Volume 217, number 2, 203–211 FEB 04822 June 1987

Review Letter

Structural features in aminoacyl-tRNAs required for recognition by elongation factor Tu

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Received 30 January 1987; revised version received 23 March 1987

In bacterial polypeptide synthesis aminoacyl-tRNA (aa-tRNA) bound to elongation factor Tu (EF-Tu) and GTP is part of a crucial intermediate ribonucleoprotein complex involved in the decoding of messenger RNA. The conformation and topology as well as the affinity of the macromolecules in this ternary aa-tRNA·EF-Tu·GTP complex are of fundamental importance for the nature of the interaction of the complex with the ribosome. The structural elements of aa-tRNA required for interaction with EF-Tu and GTP and the resulting functional implications are presented here.

Protein synthesis; aminoacyl-tRNA; Elongation factor Tu; Ternary complex

1. INTRODUCTION

During protein biosynthesis, peptide chain initiation is followed by a series of peptide chain elongation events prior to chain termination that releases the completed polypeptide. The molecular mechanisms involved in these different steps are not entirely elucidated [1,2].

In the course of elongation, the bacterial elongation factor Tu(EF-Tu) forms a complex with an aminoacyl-tRNA (aa-tRNA) in the presence of GTP, and the resulting aa-tRNA · EF-Tu · GTP ternary complex binds to the ribosomal A site during each elongation cycle. This ternary complex undergoes a series of functional states during processes such as codon-anticodon recognition, proofreading, activation of the intrinsic GTPase activity of EF-Tu and interaction with the peptidyltransferase. To understand the function of the ternary complex, knowledge of the topology of this ribonucleoprotein complex is essential since it determines the molecular interactions that can occur during the initial binding step at the ribosomal A site.

Several approaches have been used to study ternary complex formation from a qualitative and quantitative point of view, such as (i) gel filtration, (ii) affinity chromatography of aa-tRNA using immobilized EF-Tu · GTP, (iii) chemical modifications of tRNA and of EF-Tu, (iv) nuclear magnetic resonance and electron spin resonance spectroscopy, (v) oligonucleotide binding, (vi) stimulation of the GTPase activity of EF-Tu, (vii) nitrocellulose filter binding, (viii) protection of aa-tRNA by EF-Tu · GTP from deacylation and from digestion by RNase A, and (ix) fluorescence labeling of aa-tRNA and fluorescence measurements upon ternary complex formation.

The structure, function and properties of EF-Tu [3,4], and the CCA end of tRNA and its role in

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

protein biosynthesis [5,6] have been extensively described. The structural features in the aa-tRNA (aminoacyl residue, CCA end or other parts of the tRNA) required for recognition by EF-Tu·GTP are reviewed here. The recent use of a plant viral tRNA-like structure and of bovine mitochondrial serine-specific tRNAs as model 'partners' for EF-Tu·GTP has been particularly helpful in defining these features, mostly using *Escherichia coli* EF-Tu.

2. INVOLVEMENT OF THE AMINOACYL RESIDUE

A fundamental requirement for the interaction of tRNA with EF-Tu·GTP is the aminoacyl residue esterified to the 3′-terminus of the tRNA [7–10]. Protection from non-enzymatic hydrolysis of the ester linkage between the aminoacyl moiety and the tRNA is one of the most important functions of EF-Tu·GTP [11].

Dissociation constants for aa-tRNA·EF-Tu·GTP ternary complexes have values between 0.1 nM and 50 μM [12–22]. Uncharged tRNAs form very labile complexes with EF-Tu·GTP, with dissociation contants $\geqslant$0.1 mM [7,23–26]. The formation of the EF-Tu·GTP complex is a prerequisite for effective binding of aa-tRNA. Although complexes between aa-tRNA and EF-Tu·GDP have been observed [25–27] with dissociation constants of ~1 μM [24], no complexes between uncharged tRNA and EF-Tu·GDP have been detected.

Moreover, aa-tRNA rather than uncharged tRNA stimulates the intrinsic GTPase activity of EF-Tu in the presence of ribosomes [28] or of kirromycin [29].

