INTRODUCTION

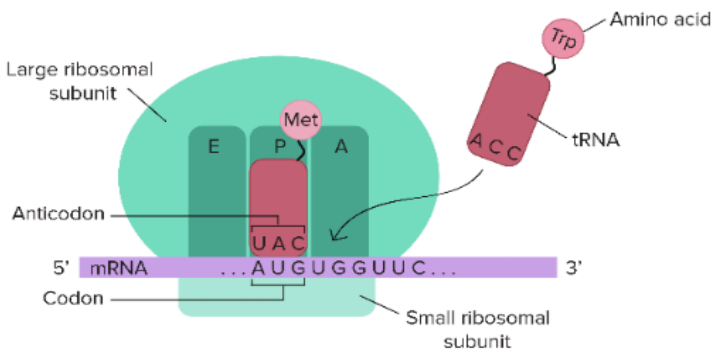
Translation is the process where polypeptide chains are formed from an mRNA. Translation is the second major step in gene expression, where the mRNA is "read" according to the genetic code, which relates the DNA sequence to the amino acid sequence in proteins. Each group of three bases in mRNA constitutes a codon, and each codon specifies a particular amino acid. The mRNA sequence is thus used as a template to assemble the chain of amino acids that form a protein.

RIBOSOME -THE TRANSLATIONAL STRUCTURE

Ribosomes are the structures where polypeptides are built. They are made up of protein and RNA. Each ribosome has two subunits, a large one and a small one. Smaller sub-units comes in contact with mRNA to initiate the process of translation. The subunits come together around an mRNA—kind of like the two halves of a hamburger bun coming together around the patty.

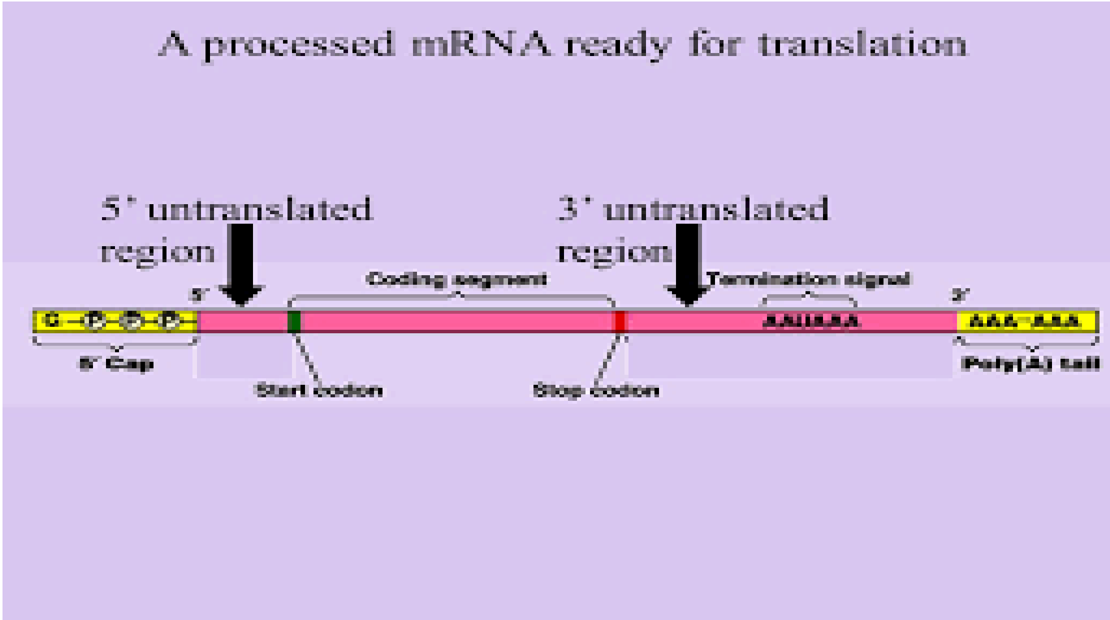
The ribosome provides a set of handy slots where tRNAs can find their matching codons on the mRNA template and deliver their amino acids. These slots are called the A, P, and E sites. Not only that, but the ribosome also acts as an enzyme, catalyzing the chemical reaction that links amino acids together to make a chain.

Translational unit in an mRNA is the region flanked by a start codon and stop codon.



