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Allosteric Control of mRNA Decoding

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The genetic code is translated by Watson–Crick-type interactions between the anticodon triplet of tRNA and the codon triplet of mRNA. The nucleobases that constitute the anticodon are presented in the tRNA structure as a three-nucleotide unit stacked on the 3′-part of the anticodon loop with the nucleobases oriented in a conformation suitable for base pairing (Figure 1). This structure does not, however, explain the details of the complicated decoding process. Where are the problems?

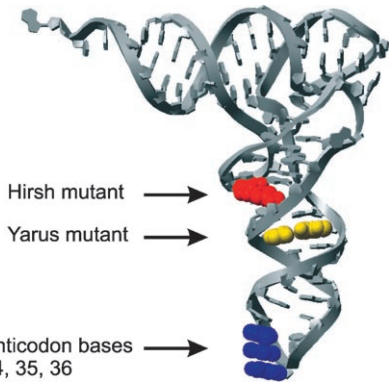

Figure 1. Structural model of tRNA in its free form (based on X-ray crystallography; PDB ID: 1EHZ) showing the anticodon bases (blue), the Hirsh mutation, which forms a U11:A24 base pair (red), and mutations that changed the meaning of the codon–anticodon interaction, identified by Yarus and co-workers at base pair 27:43 (yellow).

First, the theoretical values of the free enthalpies gained by a triplet–triplet interaction are far too low to account for the high precision with which the genetic code is translated.[1] Second, there are 64 triplet codons for twenty amino acids and there is an insufficient number of tRNAs to read these codons in the cell, that is, several codons that differ only by one nucleotide have to be recognised by a single tRNA molecule. This means that, on top of the requirement for high precision, in some cases the opposite, an inaccurate reading violating the Watson–Crick rules is required.[2] Third, there is evidence that the codon–anticodon interaction is not the only interaction governing the precise translation. Mutations in the tRNA sequences [3–5] and modifications of nucleotides[6] able to change the meaning of the codon have been identified, although the sequence of the anticodon itself was not altered.

Evidently, peptide-bond synthesis and the reading of the genetic code cannot be reduced to simple interactions between four small molecules, the two oligonucleotide triplets and two amino acid residues. The translation of mRNA takes place on a large nucleoprotein complex, the ribosome. The mass of the two codon and anticodon triplets and the two reacting amino acids constitutes less then 0.04% of the total mass of a single bacterial ribosome. Why is the ribosome so big? Beside the catalysis of peptide-bond formation, the ribosome has to perform many more functions. The ribosome recognises the initiation signals on the mRNA and starts the translation at the correct place with the appropriate reading frame. The ribosome, as a central part of the translation system, sets the pace of translation and controls the fidelity with which the amino acids are incorporated in the correct order into the growing polypeptide chain. Finally, it participates in polypeptide release and prepares for the next translation cycle with a new mRNA. During its long journey on the mRNA, the ribosome interacts with many different aminoacyl tRNAs and translation factors.

It is reasonable to assume, and was postulated several decades ago, that the tRNAs, although different in sequences, are constructed in such a manner that the overall structure of tRNA thermodynamically compensates for the different free-enthalpy changes of the particular codon–anticodon interaction. An approximately constant error rate and constant velocity of translation for all possible codon–anticodon pairs could evolve from such compensation.

There are two theoretical concepts to explain the high fidelity of codon–anticodon interactions. The direct, structural reading mechanism is based on the presumption that the codon–anticodon base pairs are placed in a frame made up from a ribosomal structure that is sufficiently stable to account for the required fidelity.[1,7] The other concept represents the kinetic proof-reading mechanism proposed originally by Ninio[8] and Hopfield[9] and experimentally substantiated by kinetic measurements on in vitro translating ribosomal systems.[10,11] The results of these studies indicated that the selection of aminoacyl tRNA for a particular codon is controlled by two thermodynamically uncoupled processes, the initial selection during binding and a proof-reading step that takes place after GTP-to-GDP hydrolysis.

