15.5B: The Mechanism of Protein Synthesis

Protein synthesis involves building a peptide chain using tRNAs to add amino acids and mRNA as a blueprint for the specific sequence.

Learning Objectives

- Describe the process of translation

Key Points

- Protein synthesis, or translation, begins with a process known as pre-initiation, when the small ribosomal subunit, the mRNA template, initiator factors, and a special initiator tRNA, come together.
- During translocation and elongation, the ribosome moves one codon 3’ down the mRNA, brings in a charged tRNA to the A site, transfers the growing polypeptide chain from the P-site tRNA to the carboxyl group of the A-site amino acid, and ejects the uncharged tRNA at the E site.
- When a stop or nonsense codon (UAA, UAG, or UGA) is reached on the mRNA, the ribosome terminates translation.

Key Terms

- **translation**: a process occurring in the ribosome in which a strand of messenger RNA (mRNA) guides assembly of a sequence of amino acids to make a protein
The Mechanism of Protein Synthesis

As with mRNA synthesis, protein synthesis can be divided into three phases: initiation, elongation, and termination.

Initiation of Translation

Protein synthesis begins with the formation of a pre-initiation complex. In *E. coli*, this complex involves the small 30S ribosome, the mRNA template, three initiation factors (IFs; IF-1, IF-2, and IF-3), and a special initiator tRNA, called fMet-tRNA. The initiator tRNA basepairs to the start codon AUG (or rarely, GUG) and is covalently linked to a formylated methionine called fMet. Methionine is one of the 21 amino acids used in protein synthesis; formylated methionine is a methione to which a formyl group (a one-carbon aldehyde) has been covalently attached at the amino nitrogen. Formylated methionine is inserted by fMet-tRNA at the beginning of every polypeptide chain synthesized by *E. coli*, and is usually clipped off after translation is complete. When an in-frame AUG is encountered during translation elongation, a non-formylated methionine is inserted by a regular Met-tRNA. In *E. coli* mRNA, a sequence upstream of the first AUG codon, called the Shine-Dalgarno sequence (AGGAGG), interacts with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template.

In eukaryotes, a pre-initiation complex forms when an initiation factor called eIF2 (eukaryotic initiation factor 2) binds GTP, and the GTP-eIF2 recruits the eukaryotic initiator tRNA to the 40s small ribosomal subunit. The initiator tRNA, called Met-tRNA<sub>i</sub>, carries unmodified methionine in eukaryotes, not fMet, but it is distinct from other cellular Met-tRNAs in that it can bind eIFs and it can bind at the ribosome P site. The eukaryotic pre-initiation complex then recognizes the 7-methylguanosine cap at the 5′ end of a mRNA. Several other eIFs, specifically eIF1, eIF3, and eIF4, act as cap-binding proteins and assist the recruitment of the pre-initiation complex to the 5′ cap. Poly (A)-Binding Protein (PAB) binds both the poly (A) tail of the mRNA and the complex of proteins at the cap and also assists in the process. Once at the cap, the pre-initiation complex tracks along the mRNA in the 5′ to 3′ direction, searching for the AUG start codon. Many, but not all, eukaryotic mRNAs are translated from the first AUG sequence. The nucleotides around the AUG indicate whether it is the correct start codon.

Once the appropriate AUG is identified, eIF2 hydrolyzes GTP to GDP and powers the delivery of the tRNA<sub>i</sub>-Met to the start codon, where the tRNA<sub>i</sub> anticodon basepairs to the AUG codon. After this, eIF2-GDP is released from the complex, and eIF5-GTP binds. The 60S ribosomal subunit is recruited to the pre-initiation complex by eIF5-GTP, which hydrolyzes its GTP to GDP to power the assembly of the full ribosome at the translation start site with the Met-tRNA<sub>i</sub> positioned in the ribosome P site. The remaining eIFs dissociate from the ribosome and translation is ready to begins.