2.1. *The α-amino group*

That the α-amino group of the aminoacyl moiety might be involved in the binding of aa-tRNA to EF-Tu·GTP is suggested by the protection of aa-tRNA in the ternary complex from chemical acetylation [30]. Various chemical modifications have been applied to investigate whether the α-amino group of aa-tRNA is essential for ternary complex formation. (i) Deamination of aa-tRNA, as reported for example for nitrous acid-treated *E. coli* Phe-tRNA^{Phe}, whose α-amino group when substituted by an α-hydroxyl group does not prohibit interaction with EF-Tu·GTP [31] although with a 100-fold lower affinity than native Phe-tRNA^{Phe} [32]; eukaryotic elongation factor 1α (EF-1α), however, is unable to interact with deaminated *E. coli* aa-tRNAs [33]. (ii) *N*-Acetylation of aa-tRNA severely diminishes binding to either EF-Tu·GTP or EF-1α·GTP [33,34]. (iii) Formylation of the α-amino group as shown with *Bombyx mori* Gly-tRNA^{Gly} results in considerable decrease in the affinity for EF-Tu·GTP as compared to the non-formylated species [18].

The experimental findings therefore suggest that the α-amino group generally favours interaction with EF-Tu·GTP, although the presence of this group is not necessary per se for binding since it can be replaced by an α-hydroxyl group. Moreover, *E. coli* Pro-tRNA^{Pro}, which lacks the primary aliphatic α-amino group, forms a stable ternary complex [19]. Chemical modifications of the α-amino group such as acetylation or formylation severely weaken the ability of the modified aa-tRNA to bind to EF-Tu·GTP.

2.2. *The side chain*

The affinities of aa-tRNAs or aa-oligonucleotides towards EF-Tu·GTP are influenced by the nature of the amino acid esterified to the 3′-terminus. This has been demonstrated convincingly using an *E. coli* Gln-tRNA^{Trp} suppressor which binds about 3-fold better to EF-Tu·GTP than the corresponding Trp-tRNA^{Trp} suppressor [15]. Similarly, misaminoacylated *E. coli* Phe-tRNA^{Lys} has a greater affinity for EF-Tu·GTP than Lys-tRNA^{Lys} [35]. Hydrolysis protection experiments with several aminoacylated and misaminoacylated tRNAs indicate that differences in the affinity for EF-Tu·GTP are influenced mainly by the chemical nature of the amino acid side chain. aa-tRNAs with apolar side chains, such as *E. coli* Trp-tRNA^{Trp} are bound much more strongly by EF-Tu·GTP than aa-tRNAs with polar side chains, such as *E. coli* Lys-tRNA^{Lys} [16]. Comparable observations have been made with different aa-oligonucleotides using the nitrocellulose filter binding assay [27]. However, RNase protection experiments with various aminoacylated and misaminoacylated *E. coli* and yeast tRNA derivatives do not confirm this model [17,18].

Depending on the amino acid esterified to the 3′-end, aa-tRNAs, aa-oligonucleotides or aa-nucleosides exhibit different effects on the accessibility of EF-Tu to modifying chemical reagents [25] and on the EF-Tu-dependent GTPase in the presence of antibiotics and ribosomes [36–38].

2.3. *DL-Amino acids*

The D-tyrosyl residue attached to *E. coli* $tRNA^{Tyr}$ can be incorporated in vitro into polypeptide chains [39] raising the question of the discrimination of D-tyrosine from ribosome-dependent incorporation into polypeptides in vivo. However, ternary complex formation with EF-Tu·GTP favours L-Tyr-$tRNA^{Tyr}$ as compared to D-Tyr-$tRNA^{Tyr}$, EF-Tu·GTP does not efficiently protect D-Tyr-$tRNA^{Tyr}$ from non-enzymatic deacylation, and dipeptide formation is faster with L-Tyr-$tRNA^{Tyr}$ than with D-Tyr-$tRNA^{Tyr}$ [40].

3. INVOLVEMENT OF THE CCA END OF tRNA

The single-stranded 3′-CCA end is essential for interaction of aa-tRNA with EF-Tu·GTP as well as for subsequent peptide bond formation ([5,6] and references therein). The 3′-end of aa-tRNA is sheltered in the ternary complex since the CCA end is protected from digestion by RNase A [15,17,18]. Various chemical and enzymatic modifications have been introduced into several nucleobase positions and into the 3′-terminal ribose and their implications on ternary complex formation examined. The most important examples are discussed below.

