

Elongation factor Tu, a GTPase triggered by codon recognition on the ribosome: mechanism and GTP consumption

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Abstract: The mechanism of elongation factor Tu (EF-Tu) catalyzed aminoacyl-tRNA (aa-tRNA) binding to the A site of the ribosome was studied. Two types of complexes of EF-Tu with GTP and aa-tRNA, EF-Tu-GTP-aa-tRNA (ternary) and (EF-Tu-GTP)₂-aa-tRNA (quinternary), can be formed in vitro depending on the conditions. On interaction with the ribosomal A site, generally only one molecule of GTP is hydrolysed per aa-tRNA bound and peptide bond formed. The second GTP molecule from the quinternary complex is hydrolyzed only during translation of an oligo(U) tract in the presence of EF-G. The first step in the interaction between the ribosome and the ternary complex is the codon-independent formation of an initial complex. In the absence of codon recognition, the aa-tRNA-EF-Tu complex does not enter further steps of A site binding and remains in the initial binding state. Despite the rapid formation of the initial complex, the rate constant of GTP hydrolysis in the noncognate complex is four orders of magnitude lower compared with the cognate complex. This, together with the results of time-resolved fluorescence measurements, suggests that codon recognition by the ternary complex on the ribosome initiates a series of structural rearrangements that result in a conformational change of EF-Tu, presumably involving the effector region, which, in turn, triggers GTP hydrolysis and the subsequent steps of A site binding.

Key words: translation, A site, codon recognition, fluorescence, stopped-flow.

Résumé : Le mécanisme de liaison de l' aminoacyl-ARNt (aa-ARNt) au site A du ribosome, catalysée par le facteur d'élargissement Tu (EF-Tu), a été étudié. Deux types de complexes entre l'EF-Tu, le GTP et l'aa-ARNt se forment in vitro selon les conditions expérimentales : un complexe ternaire, EF-Tu-GTP-aa-ARNt et un complexe quinaire (EF-Tu-GTP)₂-aa-ARNt. Généralement, lors de l'interaction avec le site A ribosomique, seulement une molécule de GTP est hydrolysée pour chaque aa-ARNt lié et chaque liaison peptidique formée. La seconde molécule de GTP du complexe quinaire est hydrolysée seulement lors de la traduction d'un segment oligo(U) de l'ARNm en présence de EF-G. L'interaction entre le complexe ternaire et le ribosome débute par la formation d'un complexe initial indépendamment du codon. Si le codon n'est pas reconnu, le complexe aa-ARNt-EF-Tu reste à l'étape initiale et il ne passe pas aux étapes suivantes de la liaison au site A. Malgré la rapidité de la formation du complexe initial, la constante de la réaction d'hydrolyse du GTP par le complexe non usuel est 4 ordres de grandeur plus petite que celle de l'hydrolyse par le complexe usuel. Ces résultats et ceux des déterminations de fluorescence en fonction du temps suggèrent que la reconnaissance du codon par le complexe ternaire sur le ribosome initie une série de réarrangements structuraux résultant en un changement de configuration d'EF-Tu, dans lequel interviendrait la région effectrice, qui déclenche l'hydrolyse du GTP et la poursuite des étapes de la liaison au site A.

Mots clés : traduction, site A, reconnaissance du codon, fluorescence, méthode à flux interrompu.

[Traduit par la rédaction]

Introduction

The well-known function of EF-Tu in protein biosynthesis is to promote the binding of the correct aa-tRNA to the ribosome

in response to the codon present in the A site. The involvement in aa-tRNA binding of a protein factor that is regulated by GTP binding and hydrolysis appears to be of key importance for the accuracy and the speed of the process. EF-Tu-GTP

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Abbreviations: EF-Tu, elongation factor Tu; aa-tRNA, aminoacyl-tRNA; HPLC, high-pressure liquid chromatography; XTP, xanthosine triphosphate; EF-G, elongation factor G; mant-dGTP, 3'-O-(N-methylanthranilyl)-2-deoxyguanosine triphosphate.

