Protein Synthesis (Translation)

MS. NEHA
Asst. Prof.(Zoology)

What is translation?

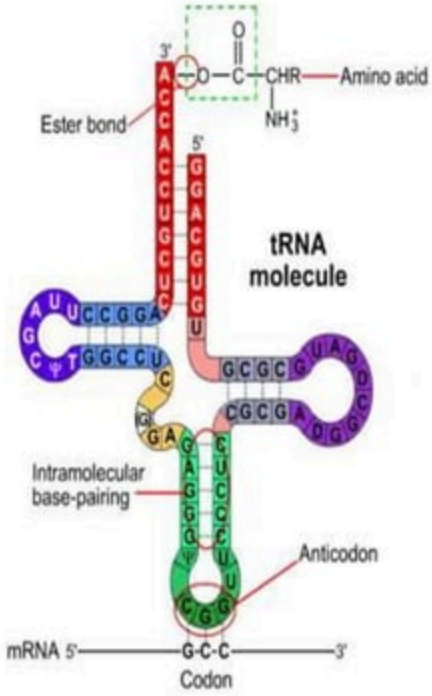
- Formation of protein from mRNA is called translation, is also known as polypeptide synthesis or protein synthesis. It is unidirectional process.
- The raw materials for protein synthesis are amino acids, mRNA, tRNAs and amino acyl tRNA synthetases.
- Ribosomes catalyze the joining of the amino acid monomers directed by the mRNA sequence.
- Amino-acyl tRNA synthetases attach amino acids to the appropriate tRNAs.
- The amino-acyl tRNA act as adaptors in the translation of the nucleic acid sequence of the mRNA into the amino acid sequence of the protein.

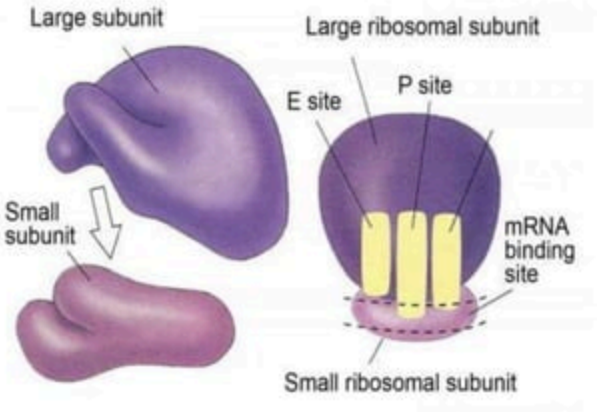
Translation occurs in three stages…

- **Initiation-** The components of the translational apparatus come together with the mRNA molecule (A tRNA carrying the first amino acid binds to the start codon).
- **Elongation-** Amino acids are brought to the mRNA as amino-acyl tRNAs and are added one at a time to a growing polypeptide chain.
- **Termination**- A stop codon in the mRNA is recognized by a protein release factor and the translational apparatus comes apart to release a completed polypeptide.

Tools of Translation...

- **Amino acids :** Twenty types of amino acids and amides constitute the *building blocks* of proteins.
- **mRNA :** It carries the coded information for synthesis of one (unicistronic) or more polypeptides (polycistronic). Its codons are recognised by tRNAs
- **tRNAs :** They picks up specific amino acid from amino acid pool and carrying over the mRNA strand.
- **Amino Acyl tRNA Synthetases :** The enzymes are specific for particular amino acids and their tRNAs.
- **Ribosomes :** The A (aminoacyl) and P (peptidyl) sites as cavities on the ribosome where charged tRNA (carrying an amino acid) molecules bind during polypeptide synthesis. The recently postulated E (exit) site is the site from which discharged tRNAs leave the ribosome. The mRNA-binding site binds a sequence near the 5 prime end of the mRNA. The binding sites are all located at or near the interface between the large and small subunits