3.1. *Base modifications*

The formation of a stable complex between aa-tRNA and EF-Tu·GTP requires an intact 3′-terminal CCA end. The length of the single-stranded 3′-end is important for binding of the aa-tRNA to EF-Tu. Thus a modified *E. coli* Phe-$tRNA^{Phe}$-CCA with a 3′-terminal sequence lengthened by one cytosine residue can form a ternary complex, albeit with low efficiency [41].

Several base substitutions or base modifications in positions 73–76 have been investigated. If the terminal adenosine residue in position 76 is replaced by formycin (F, a structural analogue of adenosine) as in yeast Phe-$tRNA^{Phe}$-CCF, the resulting tRNA can still undergo all the individual steps of elongation in vitro, although to a lower extent and at a significantly decreased rate [42].

Yeast Phe-$tRNA^{Phe}$ derivatives substituted in position 75 and 74 by 5-iodocytidine (i^5C), 2-thiocytidine (s^2C) or 5-azacytidine (n^5C) exhibit different substrate properties depending on the modification. Phe-$tRNA^{Phe}$-Ci^5CA or Phe-$tRNA^{Phe}$-i^5Ci^5CA are considerably less active [43] whereas Phe-$tRNA^{Phe}$-Cs^2CA is nearly as active as native Phe-$tRNA^{Phe}$-CCA in polypeptide synthesis [44]. Phe-$tRNA^{Phe}$-Cn^5CA and Phe-$tRNA^{Phe}$-n^5Cn^5CA are both equally active in polypeptide synthesis [45]. The s^2C in Phe-$tRNA^{Phe}$-Cs^2CA has served as a specific site of alkylation with iodoacetamide (I-acm) or iodoacetamide spin labels (I-acmSL). Both products Phe-$tRNA^{Phe}$-Cs^2C(acm)A or Phe-$tRNA^{Phe}$-Cs^2C(acmSL)A form stable ternary complexes [46]. Yeast Phe-$tRNA^{Phe}$-Cs^2CA and Tyr-$tRNA^{Tyr}$-Cs^2CA modified by alkylation with the fluorescent iodoacetamide derivative of naphthylamine sulfonic acid (I-AEDANS) both form stable complexes with *E. coli* EF-Tu·GTP [21,22]. Moreover, EF-Tu·GTP protects the 2-carbon atom of Phe-$tRNA^{Phe}$-Cs^2C(AEDANS)A from nucleophilic attack by β-mercaptoethanol and this again demonstrates that the CCA end of the aa-tRNA is shielded within the ternary complex [21].

Bisulfite-modified *E. coli* Met-$tRNA_f^{Met}$-UCA with a 5′-terminal U_1-A_{72} base pair (instead of C_1 and A_{72}) and U in place of C in position 74 interacts weakly with EF-Tu·GTP as compared to the bisulfite-treated Met-$tRNA_f^{Met}$ whose 3′-terminal CCA end has been repaired [47].

The 3′-terminal CACCA sequence of yeast $tRNA^{Phe}$ has also been modified by replacing the adenosine in position 73 by the rather bulky 1,N^6-ethenoadenosine (ϵA) analogue. The resulting Phe-$tRNA^{Phe}$-CϵACCA derivative is fully competent in ternary complex formation and in polypeptide synthesis [48].

The experimental results obtained to date can be summarized as follows. (i) The correct length of the acceptor end of the tRNA molecule and the presence of a 3′-terminal adenosine are essential for ternary complex formation. (ii) The penultimate cytidine base in position 75 is rather flexible with respect to modifications as demonstrated by

the replacement of C_{75} by 2-thiocytidine, alkylated 2-thiocytidine or 5-azacytidine which do not affect ternary complex formation; however, substitution of C_{75} by 5-iodocytidine interferes with ternary complex formation. (iii) C_{74} is apparently more sensitive to modifications since its substitution by uridine significantly weakens binding of the aa-tRNA to EF-Tu·GTP. (iv) The base in position 73 is probably not involved in ternary complex formation. (v) The single-stranded 3′-end of aa-tRNA, stabilized by stacking interactions, clearly facilitates complex formation with EF-Tu·GTP [5,6].