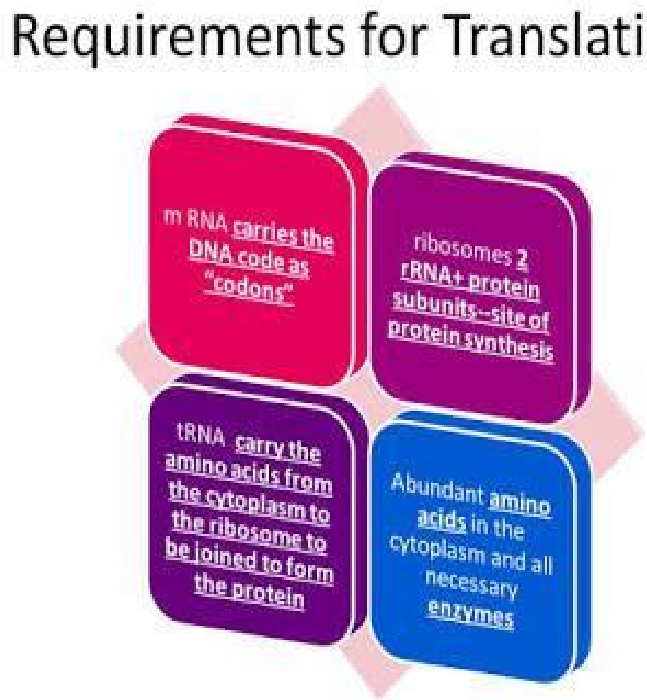
UNTRANSLATED REGION :

In a particular region, there is an area near the 5' end of the molecule that is known as the untranslated region (UTR). This portion of mRNA is located between the first nucleotide that is transcribed and the start codon (AUG) of the coding region, and it does not affect the sequence of amino acids in a protein. UTR turns out the leader sequence is important because it contains a ribosome-binding site.



Requirements for Translation

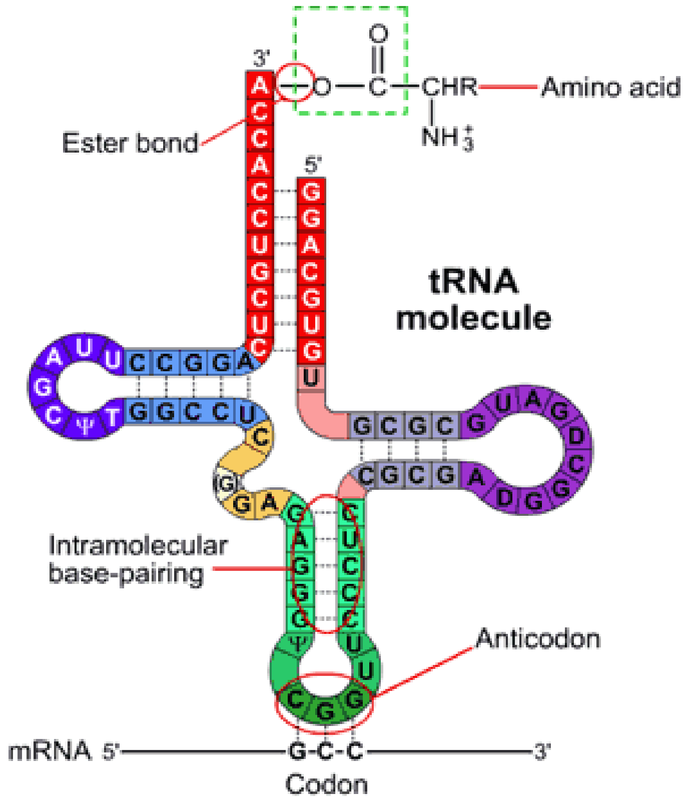
- Ribosomes
- m-RNA
- t-RNA
- AMINOACYL t-RNA SYNTHETASES
- ATP (Adenosine triphosphate)
- GTP (Guanosine triphosphate)
- ENZYMES OF TRANSLATION
- PROTEIN FACTORS