In archaea, translation initiation is similar to that seen in eukaryotes, except that the initiation factors involved are called aIFs (archaeal inititiaion factors), not eIFs.
Figure 1: **Translation initiation in eukaryotes.** In eukaryotes, a preinitiation complex forms made of the small 40S subunit, the initiator Met-tRNA\(_i\), and eIF2-GTP. This preinitiation complex binds to the 5'-m\(^7\)G cap of the mRNA with the help of other eIFs and PAB, which binds the poly(A) tail of the mRNA, and loops the tail to the cap. Once at the cap, the preinitiation complex slides along the mRNA until it encounters the initiator AUG codon. There, GTP is hydrolyzed by eIF2 and the Met-tRNA\(_i\) is loaded onto the AUG. Next, eIF5-GTP recruits the 60S large ribosomal subunit to the 40S subunit at the AUG and hydrolyzes GTP. This allows the large ribosomal subunit to assemble on top of the small subunit, generating the intact 80S ribosome, and places the Met-tRNA\(_i\) in the P site of the intact ribosome. The ribosome A site is positioned over the second codon in the mRNA reading frame, and translation elongation can begin.

**Translation Elongation**

The basics of elongation are the same in prokaryotes and eukaryotes. The intact ribosome has three compartments: the A site binds incoming aminoacyl tRNAs; the P site binds tRNAs carrying the growing polypeptide chain; the E site releases dissociated tRNAs so that they can be recharged with amino acids. The initiator tRNA, rMet-tRNA\(_i\) in *E. coli* and Met-tRNA\(_i\) in eukaryotes and archaea, binds directly to the P site. This creates an initiation complex with a free A site ready to accept the aminoacyl-tRNA corresponding to the first codon after the AUG.

The aminoacyl-tRNA with an anticodon complementary to the A site codon lands in the A site. A peptide bond is formed between the amino group of the A site amino acid and the carboxyl group of the most-recently attached amino acid in the growing polypeptide chain attached to the P-site tRNA. The formation of the peptide bond is catalyzed by peptidyl transferase, an RNA-based enzyme that is integrated into the large ribosomal subunit. The energy for the peptide bond formation is derived from GTP hydrolysis, which is catalyzed by a separate elongation factor.

Catalyzing the formation of a peptide bond removes the bond holding the growing polypeptide chain to the P-site tRNA. The growing polypeptide chain is transferred to the amino end of the incoming amino acid, and the A-site tRNA temporarily holds the growing polypeptide chain, while the P-site tRNA is now empty or uncharged.

The ribosome moves three nucleotides down the mRNA. The tRNAs are basepaired to a codon on the mRNA, so as the ribosome moves over the mRNA, the tRNAs stay in place while the ribosome moves and each tRNA is moved into the next tRNA binding site. The E site moves over the former P-site tRNA, now empty or uncharged, the P site moves over the former A-site tRNA, now carrying the growing polypeptide chain, and the A site moves over a new codon. In the E site, the uncharged tRNA detaches from its anticodon and is expelled. A new aminoacyl-tRNA with an anticodon complementary to the new A-site codon enters the ribosome at the A site and the elongation process repeats itself. The energy for each step of the ribosome is donated by an elongation factor that hydrolyzes GTP.
Translation elongation in eukaryotes: During translation elongation, the incoming aminoacyl-tRNA enters the ribosome A site, where it binds if the tRNA anticodon is complementary to the A site mRNA codon. The elongation factor eEF1 assists in loading the aminoacyl-tRNA, powering the process through the hydrolysis of GTP. The growing polypeptide chain is attached to the tRNA in the ribosome P site. The ribosome’s peptidyl transferase catalyses the transfer of the growing polypeptide chain from the P site tRNA to the amino group of the A site amino acid. This creates a peptide bond between the C terminus of the growing polypeptide chain and the A site amino acid. After the peptide bond is created, the growing polypeptide chain is attached to the A site tRNA, and the tRNA in the P site is empty. The ribosome translocates once codon on the mRNA. The elongation factor eEF2 assists in the translocation, powering the process through the hydrolysis of GTP. During translocation, the two tRNAs remain basepaired to their mRNA codons, so the ribosome moves over them, putting the empty tRNA in the E site (where it will be expelled from the ribosome) and the tRNA with the growing polypeptide chain in the P site. The A site moves over an empty codon, and the process repeats itself until a stop codon is reached.

Termination of translation occurs when the ribosome moves over a stop codon (UAA, UAG, or UGA). There are no tRNAs with anticodons complementary to stop codons, so no tRNAs enter the A site. Instead, in both prokaryotes and eukaryotes, a protein called a release factor enters the A site. The release factors cause the ribosome peptidyl transferase to add a water molecule to the carboxyl end of the most recently added amino acid in the growing polypeptide chain attached to the P-site tRNA. This causes the polypeptide chain to detach from its tRNA, and the newly-made polypeptide is released. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation initiation complex. After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction.
**Modeling translation:** This interactive models the process of translation in eukaryotes.