The work published recently by Cochella and Green in *Science*[12] adds substantial evidence for the existence and biological significance of the proof-reading mechanism and identifies aminoacyl tRNA as an active player in this process. This important investigation is based on an experiment published decades ago. Hirsh observed in 1971 that the reading of the UGA stop codon by a suppressor $tRNA^{Tyr}$, which normally possesses an anticodon CCA, is not caused by mutation in the anticodon but by a nucleotide exchange at position 24 that changes the U11:G24 base pair to U11:A24.[3] Later,

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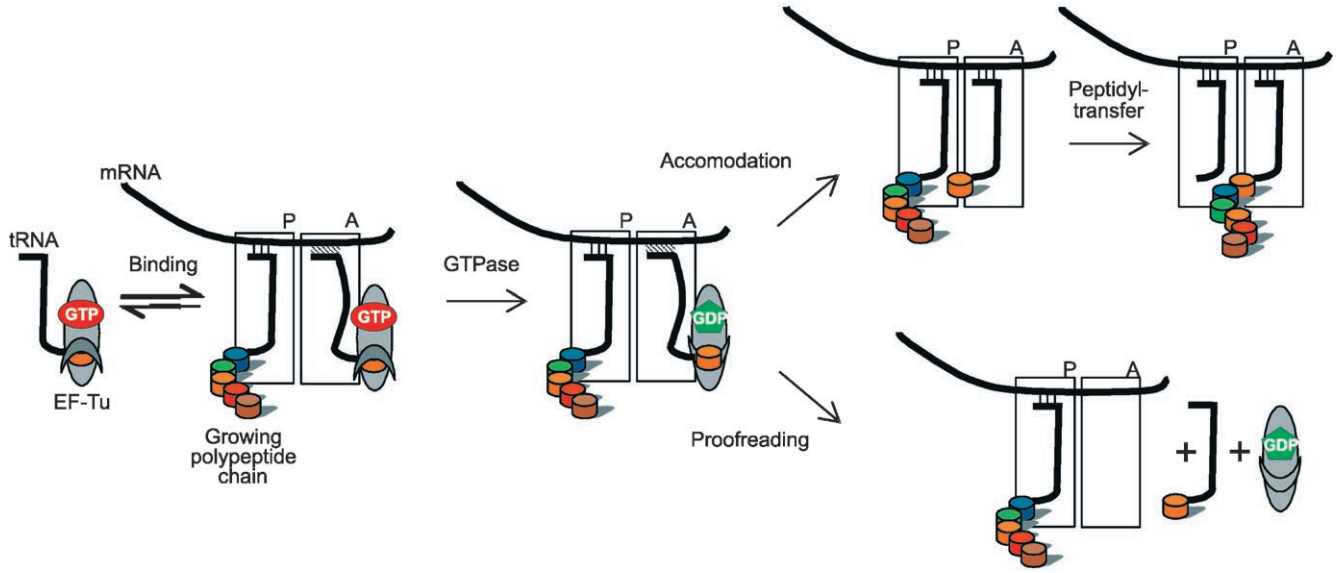

Figure 2. Proposed reaction steps leading to peptidyl transfer on the ribosome (A and P sites are indicated). The relaxation of the bent tRNA during accommodation denotes the required conformational changes of the entire molecule.

Yarus and co-workers identified other tRNA mutations that had a similar effect.[4,5] Remarkably, all these tRNA variants that affect the codon–anticodon interaction have the mutations near the junction of the D and anticodon helices, which form a coaxial helix in the three dimensional structure of tRNA (Figure 1).

