNA, GTP and ribosomes only a very small proportion of the tRNA becomes bound to the ribosome. The binding of GTP is also decreased but this decrease is not proportional to the decreased binding of deacylated tRNA. Moreover, low quantities of deacylated tRNA apparently have a stimulating effect on the binding of GTP to ribosome similar to those of aminoacyl-tRNA.

Gel filtration of incubation mixtures with ribosomes shows that radioactivities corresponding to both aminoacyl-tRNA and GTP are eluted together with ribosomes, suggesting that complex C becomes bound to this particle. Only small amounts of complex C could be demonstrated in these mixtures. Relatively high quantities of free nucleotides are also

present, the low $^{32}\text{P}/^3\text{H}$ ratio of which indicated that most of them were GDP (Fig. 10).

In the presence of saturating quantities of the binding enzyme, 50–60 pmol of GTP becomes bound/nmol of ribosomes after incubation of ribosomes in 0.2 mM-GTP and 6 mM- Mg^{2+} with this enzyme at 37°C for 30 min. An extensive cleavage of GTP to GDP occurs during this reaction. These results indicate that GTP may be bound as complex B to the ribosome even in the absence of aminoacyl-tRNA.

Discussion

During the binding reaction in prokaryotes, GTP is split to GDP and a transfer enzyme-GDP complex is released from the ribosome (Waterson *et al.*, 1970). The significance of this cleavage for the binding reaction is not yet fully understood. No cleavage of

Scheme 1. Proposed scheme for the intermediate reactions involved in the binding of aminoacyl-tRNA to rat liver ribosome

GTP apparently takes place during its combination with the T factor to form complex I or II (Ravel *et al.*, 1967). On the other hand, evidence has been presented by Lockwood *et al.* (1969) that the T factor forms a stable complex with GDP that is apparently interconvertible with the complex I through a dynamic equilibrium. The hydrolysis of GTP is ribosome-dependent (Gordon, 1969) and may be involved in the proper fixation of the aminoacyl-tRNA on the ribosomal site required for peptidyl transfer (Lucas-Lenard *et al.*, 1969). My results indicate that the cleavage of GTP occurs in particular at the ultimate steps of the binding reaction on ribosome whereas only insignificant quantities of this nucleotide are split during complex C formation and no cleavage of GTP takes place during the binding of GTP to transferase I to form complex B. Thus the mammalian transferase I is apparently not able to react with GDP.

Results presented in the preceding paper (Hradek, 1972) and the present paper indicate that a scheme different from that operating in bacterial cells can be devised for intermediate steps in the binding of aminoacyl-tRNA to rat liver ribosomes (Scheme 1). Different symbols are used for the designation of individual complexes of mammalian transferase I since these products apparently do not exactly correspond to similar complexes in bacterial systems. In mammalian cells, the combination of aminoacyl-tRNA with transferase I is apparently the initial step of the binding reaction. This conclusion is supported by the fact that (a) this complex is readily formed in the absence of GTP, (b) GTP has no effect on the extent of aminoacyl-tRNA binding to the enzyme and (c)

complex A becomes bound to ribosomes even in the absence of GTP. The significance of complex B for the mammalian binding reaction is not quite clear. Unlike the corresponding bacterial complex I, it is apparently not required as an essential intermediate for the formation of complex C. It may well be an artifact not existing in physiological conditions, since GTP becomes readily bound to complex A. However, as for the bacterial complex II, the mammalian complex C apparently represents the key intermediate in the binding of aminoacyl-tRNA to ribosomes. It may help to supply the ribosomes with GTP required for the ultimate steps of the binding reaction. The velocity with which complex C becomes attached to the ribosome indicates that it is indeed a physiological intermediate.

If GTP is required for the fixation of aminoacyl-tRNA to the acceptor site on ribosomes only as suggested by Lucas-Lenard *et al.* (1969), the binding of complex A to the ribosomes in the absence of GTP must represent the binding of aminoacyl-tRNA to ribosomal sites different from the acceptor site. Experiments by Matthaei's group (Swan *et al.*, 1969) indicate that before entering the acceptor site, aminoacyl-tRNA becomes initially attached to additional decoding sites on the ribosome.

Combinations of the binding enzyme with aminoacyl-tRNA or GTP show a relatively low interdependence. The formation and binding of complex A does not require GTP. Although aminoacyl-tRNA stimulates the binding of GTP to the transfer enzyme, for the enhancement of GTP binding to the ribosome in the presence of transferase I the presence of aminoacyl-tRNA is not essential. These facts may indicate that two different enzymes or at least two different subunits of the same enzyme are involved in this sequence of reactions, one of them reacting with GTP and the other with aminoacyl-tRNA. Multiple forms of transferase I were found in rat liver by Schneir & Moldave (1968). Only the use of labelled enzymes may help to elucidate this question.

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