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tRNA and the Guanosinetriphosphatase Activity of Elongation Factor Tu†

Guido W. M. Swart‡ and Andrea Parmeggiani*
Laboratoire de Biochimie, Laboratoire Associe du CNRS 240, Ecole Polytechnique, F-91128 Palaiseau Cedex, France
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ABSTRACT: Different sites of the tRNA molecule influence the activity of the elongation factor Tu (EF-Tu) center for GTP hydrolysis [Parlato, G., Pizzano, R., Picone, D., Guesnet, J., Fasano, O., & Parmeggiani, A. (1983) *J. Biol. Chem. 258*, 995–1000]. Continuing these studies, we have investigated some aspects of (a) the effect of different $tRNA^{Phe}$ species, including Ac-Phe-$tRNA^{Phe}$ and 3′-truncated $tRNA_{-CCA}$ in the presence and absence of codon–anticodon interaction, and (b) the effect of occupation of the ribosomal P-site by different $tRNA^{Phe}$ species. Surprisingly, we have found that 3′-truncated tRNA can enhance the GTPase activity in the presence of poly(U), in contrast to its inhibitory effect in the absence of codon–anticodon interaction. Moreover, Ac-Phe-$tRNA^{Phe}$ was found to have some stimulatory effect on the ribosome EF-Tu GTPase in the presence of poly(U). These results indicate that under specific conditions the 3′-terminal end and a free terminal α-NH_2 group are not essential for the stimulation of the catalytic center of EF-Tu; therefore, the same structure of the tRNA molecule can act as a stimulator or an inhibitor of EF-Tu functions, depending on the presence of codon–anticodon interaction and on the concentration of monovalent and divalent cations. EF-Tu·GTP does not recognize a free ribosomal P-site from a P-site occupied by the different $tRNA^{Phe}$ species. When EF-Tu acts as a component of the ternary complex formed with GTP and aa-tRNA, the presence of tRNA in the P-site strongly increases the GTPase activity. In this case, the extent of the stimulation displays variations dependent on the tRNA species. Ac-Phe-$tRNA^{Phe}$ enhances the GTPase activity more than Phe-$tRNA^{Phe}$ and $tRNA_{OH}$. Whether these differential effects are a consequence of a direct or indirect interaction between EF-Tu and the tRNA species on the ribosome remains to be verified. In conclusion, our data show that the interaction between tRNA and mRNA is pivotal for the specific binding of the ternary complex favoring the activation of the EF-Tu center for GTP hydrolysis by the ribosome.

The study of the GTPase reaction uncoupled from protein synthesis and catalyzed by elongation factor Tu (EF-Tu)[1] has yielded helpful information for an understanding of the mechanism of the coupled one. The hydrolysis of GTP is one of the key reactions for the proper functioning of the factor: formation of the complex with GTP allows EF-Tu to adopt the active conformation needed for a productive interaction with aa-tRNA and the ribosome, while hydrolysis of GTP to GDP induces a conformational transition resulting in dissociation from the ribosome [for reviews, see Kaziro (1978), Parmeggiani and Sander (1981), Bosch et al. (1983), and

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* Author to whom correspondence should be addressed.
‡ Present address: EMBL, Postfach 1022.09, Meyerhofstrasse 1, D-6900 Heidelberg, FRG.

[1] Abbreviations: EF-Tu, elongation factor Tu; GDP, guanosine 5′-diphosphate; GTP, guanosine 5′-triphosphate; P-site, ribosomal donor or peptidyl site; A-site, ribosomal acceptor site; tRNA, general name indicating several transfer RNA species; $tRNA_{OH}$, unacylated transfer RNA; aa-tRNA, aminoacyl-tRNA; $tRNA^{Phe}$, purified phenylalanine-accepting tRNA; $tRNA^{Val}$, purified valine-accepting tRNA; $tRNA_{-CCA}$, tRNA lacking the 3′-terminal CCA sequence; Ac-aa-tRNA, (*N*-acetylamino)acyl-tRNA.

