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Commentary

Aminoacyl-tRNAs

Diversity before and unity after interaction with EF-Tu:GTP

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Abstract

Evidence is presented for a new role for elongation factor EF-Tu. This involves conformational restraint or conformational selection of any aminoacyl-tRNA for channeling it to the ribosomal decoding site.

Key words: Ternary complex; New role for EF-Tu; aa-tRNA conformation

1. Protein synthesis

The turnover and delivery of molecules in the cell depends on channelling, i.e. the direct transfer, with high efficiency, of metabolic intermediates from one enzyme to another [1]. One prerequisite for channelling is that the catalysts in the pathway along which the metabolites pass are suitably organised, for example as multienzyme complexes, multifunctional enzymes or by location on membranes, so that sequential reactions can be catalysed without the dissociation of the intermediates.

The structural organization of the machinery of protein biosynthesis provides an example of such channelling, as aminoacyl-tRNAs are transferred directly from aminoacyl-tRNA synthetases (AARS) to elongation factor (EF-Tu:GTP) without dissociation into the cellular fluid.

Protein synthesis on the ribosome starts with activation and specific coupling of one out of 20 amino acids to the 3′ end of the appropriate transfer RNA (tRNA), of which there are about 100 structurally different species. This reaction is catalysed by specific aminoacyl-tRNA-synthetases, a group of 20 enzymes of different structure, which can be divided into two subgroups according to their specificity for charging the RNAs on the 2′- or on the 3′-OH group of the terminal adenosine [2,3]. During this step, the energy gained from ATP by hydrolysis is stored in an aminoacyl ester linkage at one of the two ribose hydroxyl groups of the terminal ribose of the tRNA molecule. The products of this reaction – different aminoacyl-tRNAs – are channelled to elongation factor EF-Tu:GTP, a single protein with a defined tertiary structure [4,5].

In this communication, we would like to suggest that, in addition to the transport of aminoacyl-tRNA to the ribosomal binding site and control of the fidelity of the decoding, a further important function of EF-Tu is to unify different conformations and isomers of aminoacyl-tRNA.

2. Role of EF-Tu

EF-Tu in its active form carries aminoacyl-tRNA to the ribosomal A site for the decoding of mRNA. EF-Tu is apparently released from the ribosome in the form of a binary complex with GDP, since this complex has a low affinity for the ribosome and for aminoacyl-tRNA. Both the ribosome and aminoacyl-tRNA activate the GTPase function of the EF-Tu, and their effects appear to be additive. The active EF-Tu:GTP complex is re-formed in the cell by another elongation factor, EF-Ts, which catalyses the exchange of GDP for GTP [6]. Recently, the tertiary structure of the complex EF-Tu:GTP has been determined, by using crystals of EF-Tu from *Thermus thermophilus* and *Thermus aquaticus* [4,5].

During the protein-biosynthesis cycle, the EF-Tu:GTP binds strongly to all elongator aminoacyl-tRNAs. There are significant structural differences among tRNAs, e.g. length of an extra arm or dihydrouridine loop. Up to the present, crystal structures of yeast $tRNA^{Phe}$ [8,9], $tRNA^{Asp}$ [10], and initiator $tRNA^{Met}$ [11] as well as *E. coli* $tRNA^{Ser}$ [12] and initiator $tRNA^{Met}$

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[13] have been solved. Despite close similarities among them, the three-dimensional L-shape of these tRNAs show quite substantial differences. These differences are probably important, and necessary to ensure the high specificity of the aminoacylation reaction. Consequently, EF-Tu:GTP faces aminoacyl-tRNAs which are different in respect of their tertiary structure and carry their amino acids not uniformly but either on the 2′- or on the 3′-hydroxyl group.

3. Conformation of aa-tRNA

The conformational changes of the tRNA molecule that occur during formation of ternary complex (aminoacyl-tRNA and EF-Tu:GTP) can be investigated by analysis of the lead-catalysed cleavage of tRNA. Almost 10 years ago, A. Klug and co-workers [14] showed that in the crystalline form of yeast $tRNA^{Phe}$ lead specifically cleaves only one phosphodiester bond. Pb^{2+} is co-ordinated by nucleotides 59 and 60 in the ribothymidine loop, but a cleavage occurs in the D loop at the phosphodiester bond connecting nucleotides 17 and 18. The lead cleavage pattern of yeast $tRNA^{Phe}$ in solution is different. In addition to hydrolysis at U17, two cuts occur, after nucleotides 15 and 16 [14]. This result suggests structural fluctuations in the 'corner' of native $tRNA^{Phe}$, where the T and D loops interact to stabilize the tRNA L-shape. Furthermore, it was observed that cleavage of the unmodified $tRNA^{Phe}$ transcript by lead is half as fast as that of the mature $tRNA^{Phe}$, suggesting a less ordered structure near the lead cleavage site [15].

Recently, it was demonstrated that hydrolysis of the ternary complex of aminoacyl-tRNA:EF-Tu:GTP by Pb^{2+} produced a cleavage pattern identical to that of crystalline $tRNA^{Phe}$, with cutting after nucleoside 17 [16]. This suggests that the conformation of tRNA in the crystal and in the ternary complex is the same. Thus, the EF-Tu:GTP selects the conformation of tRNA that is required for the machinery of ribosomal protein synthesis.