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forms a complex with aa-tRNA that enters the ribosomal A site rapidly. The interaction, which depends on the presence of the aminoacyl group at the 3' terminus of the aa-tRNA, involves the acceptor domain of the aa-tRNA. The affinity of the complex is very high; thus peptidyl transfer to the aminoacyl residue before GTP hydrolysis and release from EF-Tu is efficiently prevented. Provided that the anticodon of the aa-tRNA is cognate to the codon in the A site, the GTPase of EF-Tu, which otherwise is very low, is strongly stimulated. The structural transition of EF-Tu, resulting from GTP hydrolysis, in turn lowers the affinity of aa-tRNA binding by about the same factor and leads to the release of the aa-tRNA from the factor to enter the reactive state in the A site. In the event that there is no matching (or near matching) codon-anticodon interaction, GTP hydrolysis is not stimulated and the aminoacyl end of the aa-tRNA in the initial binding complex remains protected against peptide transfer until the EF-Tu-GTP-aa-tRNA complex dissociates from the ribosome. The properties of the complex pertinent for the function of screening the anticodon with respect to the match with the codon have not yet been characterized.

How cognate (or near cognate) codon anticodon interaction affects the GTPase of EF-Tu is an open question. The stimulation of the GTPase is due, at least in part, to the interaction of EF-Tu with an activating center on the 50S ribosomal subunit that is controlled by codon anticodon interaction. To establish the control, the event of codon recognition on the 30S subunit has to be communicated to the GTPase center on the 50S subunit. The molecular mechanism of this long-range communication on the ribosome has not yet been determined.

Reports suggesting that the functional complex of EF-Tu with aa-tRNA may be the quinternary complex (EF-Tu-GTP)₂-aa-tRNA (Ehrenberg et al. 1990a), in contrast with the classic ternary complex EF-Tu-GTP-aa-tRNA (Miller and Weissbach 1977; Kaziro 1978), and that two molecules of GTP are hydrolyzed during A-site binding of aa-tRNA (Ehrenberg et al. 1990a; Bilgin et al. 1992; Weijland and Parmeggiani 1993) have raised new questions about the functional interactions between EF-Tu and the ribosome during translation.

Composition of EF-Tu-GTP-aa-tRNA complex

It was suggested by Ehrenberg et al. (1990a, 1990b, 1990c) that a quinternary complex (EF-Tu-GTP)₂-aa-tRNA, rather than the generally accepted ternary complex, EF-Tu-GTP-aa-tRNA, may be the substrate for polypeptide synthesis because two molecules of GTP were found hydrolyzed by EF-Tu for each elongation cycle. The issue proved controversial, and the existence of the quinternary complex could not be confirmed by a number of groups (Bensch et al. 1991; Nissen et al. 1994; Leberman 1995). The discrepancies can be explained in part by the different temperature and buffer conditions used. Recently, it was shown that the stoichiometry in the ternary complex switches from 2:1 (EF-Tu to aa-tRNA) to 1:1 on lowering the temperature from 37°C to 0°C (Bilgin and Ehrenberg 1995). This observation is consistent with the results of Bensch et al. (1991) who, at temperatures between 0°C and 6°C, observed a 1:1 complex by the aa-tRNA protection assay, fluorescence titration, and gel filtration.

Furthermore, the relative concentration of the factor and aa-

tRNA affects the composition of the complex formed (Rodnina and Wintermeyer 1995). Using gel filtration on Superdex75 at room temperature, we observed a stoichiometry of 2:2:1 in the complex formed in excess of EF-Tu, or at stoichiometric amounts of EF-Tu and aa-tRNA, while in excess of aa-tRNA over EF-Tu, a 1:1:1 (ternary) complex was formed. Thus, not only is a stable quinternary complex formed, but its formation by binding of a second EF-Tu-GTP to an existing ternary complex appears to be favored, at least under our experimental conditions.

Replacement of GTP with some nonhydrolyzable GTP analogues (GTPγS, caged GTP), did not change the composition of the quinternary complex. In contrast, the complex containing GMPPNP during gel filtration reproducibly eluted slightly later than the quinternary complex, indicating that this analogue leads to the formation of the ternary complex. Although we have no mechanistic explanation for this observation, it is consistent with the gel filtration experiments of Nissen et al. (1994), who found a clear 1:1 stoichiometry on purification of a EF-Tu-GMPPNP-Val-tRNA^{Val} complex by HPLC. The preference for a certain type of complex, either quinternary or ternary, apparently is governed by ionic conditions, temperature, relative concentration of components, and the nature of the guanine nucleotide.