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Parmeggiani and Swart (1985)]. The role of aa-tRNA has been studied in detail: its aminoacylated 3′-terminus is important for stimulation of the catalytic center of EF-Tu, whereas (an)other tRNA region(s) including the TΨC-loop and -stem, as well as the extra loop, modulate the orientation of the 3′-end with respect to the catalytic center (Campuzano & Modolell, 1980, 1981; Bhuta & Chladek, 1980; Bhuta et al., 1981; Parlato et al., 1981, 1983; Guesnet et al., 1983; Picone & Parmeggiani, 1983). By contrast, the important role of codon–anticodon interaction has not been examined in detail. It must be mentioned that the effects of aa-tRNA or its derived fragments become particularly evident in the presence of an effector of the EF-Tu GTPase (ribosomes or the antibiotic kirromycin).

The interaction between EF-Tu·GTP and aa-tRNA, leading to codon-directed binding of aa-tRNA to the ribosomal A-site, has been considered to be involved in the above-described effects (Lucas-Lenard & Haenni, 1968; Shorey et al., 1971; Kaziro, 1978). This interaction concerns multiple points of contact: besides the 3′-end (see above; Jonák et al., 1979, 1980; Jekowsky et al., 1977; Kruse et al., 1980; Pingoud et al., 1977), the aminoacyl-acceptor arm and the TΨC-stem also interact with the factor (see above; Boutorin et al., 1981, 1982; Wikman et al., 1982). Recently, EF-Tu has been suggested to harbor an additional binding site for a second tRNA molecule (Van Noort et al., 1982, 1985). In this context an interaction between EF-Tu and peptidyl-tRNA on the ribosome has been proposed to be involved in the stimulation of the EF-Tu·GTPase center (Van Noort et al., 1986).

In this work, we have tried to define the role played on the EF-Tu ribosome dependent GTPase activity by (A) the codon–anticodon interaction in the ribosomal A-site, (B) the 3′-CCA end of tRNA, (C) the NH_2-terminal end of aa-tRNA, and (D) the occupation of the ribosomal P-site by different species of tRNA.

Materials and Methods

Sepharose 6B was from Pharmacia Fine Chemicals. All biological components were as previously reported (Chinali et al., 1977; Guesnet et al., 1983; Picone & Parmeggiani, 1983; Swart et al., 1987).

Poly(U)-programmed ribosomes without or with different $tRNA^{Phe}$ species (0.5 nmol of Phe/A_{260} unit) in the P-site ($tRNA_{OH}$, Phe-tRNA, or Ac-Phe-tRNA) were prepared as follows: 0.5 μM ribosomes, 50 μg/mL poly(U), and 0.5 μM $tRNA^{Phe}$ were incubated for 30 min at 37 °C in buffer A, containing 25 mM Tris·HCl, pH 7.8, 80 mM NH_4Cl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 7 mM 2-mercaptoethanol. Ribosomal complexes were separated from free tRNA in a centrifugation step of 3 h at 40000 rpm at 4 °C in a Beckman 50Ti rotor. The pellet was resuspended in buffer A. Alternatively, free tRNA was eliminated by gel filtration over a Sepharose 6B column (20 × 0.9 cm) in buffer A at 4 °C. For this purpose the components were preincubated at 5-fold higher concentrations. The stoichiometry of Ac-[^{14}C]Phe-tRNA per ribosome was between 0.6 and 0.7.

The GTPase activity of EF-Tu, concerning the cleavage of the γ-phosphate, was determined by measuring the liberation of $^{32}P_i$ from [γ-^{32}P]GTP with either sodium molybdate/isopropyl acetate extraction or charcoal treatment as described in the legends of the figures (Parmeggiani & Sander, 1981).

