Novel RNA interactions with the elongation factor EF-Tu: consequences for protein synthesis and tuf gene expression
Bosch, L.; Kraal, B.; Noort, J.M. van; Delft, J. van; Talens, A.; Vijgenboom, E.
Novel RNA interactions with the elongation factor EF-Tu: consequences for protein synthesis and tuf gene expression

Leendert Bosch, Barend Kraal, Johannes M. van Noort, Joost van Delft, Anneke Talens and Erik Vijgenboom

The elongation factor EF-Tu, discovered more than 20 years ago, has not suffered from a lack of attention. Despite numerous investigations, this important protein continues to yield new and interesting information about its functions, its three-dimensional structure and the organization and regulation of its two encoding genes.

During recent years novel interactions between RNA and the elongation factor EF-Tu have come to light. It is common knowledge that EF-Tu forms a ternary complex with aminoacyl-tRNA and GTP and as such plays an essential role in protein synthesis. This multifunctional translational factor, however, possesses a second tRNA-binding site which opens up as soon as the ternary complex associates with the ribosome/mRNA complex. The second site can accommodate not only aminoacyl-tRNA but also peptidyl-tRNA and non-acylated tRNA with relatively high affinities. Mutant species of EF-Tu, altered in tRNA binding and GTPase activity, act as suppressors of nonsense codons. Evidence is accumulating that EF-Tu functions as an autogenous repressor of one of its two encoding genes, tufB, by binding to the mRNA coding for EF-TuB. These EF-Tu/RNA interactions are considered in the light of the crystal structure of EF-Tu.

A preliminary model of the threedimensional structure of EF-Tu.GDP is depicted in Fig. 1. It is based on X-ray diffraction studies by LaCour, Nyborg, Clark and their colleagues. Three structural domains can be discerned. The tracing of the polypeptide chain is given here for domain I only. Cys 81, located in the loop connecting β-strand 2 and α-helix I, is exposed in EF-Tu.GDP and EF-Tu.GTP and reacts with thiol agents like N-ethylmaleimide (NEM). Binding of aminoacyl-tRNA to EF-Tu.GTP shields Cys 81, thus preventing it from reacting with the alkylating agents. This reaction with Cys 81 revealed two tRNA-binding sites on EF-Tu (Ref. 3). One is the classical binding site (site I) accessible only when GTP occupies the nucleotide binding site (also indicated in Fig. 1). Although there may be several areas of contact between aminoacyl-tRNA and site I, the 3' terminal adenosine is probably somewhere between β-strand 1 and α-helix VI. This is suggested by our finding that tRNA oxidized with periodate, when bound to site I, can be crosslinked under certain conditions to the ε-NH₂ of Lys 237 (see Fig. 1).

A second tRNA-binding site

The first indication for the second tRNA-binding site came from modification studies involving Cys 81. Whereas tRNA binding to site I prevents Cys 81 from reacting with thiol agents, binding to site II enhances the reaction. Under conditions where Cys 81 modification signals tRNA binding to site II, the 3' terminal adenosine can be crosslinked to Lys 208. This crosslinking site is at the loop connecting α-helix V and β-strand 6 (Ref. 6). Other contact areas of site II and tRNA have not been identified yet.

Perhaps the tRNA-binding site II escaped the attention of previous investigators because it becomes apparent only when EF-Tu interacts with suitable effectors, such as the ribosome or the antibiotic kirromycin. These effectors alter the conformation of EF-Tu and act as inducers of site II. Kirromycin induces site II on both EF-Tu.GTP and EF-Tu.GDP. We localized the antibiotic by its crosslinking to Lys 375 (Ref. 6), which indicates that it has close contact to the back of domain II (as shown in Fig. 1).

Of particular interest is the kirromycin-induced high affinity of peptidyl-tRNA for tRNA-binding site II. It exceeds that of aminoacyl-tRNA for site I and suggests that, on the ribosome, peptidyl-tRNA also interacts with EF-Tu.GTP through site II. Accordingly, we found that peroxidized tRNA (tRNAoxi) at the ribosomal P-site, crosslinks to Lys 208 of EF-Tu.GTP, Phe-tRNA that has entered the ribosome at the A site. No crosslinking to Lys 237 occurs under these conditions. When, however, tRNAoxi is positioned at the ribosomal A-site in the presence of EF-Tu.GTP and the ribosomal P-site is occupied by deacylated-tRNA, crosslinking to Lys 237 is observed. Crosslinking is exclusive and depends on the position of tRNA at either the ribosomal P- or A-site, irrespective of the presence of kirromycin.