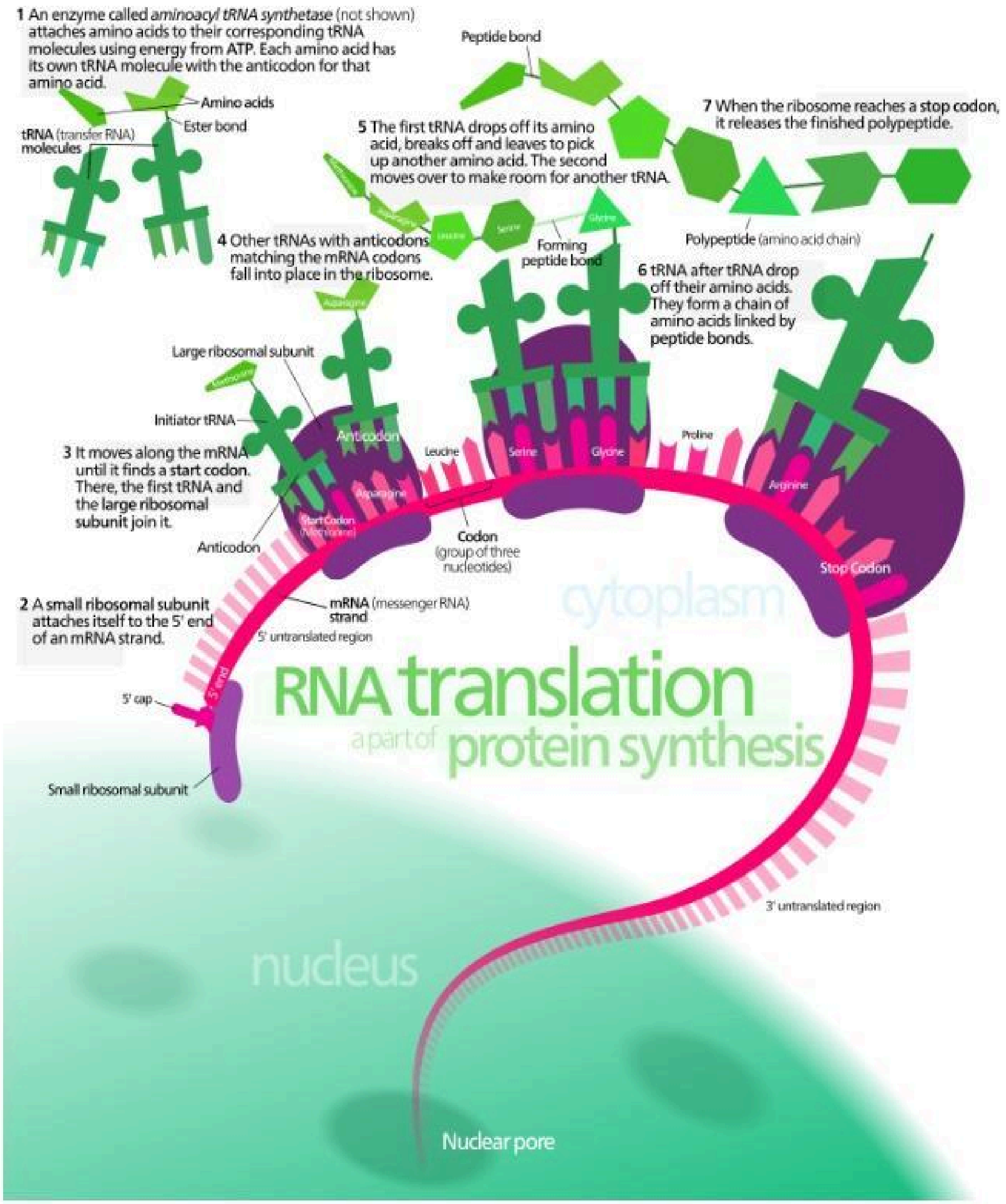
tRNA – an adaptor molecule

Proteins are made through a process called translation, where instructions stored in a piece of mRNA are used by the ribosome's to make protein. tRNAs adapt the mRNA nucleotide sequence into a protein's peptide sequence by acting as an intermediary. A tRNA is charged by attaching an amino acid to one end, it then binds the ribosome mRNA complex at the position defined by a codon.

A codon is a sequence of three nucleotides that codes for an amino acid.



OVERVIEW OF TRANSLATION



Overview of the translation of eukaryotic messenger RNA

TRANSLATION MACHINERY

It consists of ribosome's, amino acids,mRNA,tRNAs and **aminoacyl-tRNA-synthetases**. mRNA functions as a template having genetic information. Ribosome is a site of protein synthesis. RNAs are formed over DNA during transcription while protein synthesis occurs in the cytoplasm or ribosome's.

There are three reactive sites – P, A and E site. P-site (donor site) is jointly contributed by the two ribosomal subunits. A-site (acceptor site) is situated on the larger subunit of ribosome. It faces the tunnel between the two subunits. E-site (exit site) is part of larger subunit facing the tunnel site.