Results

When Codon–Anticodon Interaction Is Possible on the Ribosome, the 3′-Terminus of tRNA Is No Longer Essential for Stimulation of the EF-Tu GTPase. When a ribosomal system without kirromycin and mRNA is used, the interaction of the aminoacylated 3′-CCA end in the ternary complex does not allow the premature hydrolysis of the bound GTP at near-physiological Mg^{2+} concentrations (10 mM or less; Parlato et al., 1983). By contrast, in the presence of kirromycin tRNA always stimulates the GTPase reaction, and the observation that only 3′-truncated $tRNA_{-CCA}$ has an inhibitory effect emphasizes the essential contribution of the 3′-terminus to the observed stimulation (Picone & Parmeggiani, 1983). We wondered if this role would be equally important in the presence of the programmed ribosome. For this purpose we determined the catalytic activity of EF-Tu·GTP at 5 mM Mg^{2+} as a function of tRNA concentration with ribosomes present as positive effectors. In a control experiment, we first confirmed that $tRNA_{OH}$ and $tRNA_{-CCA}$ inhibit the EF-Tu GTPase in the absence of mRNA, forming a ternary complex with EF-Tu·GTP (Figure 1; Picone & Parmeggiani, 1983). Upon addition of poly(U), both $tRNA^{Phe}$ species stimulate the hydrolysis of GTP (Figure 1), whereas the noncognate, unacylated $tRNA^{Val}$ always inhibits the reaction (not shown). Surprisingly, despite the lack of the usual 3′-terminus, $tRNA_{-CCA}$ also stimulates the GTPase reaction. Apparently, the possibility to have codon–anticodon interaction in the A-site (opened after occupation of the P-site) changes the interaction between ribosome, EF-Tu, and/or tRNA and enhances the hydrolysis reaction. Thus, for the first time it appears that tRNA structures other than the 3′-CCA terminus are not only involved in the inhibition of the GTP hydrolysis but also in its stimulation.

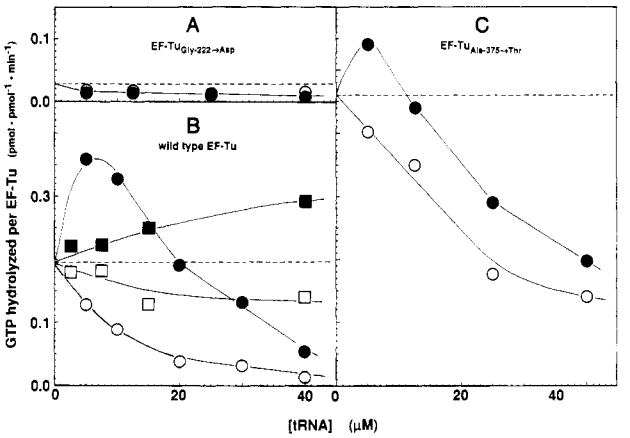

FIGURE 1: Effect of codon–anticodon interaction on the ribosome-induced GTPase activity of EF-Tu. The assay mixture (10 μL) contained 0.5 μM EF-Tu, 0.5 μM elongation factor Ts, 1.2 mM phosphoenolpyruvate, 50 μg/mL pyruvate kinase, 0.6 μM ribosomes, and tRNA as indicated without (open symbols) or with 20 μg/mL poly(U) (closed symbols). The reaction was started by the addition of 16 μM [γ-^{32}P]GTP (2500 cpm/pmol), and after 15 min at 30 °C, it was stopped in 50 μL of 1 N $HClO_4$ containing 1 mM KH_2PO_4. The dashed line indicates the amount of GTP hydrolyzed by EF-Tu without any addition of tRNA and is drawn as a reference; this quantity of GTP was independent of the absence or presence of poly(U). The experimental values were corrected for the blank, representing the amount of GTP hydrolyzed in the absence of EF-Tu and corresponding to 0.12 pmol of GTP/pmol of EF-Tu. Unacylated $tRNA^{Phe}$ (circles); $tRNA^{Phe}_{-CCA}$ (squares). Wild-type EF-Tu (panel B); EF-$Tu_{Gly-222→Asp}$ (panel A); EF-$Tu_{Ala-375→Thr}$ (panel C).

Codon–anticodon interaction similarly influences the GTPase of an EF-Tu mutant with lowered affinity for kirromycin. As shown in Figure 1C, the kirromycin-resistant EF-$Tu_{Ala-375→Thr}$ behaves as wild type; as expected, its GTPase center is more active (Fasano & Parmeggiani, 1981; Swart et al., 1982), and similar stimulatory and inhibitory profiles are observed (Figure 1C). EF-$Tu_{Gly-222→Asp}$, a mutant EF-Tu whose sensitivity to kirromycin is of a recessive nature (also

