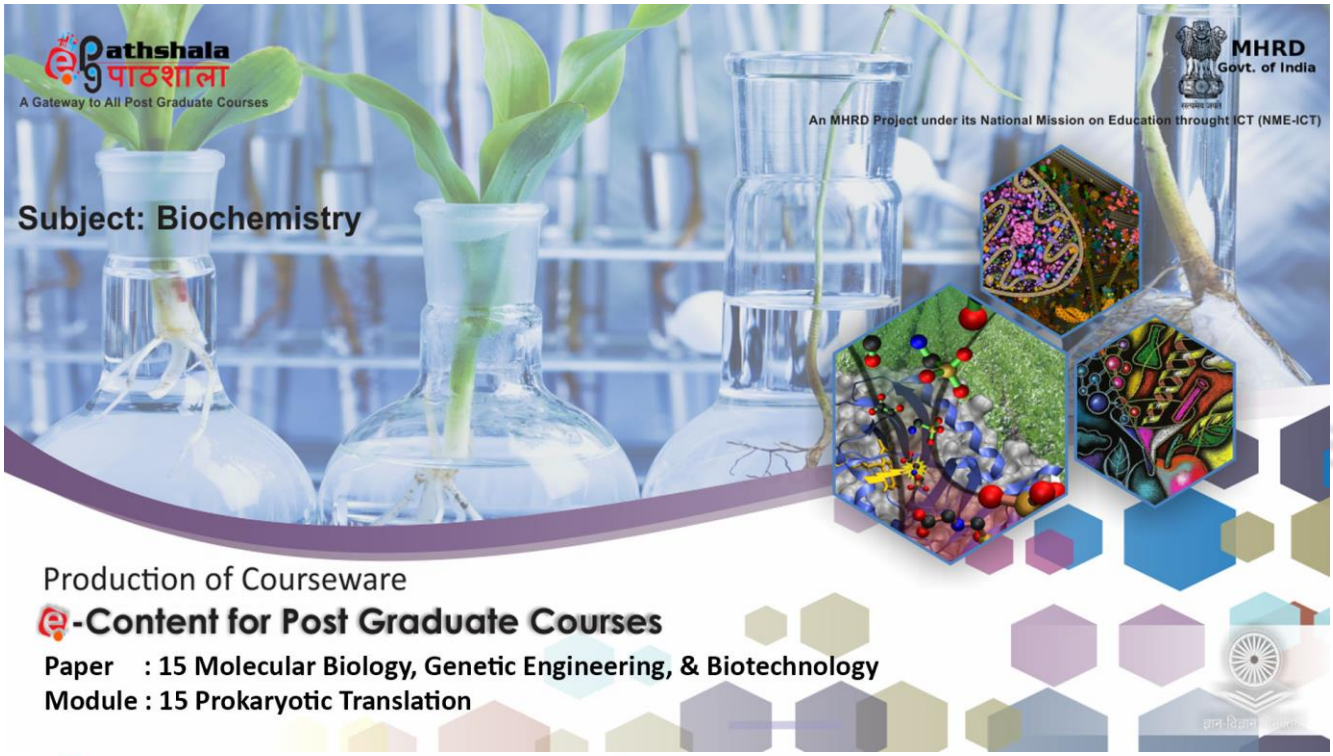

Production of Courseware

e-Content for Post Graduate Courses

Paper : 15 Molecular Biology, Genetic Engineering, & Biotechnology

Module : 15 Prokaryotic Translation

athshala पाठशाला

MHRD Govt. of India

Development Team

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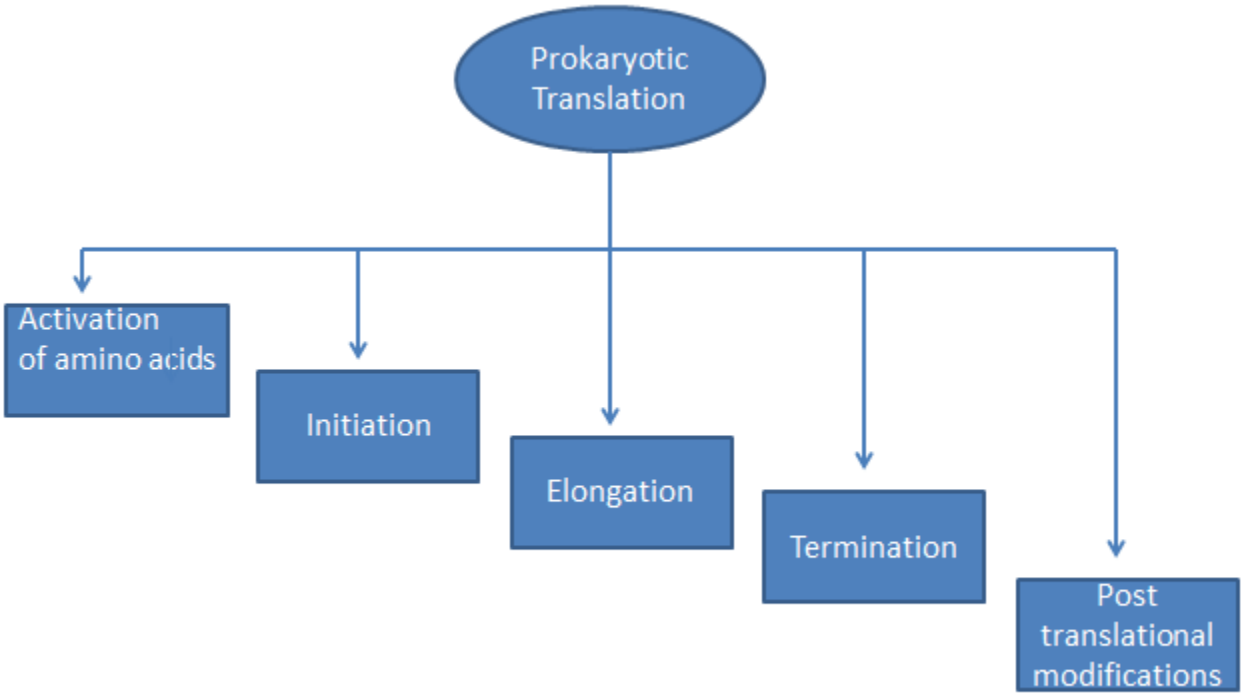
Description of Module	
Subject Name	
Paper Name	
Module Name/Title	Prokaryotic translation

1. Objectives

2. Understand the concept of genetic code
3. Understand the concept of wobble hypothesis
4. Explain the process of prokaryotic translation
5. Explain about the different types of post translational modification in prokaryotes

2. Concept Map



3.1 Introduction to protein synthesis

Translation or protein synthesis is a central process of central dogma of molecular biology. It deals with production of proteins or chains of amino acids by making use of a mRNA as a template, ribosomes as protein synthesizing machinery and tRNA's as carriers of amino acids during the translation process.

Living cells devote about 90 % of their chemical energy to synthesis of proteins and only about 10 % to other biosynthetic processes. More than 35% of the dry weight of the cell consists of ribosomes, proteins involved in translation process and tRNA molecules. This suggests that protein synthesis is an important process for the survival of microorganisms

Protein synthesis process in *E.coli* - prokaryotes occurs at a very high rate polymerizing about 100 amino acids in 5 seconds. It is a highly regulated process so that enough protein is made to meet up the cellular requirements. Along with synthesis targeting and protein degradation processes ensure optimal cellular concentration of proteins. The overview of prokaryotic translation process is as represented in figure 1.

