TRANSLATION

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Translation is a process where proteins are made by the ribosomes on the mRNA strand.

Or

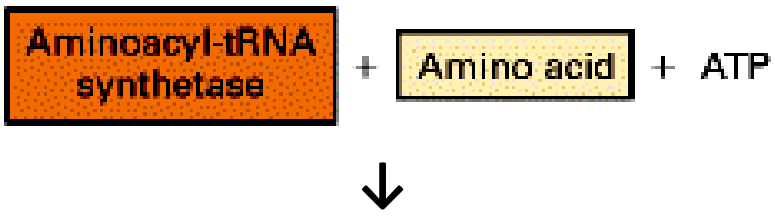
The process in the ribosomes of a cell by which a strand of messenger RNA directs the assembly or sequence of amino acids to make a protein.

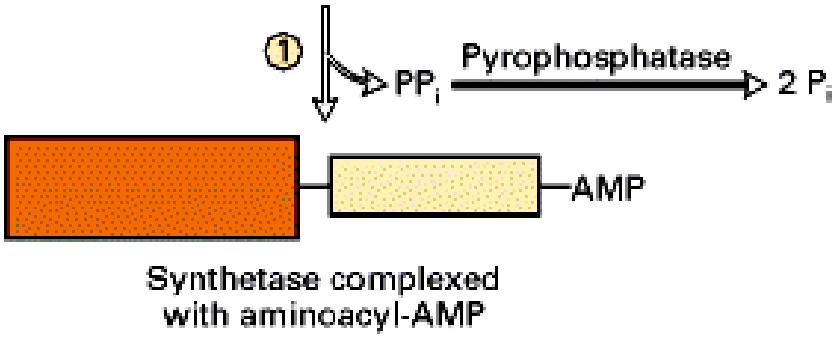
The main steps in translation are

1. Activation of amino acids
2. Transfer of amino acid to tRNA (charging of tRNA)
3. Initiation of synthesis
4. Elongation of polypeptide chain and
5. Chain termination

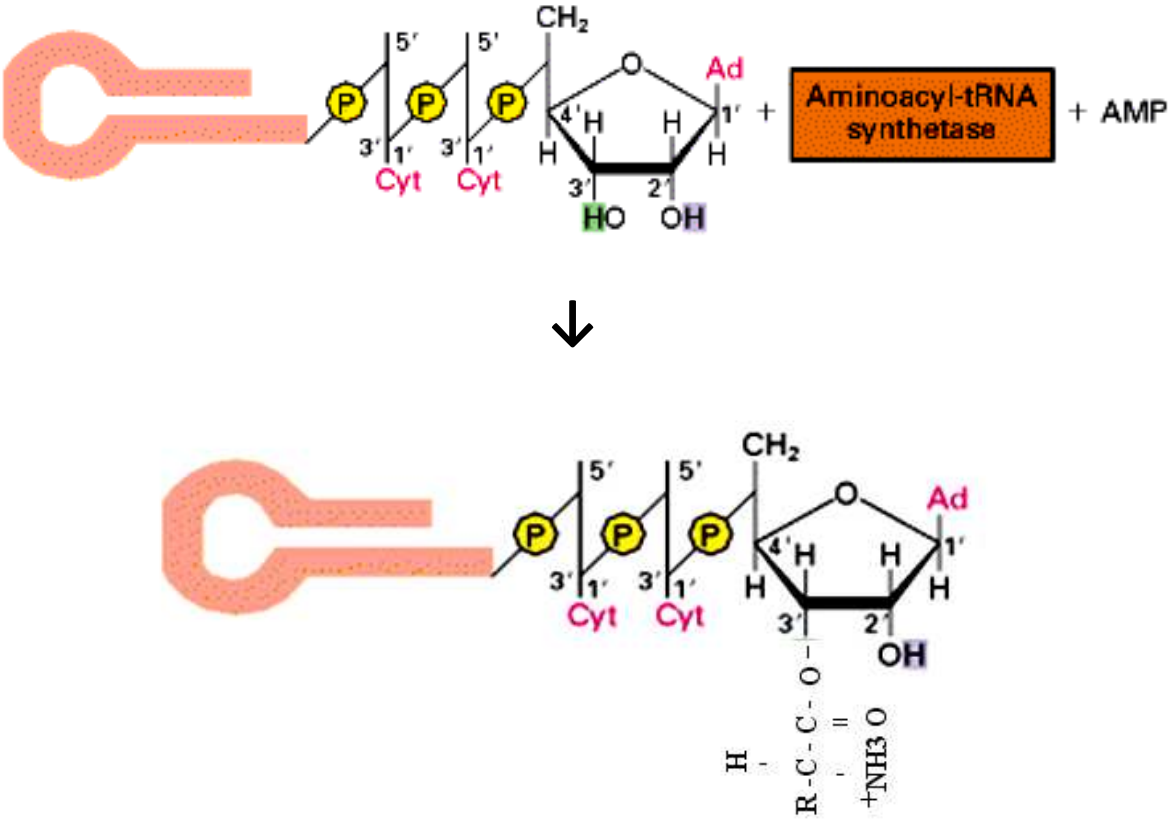
1. **Acivation of amino acids:** The first step in translation is the activation of amino acids. The 20 amino acids are commonly found in proteins are first screened to eliminate the D-isomers only the L-amino acids take part in protein synthesis.