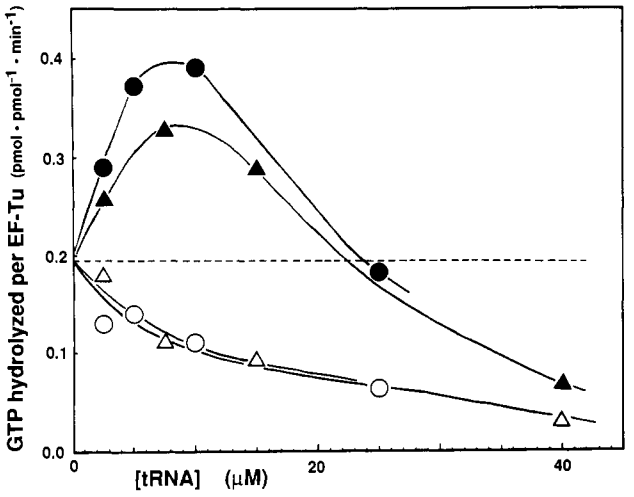

FIGURE 2: Ac-Phe-tRNA influences the EF-Tu ribosome GTPase. Conditions are identical with those in Figure 1. Ac-Phe-tRNA^{Phe} (triangles); unacylated tRNA^{Phe} (circles).

designated EF-TuBo), displays hardly any GTP hydrolysis, and the addition of uncharged tRNA further decreases the reaction, regardless of the presence or absence of mRNA (Figure 1A). This behavior goes along with our observations supporting a deficient interaction between this mutant EF-Tu and the ribosome (Swart et al., 1987).

EF-Tu·GTP Can Catalyze the Binding of Ac-Phe-tRNA to the Ribosomal A-Site. Although binding of $tRNA_{OH}$ and some analogues of peptidyl-tRNA to EF-Tu·GTP has been reported, these interactions remain negligible as compared to the K'_d values found for the complexes between EF-Tu·GTP and aa-tRNA (Pingoud et al., 1977, 1982; Sprinzl & Cramer, 1979; Tanada et al., 1982; Abrahamson et al., 1985). This is in line with the finding of Ravel et al. (1967), using the nitrocellulose filtration method, that EF-Tu·GTP does not interact with Ac-Phe-tRNA. It has been neglected so far whether Ac-Phe-tRNA^{Phe} influences the EF-Tu GTPase activity. Therefore, in this section we have investigated the effect of this tRNA species on the GTP hydrolysis reaction. A functional interaction is evident from the experiments illustrated in Figure 2, indicating that Ac-Phe-tRNA^{Phe} affects the EF-Tu ribosome GTPase in a way similar to unacylated tRNA^{Phe} (see previous section), depending on the absence or presence of poly(U). Moreover, Table I shows that Ac-Phe-tRNA can also be enzymatically bound to the A-site of P-site-blocked ribosomes. As a confirmation for its location in the ribosomal A-site, almost all this Ac-Phe-tRNA was found to be reactive to puromycin only in the presence of elongation factor G and GTP (see legend in Table I). When the concentration of AcPhe-tRNA is raised from 0.4 to 4 μM, both the nonenzymatic binding and the enzymatic binding increase. The inhibition of the GTPase observed in Figure 2 at high tRNA concentrations agrees well with this increase of the nonenzymatic binding. It can be concluded that the accessibility to EF-Tu of the ribosomal center inducing the GTPase is essential for stimulation of the reaction.

Occupation of the Ribosomal P-Site and the GTPase of EF-Tu. In view of the crucial importance of the presence and absence of codon–anticodon interaction for regulating the expression of the GTPase of EF-Tu on the ribosome, as described in the two preceding sections, we wished to test whether or not EF-Tu can recognize different tRNA species located in the P-site.

In the absence of EF-Tu all four ribosomal complexes [programmed with poly(U) and the P-site either free or occupied by $tRNA_{OH}$, Phe-tRNA^{Phe}, or Ac-Phe-tRNA^{Phe}; see Materials and Methods] display the same background activity (Figure 3). This activity is due to the presence of several nucleotidase activities that to a reduced extent remain associated with the ribosomes even after several washes at high

Table I: Enzymatic Binding of Ac-Phe-tRNA or Phe-tRNA to the A-Site of Poly(U)-Programmed Ribosomes As Mediated by EF-Tu[a]

amount of substrate bound (μM)	EF-Tu	kirromycin	Ac-Phe-tRNA bound per ribosome	Phe-tRNA bound per ribosome
0.4	−	−	0.04	0.06
	−	+	0.03	0.06
	+	−	0.11	0.39
	+	+	0.07	0.30
4	−	−	0.16	
	−	+	0.13	
	+	−	0.26	
	+	+	0.24	