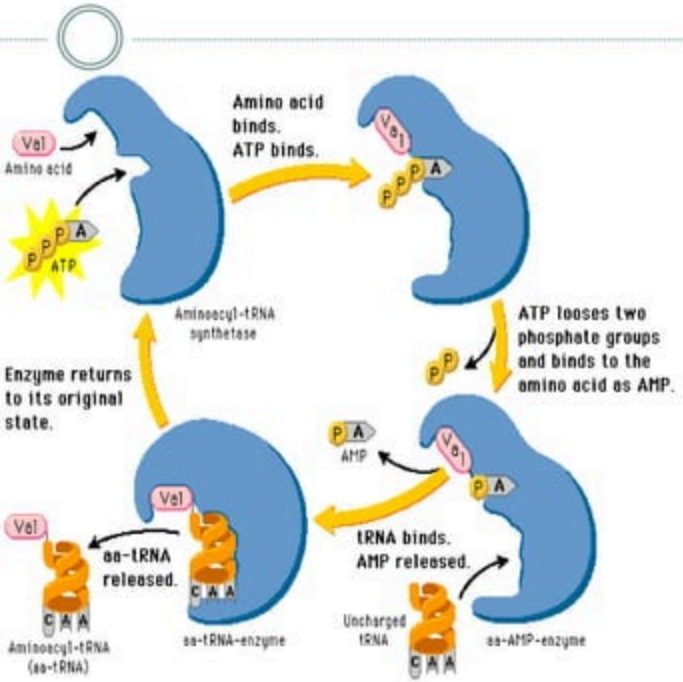

3'
5'
Amino acid
Ester bond
tRNA molecule
Intramolecular base-pairing
Anticodon
mRNA 5'
G-C-C
3'
Codon


Large subunit
Large ribosomal subunit
E site
P site
Small subunit
mRNA binding site
Small ribosomal subunit

Charging of tRNA

Step 1) The amino acid and a molecule of ATP enter the active site of the enzyme. The ATP loses pyrophosphate and the resulting AMP bonds covalently to the amino acid. The pyrophosphate is hydrolyzed into two phosphate groups.

Step 2) The tRNA covalently bonds to the amino acid to displace the AMP and the aminoacyl tRNA is then released from the enzyme



The Process of Translation

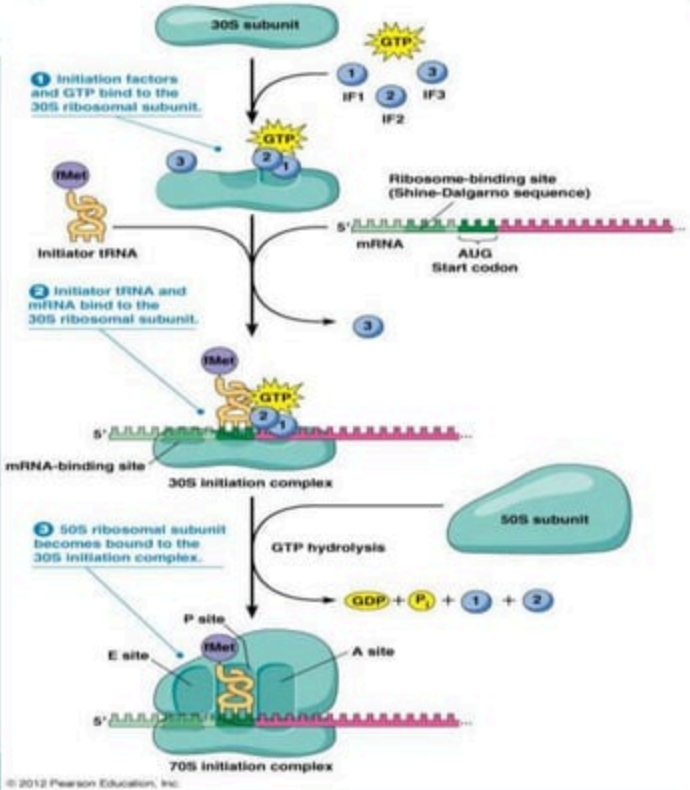
- A prokaryotic mRNA molecule encoding a single polypeptide has 5 prime non-coding leader sequence which contains a ribosome binding site
- The rest of the mRNA contains a coding sequence that starts with an AUG start codon and ends with a stop codon (UAA, UAG or UGA) and a 3 prime non-coding trailer sequence
- A eukaryotic mRNA molecule has, in addition to the above, a 5 prime cap and a 3 prime poly(A) tail. One important difference is that eukaryotic mRNAs lack a ribosome binding site (SD site)
- **N-formylmethionine (fMet)** is the modified amino acid with which every polypeptide is initiated in prokaryotes but **methionine** is the first amino acid in eukaryotes