The activation of amino acids takes place through their carboxyl groups. The each amino acid is catalized by its own specific activating enzyme, called aminoacyl tRNA synthetase (which are also called activating enzyme) to form an amino acyl adenylate (aaa) or amino acyl AMP. A high energy acyl bond is formed between the α-phosphate of ATP and carboxyl group of amino acid. The β and γ phosphates of ATP break away as inorganic phosphates. The amino acyl AMP remains bond to the activating enzyme.





2. **Transfer of amino acid to tRNA:** The transfer of the activated amino acid to tRNA (charging of tRNA) is also specific. The tRNA is named after the amino acid for which it is specific and designated as $tRNA^{val}$ is specific for valine.
   A high energy ester bond is formed between the carboxyl group of the amino acid and the 3'-hydroxyl group of the terminal adenosine of tRNA. The aminoacyl adenylate, which is attached to the activating enzyme, reacts with a specific tRNA to form an aminoacyl-tRNA complex.



Ribosome: Prokaryotic ribosome is a multicomponent particle that contains several enzyme activities needed for protein synthesis. It serves to bring together a single mRNA molecule and charged tRNA in the proper position and orientation such that

the base sequence of the mRNA molecule is translated into a series of amino acid sequence.

E. coli ribosome is well studied as an example of prokaryotic system. The ribosome is chemically composed of nucleoprotein particle consisting of two subunits. Ribosome is named by stating their sedimentation co-efficient (S-Svedberg). Intact ribosome particle is 70S and the subunits are termed 30S and 50S. The subunits are of unequal size and composition. The molecular weights of *E. coli* 70S ribosome is 2.5 x 10^6 and the subunit 50S is 1.6 x 10^6 and the 30S is 0.9 x 10^6. Both the 30S and 50S subunits can be further disassociated. i.e., 30S subunit contains one 16S rRNA molecule with 1541 nucleotides + 21 different proteins and these proteins are termed as S_1, S_2, S_3 ….*etc*., (S for Small subunit). Likewise 50S subunit contains one 5S rRNA molecule (120 nucleotides) + one 23S rRNA molecules (2904 nucleotides) + 31 different proteins. These proteins are named as L_1, L_2, L_3, …. *etc*., (L for Large subunit)