[a] The assay mixture (50 μL) contained 0.2 μM ribosomes, 40 μg/mL poly(U), 0.5 μM tRNA^{Phe}, 0.1 mM GTP, 2 mM phosphoenolpyruvate, 50 μg/mL pyruvate kinase, and 0.3 μM EF-Tu without or with 10 μM kirromycin in 50 mM imidazole–acetate, pH 7.5, 7 mM $MgCl_2$, 40 mM KCl, 50 mM NH_4Cl, and 1 mM dithiothreitol. The substrate, Phe-tRNA^{Phe} or Ac-Phe-tRNA^{Phe}, was present in the indicated amounts. Ribosomes, poly(U), and tRNA^{Phe} were preincubated at 30 °C in 10 mM $MgCl_2$ for 15 min. GTP, phosphoenolpyruvate, pyruvate kinase, and EF-Tu were preincubated at 30 °C in 12.5 mM $MgCl_2$ for 15 min. At t = 0 min the acylated tRNA was added; the incubation was for 5 min at 30 °C. Samples of 40 μL were spotted on nitrocellulose filters and washed with 3 mL of buffer. AcPhe-tRNA was added as an equimolar mixture with uncharged tRNA^{Phe}. The puromycin reaction confirmed the A-site location of Ac-Phe-tRNA on the ribosome. In addition to the binding assay, the puromycin reaction was performed on 50 μL of the reaction mixture (the total volume was 150 μL in this case), straight or after addition of 0.1 μM elongation factor G and prolonging the incubation for 10 min; the samples were cooled in ice, and then puromycin was added to a final concentration of 1 mM and allowed to react for 1 h at 0 °C. Then 240 μL of 1 M NH_4HCO_3 was added to the 60-μL mixture, and AcPhe-puromycin was extracted with 450 μL of ethyl acetate. Without the addition of EF-G, no puromycin reaction occurred at all; after the addition of EF-G, 70% of the bound Ac-Phe-tRNA reacted with puromycin.

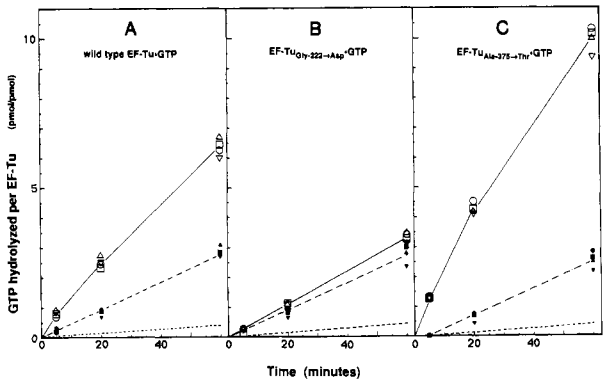

FIGURE 3: EF-Tu·GTP GTPase activity stimulated by poly(U)-programmed ribosomes with different tRNA^{Phe} species occupying the P-site. The assay mixture (100 μL) contained 0.12 μM EF-Tu, 0.12 μM elongation factor Ts, 20 μg/mL pyruvate kinase, 3.2 μM [γ-^{32}P]GTP (specific activity was 2000 cpm/pmol), 1 mM phosphoenolpyruvate, and 0.32 μM ribosomal complexes in buffer A (see Materials and Methods). The incubation was at 37 °C, and at the indicated times samples of 25 μL were taken, and the reaction was stopped in 75 μL of 1 N $HClO_4$ containing 1 mM KH_2PO_4. The plotted values are corrected for the blank (less than 1% of total radioactivity), representing the amount of GTP hydrolyzed in the absence of EF-Tu and ribosomes. The experimental curves were extrapolated to zero time. No tRNA (○); uncharged tRNA^{Phe} (□); Phe-tRNA^{Phe} (△); Ac-Phe-tRNA^{Phe} (▽). Two control curves are indicated as well: (1) ribosomes without EF-Tu (-·- and solid symbols for the different tRNA species) and (2) EF-Tu without ribosomes (- - -). Wild-type EF-Tu (panel A); EF-Tu$_{Gly-222→Asp}$ (panel B); EF-Tu$_{Ala-375→Thr}$ (panel C).

