Visualizing the protein synthesis machinery: New focus on the translational GTPase elongation factor Tu

Marina V. Rodnina[1]

Department of Physical Biochemistry, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany; and Institute of Physical Biochemistry, University of Witten/Herdecke, 58448 Witten, Germany

Three-dimensional cryoelectron microscopy (cryo-EM) is one of the few techniques capable of visualizing large, dynamic molecules. The ribosome, a molecular machine that synthesizes proteins in the cell, provides many examples of such dynamic assemblages, and the complex between the bacterial ribosome and translation elongation factor Tu (EF-Tu), stabilized by the antibiotic kirromycin, was the first to be visualized by cryo-EM (1). Since that time, the technique has progressed enormously. In this issue of PNAS, Joachim Frank and colleagues report the structure of the ribosome·EF-Tu·tRNA complex at a resolution of 6.7 Å (2), which presents a substantial advancement over previously reported cryo-EM reconstructions of that complex and provides new insights into its molecular architecture.

EF-Tu is a translational GTPase that delivers aminoacyl-tRNA (aa-tRNA) to the ribosomal decoding site. Biochemical and kinetic studies have shown that the movement of aa-tRNA into the decoding site proceeds through a number of intermediate states (3). EF-Tu in the complex with aa-tRNA and GTP is recruited by ribosomal protein L12 and placed on the ribosome in such a way that the anticodon of aa-tRNA is located in the decoding site of the ribosomal 30S subunit, whereas its 3′ end is bound to EF-Tu at the 50S subunit (1). If the incoming aa-tRNA matches the mRNA codon, the GTPase of EF-Tu is activated (3). On GTP hydrolysis, EF-Tu changes conformation, aa-tRNA is released, and its aminoacyl end accommodates in the peptidyl transferase center. Crystal structures revealed that cognate codon–anticodon interaction in the decoding site induces conformational changes of the 30S subunit (4). However, how these changes are communicated to the 50S subunit, where the G domain of EF-Tu is bound, is still not clear.

The complex studied by Villa *et al.* (2) was stalled by kirromycin, which does not interfere with GTP hydrolysis or the release of inorganic phosphate (5), but blocks the rearrangement of EF-Tu from the GTP- to the GDP-bound form. In the GTPase-activated state, aa-tRNA adopts a more open conformation, and the environment of the guanine nucleotide differs from that in the preceding and following intermediate states (6, 7). This normally transient conformation is stabilized by kirromycin binding to EF-Tu (6, 7). It is likely, therefore, that the structural arrangement of EF-Tu and aa-tRNA in the kirromycin-stalled complex is characteristic for the GTPase-activated state, although local rearrangements due to GTP cleavage and release of inorganic phosphate cannot be excluded.

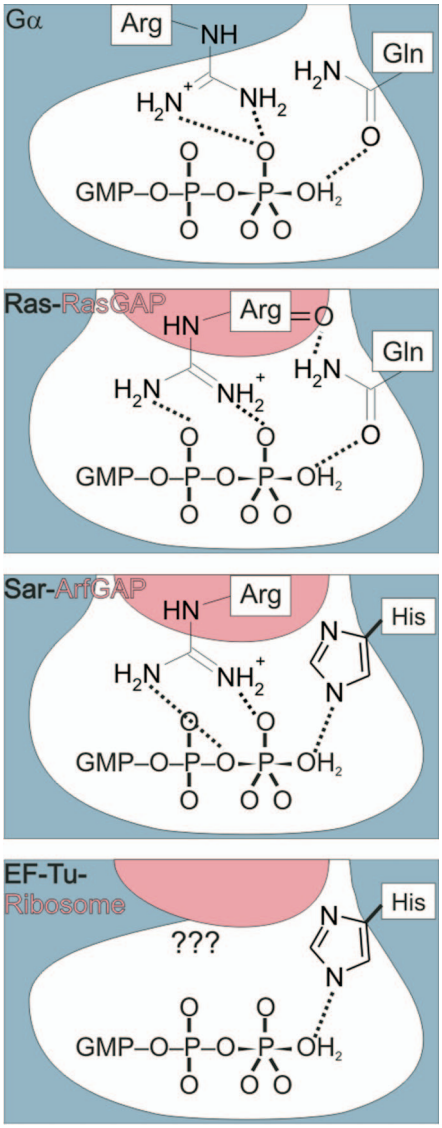

Fig. 1. Mechanism of GTP hydrolysis in the α-subunit of heterotrimeric G proteins, in Ras-RasGAP, Sar-ArfGAP (modified from ref. 16), and the EF-Tu·aa-tRNA·ribosome complex. GTP-binding protein and the GTPase-activating protein (GAP) are shown in blue and red, respectively. GTP is shown in the transition state of hydrolysis; negative charges on phosphoryl groups are omitted for clarity.