The conclusion from these studies is that both quinternary and ternary complex could be formed in vitro. It is therefore difficult to predict which type of complex is relevant in vivo. For the ratio of EF-Tu to tRNA in *Escherichia coli* cells, estimates from 1:2 to 2:1 were given (Bremer and Dennis 1987); hence, the uncertainty, or the variation, is in the range in question. Furthermore, the argument that at 37°C only a quinternary complex can be formed (Bilgin and Ehrenberg 1995) is not unambiguous, since *E. coli* cells are able to grow at lower temperatures as well. It is conceivable that both quinternary and ternary complexes are present in the cell at the same time, and it remains to be seen whether the two types of complexes differ in functional properties.

GTP consumption of EF-Tu during translation of heteropolymeric mRNA

Early studies performed in partial translation systems lacking EF-G suggest that the number of GTP molecules hydrolyzed on EF-Tu per peptide bond is close to one (Gordon 1969; Thompson and Stone 1977; Miller and Weissbach 1977; Kaziro 1978). Furthermore, in complete translation systems, one GTP was reported to be hydrolyzed by EF-Tu per cognate peptide bond formation (Ruusala et al. 1982; Kakhniashvili et al. 1986). In contrast, Ehrenberg and colleagues (Ehrenberg et al. 1990a, 1990b, 1990c; Bilgin et al. 1992; Scoble et al. 1994), using a burst technique to distinguish GTP hydrolysis by EF-Tu and by EF-G, observed that two molecules of GTP are hydrolyzed by EF-Tu per incorporated phenylalanine on poly(U)-programmed ribosomes. Moreover, the consumption of two GTPs per peptide bond was reported to be independent of the composition of the EF-Tu-aa-tRNA complex (Dincbas et al. 1995). The hydrolysis of two nucleoside triphosphates per incorporated phenylalanine was also found by Weijland and Parmeggiani (1993) who worked with mutant EF-Tu (D138N) that used XTP rather than GTP. This is another approach to avoid the problem inherent in complete protein

Table 1. GTP consumption of EF-Tu during A-site binding and translation.

mRNA	EF-G	GTP hydrolyzed or bound	
		Second codon*	Third codon
...AUGUUUACG...	–	1.0	—
...AUGUUUACG...	+	1.1	0.9
...AUGUUUUUC...	–	1.1	—
...AUGUUUUUC...	+	1.9	0.9
Poly(U)		1.0	

*The first codon is AUG. In the case of heteropolymeric mRNAs, fMet-tRNA^{fMet} is bound to the ribosomes in the presence of initiation factors. In the case of poly(U), the first tRNA is AcPhe-tRNA^{Phe} bound to the P site; the presence or absence of initiation factors and nonlabeled GTP has no influence on the measured GTP consumption by EF-Tu.

synthesis systems, i.e., the GTPase activity of EF-G, which is strongly stimulated by ribosomes and not necessarily coupled to peptide bond formation and translocation.

Recently, we designed an alternative method to measure the GTP consumption of the EF-Tu·GTP·aa-tRNA complex on ribosome binding (Rodnina and Wintermeyer 1995). We prepared the complex of EF-Tu, GTP, and aa-tRNA under conditions in which the quinternary complex is formed and then purified the complex by gel filtration. In this way, the problem of the GTPase of EF-G was avoided, since radioactively labeled GTP was present only in the stable EF-Tu complex, and exchange of GTP between the complex and EF-G was minimized by short incubation times. Equally important for obtaining unambiguous stoichiometry data is the fact that the ribosomes used were fully active in all partial reactions of initiation and elongation and that ribosomal complexes were prepared by initiation on heteropolymeric mRNAs.

We examined the GTP consumption on the A-site binding of the quinternary complex (EF-Tu·GTP)₂·aa-tRNA in the presence of either of two different heteropolymeric mRNAs or of poly(U) (Table 1). In all cases, only one of two GTPs in the complex was used per aa-tRNA bound and peptide bond formed, regardless of the mRNA used. This result varies from the results reported previously (Bilgin et al. 1992; Weijland and Parmeggiani 1993). At present, there is no entirely consistent explanation for the discrepancy. Since the ribosome system that we are using is fully active in all partial reactions, we are confident that the 1:1 stoichiometry observed is the correct one.