3.2. *Ribose modifications*

Principally three types of chemical changes in the ribose moiety have been applied: (i) scission of the ribose ring by periodate oxidation and subsequent borohydride reduction, (ii) substitution of the terminal adenosine by 2′- or 3′-deoxyadenosine [(2′d)A or (3′d)A] and (iii) substitution of the terminal adenosine by 2′- or 3′-aminoadenosine [(2′NH_2)A or (3′NH_2)A].

Oxidation and reduction of yeast $tRNA^{Phe}$ leads to $tRNA^{Phe}$(oxi-red) lacking the carbon-carbon bond between the 2′- and 3′-positions of the 3′-terminal ribose. This tRNA can still be acylated by the cognate synthetase, but cannot bind to EF-Tu·GTP [49], nor can it stimulate the EF-Tu-dependent GTPase activity [50].

In yeast Tyr-$tRNA^{Tyr}$ and Phe-$tRNA^{Phe}$ with 2′- or 3′-deoxyadenosine or 2′- or 3′-aminoadenosine at their 3′-end, the aminoacyl residue is specifically bound to either the 3′- or 2′-position. Using these modified tRNAs the effects on ternary complex formation of (i) the amino acid irreversibly fixed in either position of the ribose, (ii) lack of the vicinal hydroxyl group and (iii) replacement of the ester bond by an amide bond were investigated.

Both Tyr-$tRNA^{Tyr}$-CC(2′-d)A and Tyr-$tRNA^{Tyr}$-CC(3′d)A form ternary complexes, but with lower stability than native Tyr-$tRNA^{Tyr}$-CCA [51]. Moreover, Tyr-$tRNA^{Tyr}$-CC(3′d)A with the amino acid attached to the 2′-position interacts more strongly with EF-Tu·GTP than does Tyr-$tRNA^{Tyr}$-CC(2′d)A with the amino acid attached to the 3′-position [16,51]. Experiments with several aa-tRNAs whose amino acid was irreversibly fixed as a 2′- or 3′-aminoacylester showed that both positional isomers are recognized and bound by EF-Tu·GTP [51–53]. Analysis of the distribution of phenylalanine with respect to the 2′- or 3′-ribose position of native *E. coli* Phe-$tRNA^{Phe}$ complexed with EF-Tu·GTP indicates that the 3′-aminoacylated rather than the 2′-aminoacylated tRNA is involved in ternary complex formation [54].

If the ultimate adenosine is replaced by 2′- or 3′-aminoadenosine, again a pair of aa-tRNA isomers is obtained with the amino acid now attached to the tRNA by an amide linkage. With neither Phe-$tRNA^{Phe}$-CC(2′NH_2)A nor with Phe-$tRNA^{Phe}$-CC(3′NH_2)A from yeast can a ternary complex be found [51]. Experiments with *E. coli* Arg-$tRNA^{Arg}$-CC(2′NH_2)A and Arg-$tRNA^{Arg}$-CC(3′NH_2)A showed however that both positional analogues can participate in ternary complex formation, although with lower efficiency than native Arg-$tRNA^{Arg}$-CCA [55].

In conclusion, the vicinal hydroxyl groups of the 3′-terminal ribose of aa-tRNA do not seem to be required for ternary complex formation. On the other hand, replacement of the ester linkage by an amide between the amino acid and the tRNA may influence ternary complex formation.

4. INVOLVEMENT OF OTHER PARTS OF THE tRNA MOLECULE

4.1. *General organization of the ternary complex*

In addition to the 3′-CCA end bearing the aminoacyl residue, other structural features in the tRNA molecule are needed for strong binding of the aminoacyl-tRNA to EF-Tu·GTP as implied by the observations that the fragment UCCACCA-Ala [56], the 3′-half of the *E. coli* Val-$tRNA^{Val}$ molecule [57], or denatured *E. coli* Leu-$tRNA^{Leu}$ [33] fail to form a stable complex with EF-Tu·GTP. The molecular basis of aminoacyl-tRNA recognition by EF-Tu·GTP, has been further investigated in 'footprinting' experiments performed with yeast Phe-$tRNA^{Phe}$ [58,59], and with *E. coli* Phe-$tRNA^{Phe}$ [59,60], Glu-$tRNA^{Glu}$ [59] or Met-$tRNA_m^{Met}$ [59,60] using a variety of single- and double-strand specific ribonucleases (RNase T_1, T_2 or A, and cobra venom ribonuclease). From these studies it emerges that in addition to the single-stranded 3′-CCA end bearing the aminoacyl moiety, the EF-Tu·GTP complex efficiently protects the acceptor and the T stems of aa-tRNAs.