GTP hydrolysis occurs by an in-line attack on the γ-phosphate by a water molecule, which is clearly seen in crystal structures of EF-Tu·GTP (8). The GTPase activity of free EF-Tu is very low, indicating that the GTPase center is in its inactive conformation, and is accelerated by >5 orders of magnitude by the interaction with the ribosome (3). Sequence and structure comparisons with other GTPases indicated that His-84 may act as a catalytic residue by hydrogen bonding to the hydrolytic water and/or the γ-phosphate (Fig. 1). In fact, rapid kinetics revealed that mutation of His-84 reduced the rate of GTP hydrolysis by EF-Tu on the ribosome by 5 orders of magnitude, whereas the steps preceding GTP cleavage were hardly affected (9). However, in the ground state of EF-Tu, His-84 is oriented away from the water molecule; a simple rotation could place His-84 close to the buried water molecule, but a narrow hydrophobic gate formed by the side chains of Val-20 and Ile-61 would have to open to allow the movement (8). Villa *et al.* (2) show that this is in fact the case: in their structure the gate is open, Ile-60 and the remainder of switch I are disordered, and His-84 is repositioned toward the nucleotide. The neighboring residue Gly-83 is important for the rearrangement of the switch II region during GTPase activation because of the conformational flexibility inherent to Gly residues, as well as in GTP hydrolysis itself, probably by helping to position the catalytic water by hydrogen bonding with the main chain oxygen of Gly-83 (10).

Exactly how the opening of the hydrophobic gate is achieved is not entirely clear, but there are plausible hints from the structure. One half of the gate, Val-

Author contributions: M.V.R. wrote the paper.

The author declares no conflict of interest.

See companion article on page 1063.

[1]E-mail: rodnina@mpibpc.mpg.de or rodnina@uni-wh.de.

20, which is part of the P loop, seems to be stabilized by an interaction of the neighboring His-19 with the tetraloop motif of the sarcin-ricin loop (SRL) of the 50S subunit. The other half of the gate, Ile-60, is part of the flexible switch I region. Remarkably, the switch I region shows sufficient density to trace its position in EF-Tu on the ribosome. The conformation of switch I in the ribosome-bound EF-Tu is different from that in the GTP- or GDP-bound forms of free EF-Tu because of an interaction with 16S rRNA (junction between helices 8 and 14) on the 30S subunit. As a result of the conformational change, the position of Ile-60 may be shifted, leading to an opening of the hydrophobic gate and enabling the rotation of His-84 into the active site. Notably, the positions of His-84 and the switch I region described in the present article (2) differ from those suggested in a recent publication from the same group, where both His-84 and the switch I region residue Arg-58 were placed close to the SRL (11). The reason for this difference is not clear, but it underlines the importance of high resolution in structural studies. Arg-58, which is located in the switch I region of EF-Tu in a position homologous to that of the catalytic arginine in Gα proteins, is not essential for GTP hydrolysis by EF-Tu (12), in keeping with its position in the present structure (2).

The tRNA is known to play an active role in controlling the rate of the GTPase activation. The present study (2), together with the previous cryo-EM reconstructions of the same complex and the X-ray structures of the ribosome (4), identified several potentially important contacts that may help to induce and stabilize the active conformation of EF-Tu on the ribosome. On codon recognition, the 30S subunit switches to a closed conformation, in which the codon–anticodon duplex is

The ribosome provides examples of dynamic assemblages.

stabilized, presumably involving an interaction with protein S12 (2, 11). It is possible that the contact between the switch I region of EF-Tu and helices 8 and 14 in the 30S subunit requires the closed conformation of the 30S subunit, which would imply a direct link between changes in the decoding center and GTPase activation. The interactions at the decoding center in the 30S subunit and at the L11-binding region on the 50S subunit (2, 11) seem to favor a particular orientation of the tRNA. At the same time, EF-Tu appears to be fixed by its interactions with the ribosome. As a consequence, the aa-tRNA is distorted and shifted relative to EF-Tu, in particular, with respect to the contact with Asp-86 in the switch II region (2). This shift may facilitate the movement of His-84; however, the side chain of Asp-86 is probably not important, because mutations of Asp-86 did not impair EF-Tu function in protein synthesis (12).