The completion of the elongation cycle by EF-G-dependent translocation did not increase the GTP consumption by EF-Tu as long as heteropolymeric mRNA sequences are translated. We concluded that generally only one of the two GTPs in the quinternary complex is hydrolyzed during one full elongation cycle. Both GTPs of the quinternary complex were hydrolyzed only on incorporation of the first Phe when adjacent Phe codons, UUUUUC, were translated in the presence of EF-G. This result is in keeping with the 2:1 stoichiometry obtained when poly(U) is translated (Ehrenberg et al. 1990a, 1990b; Scoble et al. 1994; Weijland and Parmeggiani 1993). It is likely that the hydrolysis of the additional GTP (or XTP) is due to the translation of the stretch of uridines.

The present results show that the hydrolysis of two molecules of GTP per amino acid incorporated is the exception rather than the rule and that it requires (i) a homopolymeric stretch of the mRNA and (ii) the action of EF-G. The latter feature strongly suggests that the hydrolysis of the second GTP is related to the translocation that follows A-site binding and peptide bond formation. It is not likely to be involved in the mechanism of A-site binding or in controlling the accuracy of aa-tRNA selection, as proposed previously (Weijland and Parmeggiani 1994).

Homopolymeric stretches in mRNA are known to be slippery in that they are prone to ribosomal frame-shifting by also providing the cognate codon in the +1 or –1 frame (Atkins et al. 1990; Fu and Parker 1994). There is also evidence suggesting that EF-Tu has a role in frame-shift suppression (Hughes et al. 1987; Vijgenboom and Bosch 1989; Tuohy et al. 1990). On the basis of these results, our present working hypothesis to explain the increased GTP consumption by EF-Tu during translation of homopolymeric mRNA stretches is that it may be related to the reversal of a shift of the anticodon of the peptidyl-tRNA on the mRNA that took place in the transition state of translocation. In this restricted sense, an interaction of EF-Tu·GTP with peptidyl-tRNA on the ribosome (Sprinzl 1994) may be involved.

A frame-shift correction function implies that the second EF-Tu·GTP of the quinternary complex remains bound to the ribosome while EF-G·GTP enters and promotes translocation. The binding of the two factors has generally been described as exclusive, although in most cases the competition between EF-G·GTP and EF-Tu·GTP·aa-tRNA has been demonstrated, leaving open the situation with EF-G·GTP and EF-Tu·GTP. Furthermore, recent evidence suggesting a synergism of EF-Tu and EF-G in promoting (uncoupled) GTP hydrolysis on vacant ribosomes (Mesters et al. 1994a) can be interpreted as indicating the simultaneous presence of both factors on the ribosome. Our current experiments are aiming at substantiating this (at present rather speculative) model that implies a novel function of EF-Tu in helping to maintain the correct frame in translation.

Codon recognition in the A site by the EF-Tu·GTP·aa-tRNA complex is preceded by the formation of an initial complex

To study the events during A-site binding that lead to GTP hydrolysis, we have established the use of rapid kinetic techniques (Rodnina et al. 1993, 1994). GTP hydrolysis and peptide bond formation were followed by the quench-flow technique, and the conformational changes of both aa-tRNA and EF-Tu were followed by monitoring the fluorescence changes of fluorophores in the aa-tRNA or on GTP using the stopped-flow technique. The evidence obtained suggests at least six distinct steps in the sequence of A-site binding (Table 2).

The first step in the sequence of interactions between the ribosome and the complex EF-Tu·GTP·aa-tRNA is the condon-independent formation of an initial complex (Rodnina et al. 1993, 1996) that is reported by fluorescent groups located in the central part of the tRNA molecule. The formation of the complex requires neither the presence of the

Table 2. Kinetic steps during A-site binding of ternary complex.