This important observation can be explained by the slightly modified proofreading model shown in Figure 2. Aminoacyl tRNA bound in a ternary complex with elongation factor Tu and GTP approaches the programmed, but still vacant A site of ribosomes. Binding to the A site is dependent on the codon–anticodon interaction and leads to activation of GTPase and GTP hydrolysis. This, however, is not the final decoding step that opens the way for peptide-bond formation. Instead, branching occurs.[11,13] The aminoacyl tRNA is either accommodated into the peptidyl transferase centre (cognate aminoacyl tRNA), this leads to the synthesis of a new peptide bond, or rejected from the A site (near-cognate aminoacyl tRNA). The branching point of the pathways lies after GTP hydrolysis and is the critical step in increasing the fidelity of translation. Cochella and Green compare the rates of the reactions defined in Figure 2 for Trp-tRNA^{Trp} G24 (wild-type) and Trp-tRNA^{Trp} A24 (the Hirsh suppressor) on ribosomes programmed with the AUG stop codon. They demonstrate that the advantage of Trp-tRNA^{Trp} G24 over Trp-tRNA^{Trp} A24 in competing for this near-cognate codon is based on an acceleration of GTPase activation as well as accommodation in the case of the Hirsh mutant. Thus, the codon–anticodon interaction is not determined exclusively by base pairing between the respective triplets. The conformation of the tRNA molecule allosterically controls GTPase activation and the accommodation of aminoacyl tRNA into the A site as well. The accommodation involves interactions of ribosomes with parts of tRNA distant from the anticodon.

Electron microscopy snap shots of aminoacyl tRNA–ribosome complexes taken at different stages of the reaction pathway provide independent structural evidence for the existence of the accommodation step. A significant conformational change in the elbow region of aminoacyl tRNA was identified during the transition from initial A-site binding to the reactive position in the peptidyl transferase centre.[14] This motion is related to a positioning of the D and anticodon arms of the tRNA on the ribosome and implies a bending of these coaxial helices (Figure 2). It suggests a mechanism by which the mutations in these distant regions could influence the decoding process. Frank and colleagues imply that the aminoacyl tRNA is a "molecular spring" that is relaxed during the decoding, that is, accommodation, process.

The role of nucleotide modifications in tRNAs has been intensively investigated in the past. There is strong evidence in support of the fine tuning of the codon recognition by modifications in anticodon and anticodon loops. Such modifications are able to exclude or facilitate wobble interactions involving the third codon letter.[6] The perception that accommodation and the tRNA "spring effect" might be affected by mutations located far outside the anticodon loop allows speculation about the role of numerous modified nucleotides scattered in the tRNA sequences. It is possible that at least some of them also contribute to the correct decoding of the genetic code and tune the structure of individual tRNAs to fit into the complicated assembly of molecules that constitute the translation system.

Keywords: decoding · mRNA · ribosomes · translation · tRNA

[1] J. M. Ogle, V. Ramakrishnan, *Annu. Rev. Biochem.* **2005**, *74*, 129–177.
[2] F. H. Crick, *J. Mol. Biol.* **1966**, *19*, 548–555.
[3] D. Hirsh, *J. Mol. Biol.* **1971**, *58*, 439–458.
[4] D. Smith, M. Yarus, *J. Mol. Biol.* **1989**, *206*, 503–511.
[5] D. W. Schultz, M. Yarus, *J. Mol. Biol.* **1994**, *235*, 1381–1394.

[6] P. F. Agris, *Nucleic Acids Res.* **2004**, *32*, 223 – 238.
[7] V. Ramakrishnan, *Cell* **2002**, *108*, 557 – 572.
[8] J. Ninio, *Biochimie* **1977**, *59*, 759 – 760.
[9] J. J. Hopfield, *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 4135 – 4139.
[10] R. C. Thompson, *Trends Biochem. Sci.* **1988**, *13*, 91 – 93.
[11] T. Pape, W. Wintermeyer, M. V. Rodnina, *EMBO J.* **1998**, *17*, 7490 – 7497.
[12] L. Cochella, R. Green, *Science* **2005**, *308*, 1178 – 1180.
[13] J. F. Eccleston, D. B. Dix, R. C. Thompson, *J. Biol. Chem.* **1985**, *260*, 16 237 – 16 241.
[14] J. Frank, J. Sengupta, H. Gao, W. Li, M. Valle, A. Zavialov, M. Ehrenberg, *FEBS Lett.* **2005**, *579*, 959 – 962.

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