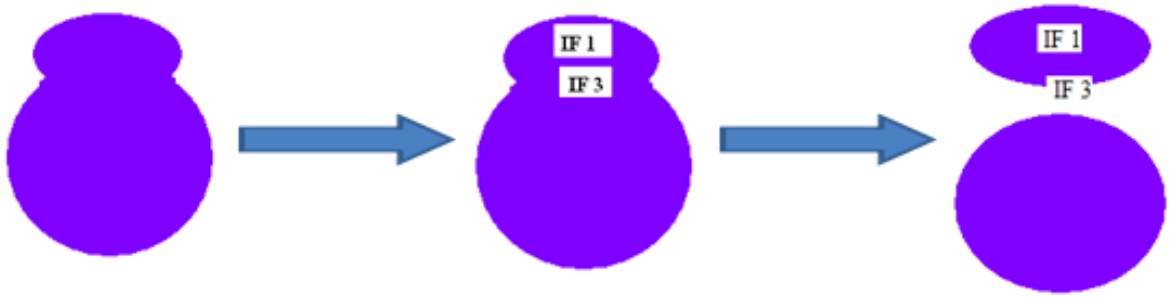
Protein Synthesis: Protein synthesis can be divided into three stages

a) Polypeptide chain initiation.
b) Chain elongation and
c) Chain termination.

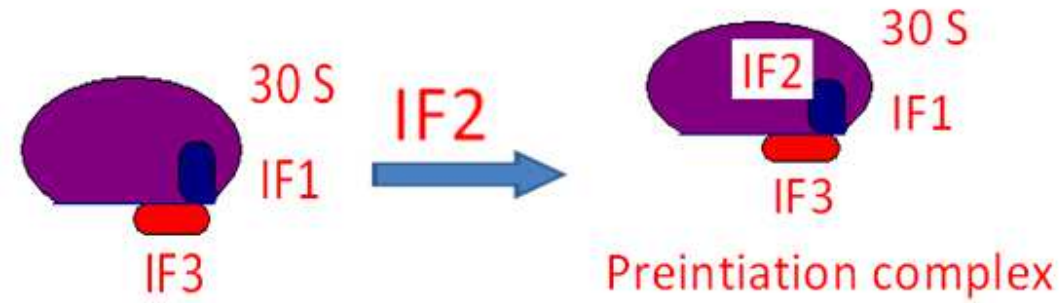
Initiation of Translation: The main features of the initiation stem are binding of mRNA to the ribosome, selection of the initiation codon and binding of charged tRNA bearing the first amino acid (methionine).

The first stage in initiation of translation is formation of pre-initiation complex. This complex is formed with co-operative interaction of three non ribosomal proteins (IF_1, IF_2 and IF_3) called initiation factors. Formation of pre-initiation complex requires free 30S subunit. This subunit is supplied by IF_1 and IF_3. IF_1 and IF_3 act in disassociating the 30S subunit and 50S subunit from the intact 70S ribosome. IF_1 binds to the 30S subunit whereas IF_3 binds at a point on 30S subunit such that the 50S subunit cannot associate with 30S subunit to form 70S unit. IF_2 now recognizes this assembly and get bound to 30S. Now this whole complex is called pre-initiation complex. IF_3 binds close to 3'OH terminal of 16S rRNA. It also denatures base paired region at this point such that a peculiar sequence called Shine-Dalgarno sequence get exposed. So that mRNA can come and bind to the 30S subunit. This shine-dalganno sequence is a conserved sequence. Now IF_2 mediates binding of one molecule of GTP to it and also one molecule of charged tRNA *i.e*., $tRNA^{met}$ binds, IF_3 leaves the 30S subunit. Release of IF_3, frees the site on 30S subunit such that 50S subunit attaches and an intact 70S ribosome is formed. During this process by unknown mechanism IF_1 falls off the 30S subunit. At this point the S and L proteins of 30S and 50S subunits align in such a way that two sites P and A are formed on 70 S ribosome. P site is referred as peptidyl site