Evidence for conformational changes of aminoacyl-tRNA upon binding to EF-Tu:GTP came also from fluorescence studies. Fluorescein was attached covalently to the 4-thiouridine (s^4U) at position 8 in each of four tRNAs, specific for alanine, methionine, phenylalanine and valine [17]. Although the probes were attached to chemically identical sites, their emission intensities varied by a factor approaching 3, indicating different conformations of the aminoacyl-tRNAs. Upon association of EF-Tu:GTP with 4-thiouridine-labelled Ala- tRNA, Met-tRNA and Phe-tRNA emission intensities increased by 244%, 57% and 15%, respectively, but Val- tRNA showed no fluorescence change upon binding EF- Tu:GTP. Upon association of these fluorescence-labelled aa-tRNAs with EF-Tu:GTP, their emission intensities became nearly equal again, in spite of the great differences in the emission intensities of the free aa-tRNAs.

Thus the binding of EF-Tu:GTP induced or selected a conformation of the aa-tRNA that was very similar, and possibly the same, for each aminoacyl-tRNA species. It appears that EF-Tu functions, at least in part, by minimizing the diversity of conformations of aminoacyl-tRNA, as a prior step to their binding at the ribosomal decoding site. As already mentioned, tRNA can be aminoacylated at either the 2′- or the 3′-hydroxyl group. The ester bond is labile, but it becomes stabilized in the aa-tRNA:EF-Tu:GTP ternary complex. Recently, it has been shown by NMR measurements that aminoacyl-tRNA in the complex with EF-Tu:GTP is not attached to tRNA by a normal ester bond to either the 2′- or 3′-hydroxyl group, but instead there is an intermediate structure containing a stable covalent linkage to both vicinal hydroxyls of the terminal adenosine, in a manner resembling carboxylic acid orthoesters [18]. The orthoester acid intermediate is released as 3′-aminoacyl-tRNA when it enters the ribosomal A-site and EF-Tu:GDP dissociates from the ribosome.

4. New role for EF-Tu

The established functions of EF-Tu thus appear to be joined by a new one, that of conformational constraint, or conformational selection of the aa-tRNA. That a protein should restrict the flexibility of a conformationally labile substrate is of course not new, but it is interesting that the various binding partners (different aa-tRNAs) of the EF-Tu:GTP complex should be so diverse, and that it should be the EF-Tu complex that establishes unity among them, so that each aa-tRNA can bind correctly to the ribosome. This exemplifies a kind of channelling different from that referred to at the beginning – a channelling not of position but of conformation.

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References

[1] Negrutskii, B.S. and Deutscher, M.P. (1991) Proc. Natl. Acad. USA 88, 4991–4995.
[2] Sprinzl, M. and Cramer, F. (1975) Proc. Natl. Acad. Sci. USA 73, 3049–3053.
[3] Eriani, G., Delarue, M., Poch, O., Ganglott, J. and Moras, D. (1990) Nature 347, 203–206.
[4] Berchold, H., Reshetnikova, L., Reiser, C.O.A., Schirmer, N.K., Sprinzl, M. and Hilgenfeld, R. (1993) Nature 365, 126–132.
[5] Kjeldgaard, M., Nissen, P., Thimp, S. and Nyborg, J. (1993) Structure 1, 35–50.
[6] Clark, B.F.C., Kjeldgaard, M., Barciszewski, J. and Sprinzl, M. (1994) Transfer RNA (Söll, D. and RajBhandary, T. eds.) American Society of Microbiology, in press.

[7] Holbrook, S.R., Sussman, J.L., Warrant, R.W. and Kim, S.H. (1978) J. Mol. Biol. 123, 631–660.
[8] Sussman, J.L., Holbrook, S.R., Warrant, R.W., Church, G.M. and Kim, S.H. (1978) J. Mol. Biol. 123, 607–630.
[9] Hingerty, B., Brown, R.S. and Jack, A. (1978) J. Mol. Biol. 124, 523–534.
[10] Westhof, E., Dumas, P. and Moras, D. (1985) J. Mol. Biol. 184, 119–145.
[11] Baravappa, R. and Sigler, P.B. (1991) EMBO J. 10, 3105–3111.
[12] Price, S., Cusack, S., Borel, F., Berthet-Colominas, C. and Leberman, R. (1993) FEBS Lett. 324, 167–170.
[13] Wook, N.H., Roe, B.A. and Rich, A. (1980) Nature 286, 346–351.
[14] Brown, R.S., Dewan, I.C. and Klug, A. (1985) Biochemistry 24, 4785–4801.
[15] Behlen, L.S., Sampson, J.R., Renzo, A.B. and Uhlenbeck, O.C. (1990) Biochemistry 29, 2515–2523.
[16] Otzen, D., Barciszewski, J. and Clark, B.F.C. (1993) Biochem. Mol. Biol. Int. 31, 95–103.
[17] Janiak, F., Dell, V.A., Abrahamson, I.K., Watson, B.S. and Johnson, A.E. (1990) Biochemistry 29, 4268–4277.
[18] Förster, C., Limmer, S., Zeidler, W. and Sprinzl, M. (1994) Proc. Natl. Acad. Sci. USA, in press.