Translation in prokaryotes

Initiation

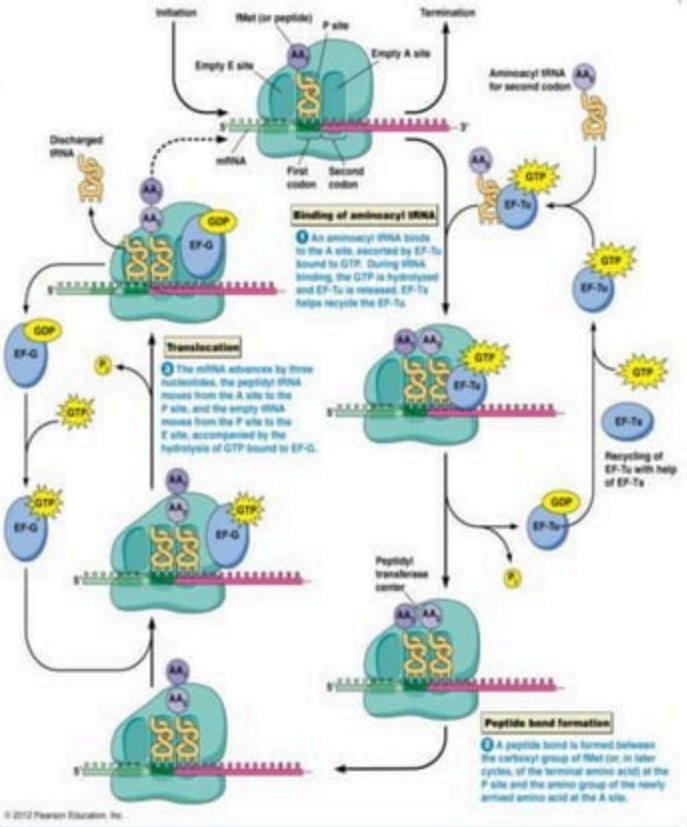
Formation of the 70S translation initiation complex occurs in three steps:-

- Three initiation factors (IF) and GTP bind to the small ribosomal subunit.
- The initiator aminoacyl tRNA and mRNA are attached. The mRNA-binding site is composed of a portion of the 16S rRNA of the small ribosomal subunit. The 3' end of the 16S rRNA bears a pyrimidine-rich stretch that base pairs with the Shine-Dalgarno sequence of the mRNA)
- The large ribosomal subunit joins the complex. The resulting 70S initiation complex has fMet-tRNA^{fMet} residing in the ribosome's P site



