Long-range signalling in activation of the translational GTPase EF-Tu

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Elongation factor Tu (EF-Tu) is a GTPase that delivers aminoacyl-tRNA (aa-tRNA) to the ribosome during protein synthesis. The factor is activated in response to the correct recognition of the mRNA codon by the anticodon of the aa-tRNA. The mechanism of signalling between the decoding centre of the ribosome and the site of GTP hydrolysis in EF-Tu where GTP hydrolysis takes place is not known. New high-resolution cryo-electron microscopic (cryo-EM) structures of the ribosome complex with aa-tRNA and EF-Tu blocked by the antibiotic kirromycin provide insights into the mechanistic details of the long-range signalling responsible for GTPase activation.

Ribosomes synthesize proteins from aa-tRNA according to the sequence of codons in the mRNA. The ternary complex EF-Tu·GTP·aa-tRNA initially binds to the ribosome through contacts of EF-Tu with the ribosomal protein L7/12, followed by a rapid and reversible codon reading step (Figure 1; reviewed in Rodnina *et al* (2005)). Formation of the fully complementary codon–anticodon duplex induces local and global conformational changes at the ribosomal decoding centre that lock the aa-tRNA in the codon-bound state (Ogle and Ramakrishnan, 2005) and prepare EF-Tu for rapid GTP hydrolysis. The crystal structures of EF-Tu and mutational analysis showed that a histidine residue in EF-Tu, His84 in *Escherichia coli* EF-Tu, is the active site residue that stabilizes the transition state of the reaction and positions the hydrolytic water molecule for attack on the γ-phosphate of GTP (Berchtold *et al*, 1993; Daviter *et al*, 2003). The intrinsic GTPase activity of EF-Tu is low, because His84 is oriented away from the GTP-binding pocket and prevented from entering the active site by a hydrophobic gate formed by the side chains of Val20 and Ile61 of EF-Tu. Upon GTPase activation of EF-Tu on the ribosome, His84 has to move toward the γ-phosphate, and this movement should be induced only when a correct codon–anticodon complex is formed. The new high-resolution cryo-EM reconstructions (Schuette *et al*, 2009; Villa *et al*, 2009) suggest how this may be achieved.

The activated state of EF-Tu is short-lived but can be stabilized by addition of the antibiotic kirromycin. The antibiotic does not affect any of the steps up to GTP hydrolysis and release of inorganic phosphate but blocks the following domain rearrangement of EF-Tu from the GTP- to the GDP-bound form, which in the absence of kirromycin results in the release of aa-tRNA from the factor (Kothe and Rodnina, 2006). Consistent with this view, the cryo-EM reconstruction shows distinct densities for GDP and kirromycin, whereas the contact between the acceptor end of the tRNA and EF-Tu is maintained (Schuette *et al*, 2009). Compared with the free ternary complex, the tRNA in the kirromycin-stalled complex is distorted and the orientation of functionally important regions of EF-Tu, including the P loop and switch I and II regions is changed. Notably, the distortion of aa-tRNA appears far more complex than anticipated earlier and extends throughout the tRNA molecule (Schuette *et al*, 2009). It seems very likely that the distortion of the tRNA leads to the movement of the switch I region of EF-Tu which, in turn, allows a rotation of His84 of EF-Tu toward the γ-phosphate of GTP, but the details of the models as to how exactly the conformational rearrangement is induced are somewhat different in the two reports (Schuette *et al*, 2009; Villa *et al*, 2009). The structures themselves do not provide information about the sequence of events; thus, the more plausible model is the one that fits best to previous biochemical and biophysical data on the mechanism of decoding and GTPase activation.

Frank and colleagues (Villa *et al*, 2009) suggest that the interaction between the elbow region of aa-tRNA and the L11-binding region of 23S rRNA is established at an early stage of ternary complex binding followed by codon recognition and 30S domain closure. However, kinetic measurements suggested that the codon recognition precedes and—under the conditions of the pre-steady-state experiments—is rate limiting for the formation of the open tRNA intermediate (Rodnina *et al*, 1994). Similarly, single molecule FRET (smFRET) studies show that codon reading precedes the formation of the kirromycin-stalled state (reviewed in Marshall *et al* (2008)). Notably, the smFRET measurement show that the antibiotic thiostrepton, which binds to the L11-binding region and probably blocks the access the elbow region of aa-tRNA, does not affect the preceding codon reading step (Marshall *et al*, 2008);
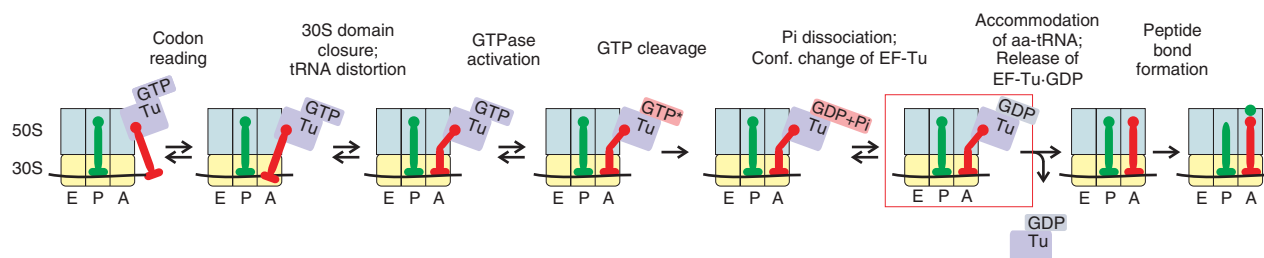
Figure 1 Sequence of steps during GTPase activation. For explanations, see text. The distortion of aa-tRNA is indicated by a kink. E, P, A, tRNA binding sites of the ribosome. GTP* denotes the GTPase-activated conformation of EF-Tu. The structure of the kirromycin-stabilized intermediate indicated by the red frame was solved by cryo-EM.