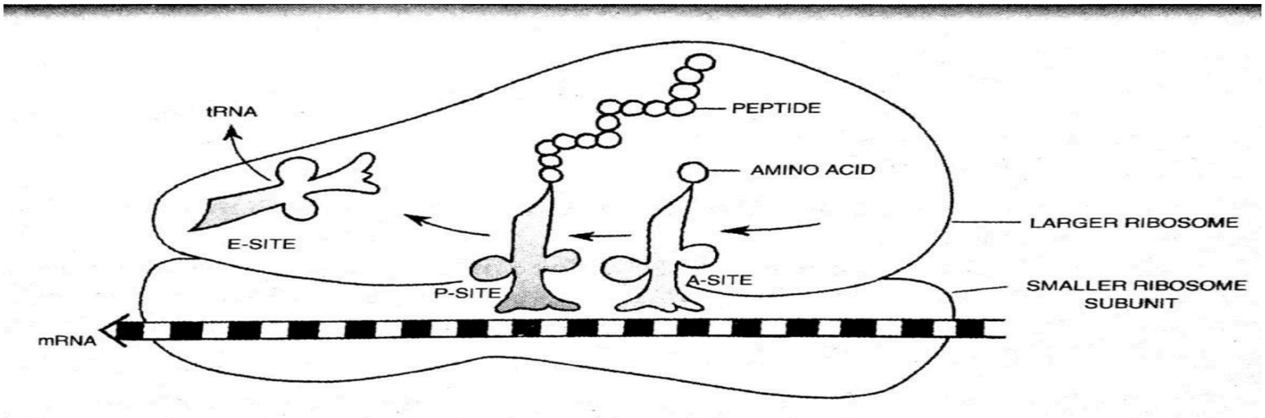
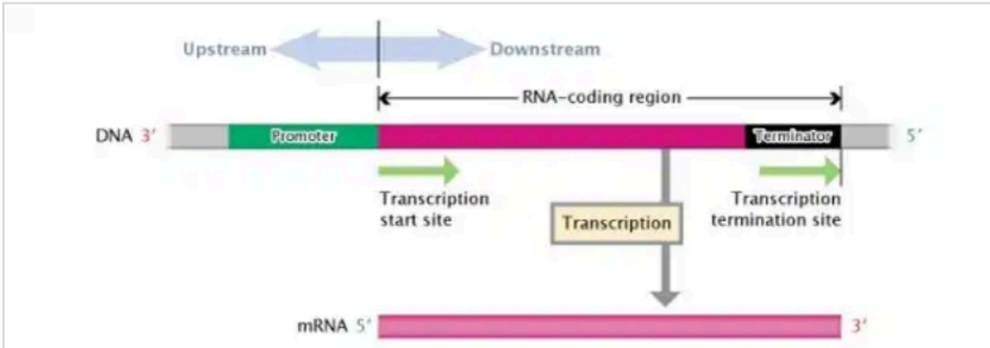
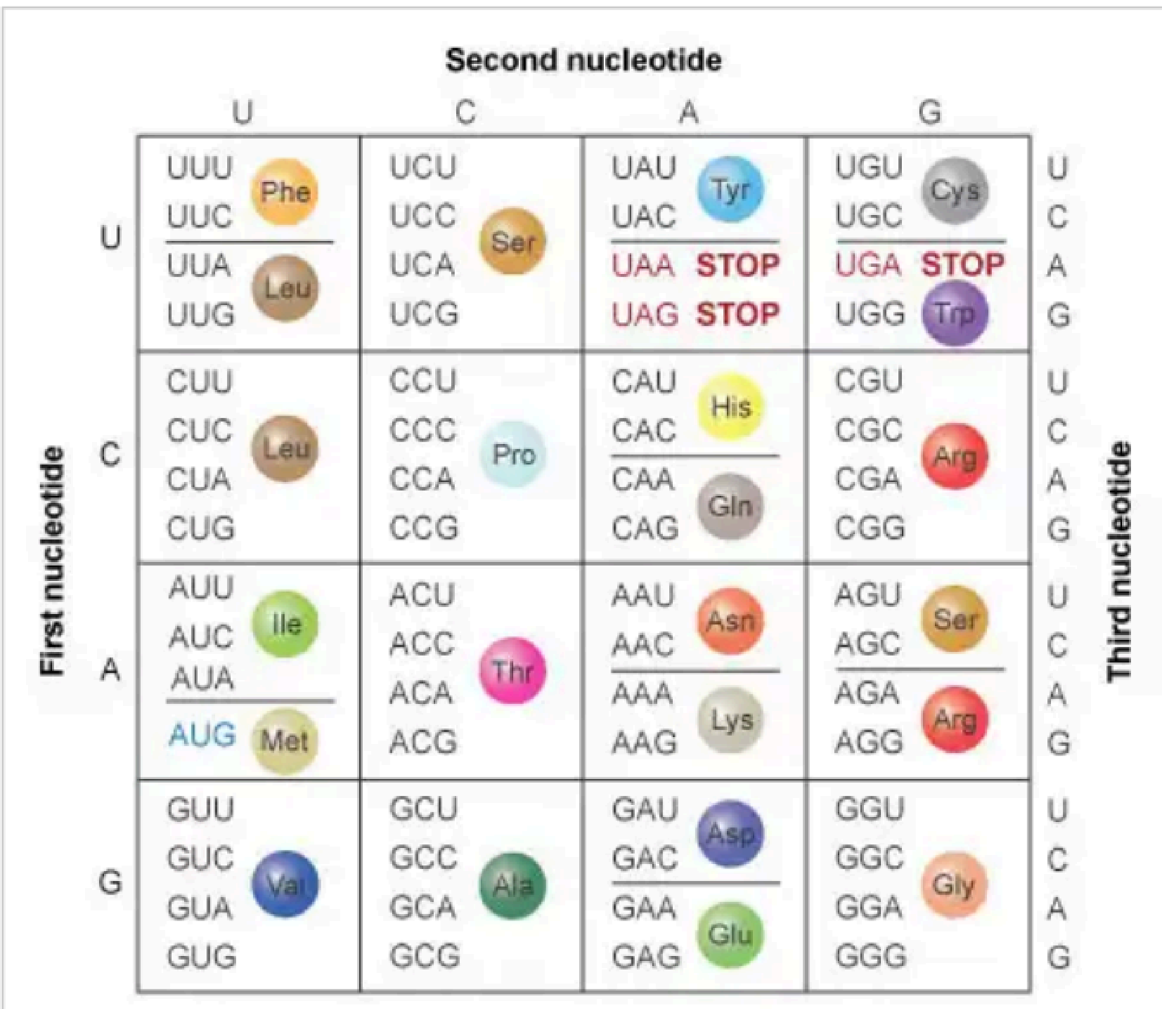