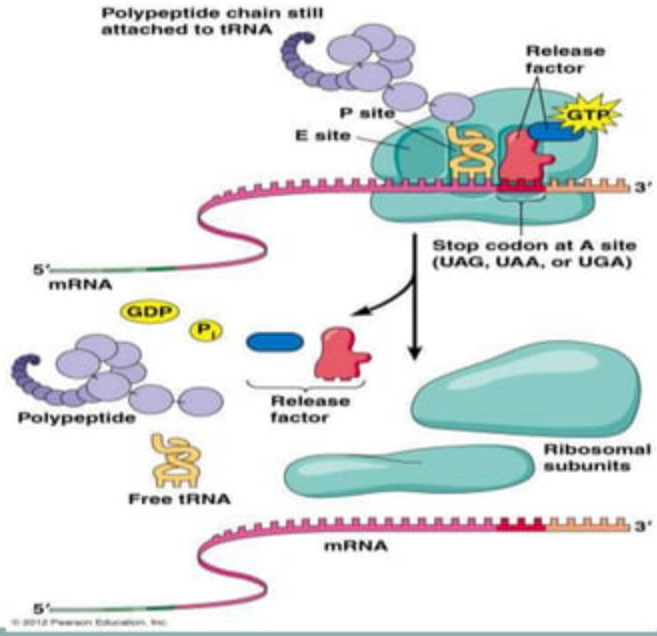
Chain elongation

- Elongation begins with the binding of the second aminoacyl tRNA at the ribosomal aminoacyl (A) site. The tRNA is escorted to the A site by the elongation factor EF-Tu, which also carries two bound GTPs. As the tRNA binds, the GTPs are hydrolyzed and EF-Tu is released. EF-Ts helps recycle the EF-Tu.
- A peptide bond is formed between the carboxyl group of the terminal amino acid (or fMet in the first cycle) at the P site and the amino group of the newly arrived amino acid at the A site. This reaction is catalyzed by the peptidyl transferase activity of the 23S rRNA molecule in the large ribosomal subunit.
- After EF-G-GTP binds to the ribosome and GTP is hydrolyzed, the tRNA carrying the elongated polypeptide translocates from the A site to the P site. The discharged tRNA moves from the P site to the E (exit) site and leaves the ribosome. As the peptidyl tRNA translocates, it takes the mRNA along with it. Consequently, the next mRNA codon is moved into the A site, which is open for the next aminoacyl tRNA. These events are repeated for each additional amino acid.



Termination

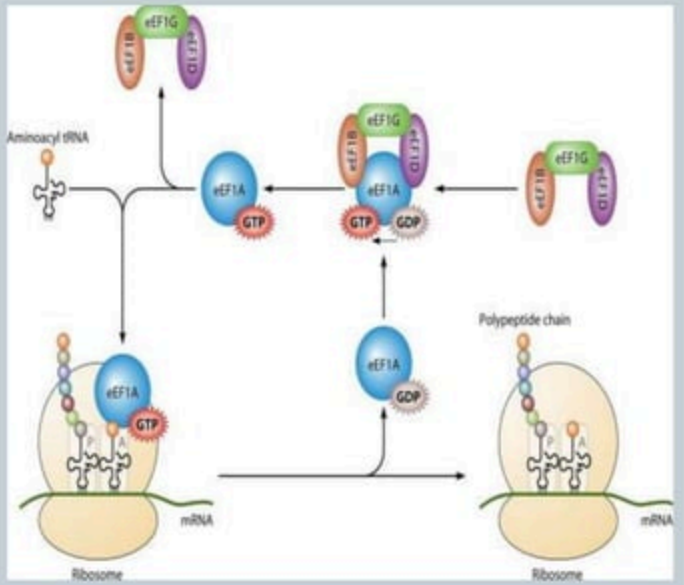
Depends on release factors that recognize the three stop codons. When a stop codon (UAG, UAA, or UGA) arrives at the A site, it is recognized and bound by a protein release factor. This protein causes the polypeptide to be transferred to a molecule of water to cause its release from the tRNA and the dissociation of the other components of the elongation complex.



Translation in Eukaryotes

Initiation

- In eukaryotes, translation includes a different set of initiation factors (eIFs), a slightly different assembly pathway
- eIF2 binds the initiator tRNA ^{met} before the small ribosomal subunit. This complex can attach to the 5 prime cap structure of the mRNA.
- As there is no Shine-Dalgarno sequence, the ribosome begins translation at a AUG that is located within the Kozak consensus sequence (often a good match to CAAA**AUG**).



Chain elongation

- The process of elongation in eukaryotes is same as in prokaryotes
- But elongation factors are different- eEF1 and eEF2 in eukaryotes.

Termination

- Termination factor eRF1 is required for termination in eukaryotes
- Procedure is same as in prokaryotes