PROTEIN BIOSYNTHESIS

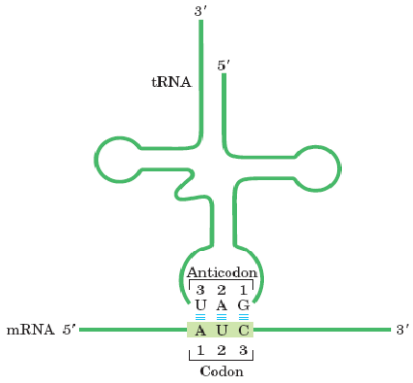
It is synthesis of polypeptide in ribosomes. The sequence of amino acids in polypeptide is dictated by the sequence of nucleotides in mRNA. The particular amino acid sequence of a protein is constructed though the translation of information encoded in mRNA. This process is carried by ribosomes. The ribosomes are ribo-nucleo-proteins that are essential for biosynthesis of protein. Each amino acid in a protein is specified by a specific ribonucleotide triplet. These ribonucleotide triplet are called codons.

Genetic Code: the genetic information resides in the linear sequence of deoxy-ribonucleotides in DNA, which is transcribed into mRNA. This mRNA guides the protein synthesis that is called translation.

The sequence of nucleotides of mRNA dictates the arrangement of amino acids in protein. A triplet of consecutive nucleotide is an mRNA chain is called codon that specifies an amino acid. This triplet code of nucleotides is referred to a genetic code.

FEATURES OF GENETIC CODE

1. The genetic code is degenerate: it has multiple codons for every amino acids.
2. The codons AUG signals the initiation of translation (AUG also specifies or codes for Methionine).
3. The triplet codons UAA, UGA and UAG are signals for termination of translation. These are also called non-sense codons or stop codons.
4. Each t-RNA molecule carrying a particular amino acid contains a specific sequence complementary to codon that is termed as anticodon.



The protein synthesis occurs in five stages in cytosol:

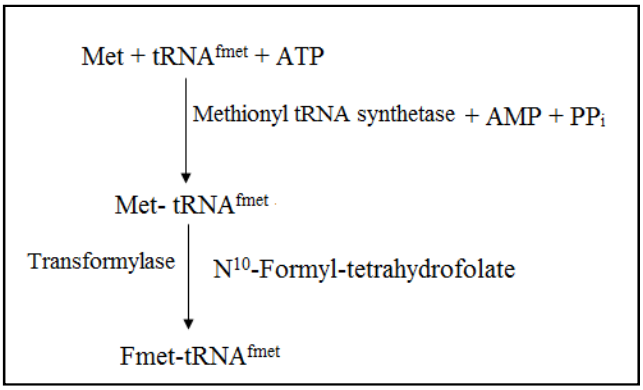
1. Activation of amino acids.
2. Initiation of polypeptide chain.
3. Elongation.
4. Termination.
5. Folding and post-translational processing.

1. Activation of amino acids:

$$\text{Amino Acid} + \text{tRNA}^{aa} + \text{ATP} \xrightarrow{\text{Amino-acyl-tRNA synthetase}} \text{Amino Acyl-tRNA}^{aa} + \text{AMP} + PP_i$$

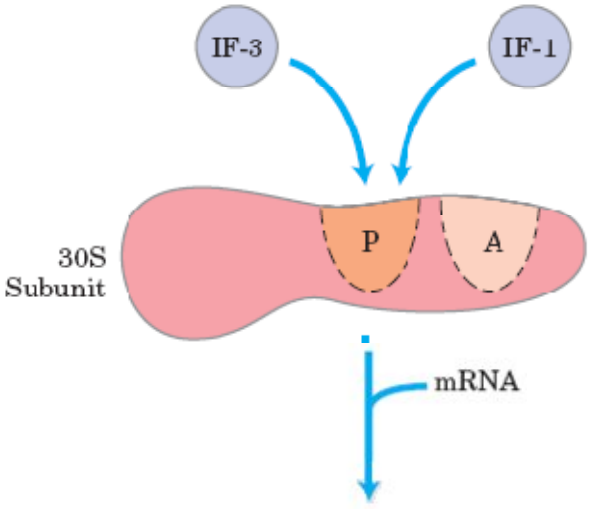
The Enzyme required for activation of amino acids for protein biosynthesis is Amino-acyl-tRNA synthetase. As the start codon or initiation codon is AUG that also codes for methionine, all the polypeptides begin with methionine.