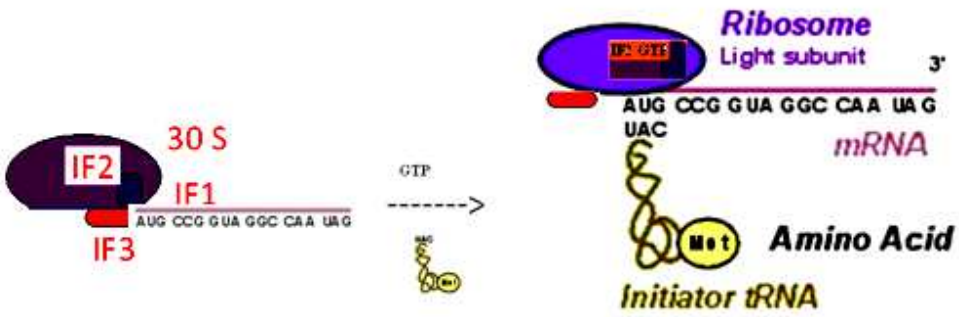
and A site as aminoacyl site where fresh charged tRNA come and get bound to previous amino acid in P site by a formation of peptide bond. When both the sites are formed the formyl methionine tRNA ($tRNA^{fmet}$) occupies the P site. IF_2-GTP occupies the A site. By an unknown mechanism GTPase activity is observed with disassociation of GTP in to GDP + Pi, formation of IF_2-GDP complex makes an unstable binding of IF_2 to 30S subunit. Thus now P site is occupies by $tRNAf^{met}$ and A site is vacant.



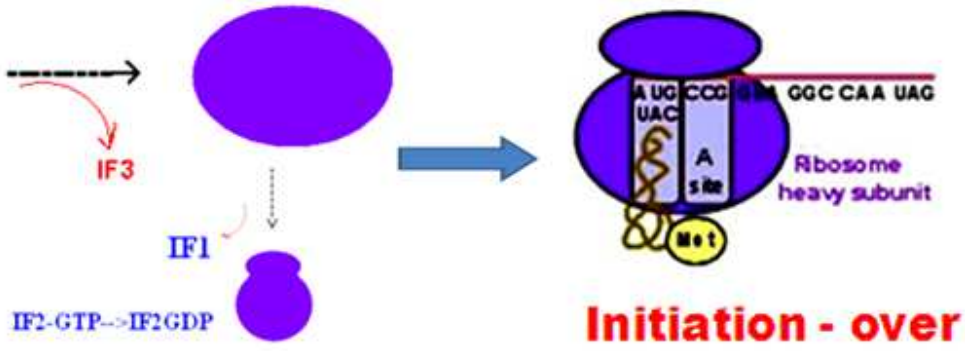
70S Intact Ribosome Binding of Initiation Factors Separation of 30 & 50 S subunits



Binding of IF 1, IF2 and IF3



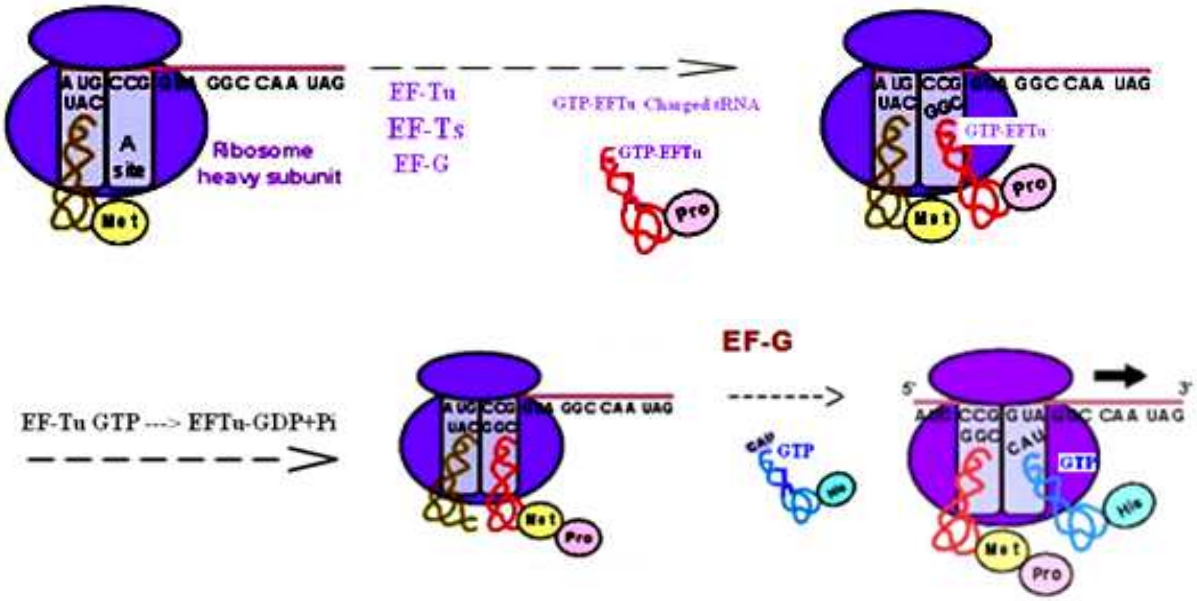
Attachment of mRNA Binding of initiator tRNA