Thus, within the ternary complex, the T loop of

the aminoacyl RNA domain (structure formed by the acceptor stem and the T stem and loop) remains accessible, possibly for interaction with ribosomal RNA, and the anticodon RNA domain (structure formed by the D and the anticodon stems and loops) is still available for codon-anticodon interaction on the ribosome. This overall structural organization of the ternary complex is supported by several other studies that have focused on the parts of the tRNA molecule not involved in ternary complex formation, as discussed below.

4.2. *The T loop*

Apart from the 'footprinting' studies mentioned in section 4.1, experimental data indicating the non-involvement of the T loop in ternary complex formation are still lacking. Interestingly bovine mitochondrial Ser-tRNA^{Ser}s can form a ternary complex with EF-Tu·GTP [61]. Indeed, these tRNAs lack the conserved TΨC sequence in their T loop; thus, this region is not essential for ternary complex formation. The *Staphylococcus epidermis* Gly-tRNA^{Gly} involved in cell wall synthesis which also lacks the conserved TΨC sequence also forms a complex with EF-Tu·GTP although with very low efficiency [17,18].

4.3. *The variable loop*

In *E. coli* Phe-tRNA^{Phe}, the variable region is protected by EF-Tu·GTP against nuclease digestion. However, when its base X_{47} is labeled with fluorescamine, no change in fluorescence is observed upon ternary complex formation [62], suggesting that the variable region although possibly 'covered' to some extent by EF-Tu, is probably not in very close contact with the factor. In any event, the variable region is not believed to be essential for ternary complex formation.

4.4. *The anticodon stem and loop*

The anticodon stem and loop of aa-tRNA in the complex with EF-Tu·GTP remain accessible to double- and single-strand specific nucleases. Several other lines of evidence also suggest that EF-Tu does not interact with the anticodon region of aminoacyl-tRNAs: (i) cleavage of the phosphodiester bond after modified U_{34} the first base of the anticodon of *E. coli* tRNA^{Val} [57] or after A_{35} the second base of the anticodon of yeast tRNA^{Phe} [63] and recombination of the respective 3′- and 5′-halves does not alter the ability of these aa-tRNAs to interact with EF-Tu·GTP; (ii) fluorescence emission of the Y base in the anticodon region of yeast tRNA^{Phe} is unaffected by interaction with EF-Tu·GTP [11], even removal of the Y base does not impair ternary complex formation [64]; (iii) chemical modifications such as cyanoethylation of I_{34} the first base of the anticodon of *E. coli* tRNA^{Arg} [65] or kethoxal modification of G_{34} the first base of the anticodon of *E. coli* tRNA^{Phe} [66], do not affect ternary complex formation; (iv) *E. coli* tRNA^{Phe} and tRNA^{Glu} which have complementary anticodons can form dimers; interestingly, these aa-tRNAs when present in ternary complexes with EF-Tu·GTP can still form dimers [67], indicating that the anticodon region remains 'free' in the ternary complex.

4.5. *The D stem and loop*

The D stem and loop are not shielded by EF-Tu·GTP as shown by footprinting experiments. Moreover, bovine mitochondrial Ser-tRNA$^{Ser}_{GCU}$ which completely lacks the D stem and loop interacts with EF-Tu·GTP [61].

Concerning the unpaired region located to the 5′-side of the D stem, covalent attachment of fluorescein to s^4U_8 in *E. coli* tRNA^{Phe} has no effect on ternary complex formation [68] and photochemical cross-linking of s^4U_8 to C_{13} in *E. coli* Val-tRNA^{Val} does not alter the ability of the aa-tRNA to bind to EF-Tu·GTP [57].

4.6. *The 5′-terminal base-pair*

E. coli tRNA$_f^{Met}$ is one of few tRNAs devoid of a hydrogen-bonded base at the 5′-terminus. This unusual structural feature has been correlated with the observation that *E. coli* Met-tRNA$_f^{Met}$ seems not to be an efficient ligand for *E. coli* and *Bacillus stearothermophilus* EF-Tu·GTP as measured by gel filtration [47,69] and affinity chromatography [70]. In contrast to *E. coli* Met-tRNA$_f^{Met}$, yeast Met-tRNA$_i^{Met}$, which possesses an A_1-U_{72} base-pair can bind to *E. coli* EF-Tu·GTP but only weakly to yeast EF-1α·GTP as shown by gel filtration [71,72].