A number of questions concerning the mechanism of GTPase activation in EF-Tu remain open. The interaction of EF-Tu with protein L12 accounts for a 2,500-fold stimulation of GTP hydrolysis (13). The contact between L12 and EF-Tu involves the C-terminal domain of L12 and helix D of EF-Tu (13, 14), which is far away from the nucleotide-binding pocket, indicating an indirect effect. As in the previous cryo-EM and X-ray structures, the L12 stalk of the ribosome is not resolved in the present cryo-EM reconstruction (2) and, thus, the structural basis for the strong effect of L12 on the GTPase of EF-Tu is not clear. Also, the role of the SRL remains obscure. Single-molecule FRET measurements indicated that the cleavage of the SRL does not abolish the formation of the GTPase-activated state but impedes subsequent steps (15), a result that is difficult to reconcile with the present structure. Finally, it is not known whether groups other than His-84 take part in catalysis (indicated by question marks in Fig. 1); extensive mutagenesis on *Escherichia coli* EF-Tu did not identify any other amino acid crucial for GTP hydrolysis. Clearly, the present cryo-EM structure (2) provides significant new insight into the structure of an intermediate complex that is very close to the GTPase-activated state. However, to answer the open questions regarding the exact mechanism of GTPase activation will require even higher resolution and—preferably—the isolation of the true GTPase transition state.

1. Stark H, *et al.* (1997) Visualization of elongation factor Tu on the *Escherichia coli* ribosome. *Nature* 389:403–406.
2. Villa E, *et al.* (2009) Ribosome-induced changes in elongation factor Tu conformation control GTP hydrolysis. *Proc Natl Acad Sci USA* 106:1063–1068.
3. Rodnina MV, Gromadski KB, Kothe U, Wieden HJ (2005) Recognition and selection of tRNA in translation. *FEBS Lett* 579:938–9342.
4. Ogle JM, Ramakrishnan V (2005) Structural insights into translational fidelity. *Annu Rev Biochem* 74:129–177.
5. Kothe U, Rodnina MV (2006) Delayed release of inorganic phosphate from elongation factor Tu following GTP hydrolysis on the ribosome. *Biochemistry* 45:12767–12774.
6. Rodnina MV, Fricke R, Kuhn L, Wintermeyer W (1995) Codon-dependent conformational change of elongation factor Tu preceding GTP hydrolysis on the ribosome. *EMBO J* 14:2613–2619.
7. Rodnina MV, Fricke R, Wintermeyer W (1994) Transient conformational states of aminoacyl-tRNA during ribosome binding catalyzed by elongation factor Tu. *Biochemistry* 33:12267–12275.
8. Berchtold H, *et al.* (1993) Crystal structure of active elongation factor Tu reveals major domain rearrangements. *Nature* 365:126–132.
9. Daviter T, Wieden HJ, Rodnina MV (2003) Essential role of histidine 84 in elongation factor Tu for the chemical step of GTP hydrolysis on the ribosome. *J Mol Biol* 332:689–699.
10. Knudsen C, Wieden HJ, Rodnina MV (2001) The importance of structural transitions of the switch II region for the functions of elongation factor Tu on the ribosome. *J Biol Chem* 276:22183–22190.
11. Li W, *et al.* (2008) Recognition of aminoacyl-tRNA: A common molecular mechanism revealed by cryo-EM. *EMBO J* 27:3322–3331.
12. Knudsen CR, Clark BF (1995) Site-directed mutagenesis of Arg58 and Asp86 of elongation factor Tu from *Escherichia coli*: Effects on the GTPase reaction and aminoacyl-tRNA binding. *Protein Eng* 8:1267–1273.
13. Diaconu M, *et al.* (2005) Structural basis for the function of the ribosomal L7/12 stalk in factor binding and GTPase activation. *Cell* 121:991–1004.
14. Kothe U, Wieden HJ, Mohr D, Rodnina MV (2004) Interaction of helix D of elongation factor Tu with helices 4 and 5 of protein L7/12 on the ribosome. *J Mol Biol* 336:1011–1021.
15. Blanchard SC, Gonzalez RL, Kim HD, Chu S, Puglisi JD (2004) tRNA selection and kinetic proofreading in translation. *Nat Struct Mol Biol* 11:1008–1014.
16. Bos JL, Rehmann H, Wittinghofer A (2007) GEFs and GAPs: Critical elements in the control of small G proteins. *Cell* 129:865–877.