Outside the ribosome, however, both crosslinking sites are accessible to tRNA when kirromycin acts as the allosteric effector. These data illustrate the specificity of the interaction between ribosome-bound tRNA and EF-Tu. They strongly suggest that the two tRNA-binding sites of EF-Tu are functionally and topographically distinct and fulfil an important role in polypeptide synthesis on the ribosome.

EF-Tu and aminoacyl-tRNA selection on the ribosome

Aminoacyl-tRNA selection on the ribosome is thought to occur in at least...
two discriminating steps (for review see Ref. 10). The first involves initial recognition of the ternary complex EF-Tu.GTP.aminoacyl-tRNA and the second (after GTP hydrolysis), rejection or retention of aminoacyl-tRNA before peptide bond formation. The latter step is called proofreading.

Hydrolysis of GTP is mediated by EF-Tu (Ref. 11) and the GTPase center of EF-Tu is activated by the same effectors that induce tRNA-binding site II: the ribosome or kirromycin12. Activation in the presence of kirromycin is increased by adding tRNA. Very recently we found (van Noort et al., unpublished) that this increase is due to binding of tRNA to site II of EF-Tu, whereas binding of aminoacyl-tRNA to site I does not influence the GTPase. Although the implications of this are not yet fully understood, they are probably highly significant for the selection of aminoacyl-tRNA on the ribosome.

Before entering the ribosome, aminoacyl-tRNA occupies the classical tRNA-binding site of EF-Tu and GTP hydrolysis does not occur. Only upon interaction with the ribosome, does EF-Tu undergo a conformational transition which opens up tRNA-binding site II; we do not yet know exactly when this occurs. There is evidence that codon/anticodon interaction alters the conformation of aminoacyl-tRNA (Ref. 13). This may force EF-Tu on the ribosome to adapt its own conformation, thereby disclosing tRNA-binding site II. Only then can peptidyl-tRNA bind to site II: whereupon GTP hydrolysis ensues, EF-Tu.GDP releases the 3' end of aminoacyl-tRNA and eventually leaves the ribosome5. Non-cognate aminoacyl-tRNA does not change its conformation because codon/anticodon interaction does not occur and site II of EF-Tu remains closed. The residence of aminoacyl-tRNA in site I as such does not activate the GTPase and permits the ternary complex to leave the ribosome without expending energy through GTP hydrolysis. Obviously, the postponement of GTP hydrolysis until proper codon/anticodon interaction has taken place is energetically advantageous. This sequence of events is now open to further experimental verification.

**Mutant EF-Tu and suppression of nonsense codons**

We have seen that EF-Tu.GTP interacts on the ribosome with aminoacyl-tRNA through tRNA-binding site I and with peptidyl-tRNA through tRNA-binding site II (see Fig. 2). This dual interaction of EF-Tu with the two tRNAs may contribute significantly to the proper positioning of the latter on

---

**Fig. 1.** (a) Three-dimensional representation of the overall shapes of the domains of EF-Tu. The position of GDP in domain I is shown by the dotted region. The dotted lines of the symbol for residue 357 in domain II indicate that this residue is located at the backside of the domain. (b) Structural cartoon of domain I with arrows representing β-strands and cylinders representing α-helices. The model is based on X-ray diffraction studies of mildly trypsinized EF-Tu. GDP by LaCour et al.1

