Protein Biosynthesis

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Correction of Errors during Protein Synthesis

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The precise translation of genetic information, coded by nucleic acids, into protein sequence is essential for cell survival. Erroneous polypeptides are usually unable to fold correctly, remain inactive, and are finally degraded in the cell. For the synthesis of a linear chain composed of several hundreds amino acids, translation must proceed with high precision. The error rate of translation in vivo has been estimated to be about one error per 10000 amino acid residues.[1]

The elongation of a polypeptide by a single amino acid residue can be divided into several distinct chemical steps.[2] It is clear that none of these steps can proceed with absolute precision. The genetic code is translated by Watson–Crick-type interactions between the anticodon triplet of tRNA and the codon triplet of mRNA. In this major step, the chemical nature of the Watson–Crick base pair determines which anticodon is selected for elongation. The difference between a perfect fit and a mismatch in one base pair of the alternative triplet may be very small. In an another important step, the charging of tRNA with the cognate amino acid, small differences in the chemical structure of the amino acids, for example, isoleucine and valine[3] are used for discrimination. In these steps the specificity of the recognition process cannot account for the high fidelity needed for faithful translation of the genetic information. How can this problem be resolved?

Proteins are synthesized on large nucleoprotein complexes called ribosomes. Although more than 150 molecules (proteins, RNA molecules, nucleotides) participate in this process, the active center of the ribosome is composed entirely of RNA. The determination of the three-dimensional structure of the whole ribosomal complex has provided new insight into ribosome function.[4]

It is well known that some biochemical processes in which linear polymers like nucleic acids or polypeptides are synthesized use multistep mechanisms to increase the overall precision of the elongation process. After the selection in the first step, the quality of the product is checked in the second step and, if necessary, corrected. However, this proofreading mechanism functions only when the second step is separated from the first one by a sufficiently high thermodynamic barrier to prevent reversibility.[5]

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For a long time it was believed that in the case of polypeptide synthesis on ribosomes such a proofreading step takes place only during the codon-dependent selection of a particular aminoacyl-tRNA in the aminoacyl (A) site of the ribosomes, that is, before the new peptide bond is formed (pretransfer proofreading).[5,6] Zaher and Green[7] now provide evidence that even after chemical incorporation of the wrong amino acid into the growing polypeptide a mistake can be corrected (posttransfer proofreading).

The codon–anticodon interaction requires the perfect Watson–Crick base pairing of the first and second codon letter with the corresponding nucleotides in the tRNA anticodon. The interaction with the third nucleotide can, in some cases, violate this rule (wobble). For precise translation, nine nucleotides of mRNA are placed on the three decoding tRNA sites (A, P, and E site) of the small 30S ribosomal subunit (Figure 1). At least two of these three sites are simultaneously occupied by tRNAs that are selected through the codon–anticodon interaction (Figure 1a). According to allosteric three-site model formulated by Nierhaus, the A site

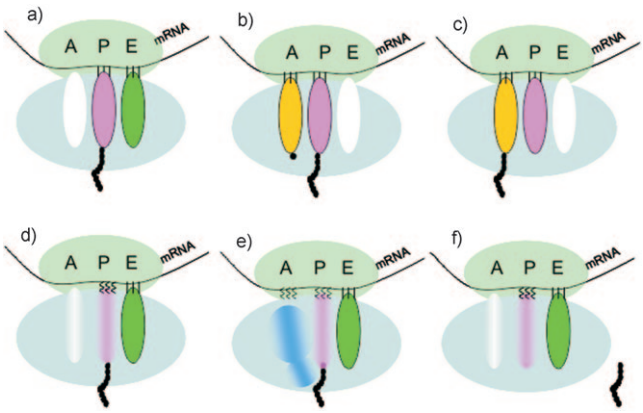

Figure 1. The ribosome in different states during normal elongation (a–c) and during posttransfer error correction (d–f). The programmed ribosome with correct codon–anticodon interactions in the P and E sites (a). The ribosome after EF-Tu-dependent binding of aminoacyl-tRNA in the A site (b) and peptidyl transfer (c). The A, P, and E sites occupied by the corresponding tRNAs are yellow, purple, and green, respectively; empty sites are white. Sharp outlines indicates the correct conformation of the site. If an incorrectly coded aminoacyl-tRNA escapes the first (pretransfer) proofreading step, it can land as an incorrectly coded peptidyl-tRNA in the P site (d). The structure of the binding site is affected (fuzzy outline), leading to preferential binding of release factors (blue in e) to the A site. Peptidyl-tRNA in the P site is hydrolyzed and the peptide is released (f).