Step	Observed by*	k_{app} (s ⁻¹)†
Initial binding	D loop of tRNA ^{Phe} (StF) U8 of tRNA ^{Phe} (StF)	45
Codon recognition	AC loop of tRNA ^{Phe} (StF)	23
Conformational change	D loop of tRNA ^{Phe} (StF) EF-Tu·mant-dGTP (StF)	20 18
GTP hydrolysis	GTPase (QF)	12
Rearrangement of and aa-tRNA release from	EF-Tu·mant-dGTP (StF)	6
EF-Tu·GDP	D loop of tRNA ^{Phe} (StF)	6
Peptide bond formation	AcPhePhe (QF)	3

*StF, fluorescence stopped-flow technique; QF, quench-flow technique.

†0.3 μM ribosomes, 20°C, 10 mM MgCl₂.

correct codon in the A site nor the presence of mRNA in general. The binding site is not physically coinciding with the A site, since preoccupation of the A site does not inhibit the formation of the initial complex. The initial complex is labile, the rate constant of binding and dissociation being 6×10^7 M⁻¹ s⁻¹ and 22 s⁻¹, respectively, for EF-Tu·GTP·Phe-tRNA^{Phe} and EF-Tu·GTP·Leu-tRNA^{Leu} (20°C, 10 mM Mg²⁺). The formation of the complex strongly depends on the concentration of Mg²⁺, and about five Mg²⁺ ions are involved in the interaction. The extrapolation of the rate constants to physiological conditions with respect to Mg²⁺ concentration (3 mM) and temperature (37°C) yields rate constants for the formation and dissociation of the initial complex of about 10⁶ M⁻¹ s⁻¹ and 70 s⁻¹, respectively.

During the initial binding of the ternary complex, the anticodon is screened for codon recognition, and a nonmatching ternary complex is rejected. It has been shown previously that, even at a several hundred-fold excess of noncognate ternary complexes over the cognate one, the synthesis of poly(Phe) in the poly(U) system was not inhibited (Bilgin et al. 1988), albeit at rather low concentrations. We have performed computer simulations using the kinetic parameters of the initial binding of both cognate and noncognate ternary complexes and the rate constants of subsequent steps of cognate ternary complex binding (Rodnina et al. 1996) to check the competition by noncognate ternary complexes at in vivo concentrations (Bremer and Dennis 1987). Assuming concentrations of ribosomes and cognate ternary complex of 0.25 and 0.01–0.1 mM, respectively, we found that the rate of incorporation of a cognate amino acid is inhibited by 50% at 1 mM concentration of noncognate ternary complexes and by 80% at 2.4 mM. At those concentrations, where initial binding of a cognate ternary complex is running at a rate of 300 s⁻¹, even a fivefold inhibition to 60 s⁻¹ would probably not make the initial binding the rate-limiting step of elongation.

GTP hydrolysis is triggered by a conformational change of EF-Tu induced by codon recognition

The following step of codon recognition leads to a series of conformational changes in both aa-tRNA and EF-Tu. Reading

the codon in the A site leads first to the rearrangement of the anticodon loop of the aa-tRNA. The emission spectrum of wybutine in tRNA^{Phe} is blue shifted on binding the codon triplet in the A site (Paulsen et al. 1982); this indicates that, by binding to the codon, the stacking interactions of wybutine with the neighboring adenines are changed, i.e., that the conformation of the anticodon loop changes. The conformational change induced by the codon binding is further transmitted through the tRNA molecule in the region of the D loop (Rodnina et al. 1993). On the basis of the fluorescence and quenching data, it is likely that the structure of the D loop is unfolded, resulting in a transient tRNA intermediate that has the D loop more exposed to the solvent excess than in the ternary complex, or bound to the A site (Rodnina et al. 1994). Finally, the conformation of the G domain of EF-Tu is altered, as observed by a fluorescent GTP derivative, mant-dGTP (Rodnina et al. 1995). Thus, recognition of a cognate codon provides a signal that is transmitted to originate a series of coupled conformational changes of aa-tRNA and EF-Tu in the complex.