**Fig. 2.** Model of a translating ribosome illustrating the dual interaction of EF-Tu.GTP with aminoacyl-tRNA at the ribosomal A-site and with peptidyl-tRNA at the ribosomal P-site.
the ribosome. Interestingly, mutations altering tRNA binding of EF-Tu influence the reading of the messenger RNA. We found that E. coli cells harbouring mutations in both tufA and tufB, the two EF-Tu genes, suppress all three nonsense codons, UAG, UAA and UGA (Ref. 14) (for some representative experiments see Table I). The product of the mutant tufA, designated EF-TuA2, has Ala 375 replaced by Thr (Ref. 15) (see Fig. 1). This replacement has lowered the binding constants of Phe-tRNA and Tyr-tRNA for site I of EF-TuA.GTP three- and six-fold, respectively16. We do not know whether site II is also affected. The GTPase centre is slightly influenced17. The product of the mutant tufB, designated EF-TuB, has Asp substituted for Gly 222 and has a defective tRNA-binding site II (Refs 15, 18). Its GTPase activity is drastically reduced (compare the next paragraph). Surprisingly, nonsense suppression requires the combined action of both EF-TuA2 and EF-TuB, in the cell (Table I). This raises the interesting question whether translation of a sense codon also occurs by cooperation of two EF-Tu molecules on the ribosome. If so, this would shed new light on the mechanism of translation.

**Mutant EF-TuB and the oncogene product p21 ras**

A GTP-binding protein that has attracted much attention recently is the human bladder protein p21 ras. The encoding gene becomes an oncogene by a point mutation replacing Gly 12. Human tumor cell lines and chemically induced rodent tumors contain such activated ras genes, capable of inducing morphological and tumorigenic transformation when introduced into preneoplastic immortal cell lines (for review see Ref. 19). The p21 ras protein binds GDP and GTP with high affinities comparable to that of EF-Tu for GDP. Gly 12 is in a region that shares sequence similarity with the nucleotide-binding site of other proteins. Models for this region of p21 ras have been suggested. Normal p21 ras has GTPase activity but this is grossly impaired in the oncogene protein20. Neither the extent nor the specificity of nucleotide binding is altered by the mutation conferring transforming activity. It has been speculated that p21 ras acts as a transducer of signals to the adenylate cyclase and that loss of GTPase activity results in abnormally high intracellular cyclic AMP concentrations.

![(from the text)](image)

**Table I. Suppression of nonsense codons by mutant EF-Tu, measured as $\beta$-galactosidase activity**

<table>
<thead>
<tr>
<th>Strain*</th>
<th>UAG–189</th>
<th>UAA–189</th>
<th>UGA–189</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV2.AB</td>
<td>0.8±0.4</td>
<td>0.8±0.4</td>
<td>360±85</td>
</tr>
<tr>
<td>EV5.AB</td>
<td>7.4±0.4</td>
<td>6.1±0.4</td>
<td>132±170</td>
</tr>
<tr>
<td>EV3.AB</td>
<td>1.7±0.4</td>
<td>1.3±0.4</td>
<td>159±26</td>
</tr>
<tr>
<td>EV8.AB</td>
<td>2.0±0.6</td>
<td>1.2±0.4</td>
<td>260±47</td>
</tr>
</tbody>
</table>

* Nonsense suppression has been measured by assaying the $\beta$-galactosidase activity of cells harbouring a chromosomal lac proB deletion and an F' episome containing a fused lacI and lacZ gene. The latter episome carries one of the three nonsense codons in the I portion of the fusion at position 189. These nonsense codons cause termination of translation before the Z-encoded portion of the protein is formed. Suppression of the nonsense codons permits translation of the lacZ coding sequence. As a result, a hybrid repressor-$\beta$-galactosidase molecule is synthesized. The hybrid protein has a normal $\beta$-galactosidase activity. The latter activity is expressed in arbitrary units.

* After the dash the codon position is indicated. Similar experiments have been performed with nonsense codons at position 220 in the I portion of the lacI/lacZ fusion.

* The symbols A and B, stand for wild-type tufA and tufB products (sensitive to kirromycin). A indicates a tufA product resistant to kirromycin; B indicates a product of mutant tufB, recessive to kirromycin resistant tufA. The F' episome (compare a) has been introduced into the four strains indicated.

Interestingly, the effects of p21 ras and the EF-TuB (Gly 222→Asp) mutations are very similar; both mutations affect a Gly residue in the GTP-binding region (Fig. 1). The affinity of EF-TuB, for GTP is hardly affected but the GTPase activity of the mutant protein is drastically reduced (Swart et al., unpublished results).

