Let's see how tmRNA rescues a stuck ribosome

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In the current issue, Weis *et al* (2010a) and Fu *et al* (2010) provide cryo-electron microscopy snapshots of different states of the bacterial ribosome-rescuing complex with tmRNA. This regulatory RNA molecule remarkably carries both tRNA- and mRNA-like elements that have to move through the ribosome machinery when it is stalled on an mRNA lacking a termination codon. The comparison of three intermediate states gives novel insights into the mechanism of tmRNA translocation and transient accommodation on the ribosome, and into *trans*-translation—the template switching from a defective mRNA to the short coding region of the tmRNA, which allows rescuing the stuck ribosome.

During the process of protein synthesis, the ribosome machinery may stall on an incomplete mRNA for which the stop codon is missing. Bacteria use a particular RNA molecule to rescue the ribosome: tmRNA (also called SsrA), which is rather large (~260 to 430 nucleotides depending on bacterial species) and contains a tRNA-like domain (TLD) that can be alanine-charged at the 3' CCA end, a series of pseudo-knot structures (PK1–PK4) and a partially hidden open reading frame (ORF; see Figure 1A) coding for a peptide tag that targets the incorrectly formed nascent peptide for degradation. During the process of template switching (the so-called *trans*-translation, which also involves protein SmpB), the tmRNA molecule binds to the ribosome, replaces the defective mRNA, adds an alanine residue to the nascent peptide and takes over the mRNA role by providing its own short ORF (which contains a regular stop codon) for translation; in this way the ribosome is rescued and the defective mRNA and the tagged polypeptide gets degraded. Promoted by elongation factor Tu (EF-Tu), the binding of tmRNA to the ribosome results in a pre-accommodated state, where the TLD part of the tmRNA is bound in the aminoacyl (A) site of the ribosome, as visualised by an earlier cryo-electron microscopy (cryo-EM) study (Valle *et al*, 2003). In this complex, the pseudo-knot structure forms an almost closed ring that surrounds the head of the 30S ribosomal subunit (Figure 1B). After transfer of the alanine residue from the TLD CCA end (Figure 1A) to the nascent polypeptide, the TLD needs to translocate to the peptidyl (P) site of the ribosome to free the A site for the first tRNA that will base pair with the resume codon of the ORF (Figure 1A).

In an exciting cryo-EM study of reconstituted (*T. thermophilus*) or endogeneous (*E. coli*) ribosome complexes, two

papers in this issue by Weis *et al* (2010a) and Fu *et al* (2010) now address the long-standing question of how the tmRNA translocates through the ribosome to adopt the translation resume position. Both studies describe the state in which the TLD of the tmRNA is translocated into the P-site (Figure 1B). The comparison of the pre-accommodated state in the presence of EF-Tu (Valle *et al*, 2003), with the accommodated state when the TLD is fully positioned into the A-site (Weis *et al*, 2010b), shows that the large RNA structure follows the TLD movement during the translocation process from the A to the P-site (Figure 1C and D). The tmRNA provides sufficient flexibility to do so through conformational changes of the part of the pseudo-knot structure that is located over the A and P sites (Figure 1D), resulting in an opening of the initially almost closed ring. In addition, the ORF serves as a flexible linker between the 5' helix H4/PK1 and helix H5 (next to PK2; Figure 1A and B). In other words, when the TLD translocates into the P site, it appears to stretch out the central ORF region and the nucleotides 5' to it in order to position it into the mRNA channel; this could be accompanied by a transient opening of the 30S subunit beak, during which the defective mRNA could be released. PK4 and PK1 follow the movement of the TLD towards the P site, such that the 5' end of the ORF gets positioned into the P site (Figure 1B). In contrast, PK2 and PK3 remain almost in the same position next to the mRNA entry site (ribosomal proteins S3, S4 and S5) and hold the 3' end of the ORF. Interestingly, Weis *et al* (2010a) provide insights into an intermediate state showing that the linker (helix H2) between the TLD and PK4 can move through one of the inter-subunit bridges (B1a) when it opens transiently on a rotational movement of the subunits (Figure 1D), a conformational transition of the ribosome well-known during tRNA translocation (reviewed by Korostelev *et al*, 2008). Both papers in this issue show that the global structure of the tmRNA is conserved through the process, that is, there is no major RNA unfolding required for tmRNA translocation—despite of the apparently big size of the tmRNA molecule—because most parts are outside of the ribosome anyway. As the stop codon is buried in helix H5 (Figure 1A), the 3' part of the ORF hidden in helix H5 will have to unfold at a later stage (possibly once the TLD has also translocated to and through the exit (E) site of the ribosome, Figure 1D); this may actually take place when the H5 region enters the mRNA channel, where the helicase activity of ribosomal proteins S3 and S4

Figure 1 (A) Secondary structure of *T. thermophilus* tmRNA (from Weis *et al*, this issue). Indicated are pseudo-knots, PK, the 5'-3' orientation of the ORF and the tRNA-like domain (TLD). Contacts with the ribosome are indicated in grey: A and P sites, inter-subunit bridge B1a. (B) Cryo-EM structure of the 70S/tmRNA complex in the resume state (Fu *et al*, this issue); only the 30S subunit is shown, viewed from the inter-subunit side. Indicated are PK's, beak of the 30S subunit head, ribosomal proteins S3, S4, S5 at the back of the 30S, 5'-3' orientation of the ORF, peptidyl transferase centre (PTC) on the 50S. (C) Comparison of different 70S/tmRNA complexes (only the 30S subunit is shown): the pre-accommodated state (left, in orange) in the presence of EF-Tu superposed to the P-site translocated state (in blue; Fu *et al*, this issue), and the A-site accommodated state (right, in red) superposed to the P-site translocated state (in blue; Weis *et al*, this issue). (D) Comparison of the 70S/tmRNA complexes (top view) before (left), 'during' (middle) and after (right) translocation (Weis *et al*, this issue). A rotational movement of the subunits (middle, intermediate state before the translocation, zoomed view) allows helix H2b-c to move through inter-subunit bridge B1a and transfer the TLD to the P-site.

could promote unfolding of structured 5' mRNAs (Takyar *et al*, 2005). The two papers also provide insights into the role of protein SmpB, suggesting that it interacts with the ORF upstream of the first codon to indirectly position the resume codon into the A-site (Figure 1D). This may be based on the interactions with the non-coding triplet in the P site, thus replacing at least, in part, the interactions of a standard P site tRNA that normally sets the correct mRNA reading frame. Interestingly, mutations in the -1 triplet can cause frame shifts (Miller *et al*, 2008). How the specific interaction of SmpB with the codon bases may look like remains to be investigated. In the endogenous complex, a tRNA

molecule is found in the A site (Figure 1D)—and indeed it is predominantly an Ala-tRNA (Fu *et al*, 2010), as expected from the first coding triplet (GCA or GCC) in the tmRNA ORF (Figure 1A).

Taken together, the available cryo-EM structures show that a first small movement of the tmRNA occurs during the transition from the pre-accommodated state (with EF-Tu; Valle *et al*, 2003) to the accommodated state (Weis *et al*, 2010b), followed by a major movement because of the TLD translocation from the A-site to the P-site (Fu *et al*, 2010; Weis *et al*, 2010a; Figure 1C and D). It can be expected that yet another large conformational change occurs on translocation

to the E site, possibly yet again without affecting much of the global pseudo-knot structure—future studies will address this last snapshot of ribosome rescue.

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Conflict of interest

The author declares that he has no conflict of interest.

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