Fig. 6.35. Ribosome and Reactive Sites

TRANSCRIPTION UNIT



GENETIC CODES



TRANSLATION MECHANISM:

1. Activation of Amino Acids :

   It is carried out by **activating enzymes**, known as **aminoacyl-tRNA-synthetase**. It produces **amino-acyl-adenylate-enzyme** complex.

$$\underset{\text{amino acid}}{AA} + ATP + \underset{\text{amino acyl tRNA synthetase}}{E} \xrightarrow{Mg^{2+}} AA \sim AMP - E + \underset{\text{Pyrophosphate}}{PPi}$$

2. Charging or Aminoacylation of tRNA :

   The complex reacts with tRNA specific for the amino acids to form aminoacyl- tRNA complex. Enzyme and AMP are released. tRNA complexed with amino acid is sometimes called **charged tRNA.** The amino acid is linked to 3-OH-end of tRNA through its –COOH groups.

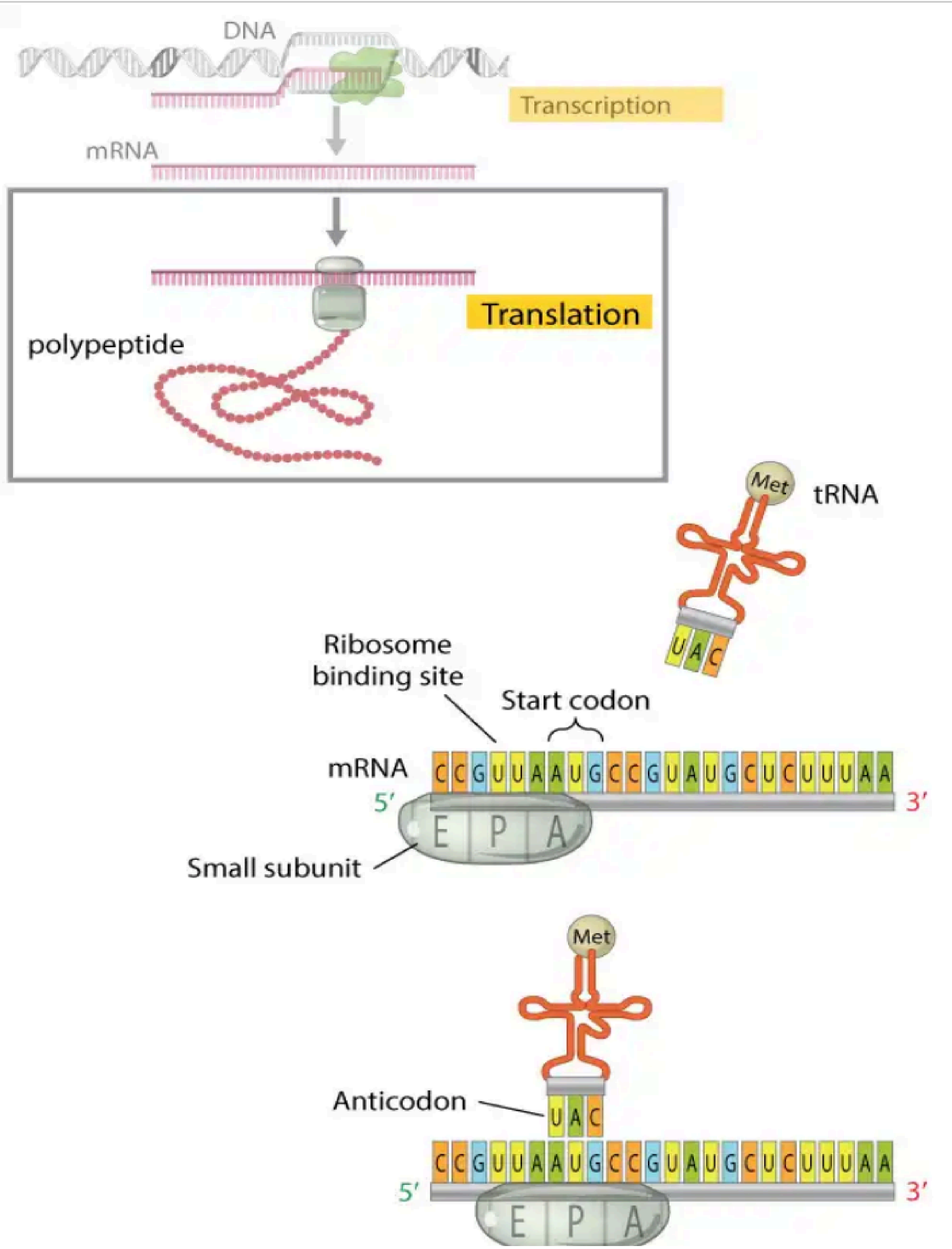
$$\underset{\text{aminoacyl adenylate enzyme}}{AA \sim AMP - E} + tRNA \longrightarrow AA\text{-}tRNA + AMP + E$$

3. INITIATION :

It requires factors called **initiation factors.** These are three initiation factors in prokaryotes – IF3, IF2, and IF1. Eukaryotes have nine initiation factors – eIF2, eIF3, eIF1, eIF4A, eIF4B, eIF4C, eIF4D, eIF5, eIF6. Out of these **IF3** or **eIF2** is attached to smaller sub units of ribosome's in the dissociated state. **GUANOSINE TRIPHOSPHATE** is required. mRNA is attaches itself to smaller subunits of ribosome's in the region of its cap. The cap has nucleotides complementary to the nucleotides present in the 3' end of rRNA. The attachment is such that initiation codon of mRNA comes to lie at p-site.