**EF-Tu regulates the expression of tufB**

The two EF-Tu genes, tufA and tufB, are arranged in two different transcription units: the str operon with the genes coding for S12, S7, EF-G, EF-TuA at position 72 min of the E. coli chromosome; and the tRNA/tufB operon with the genes coding for tRNA4, tRNA2, tRNA1 and EF-TuB at position 88 min (Ref. 21). Studies of the regulation of the expression of tufA and tufB are hampered because the corresponding gene products, EF-TuA and EF-TuB, differ only in the C-terminal amino acid22. Advantage can be taken of a mutation affecting one of the tuf genes that permits distinction and separation of the tuf products, provided it does not upset gene regulation. A mutation in tufB, substituting Asp for Gly 222 in domain I of EF-TuB, (see Fig. 1), permits separate determination of the intracellular concentrations of EF-TuA and EF-TuB. Such determinations revealed that the ratio of EF-TuA and EF-TuB concentrations in the cell remain constant under varying steady-state growth conditions. This means that there is coordinate regulation of the expression of tufA and tufB. Another mutation affecting tufA, and substituting Thr for Ala 375 in domain II disturbs the regulation of tufB, indicating that EF-Tu itself is involved in the regulation of tufB. Altering tuf gene dosage and in-vitro
other studies did not reveal any effect of cotranscript of 1800 bases yields a
of
studies of EF-Tu in a coupled transcription/translation system, showed that
EF-Tu acts as an autogenous repressor of tufB (not of tufA).23-24
TufB is cotranscribed with four upstream tRNA genes. Processing of the
cotranscript of 1800 bases yields a transcript of 1300 bases (Fig. 3). Transcrip-
tion from the secondary promoter P2 is negligible. Assays of the intra-
cellular concentration of tRNA encoded by the Tyr2 gene upstream of tufB and
other studies did not reveal any effect of the autogenous repressor on transcription of the
tRNA/tufB operon or on the processing of its cotranscript. Recent in-vitro
studies revealed that EF-Tu acts as an autogenous repressor by feed-
bac inhibition of translation. Deletion of the four tRNA genes of the operon results
in a sharp increase in the intra-
cellular EF-TuEFTuA ratio (van
Delft et al., unpublished results).

The mutation Gly 222—→Asp of EF-
TufB, does not affect autogenous repression (see above). More strikingly,
EF-Tu that has undergone a deletion of α-helices I, II and III and β-strands 3
and 4 (see Fig. 1) is an active repressor. Obviously, this deletion radically mutates
domain I and destroys the nucleo-
tide binding site (van Delft et al.,
unpublished).
We conclude that an intact tertiary structure of domain I is not required for autogenous repression, nor is the binding of GTP. The point
mutation Ala 375—→Thr in domain II
(EF-TuAR) significantly affects the repressor function.24 This may implicate
domain II rather than domain I in the
binding of the cotranscript but it remains for further studies to define the
interaction of EF-Tu with the cotranscript and to explain how this interac-
tion interferes with the translation but not with the processing of the
cotranscript.

EF-Tu is not the only regulator of
tufB expression. Our recent experi-
ments25 indicate that transcription from the P1 promoter of tRNA/tufB operon
(Fig. 3) is submitted to the same regu-
latory mechanism as transcription of the ribosomal RNA genes arranged in the
rrn operons. Nomura and his colleagues demonstrated26 that a product of the rrn
operons represses transcription of rRNA and tRNA operons. Interestingly, the
second regulatory mechanisms: re-
pression of transcription and feedback
inhibition of translation also govern the
regulation of rRNA and ribosomal protein synthesis, albeit separate
operons are involved. In EF-TuB syn-
thesis, these two mechanisms act at one
and the same operon. Additional
mechanisms are not excluded, however.