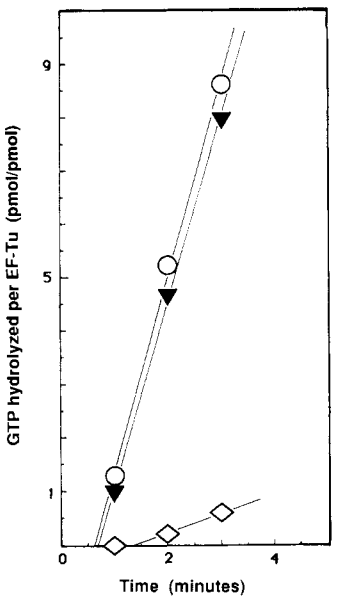

FIGURE 4: GTPase activity of EF-Tu·GTP·kirromycin and occupation of the ribosomal P-site. Conditions are identical with those in Figure 3; kirromycin was 20 μM. The control curve of EF-Tu·GTP·kirromycin without ribosomes is also shown (◇). Mutated EF-Tu factors were not tested. No tRNA (○); Ac-Phe-tRNA (▼).

NH_4Cl concentrations and isolation of "tight couple" 70S ribosomes (Kakhniashvili et al., 1980; A. Parmeggiani, unpublished results). The catalytic activity of EF-Tu·GTP is stimulated by all four complexes to the same level. EF-Tu·GTP seems to be able to distinguish neither between programmed ribosomes with an occupied or free P-site nor between the different tRNA species located in the P-site. Addition of kirromycin to wild-type EF-Tu does not modify the described pattern. Apparently, also EF-Tu·kirromycin is unable to recognize a difference in the ribosomal complexes (Figure 4).

When the ternary complex EF-Tu·GTP·Phe-tRNA is added, GTP turnover becomes much faster, and now the EF-Tu GTPase is differently expressed: ribosomes with an occupied P-site are more effective in stimulating the GTPase activity than those with an unoccupied one (Figure 5). Some distinction also occurs between the different tRNA species; among them, Ac-Phe-tRNA is the best effector. A similar effect was observed in an assay optimizing the in vitro protein synthesis, when the P-site of poly(U)-programmed ribosomes was preoccupied with Ac-Phe-tRNA (Wagner et al., 1982).

Mutant EF-Tu species were also tested for their ability to distinguish the different ribosomal complexes and found to behave the same as wild type. Kirromycin-resistant factors have a higher intrinsic GTPase activity (Fasano & Parmeggiani, 1981; Swart et al., 1982), and GTP hydrolysis is more readily stimulated by ribosomes (Figure 3C). Since Phe-$tRNA^{Phe}$ is not added in saturating amounts and the kirromycin-resistant EF-$Tu_{Ala\text{-}375\rightarrow Thr}$ has lower affinity for aa-tRNA than the wild-type factor (Sam et al., 1985), slightly less GTP is hydrolyzed when the ternary complex is added (Figure 5C). As expected, the GTPase activity of EF-$Tu_{Gly\text{-}222\rightarrow Asp}$ is hardly stimulated by ribosomes (Figure 3B; Swart et al., 1987); nevertheless, its ternary complex can somewhat distinguish the different ribosomal complexes (Figure 5B).

This series of experiments well depicts the complexity of the mechanisms coordinating the effects of ribosomes and aa-tRNA on the catalytic center of EF-Tu. The accessibility of the ribosomal A-site plays an important role for the action of