Aminoacyl tRNA complex specific for the initiation codon reaches the p-site. Anti-codon establishes temporary hydrogen bond with the initiation codon of mRNA. The codon- anticodon reaction occurs in the presence of initiation factors **eIF3** in eukaryotes and **IF2** in prokaryotes .It requires energy provided by GTP.

$$\text{40S subunit} + \text{mRNA} \xrightarrow{\text{eIF2}} \text{40S} - \text{mRNA}$$


DNA
Transcription
mRNA
Translation
polypeptide
Met
tRNA
UAC
Ribosome
binding site
Start codon
mRNA
CCGUUAAUGCCGUAUGCUCUUUAA
5′
3′
E P A
Small subunit
Met
Anticodon
UAC
CCGUUAAUGCCGUAUGCUCUUUAA
5′
3′
E P A

4. ELONGATION :

An aminoacyl tRNA complex reaches the A-site and attaches in mRNA codon next to initiation codon with the help of its anticodon. The step requires GTP and an **elongation factor** in eukaryotes and EF-Tu as well as EF-Ts in prokaryotes. It has been found out that in E-coli the most abundant protein is elongation factor. A peptide bond is established between the carboxyl group of amino acid attached to tRNA at P-site and amino acid attached to tRNA at A-site. The reaction is catalyzed by enzyme **peptidyl tranferase** which is an RNA-enzyme. Due to this, NH2 group of the first amino acid is blocked from getting involved in peptide bond formation with another amino acid. In the process the connection between tRNA and the amino acid at the P-site breakes. The free tRNA of the P-site slips to E-site and from there to the outside of ribosome with the help of G-factor. The A-site carries peptidyl tRNA complex.