Viral RNA replicase

Beside its role in protein synthesis, EF-Tu functions in viral RNA repli-
cation as a host-activated subunit of the replicase. We do not know whether EF-
Tu directly interacts with the viral RNA template. Modification of Cys 81 with
TPCK, which prevents RNA binding to site I (but not to site II, van Noort et al., unpublished results), does not abolish the replicase subunit function of
EF-Tu (Ref. 27). EF-Tu.GTP, cross-
linked at site I via His 66 to ε-N-Br-
acyetyllysyl-tRNA, still exhibits poly(A)-
dependent binding to ribosomes and successfully performs GTP hydrolysis.
However, the complex does not allow subsequent transpeptidation in the
protein chain elongation cycle. On the other hand, the crosslinked complex can be reconstituted into active viral replicase. Finally EF-Tu can be covalently linked to EF-Ts without loss of ability to
work in QB replicase.27

All these data can be readily interpreted by excluding binding of EF-Tu to
viral RNA. However, the discovery of a second tRNA-binding site on EF-Tu and
the binding of the latter protein to the cotranscript of the tRNA/tufB operon call for caution in this respect. These novel RNA interactions might
share common recognition sites with a
potential viral RNA binding to this
three-domain protein.

Acknowledgements

The research of the authors is sup-
ported by the Netherlands Foundation
for Chemical Research (SON) which is
subsidized by the Netherlands Organiz-
ation for the Advancement of Pure Research (ZWO). Parts of this work
have been performed in collaboration with
Dr s A. Parmeggiani and G. Swart
(Palaisseau, France) and Drs T.
LaCour, J. Nyborg and B. F. C. Clark
(Aarhus, Denmark).

References

1. Rubin, J. R., Morikawa, K., Nyborg, J.,
LaCour, T. F. M., Clark, B. F. C. and Miller,
3. van Noort, J. M., Duisterwinkel, F. J., Jonak,
J., Sedlacek, J., Kraal, B. and Bosch, L.
(1982) EMBO J. 1, 1199-1205.
Molecular Mechanisms of Protein Biosynthesis
95-127
6. van Noort, J. M., Kraal, B., Bosch, L.,
LaCour, T. F. M., Nyborg, J. and Clark,
3969-3972
7. van Noort, J. M., Kraal, B. and Bosch, L.
(1985) Proc. Natl. Acad. Sci. USA 82,
3122-3126
8. Bosch, L., Kraal, B., van der Meide, P. H.,
Duisterwinkel, F. J. and van Noort J. M.
(1983) in Protein Synthesis Translational and Posttranslational Events (A. K. Abraham
et al., eds), pp. 165-181, The Humana Press
Gene Function in Prokaryotes (Beckwith, J.,
Davis, J. and Gallant, J. A., eds) pp. 23-63,
Cold Spring Harbor Laboratory
11. Wolf, H., Chinati, G. and Parmeggiani, A.
4910-4914
Cell. Biochem. 35, 129-138
Biol. 167, 205-209
14. Vijgenboom, E., Vink, T., Kraal, B. and
Bosch, L. (1985) EMBO J. 4, 1049-1052
15. Duisterwinkel, F. J., Kraal, B., de Graaf,
J. M., Talens, A., Bosch, L., Swart, G. W. M.,
Parmeggiani, A., LaCour, T. F. M., Nyborg,
J. and Clark, B. F. C. (1984) EMBO J. 3,
113-120
Leiden
17. Swart, G. W. M., Kraal, B., Bosch, L. and
Parmeggiani, A. (1982) FEBS Lett. 142,
101-106
18. Duisterwinkel, F. J., de Graaf, J. M., Schretlen,
P. J. M., Kraal, B. and Bosch, L. (1981)
Eur. J. Biochem. 117, 7-12
19. Land, M., Parada, F. and Weinberg, R. A.
22. Arau, K., Clark, B. F. C., Duffy, L., Jones,
M. D., Kaziro, Y., Laursen, R. A., L'Italien,
J., Miller, D. L., Nagarikati, S., Nakamura,
S., Nelsen, K. M., Petersen, E. T., Takahashi,
USA 77, 1326-1330
23. van der Meide, P. H., Vijgenboom, E.,
Biochem. 130, 397-407
24. van der Meide, P. H., Kastelein, R. A.,
Biochem. 130, 409-417
25. van Delft, J. H. N., Talens, J., de Jong, P.
and Bosch, L. (1984) EMBO-NATO Work-
sop on Function, Structure and Genetics of
Prokaryotic and Eukaryotic Elongation
Factors, Thiverval-Grignon, France, Abstr.
Annu. Rev. Biochem. 49, 525-548