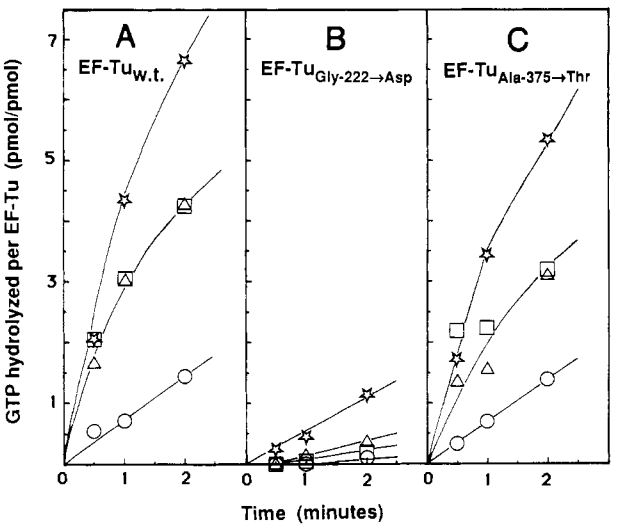

FIGURE 5: EF-Tu·GTP·Phe-tRNA GTPase activity stimulated by poly(U)-programmed ribosomes with different $tRNA^{Phe}$ species occupying the P-site. Conditions are identical with those in Figure 3; Phe-tRNA was added to 0.5 μM. No tRNA (○); uncharged $tRNA^{Phe}$ (□); Phe-$tRNA^{Phe}$ (△); Ac-Phe-$tRNA^{Phe}$ (☆).

ribosomes and aa-tRNA. The ability of EF-Tu as part of the ternary complex to recognize the different species of tRNAs located in the P-site appears to be limited, at least with the GTPase activity as a probe.

Discussion

Our view on the stimulation of the catalytic center on EF-Tu considers the ribosome as the most important effector of the GTPase reaction, while tRNA, mRNA, and ionic environment are more involved in effecting an optimal orientation for the interaction between ribosomes and EF-Tu needed for protein synthesis. Under physiological conditions the induction of the EF-Tu GTPase is almost completely dependent on the accessibility of the ribosomal center that stimulates the hydrolysis reaction and probably makes part of the A-site. This accessibility is conditioned by occupation of the P-site and presence of mRNA, enabling codon–anticodon interaction in the A-site. A nonphysiological condition resulting in opening of the ribosomal center is a raising of the Mg^{2+} concentration. When the above conditions are fulfilled (or in the presence of kirromycin), aa-tRNA can effectively stimulate GTP hydrolysis, and its 3′-terminus plays an important role in this action. Under physiological conditions, the overall conformation of aa-tRNA in the ternary complex is such that the 3-′terminus is unable to stimulate GTP hydrolysis. tRNA structures other than the 3′-end are thought to be responsible for this effect, by controlling the orientation of the 3′-end and via long-range effects mediated by EF-Tu (Parmeggiani et al., 1984).

Whereas previous reports (Parlato et al., 1983) pointed to the negative role of the latter structures on the EF-Tu GTPase, this work shows that they can also act as positive effectors of the reaction under certain experimental conditions. Interestingly, the presence of a free NH_2 group on the aminoacyl residue of aa-tRNA does not seem to be an absolute requirement for enhancing the EF-Tu GTPase in the presence of codon–anticodon interaction. This observation emphasizes the prominent role played by the latter interaction in the stimulation of the EF-Tu GTPase. This study does not question the crucial role of a free NH_2 terminus in aa-tRNA for an efficient and physiological stimulation of the GTPase reaction. As shown in Table I, five times more Phe-$tRNA^{Phe}$ than Ac-Phe-$tRNA^{Phe}$ was enzymatically bound to the ribosome under comparable conditions. Our results point to the

complexity of the interaction between EF-Tu, aa-tRNA, and the ribosome; we feel that partial results obtained in specific systems should be interpreted in their own context and not be directly extrapolated to the physiological situation. Clearly, further investigations of the different parameters are needed for the understanding of the molecular mechanisms regulating GTP hydrolysis.

Conditions decreasing the accessibility of the ribosomal A-site also inhibit the splitting of GTP. At low Mg^{2+} concentrations when unprogrammed ribosomes are present, this is the case for each tRNA species, forming a ternary complex with EF-Tu·GTP. Indeed, in the absence of mRNA, the A-site should not be accessible and the ribosomal P-site should only bind $tRNA_{OH}$ or (analogues of) peptidyl-tRNA (De Groot et al., 1971; Lill et al., 1986; Nierhaus et al., 1986). These conclusions are supported by Guesnet et al. (1983): at 5 mM Mg^{2+} in the absence of mRNA, the stimulation by aa-tRNA of the GTPase activity of EF-Tu·kirromycin is independent of the absence or presence of ribosomes; only at 10 mM Mg^{2+} or higher, ribosomes substantially enhance the reaction. The addition of mRNA causes opening of the A-site (after filling of the P-site), and as a consequence, all cognate tRNA species stimulate the hydrolysis of GTP, but only aa-tRNA can optimally interact with EF-Tu as well as with the ribosome. At high tRNA concentrations the anomalous, cognate ternary complexes have to compete for A-site binding with the corresponding free tRNA species, and this competition turns the initially observed stimulation again into inhibition (this paper).