The fluorescence of the GTP derivative, mant-dGTP, is increasing on binding to EF-Tu. However, it is not affected further by binding of aa-tRNA or kirromycin and by hydrolysis; the EF-Tu·mant-dGTP·Phe-tRNA^{Phe} and EF-Tu·mant-dGDP complexes have the same fluorescence (Rodnina et al. 1995). According to the structural studies, the ribose moiety of the nucleotide in EF-Tu is positioned such that the 2'- and 3'-hydroxyl groups are pointing towards the solvent and should not be protected in the complex with the protein (Kjeldgaard and Nyborg 1992). Thus, the mant group at the 3' position in mant-dGTP is also expected to be exposed in the complex with EF-Tu, in accordance with fluorescence quenching data (Eccleston et al. 1989; Rodnina et al. 1995). On binding to the ribosome, mant-dGTP in the complex with EF-Tu and aa-tRNA shows a transient increase in fluorescence. However, this effect is not caused by the protection by the ribosome, since the accessibility of the fluorescent group to the solvent, as judged from transient quenching experiments, remains the same on binding to the A site as in the ternary complex (Rodnina et al. 1995). Thus, the observed conformational change represents the structural rearrangement of the G domain of EF-Tu on binding of the ternary complex to the A site. It reflects the formation of a structural intermediate of EF-Tu that requires correct codon recognition and precedes GTP hydrolysis but corresponds neither to the GTP nor the GDP form of EF-Tu. These data suggest that the observed conformational change in EF-Tu corresponds to the structure of the factor in the activated state before GTP hydrolysis.

Interestingly, kirromycin is keeping EF-Tu in the high fluorescence state on the ribosome (Rodnina et al. 1995). This suggests that the antibiotic freezes the factor not only in a GTP-like conformation with respect to the arrangement of the structural domains of the protein (Parmeggiani and Swart 1985; Mesters et al. 1994b), but in a conformation of the G domain that represents the activated state of the enzyme with respect to GTP hydrolysis (GTPase state). In this state, the emission spectrum of many is blue shifted about 7 nm, indicating a more hydrophobic environment of the fluorophor relative to the initial state (Rodnina et al. 1995). According to the crystal structure of EF-Tu·GMPPNP from thermophiles (Berchtold et al. 1993; Kjeldgaard et al. 1993), the ribose moiety of the nucleotide is located close to the effector region (amino acids 41–62), the closest

Table 3. Binding and GTPase of EF-Tu-GTP-Phe-tRNA^{Phe} on poly(U)- and poly(A)-programmed ribosomes.

mRNA	Binding ($\mu\text{M}^{-1}\cdot\text{s}^{-1}$)	GTPase (s^{-1})
Poly(U)	70	>12*
Poly(A)	60	2×10^{-3}
No ribosomes		2×10^{-5}

*Value obtained at nonsaturating amounts of ribosomes (0.3 μM).

amino acid being Tyr 47 (or Phe 46 in the *E. coli* protein). Thus, it is conceivable, though at present speculative, that it is a movement of the effector region towards the GTP site in the G domain that causes the observed fluorescence change and constitutes the trigger of GTP hydrolysis.

The hydrolysis of GTP in the complex follows the conformation rearrangements described above and proceeds with rate of about 12 s^{-1} under our experimental conditions (20°C, 10 mM Mg^{2+}) (Rodnina et al. 1995). To understand the functional role of quinternary versus ternary complex, it is interesting to compare the rates of the ribosome-stimulated GTP hydrolysis in both types of complexes. As described above, the composition of the EF-Tu-GTP complex with aa-tRNA depends on the relative amounts of factor and tRNA (Rodnina and Wintermeyer 1995). At the conditions used in this experiment, the quinternary complex (EF-Tu-GTP)₂-Phe-tRNA^{Phe} is formed. Control experiments, using the true ternary complex, have revealed the same kinetic picture. While a cognate ternary complex from initial binding rapidly proceeds through codon recognition and GTP hydrolysis, the binding of a non-cognate ternary complex, which leads to the initial binding complex only, does not promote subsequent fast GTP hydrolysis. In fact, the rate constant of GTP hydrolysis in the non-cognate complex is $2 \times 10^{-3}\text{ s}^{-1}$ (Table 3), four orders of magnitude lower than that of the cognate ternary complex, despite the rapid formation of the noncognate initial complex. This suggests that codon-anticodon recognition, which takes place after the formation of the initial binding complex, provides a specific signal that triggers fast GTP hydrolysis by EF-Tu on the ribosome.