(white field with sharp outline in Figure 1) is correctly programmed for the binding of the new aminoacyl-tRNA only when the P and E sites are structured in a way defined by the correct codon–anticodon interaction and the body of tRNA is correctly placed into the respective site.[8]

The new aminoacyl-tRNA is transferred to the programmed A site with participation of the elongation factor EF-Tu·GTP.[9] Simultaneously, the tRNA bound at the E site is released. In this process (a→b in Figure 1) and kinetic proofreading can occur to eliminate incorrectly placed aminoacyl-tRNAs before the new peptide bond is formed. If a correct aminoacyl-tRNA is bound to the A site (indicated in Figure 1 by three straight lines between the anticodons of tRNAs and mRNA and by the sharply outlined binding sites), the peptide bond forms spontaneously leading to the pre-translocational complex (Figure 1 c). Now, the peptidyl-tRNA is located on the A site and a stripped tRNA is in the P site. This complex is moved in a process called translocation, and a complex analogous to that in Figure 1 a is formed and presents a new codon (not shown).

Using model reactions with short mRNAs in vitro, Zaher and Green studied the effects of mismatches (Figure 1 d–f) in the P and E sites on the decoding properties of the A site. They demonstrated that the codon–anticodon mismatch in the P site (Figure 1 d) or in both P and E sites (not shown) results in enhanced release-factor-dependent hydrolysis of the peptidyl residue from peptidyl-tRNA bound to the P site. Normally, such a hydrolysis takes place only when a nonsense codon (one of the three codons for which no aminoacyl-tRNA exists) is placed in the A site. In this case termination factors, which are always present in the cell, enter the A site and terminate the polypeptide synthesis by hydrolysis of the ester bond in P-site-bound peptidyl-tRNA. The work of Zaher and Green now shows that a mismatch in the P site (or P and E sites) reprograms the A site to be susceptible for release-factor binding and hydrolysis of the peptidyl-tRNA ester bond (Figure 1 e). The hydrolyzed peptide is then released from the ribosome (Figure 1 f). This sequence of reactions represents a novel mechanism for the posttransfer correction of an erroneous peptide bond.

The precise molecular mechanism of this posttransfer "quality control" remains unclear. The three tRNA binding sites are not isolated structural units as the schemes of ribosomes with three tRNA binding sites may suggest. In reality, the structure of one site may depend on occupancy of the other. There is evidence indicating that the codon–anticodon interaction depends on additional molecular contacts of the ribosomal binding sites with the tRNA body.[10,11] The ribosomal RNA provides the main structural features for the binding of tRNAs, and 16S RNA contributes to the structural alignment of mRNA triplets to be decoded. Therefore, an ill-defined codon–anticodon interaction in the P site may disturb the structure of the A site through changes in the ribosomal RNA structure (indicated by fuzzy outlines in Figure 1 d–f). As a consequence, the affinity of the aminoacyl-tRNA to the A site decreases and the binding of the release factors to the A site is promoted.[7]

There are also other possibilities for posttransfer error correction during translation. It is known that the misreading of codons can lead to ribosomal frameshifting. Such an event results in reading-frame alteration and leads sooner or later to the appearance of a nonsense codon in the A site followed by release-factor-dependent termination. Another possibility is the drop-off of a peptidyl-tRNA as a result of an incorrect codon–anticodon interaction. Peptidyl-tRNA is then hydrolyzed outside of the ribosome by peptidyl-tRNA hydrolase.[12]

The posttransfer proofreading mechanism described by Zaher and Green for ribosomal translation is known for DNA polymerases. These enzymes, which are responsible for the faithful replication of DNA, are able to cut out erroneously incorporated nucleotide. The elongation step is then repeated on the polynucleotide shortened by one residue. The post-transfer proofreading mechanism in ribosomal translation is energetically more expensive than the correction used by DNA polymerase. In the posttransfer proofreading step during polypeptide synthesis the complete polypeptide is discarded and finally degraded. Since functional polypeptides are shorter than DNA by orders of magnitude, this wasteful mechanism is acceptable for protein synthesis but not for DNA replication.

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[1] F. Bouadloun, D. Donner, C. G. Kurland, *EMBO J.* **1983**, *2*, 1351–1356.
[2] M. V. Rodnina, K. B. Gromadski, U. Kothe, H. J. Wieden, *FEBS Lett.* **2005**, *579*, 938–942.
[3] L. Pauling, *Festschrift Arthur Stoll*, Birkhäuser, Basel, **1958**, pp. 597–602.
[4] A. Korostelev, H. F. Noller, *Trends Biochem. Sci.* **2007**, *32*, 434–441.
[5] J. J. Hopfield, *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 4135–4139.
[6] J. Ninio, *Biochimie* **1975**, *57*, 587–595.
[7] H. S. Zaher, R. Green, *Nature* **2009**, *457*, 161–166.
[8] K. H. Nierhaus, *Biochimie* **2006**, *88*, 1013–1019.
[9] E. Villa, J. Sengupta, L. G. Trabuco, J. LeBarron, W. T. Baxter, T. R. Shaikh, R. A. Grassucci, P. Nissen, M. Ehrenberg, K. Schulten, J. Frank, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 1063–1068.
[10] D. Smith, M. Yarus, *J. Mol. Biol.* **1989**, *206*, 503–511.
[11] L. Cochella, R. Green, *Science* **2005**, *308*, 1178–1180.
[12] R. P. Anderson, J. R. Menninger, *Mol. Gen. Genet.* **1987**, *209*, 313–318.