How can the results reported in this paper be correlated with the recently proposed model (Van Noort et al., 1986) that on the ribosome the GTPase of EF-Tu may be triggered by an interaction with peptidyl-tRNA located in the P-site? In this regard, we have examined whether EF-Tu can recognize a free from an occupied P-site in poly(U)-programmed ribosomes. In the absence of ternary complex, the results were rather clear, and at least under the chosen conditions, EF-Tu was not able to distinguish either the occupation of the P-site or the different species of tRNA herein located, as shown by the lack of any modification on the EF-Tu GTPase. Therefore, in this condition the hydrolysis of GTP appears not to be influenced by a tRNA molecule situated in the P-site. This is true also in the presence of kirromycin, which has been reported to expose a tRNA binding site of EF-Tu different from that involved in the formation of the ternary complex (Van Noort et al., 1982).

A different picture becomes evident when EF-Tu acts as a part of the ternary complex. The GTP hydrolysis is greatly enhanced. Here, there are also some differences in the extent of the evoked GTPase activity, depending on whether Ac-Phe-$tRNA^{Phe}$ or Phe-$tRNA^{Phe}$ or $tRNA_{OH}$ had been located in the ribosomal P-site. Therefore, in this condition peptidyl-tRNA or in general tRNA in the P-site may interact directly with EF-Tu, as proposed by Van Noort et al. (1986). Alternatively, these effects may be indirect and mediated by the ribosome. Indeed, different tRNA species display differential affinities for binding to the ribosomal P-site and influence to a different extent the accessibility of the ribosomal A-site to the ternary complex (De Groot et al., 1971; Lill et al., 1986; Nierhaus et al., 1986). Our experimental system does not distinguish between these two possibilities.

It is noteworthy that our results with truncated tRNA show that a direct interaction between the 3′-end of peptidyl-tRNA and EF-Tu is not condition sine qua non for GTP hydrolysis. This conclusion is in agreement with older observations of the literature. When GTP is replaced by its nonhydrolyzable analogue GMPPCP, EF-Tu·GMPPCP remains associated with the ribosome. In this condition, peptidyl-tRNA, though unreactive with aa-tRNA, conserves its reactivity to puromycin (Haenni & Lucas-Lenard, 1968; Shorey et al., 1971; Kaziro, 1978). In the presence of kirromycin peptide bond formation is inhibited because EF-Tu·GDP does not dissociate from the ribosome; here again, peptidyl-tRNA remains reactive to puromycin (Wolf et al., 1974, 1977). Of course, the possibility of an interaction between EF-Tu and a peptidyl-tRNA region other than the 3′-terminus is not excluded.

We feel that at present too extensive speculations about the kind of interaction between EF-Tu and tRNA in the P-site are premature. Further work is required to elucidate the control mechanisms of the EF-Tu GTPase on the ribosome, a process that due to its crucial importance in the elongation cycle is obviously exposed to a complex regulation at different levels.

We like to propose that with respect of the GTPase activity the relative orientation of EF-Tu and aa-tRNA in the ternary complex is reflected in at least two different conformations. In one conformation the 3′-end of intact aa-tRNA does not stimulate the GTPase; this configuration occurs for the ternary complex in solution. When the conditions for opening of the ribosomal A-site are fulfilled (or in the presence of kirromycin), a conformational transition takes place, changing the positioning of the 3′-terminus, so that it can now stimulate the hydrolysis reaction [compare Parlato et al. (1983)].

Conclusions

The arguments based on the presently available data emphasize the importance of the ribosomal A-site as well as of the different regions of tRNA for the regulation of the EF-Tu GTPase activity. A second molecule of tRNA located in the ribosomal P-site influences the EF-Tu-dependent GTP hydrolysis via an interaction that may be indirect or direct but not necessarily involving its 3′-CCA terminus. The correct interaction of the ternary complex on the ribosome appears to be the fundamental event for triggering the EF-Tu-dependent GTPase reaction during polypeptide synthesis.

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