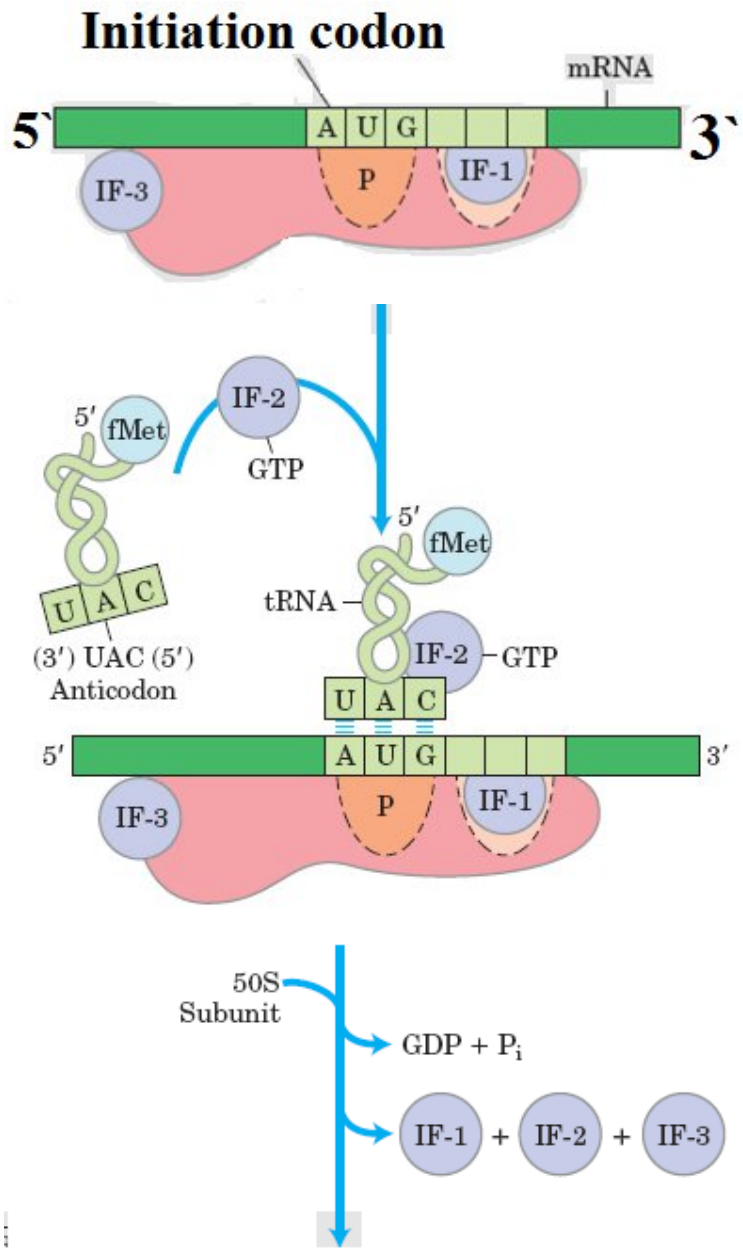
In case of bacteria, the first the amino acid is N-formyl-methionine instead of methionine. The reaction of activation of formyl methionine in bacteria proceeds in following manner:

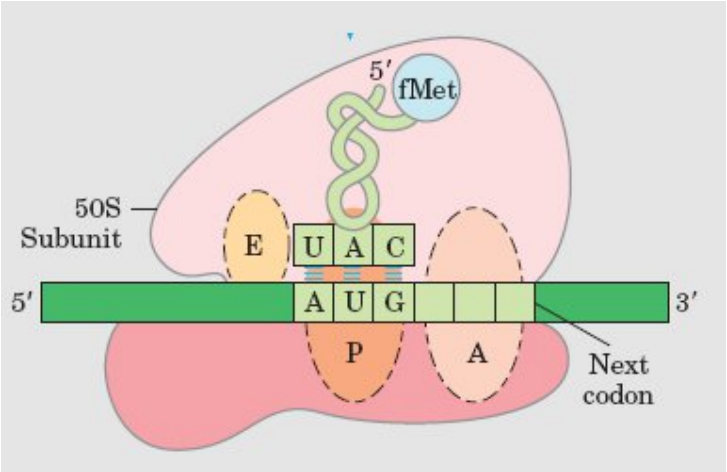


The tRNA having attached amino acid to it is called charged tRNA or amino acyl-tRNA.

2. Initiation of translation in bacteria: the components that participates in the initiation of translation are mRNA, 30s ribosomal subunit, 50s ribosomal subunit, 50s ribosomal subunit, fmet-tRNA^{fmet} (initiating tRNA) and initiation factors (IF1, IF2, IF3). The 30s ribosomal subunit have two sites called A site (amino acyl site) and P site (Peptidyl site). The 50s ribosomal subunit possess one site called E site (Exit site). The A site and P site bind to amino acyl tRNA whereas E site binds to uncharged tRNA.
   Steps of initiation of protein biosynthesis:
   a. The IF1 and IF3 binds 30s ribosome. The IF1 binds the A site to prevent the entry of first tRNA to A-site.
   b. Binding of IF2-GTP to fmet-tRNA^{fmet} complex. This complex is taken to P site of 30s ribosome.
   c. After the initiating tRNA has attached the P-site, all the factors released and GTP bound to IF2 is hydrolyzed to GDP.
   d. Binding of 50s ribosomal subunit to 30s subunit forming 70s subunit that is called initiation complex.




Initiation codon
mRNA
5`
3`
A U G
IF-3
P
IF-1
5′ fMet
IF-2
GTP
U A C
(3′) UAC (5′)
Anticodon
tRNA
IF-2 GTP
5′
3′
50S
Subunit
GDP + P_i
IF-1 + IF-2 + IF-3



3. Elongation: The requirements of elongation step are as follows:
    a. Initiation complex.
    b. Elongation factors (Ef-Tu, Ef-Ts, Ef-G).
    c. GTP
    d. Amino acyl-tRNA.

There are three steps under elongation:

Step I: Binding of an incoming amino-acyl-tRNA

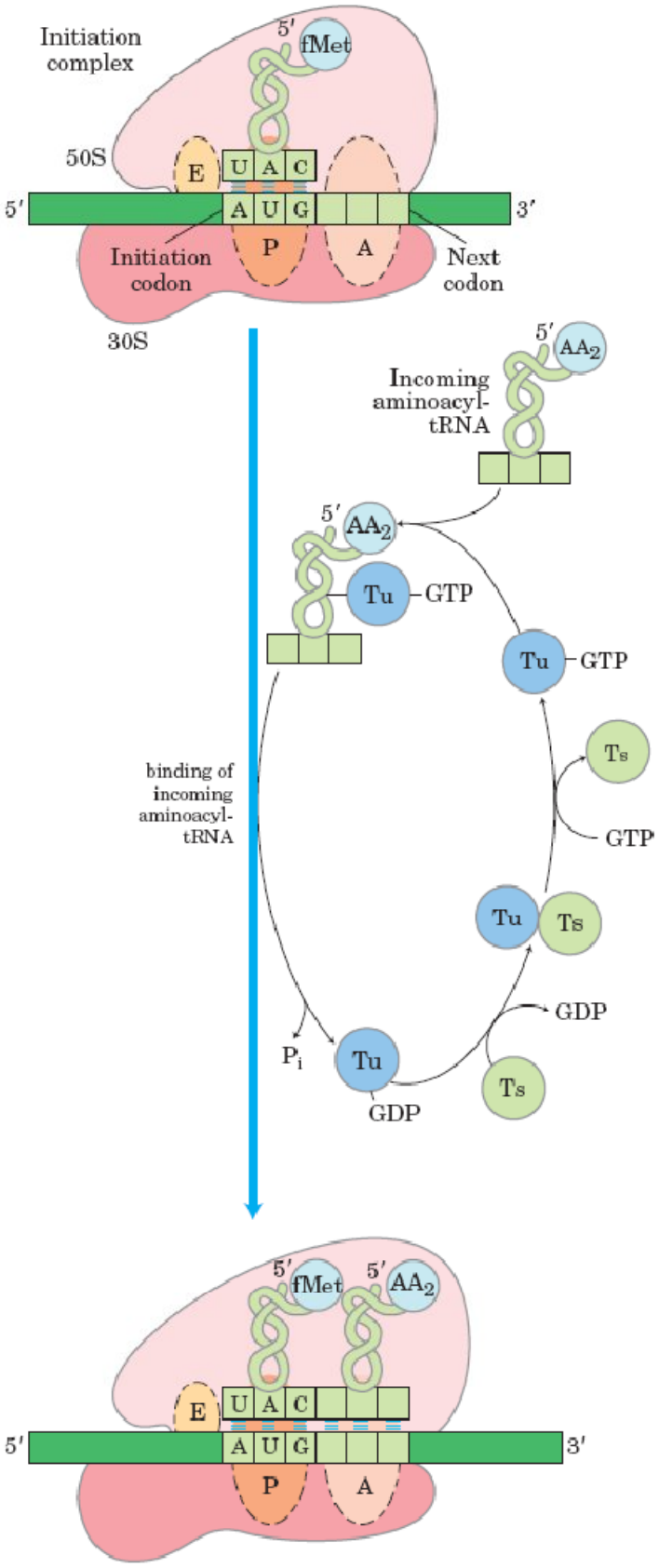
The appropriate incoming amino-acyl-tRNA binds to a complex of GTP bound EF-Tu. The resulting amino-acyl-tRNA-EF-Tu-GTP complex binds to the A site of initiation complex. The GTP is hydrolyzed forming EF-Tu-GDP that is released from ribosomes.