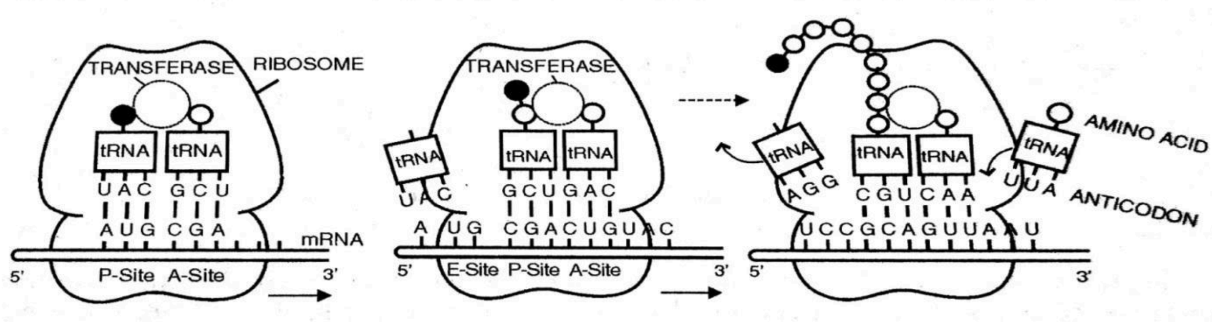


Fig. 6.36. Elongation of the polypeptide chain.

Soon after the establishment of first peptide linkage and slipping of the freed tRNA of P-site, the ribosome rotates slightly. The process is known as **translocation**. It requires a factor called **translocase** and energy from GTP. As a result of translocation the A-site codon along with peptidyl-tRNA complex reaches the P-site. A new codon is exposed at the A-site. It attracts a new aminoacyl tRNA complex. The process of bond formation and translocation is repeated. One by one all the codons of mRNA are exposed at the A-site and get decoded through incorporation of amino acids in the peptide chain. The peptide chain elongates. The elongated peptide chain lies in the groove of the larger sub unit of ribosome to protect itself from cellular enzymes because it is prone to breakdown due to its extended nature.

5. TERMINATION :

Polypeptide synthesis is terminated when a stop codon of mRNA reaches the A site. There are three stop codons - UAA, UAG and UGA. These codons are not recognized by any of the tRNAs. Therefore no more aminoacyl tRNA reaches the A-site. The P-site tRNA is hydrolysed in the complete polypeptide is released in the presence of GTP-dependent **release factor**. It is single in eukaryotes and double in prokaryotes. In prokaryotes RF1 is specific for UAG and UAA. RF2 is specific for UAA and UGA. GTP dependent RF3 is required for releasing the RFs from ribosome. Ribosome moves over the stop codon and slips off the mRNA chain. The two sub-units of ribosome separate in the presence of dissociation factor.