Interestingly, modified *E. coli* Met-tRNA$_f^{Met}$ with U_1-A_{72} [47] or C_1-G_{72} [70] binds much more strongly to *E. coli* EF-Tu·GTP than native Met-

$tRNA_f^{Met}$ with a non base-paired C_1 and A_{72}. Thus it has been concluded that pairing between the 5′-terminal base and the fifth base from the CCA end is important for stable ternary complex formation [47,70]. However, in bovine mitochondrial $tRNA_{UGA}^{Ser}$ the two 5′-terminal nucleotides are not base-paired. Since Ser-$tRNA_{UGA}^{Ser}$ can bind to EF-Tu·GTP [61], the presence of a base-pair at the end of the acceptor stem is not absolutely required for ternary complex formation.

4.7. *The 5′-terminal phosphate*

It was proposed that an intramolecular salt bridge between the α-amino group of the amino acid and the 5′-terminal phosphate of the tRNA could determine the conformation of the aminoacyl residue on the aa-tRNA and influence binding to EF-Tu·GTP [47]. However, yeast $tRNA^{Phe}$ whose 5′-terminal phosphate has been removed binds as well to EF-Tu·GTP as native Phe-$tRNA^{Phe}$ [73].

4.8. *Possible conformational changes in aa-tRNA upon binding to EF-Tu·GTP*

The anticodon RNA domain is not 'covered' by EF-Tu in the ternary complex [58–60]. However, enhancement as well as protection of certain cleavage sites in the anticodon RNA domain suggest a structural rearrangement of this region upon ternary complex formation. To further investigate conformational changes in aa-tRNA upon binding to EF-Tu·GTP two different approaches were used: (i) *E. coli* Arg-$tRNA^{Arg}$ and yeast Tyr-$tRNA^{Tyr}$ have been modified by spin labeling at position s^2C_{32} [74] and i^6A_{37} [75], respectively. The immobilization of the spin labels upon ternary complex formation indicates a conformational change of the anticodon region; (ii) *E. coli* $tRNA^{Phe}$ has been labeled using fluorescein at position s^4U_8. The increase in the intensity of fluorescence emission of the modified Phe-$tRNA^{Phe}$ upon binding to EF-Tu·GTP results most probably from a conformational change near the s^4U_8 base rather than from direct interaction between the label and EF-Tu·GTP as shown by fluorescence quenching measurements [68]. Structural changes in the 3′-terminus of aa-tRNA in the ternary complex are further confirmed by improved or diminished binding of oligonucleotides complementary to the CCA end of a number of *E. coli* tRNAs [76]. To monitor conformational changes, ethylnitrosourea has also been used as a chemical probe for the accessibility of various phosphodiester bonds of yeast Phe-$tRNA^{Phe}$ in the ternary complex. Some phosphodiester bonds are labilized whereas others reveal an enhanced stability towards the alkylating agent [77]. From these studies it is however not entirely clear to what extent a structural rearrangement takes place within the tRNA molecule.

4.9. *The positioning of aa-tRNA on EF-Tu·GTP and its GTPase activity*

Several experiments indicate that aa-tRNAs can stimulate the GTPase activity of EF-Tu (i) in the presence of kirromycin and under appropriate ionic conditions, (ii) in the presence of ribosomes, and (iii) in the presence of kirromycin and ribosomes [78]. Even the trinucleotide CCA bearing an aminoacyl residue, or tRNA lacking the CCA end can stimulate the kirromycin-induced GTPase activity of EF-Tu [28].

These observations and the data discussed above (section 4.1) have led to the proposal that the aminoacyl-tRNA binding site on EF-Tu·GTP comprises a site for the positioning of the single-stranded 3′-end with the aminoacyl residue, and a site for the positioning of the continuous helix formed by the acceptor and the T stem [79].