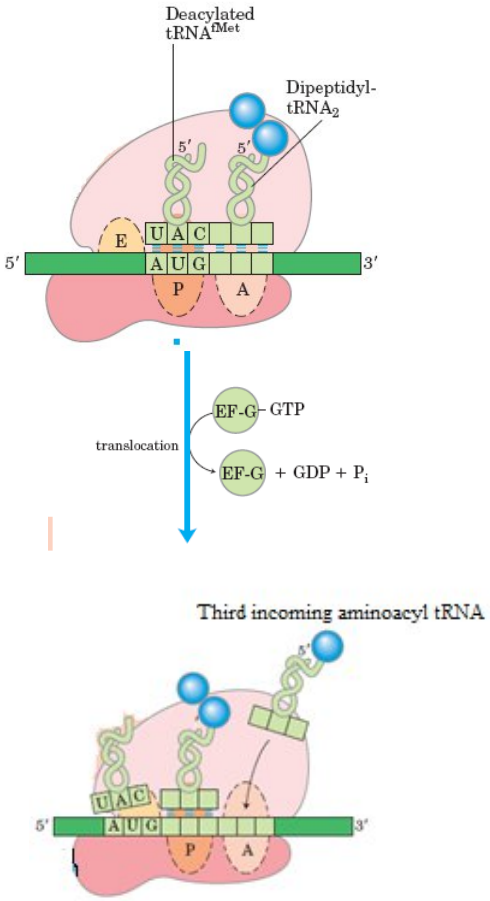
Step 2: Peptide bond formation

The peptide bond is now formed between the two amino acids bound by their tRNAs to A and P sites. This occurs by the transfer of the amino acids in P site from its tRNA to amino group of second amino acid in the A site. This peptide bond formation occurs by an enzymatic activity called peptidyl transferase. This enzymatic activity is present in the 23s rRNA present in ribosomes.

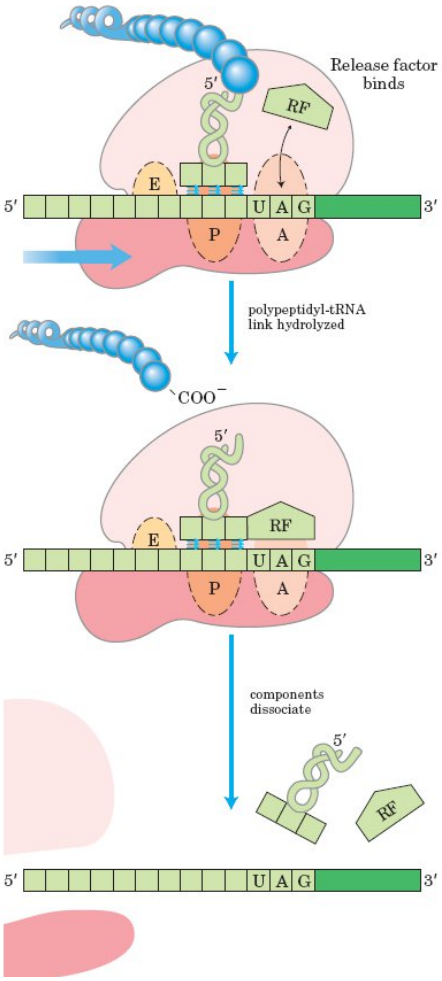
Step 3: Translocation

The ribosome moves one codon towards the 3`end of mRNA. This moves the uncharged tRNA to the E-site from P-site and tRNA bound to polypeptide moves the P-site from A site. The translocation is an activity of EF-G (also called translocase). The EF-G is bound to GTP. The GTP is hydrolyzed to GDP yielding energy that is used for translocation. After the translocation the A site becomes empty and is ready to accept new amino-acyl tRNA.


Initiation complex
5′
fMet
50S
E
U A C
5′
A U G
3′
Initiation codon
P
A
Next codon
30S
5′
AA2
Incoming aminoacyl-tRNA
5′
AA2
Tu
GTP
Tu
GTP
Ts
GTP
binding of incoming aminoacyl-tRNA
Tu
Ts
GDP
Pi
Tu
GDP
Ts
5′
fMet
5′
AA2
E
U A C
5′
A U G
3′
P
A



4. Termination and release of polypeptide: the completion of polypeptide chain is signalled by a termination or stop codon (UAA, UGA, UAG). The polypeptide is released from the ribosome aided by proteins called release factors. In bacteria, once a stop codon occupies the A-site in the ribosome, the three release factors, RF-1, RF-2 and RF-3 contributes to transfer of polypeptide chain to water molecule, release of polypeptide from last tRNA and dissociation of 30s and 50s ribosomal subunits.



5. Folding and post-translational processing: in order to achieve its biological active form the new polypeptide must fold into its proper three dimensional conformation. Before or after folding, the new polypeptide may undergo enzymatic processing such as
    - Removal of one or more amino acids
    - Addition of acetyl, phosphoryl, methyl, carboxyl or other groups to certain amino acids in polypeptide.
    - Proteolytic cleavage.
    - Attachment of oligosaccharide or prosthetic group.