similarly, pre-steady-state measurements suggest that the kirromy-cin-stalled open aa-tRNA intermediate is not formed in the presence of thiostrepton (MV Rodnina, unpublished data). Thus, a distortion of the tRNA molecule due to the interactions with the L11-binding region of 23S rRNA is unlikely to be an early event in aa-tRNA binding to the ribosome.

A different order of events is suggested by Spahn, Ramakrishnan and colleagues (Schuette et al, 2009). They suggest that the tRNA first enters the decoding site on the 30S subunit in a non-distorted conformation (Figure 1). The authors point out that the relative orientation of codon and anticodon in this early complex would not be optimal but might allow transient probing of the mRNA codon. This model is in excellent agreement with the smFRET data that observed rapid and reversible A-site sampling by incoming cognate and near-cognate aa-tRNAs after the initial codon-independent binding of the EF-Tu·aa-tRNA complex to L7/L12 (Marshall et al, 2008). Domain closure of the 30S subunit on the correct codon–anticodon complex would force the anticodon stem into the accommodated orientation, thus imposing strain on the tRNA molecule, which at the other end is still held by the interactions of the acceptor stem with EF-Tu (Schuette et al, 2009).

The sequence of the following steps is similar in the two models (Schuette et al, 2009; Villa et al, 2009). The tRNA distortion affects the relative orientation between tRNA and EF-Tu, which probably allows for the following rearrangements to occur. During the activation, the environment of the fluorescence reporter group attached to the ribose of GTP (mant-GTP) is altered (Rodnina et al, 1995). Given that in EF-Tu·GTP·aa-tRNA, the switch I region is located in close proximity of GTP, the change in mant-GTP fluorescence may reflect the movement of the switch I region away from the nucleotide binding pocket toward the 30S subunit where it interacts with A344 in the shoulder. This interaction opens one wing of the hydrophobic gate, whereas the other wing is held fixed by the interaction between SRL and the P loop, and reorients His84 toward the nucleotide.

The crucial question to answer is how the fidelity of signalling is controlled. Non-cognate ternary complexes do not go past the transient binding to L7/12 and are rejected before any of the following rearrangements occur to a significant extent (Rodnina et al, 2005; Marshall et al, 2008). However, both cognate and near-cognate aa-tRNA sample into the decoding centre and interact with the mRNA. The resulting conformational changes of the 30S subunit are different for the cognate and near-cognate ternary complex (Ogle and Ramakrishnan, 2005). It is possible that the ribosome cannot impose strain on a near-cognate tRNA, because the mis-matched codon–anticodon complex is not stabilized sufficiently by the interactions with the decoding centre and the domain closure is not induced. As a consequence, the relative orientation of the tRNA and EF-Tu is not changed, preventing the movement of the switch I region and GTPase activation. Alternatively, near-cognate aa-tRNA may be distorted to some extent, allowing the switch I region to sample toward the h8/14 junction at the 30S subunit shoulder. However, the position of the shoulder depends on codon recognition; in the complex with the near-cognate aa-tRNA the contact may be impaired, resulting in unsuccessful GTPase activation attempts.

One remaining question concerns the role of the SRL. Both cryo-EM reconstructions reveal that if EF-Tu were in the GTP-bound form, His84 would clash with the SRL (Schuette et al, 2009; Villa et al, 2009). This suggests that the interaction between EF-Tu and the SRL as seen in the kirromycin-stalled complex can form only after the GTPase activation. Such an interpretation is fully consistent with the smFRET results, which indicate that the cleavage of SRL does not affect the formation of the GTPase-activated state but slows down the following reactions (Marshall et al, 2008). However, if not the SRL, what are the interactions of EF-Tu with the ribosome that aid aa-tRNA distortion and fix the position of EF-Tu in a strained complex? The interactions of EF-Tu with L7/12 (which are not resolved in the present structures, probably because the interaction is transient (Rodnina et al, 2005)) and of domain II of EF-Tu with the 30S subunit may serve this role. Although this is a question that remains to be answered in future work, the new cryo-EM structures provide an important step toward understanding the signalling in translational GTPases and the quality control of translation.

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