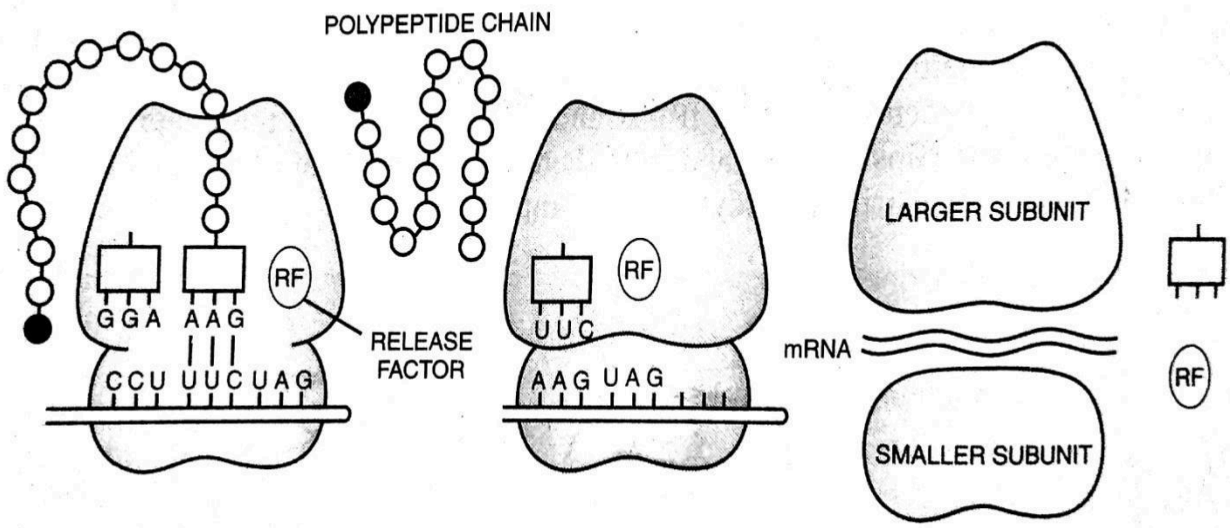


Fig. 6.37. Termination of polypeptide chain.

In prokaryotes, formylated methionine is commonly the initiating amino acid. It is ether deformylated or sometimes removed from polypeptide. The initiating methionine is usually not retained in eukaryotes. At a time several polypeptides are synthesized from the same mRNA strand. Each ribosome of a polyribosome forms the same type of polypeptide. Formation of a number of copies of the same polypeptide simultaneously from an mRNA with the help of a polysome is called **translational amplification**.

POST-TRANSLATIONAL PROCESSING

After translation, the polypeptide is further processed to be functional.

Many eukaryotic proteins require post-translational processing to be functional, while in E. coli many of the post-translational processing steps are not performed.

- **Several types of processing:**

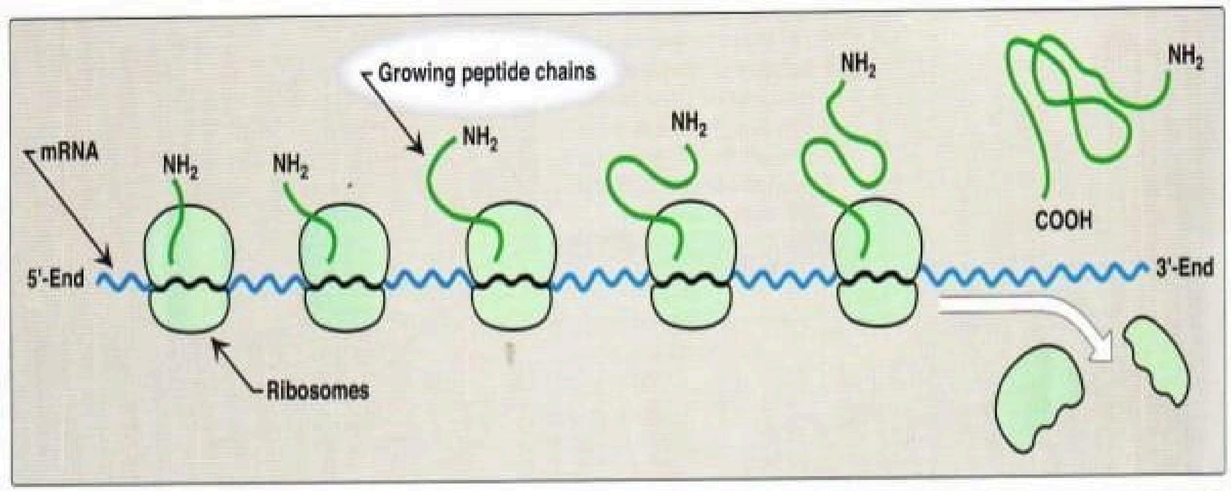
1. Folding into proper conformation, which can be assisted by chaperone proteins.

2. Signal peptide cleavage- Here, many proteins carry signal sequences for intracellular translocation and secretion, these signals are cleaved during secretion.

3. Glycosylation - It is the process of addition of sugar to the protein, it is very common in eukaryotic proteins, it would affect the protein function, stability, immunogenicity.

4. Phosphorylation : It’s a biochemical process that involves the addition of phosphate to an organic compound.

5. Proteolytic processing : It is a major form of post translational modification which occurs when a protease cleaves one or more bonds in a target protein to modify its activity. This processing may lead to activation, inhibition or destruction of the protein activity.



A polyribosome consists of several ribosomes simultaneously translating one mRNA.

END PRODUCTS OF TRANSLATION

End Product

- The end products of protein synthesis is a **primary structure of a protein**.
- A sequence of **amino acid** bonded together by **peptide bonds**.