Formation of 70 S ribosome

Chain Elongation:

Three non ribosomal proteins i.e., elongation factors EF-Tu, EF-Ts and EF-G are required for elongation of polypeptide chain. These proteins are present in the cell. In the first step GTP binds to EF-Tu to form a binary complex. Binding of GTP brings a conformational change in EF-Tu such that a charged tRNA molecule binds to the binary complex consists of EF-Tu + GTP + tRNA + Amino Acid.



This ternary complex binds to A site. Now GTPase activity triggered in presence of EF-Tu which results in formation of (EF-Tu GTP) into EF-Tu GDP + Pi. EF-Tu GDP is unstable and gets released off from the charged tRNA thus leading to disassociation of ternary complex. Now charged tRNA is free in A site of ribosome. In 50S subunit

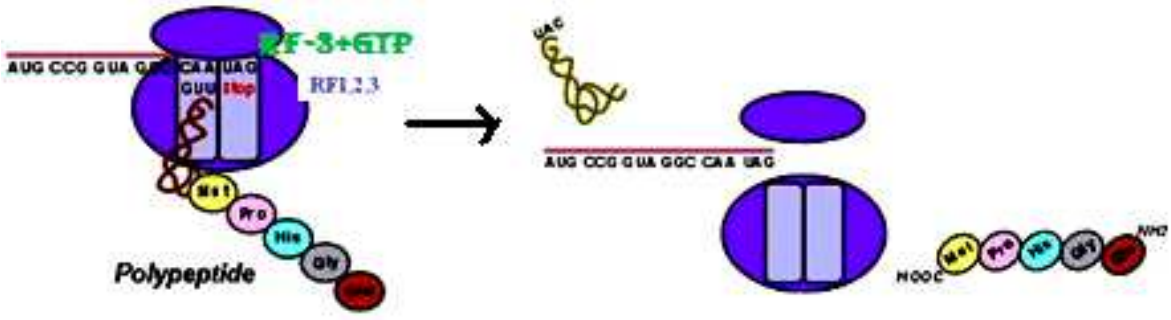
the L proteins aligh in such a way that to creat an active center known as peptidyl transferase center. At this center a peptide bond is formed between the amino acid in P site and that present in A site by peptidyl transferase.

The reaction is as follows

P SITE		A SITE		P SITE	A SITE
Fmet-COO tRNA	+	NH2-AMINOACID tRNA	----------> Peptidyl transferase tRNA Deacylase	Uncharged tRNA	Fmet-AMINOACID tRNA

Formation of peptide bond leads to cleavage of the bond between fmet (amino acid) and tRNA holding it. This cleavage is brought about by tRNA deacylase which is a ribosomal component. tRNA without the amino acid is now totally unstable and poorly bound in P site. So it immediately disassociated from the 70 S ribosome. The peptidyl tRNA in A site is weekened. But it will be stable and strong if it is present in P site. Hence by the involvement of EF-G translocation of peptidyl tRNA from A site to P site occurs. Now again the A site is free and the cycle continues until termination codon occupy the A site.

Chain Termination: Mechanisms involved during chain termination is unclear. However, termination is triggered when termination codon occupy the A site of the 70S ribosome. To begin with releasing factor RF_3 binds to GTP to form RF3-GTP complex. This complex binds to 70S ribosome which intern triggers binding of RF_1, RF_2 and RF_3 together act in releasing of fresh/new/nascent polypeptide from the ribosome unit.



Thus synthesized polypeptide is made stable/functional by post translational modifications.
