A twisted tRNA intermediate sets the threshold for decoding

MICHAEL YARUS,¹ MIKEL VALLE,² and JOACHIM FRANK^{2,3}

¹Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347, USA

²Howard Hughes Medical Institute, Health Research, Inc., Albany, New York 12201-0509, USA

³Department of Biomedical Sciences, State University of New York, Albany, Albany, New York 12201-0509, USA

ABSTRACT

Putting together consistent cryo-EM structure, transient kinetic and mutant tRNA suppressor data, it appears that a deformed or wagging aminoacyl-tRNA is the critical transitional structure examined by the ribosome during decoding at the A site. The unusual conformation may be required for effective proofreading of the codon–anticodon complex.

Once information gets into peptides, you cannot get it back out. A corollary to this famous adage is that the precision of translation itself must be determined before the peptide bond forms. New findings unite very diverse experiments to confirm that transfer RNA (tRNA) deformation accompanies and helps to guide those early steps of translation that must be accurate—from the binding of an aminoacyl-tRNA up to its incorporation at the ribosome's A site.

The newest information is structural (Valle et al. 2002), from a cryo-EM reconstruction of *Escherichia coli* 70S ribosomes carrying bound phe-tRNA:EF-Tu:GTP in the presence of kirromycin. The latter antibiotic allows hydrolysis of GTP to GDP but then jams further progress by bridging domains of EF-Tu that otherwise close like scissors. One therefore gets a view of EF-Tu and tRNA that represents the structure before the incoming aminoacyl end enters the peptidyl transferase center. As will be evident in Figure 1a, the entire factor–tRNA structure can be seen (red), but the anticodon arm of the free tRNA or EF-Tu:aa-tRNA structure cannot be fit to the observed electron density (Fig. 1b). Instead, the anticodon arm must be bent. In addition, the D and TΨ loops of the tRNA have moved, apparently to contact the GTPase-associated center (GAC) of the 23S rRNA (around nt 1060–1075 in *E. coli*). This alteration suggests that the tRNA structure may help to signal the need for GTPase activity from the decoding center to the GTPase domain of EF-Tu (see below). Colored beads on the purple tRNA structure highlight the nucleotides discussed next.

Rapid kinetic analysis agrees with this picture: During EF-Tu:aa-tRNA association with ribosomes, a change in the conformation of the D-loop (blue beads in Fig. 1b) is the first detectable event (Pape et al. 1998). This altered tRNA shape is then resolved when the tRNA is released by EF-Tu:GDP and enters the peptidyl transferase center. The rates at which tRNAs transit these steps are dramatically different for tRNAs that match their codons or that have one mispair or that do not match at all (for review, see Rodnina and Wintermeyer 2001). Therefore these in vitro kinetics suggested that D-loop shape changes during codon recognition, GTPase activity, and accommodation to the peptidyl transferase center are essential for accurate decoding (Rodnina and Wintermeyer 2001). Note that dissociation before peptide transfer also appears influential in the coding decision.

Similar ideas arose from directed mutation of suppressor tRNAs, particularly from tRNA mutations that enable unusual coding at the first and third (wobble) codon positions (Schultz and Yarus 1994). These mutations taken together (green beads, Fig. 1b) define a conjoined D-loop–anticodon arm tRNA domain whose conformation is apparently crucial (Yarus and Smith 1995) to acceptance or rejection by the ribosome. For example, a combination of D-arm and anticodon arm mutations made it possible to read a normally forbidden first position wobble pair with about 50% efficiency (Schultz and Yarus 1994). This suggested that altered tRNA conformations are more permissive, and in particular that the D-arm–anticodon arm domain must “waggle” during coding decisions (Yarus and Smith 1995).

These conformational shifts can be explained by the logic of proofreading (Yarus and Smith 1995). The codon–anticodon match must first be tested while the aminoacyl arm

Reprint requests to: Michael Yarus, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347, USA; e-mail: yarus@stripe.colorado.edu.

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FIGURE 1. (a) Phe-tRNA bound to EF-Tu: GDP (red) on the 70S ribosome (50S subunit, blue, 30S subunit, yellow). The complex has been stalled with kirromycin just after GTP hydrolysis but before EF-Tu rearrangements. (b) Ribbons representation of the Phe-tRNA (purple) and EF-Tu (orange) inside the semitransparent cryo-EM density of the ternary complex. The beads on the tRNA label mutations that alter the accuracy of translation (dark and light green) and sites of reporter fluorophores used in kinetic experiments (blue). (DC) decoding center; (GAC) GTPase-associated center.

is outside the peptidyl transferase center, on EF-Tu. The codon–anticodon match is tested again after GTP hydrolysis drops the aminoacyl arm of a tRNA into the A site, before the peptide bond is formed. Throughout these displacements of the tRNA’s aminoacyl arm, the anticodon must be in the same place, bound to the codon (at DC in Fig. 1b). Accordingly, we have cryo-EM structures that clearly indicate deformation of the aminoacyl-tRNA anticodon arm and D/T Ψ loop structures immediately after recognition of the tRNA’s codon–anticodon match (that is, just after GTP hydrolysis by EF-Tu). We have kinetics that imply that D-loop deformation is likely to be a universal early step during successful encounters with the ribosomal decoding site *in vitro* (before EF-Tu departs). Finally, we have genetic data indicating that easier deformation of the proximal anticodon arm and D-arm lowers the threshold for biologically acceptable coding in the steady state within

growing cells. Separately and especially taken together, these findings show that ribosomes deform incoming tRNAs while discriminating among them. The ribosome distorts the tRNA in order to proofread, and may cleverly exploit successful distortion to help communicate a codon–anticodon match to EF-Tu GTPase (Rodnina and Wintermeyer 2001).

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