5. A VIRAL tRNA-LIKE STRUCTURE AS MODEL 'PARTNER' FOR EF-Tu

The 3′-region of the genomic RNAs of several plant viruses can interact with many tRNA-specific proteins and therefore possesses tRNA-like properties [80,81]. Since folding of the viral tRNA-like region differs greatly from that of tRNAs, these viral RNAs have served as model 'substrates' to investigate the RNA conformational requirements of certain tRNA-specific enzymes ([82] and references therein).

The 3′-terminal tRNA-like structure in turnip yellow mosaic virus (TYMC) RNA can be adenylated by the tRNA nucleotidyltransferase and subsequently aminoacylated by the valyl-tRNA synthetase. Moreover, TYMV Val-RNA can form a complex with EF-Tu·GTP [79]. One unique feature of the tRNA-like structure (~86

nucleotides) in TYMC RNA is that the acceptor stem is formed in a completely unconventional manner by folding of only the 3′-part without participation of the 5′-side of the tRNA-like region [83–85]. Thus, as opposed to what is observed in tRNAs, the aminoacyl RNA domain (~45 nucleotides) constitutes an independent region in the tRNA-like structure of this viral RNA. Because of this peculiar folding, affinity chromatography of TYMV Val-RNA fragments on immobilized EF-Tu·GTP has demonstrated that the aminoacylated aminoacyl RNA domain is sufficient for ternary complex formation [79]. These results provide the first direct evidence that the anticodon RNA domain is not required for interaction with EF-Tu·GTP.

Although the structural features in aa-tRNAs required for recognition by bacterial EF-Tu·GTP are now fairly well understood, few data are available concerning the recognition of aa-tRNAs by eukaryotic EF-1α and GTP. The study of the recognition of TYMV Val-RNA with wheat germ EF-1α and GTP revealed that the valylated aminoacyl RNA domain fulfills the requirements for complex formation. Consequently, it has been suggested that the aminoacylated aminoacyl RNA domain is essential and sufficient also in aa-tRNA for efficient interaction with eukaryotic EF-1α·GTP as it is for bacterial EF-Tu·GTP [82].

6. CONCLUDING REMARKS

The primary structure of *E. coli* EF-Tu has been determined on the basis of its DNA [86] and protein sequence [87] and considerable progress has been made in the elucidation of the tertiary structure of the EF-Tu·GDP complex by means of X-ray crystallography [88,89]. The three-dimensional structures of various tRNAs have been resolved [90] and besides tRNA·aminoacyl-tRNA synthetase complexes [91], the aa-tRNA·EF-Tu·GTP complex may become an additional prominent model system for the investigation of the principles of RNA protein interactions.

EF-Tu is a uniquely interesting protein in view of the great number of different components with which it interacts: guanosine nucleotides (GDP, GTP, ppGpp, pppGpp), antibiotics (kirromycin, aurodox), proteins (elongation factor Ts, the subunits of the replicases of bacteriophage Qβ and f2 and possibly ribosomal proteins), nucleic acids (aa-tRNAs and presumably ribosomal RNA) and finally the peptidyltransferase center [3]. Furthermore, EF-Tu is a macromolecule of increasing attraction because of its function as a G-nucleotide binding protein [92].

Concerning recognition of aa-tRNA by EF-Tu·GTP, the following schematic picture can be drawn for the ternary complex. A rather flexible aa-tRNA binds to an allosterically controlled EF-Tu·GTP complex covering the aminoacyl moiety, the 3′-CCA end and the continuous helix of the aminoacyl RNA domain; the T loop and the anticodon RNA domain are free and accessible for interaction with the ribosomal A site and the messenger RNA. However, the description at the molecular level of the interaction between aa-tRNA and EF-Tu·GTP will have to await crystallisation of the ternary complex.

ACKNOWLEDGEMENTS

We are greatly indebted to Anne-Lise Haenni and Mathias Sprinzl for critical contributions, continuous support and stimulating discussions. We also thank François Chapeville for his constant encouragements. H.G.F. is grateful to the French-German Scientific Program (PROCOPE) for financial support during his stay at the Institute Jacques Monod and to the 'Deutsche Forschungsgemeinschaft' (SFB 213, D-3) for additional support. R.L.J. is grateful to the 'Ministère de la Recherche et de la Technologie' for a stipendium. This work was partly supported by a grant from the 'Ecole Pratique des Hautes Etudes'.

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