A conformational signal created by codon-anticodon interaction is transmitted to EF-Tu and triggers GTP hydrolysis

The question remains as to how codon-anticodon interaction, conformational change of EF-Tu, and GTP hydrolysis are related to each other. Since the formation of the observed transient intermediate of EF-Tu strictly requires codon-anticodon interaction, we propose that the conformational change of the anticodon loop, and possibly also of the mRNA, that is induced by codon-anticodon interaction creates a conformational signal that is transmitted to EF-Tu. Previously, the ribosome has been implicated in signalling the event of cognate codon-anticodon interaction to EF-Tu and in activating the GTPase, for example, through structural rearrangements of 23S rRNA (Tappich and Dahlberg 1990; Moazed et al. 1988; Noller 1991) and (or) 16S rRNA (Powers and Noller 1994). Our results suggest that the tRNA may be involved as well. In the crystal structure of the ter-

nary complex, interactions of the acceptor domain of the tRNA with interface residues of domains I and II, as well as of the acceptor and T stems with domain III of EF-Tu, are seen (J. Nyborg, personal communication). The affinity of the interaction is high, thereby keeping the aminoacyl end protected by EF-Tu until codon recognition and subsequent GTP hydrolysis bring about the conformational change to dissociate the complex (Johnson et al. 1986). The interactions between the acceptor domain of the tRNA and the factor may function in the direct communication between the two molecules, thereby providing a potential link between codon-anticodon interaction and GTP hydrolysis by EF-Tu. It has been shown that binding of the codon, or a tRNA with a complementary anticodon, induces a conformation of the tRNA in which tertiary structure interactions between D and T loops are opened (Rigler and Wintermeyer 1983; Moras et al. 1985). Thus, the acceptor domain of the tRNA, on codon-anticodon interaction, may become free to move relative to the anticodon domain and change the interactions with the factor. In this model, the tRNA that has recognized the cognate codon in the A site is acting as an effector of EF-Tu. Presumably, the ribosome is also involved, possibly by providing binding interactions that promote the formation of the activated structure of EF-Tu.

The alternative model is that the rate constants characterizing the ribosome's interaction with EF-Tu, including the rate constant of GTP hydrolysis, are independent of the nature of aa-tRNA or the codon exposed in the A site (Thompson 1988). This conclusion was based on the determinations of the rate constants of ribosome-induced GTP hydrolysis in cognate (Phe-tRNA^{Phe}, 25 s^{-1}) and near-cognate (Leu-tRNA^{Leu2}, 4 s^{-1}) ternary complexes compared with GTP hydrolysis by the binary complex EF-Tu-GTP ($>1\text{ s}^{-1}$) (Thompson and Dix 1982; Thompson et al. 1986). Our data clearly show that the ribosome interactions of the noncognate ternary complex and the binary complex are entirely different. The rate constants of binding and dissociation of the noncognate ternary complex are $6 \times 10^7\text{ M}^{-1}\cdot\text{s}^{-1}$ and 22 s^{-1} , respectively; for the binary complex EF-Tu-GTP, the values for the corresponding constants were $5 \times 10^4\text{ M}^{-1}\cdot\text{s}^{-1}$ and 10^{-2} s^{-1} , respectively, determined at somewhat different, but comparable, conditions (Thompson et al. 1986). The most important difference, however, comes from the measurements of the rate constants of GTP hydrolysis for the two types of complexes. For the binary complex, the rate of GTP hydrolysis is limited by the rate of the binding of EF-Tu-GTP to the ribosome ($5 \times 10^4\text{ M}^{-1}\text{ s}^{-1}$). In contrast, for the ternary complex, the rate of the bimolecular reaction ($6 \times 10^7\text{ M}^{-1}\text{ s}^{-1}$) is not limiting at the concentrations that we have used. The rate constant of ribosome-induced GTPase in the noncognate ternary complex is $2 \times 10^{-3}\text{ s}^{-1}$, only 40 times faster than the intrinsic GTPase in the unbound complex ($5 \times 10^{-5}\text{ s}^{-1}$) (Table 3). In comparison, in the presence of correctly programmed ribosomes, the rate of GTP hydrolysis is 12 s^{-1} , which is four orders of magnitude faster than the intrinsic GTPase. This provides further evidence that codon recognition constitutes a necessary signal for triggering fast GTP hydrolysis in the ternary complex on the ribosome.

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