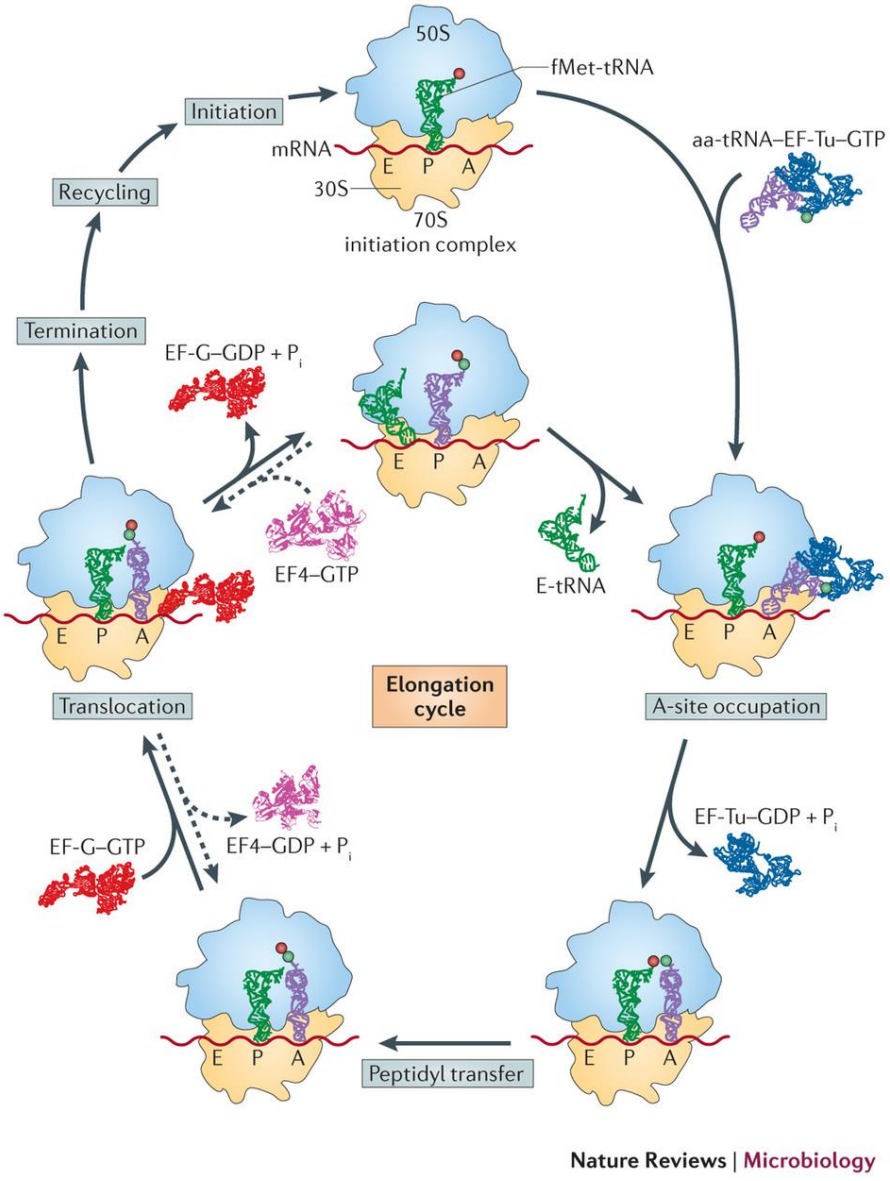


Figure 1. Schematic representation of prokaryotic translation process

3.2 Components of translation: Different components required for the translation process are as described below.

3.2.1 mRNA (messenger RNA)

Ribosomes are involved in translating the genetic message of mRNA into proteins. The mRNA is read in 5'→3' direction producing a corresponding polypeptide chain in N terminal to C terminal direction.

3.2.2 tRNA (transfer RNA)

Transfer RNA molecules act as adapters or carriers of aminoacids. It has a clover leaf like structure (Figure 2) with the 3' OH end of the tRNA bonded to the amino acid. It contains an anticodon arm, which carries anticodons on it which are involved in bonding to specific codons on mRNA during the translation process. The anticodon sequence is complementary to the codon for that aminoacid. For example 5'GCA3' is a codon for alanine: the anticodon then is CGU, but in the 3' to 5' direction.

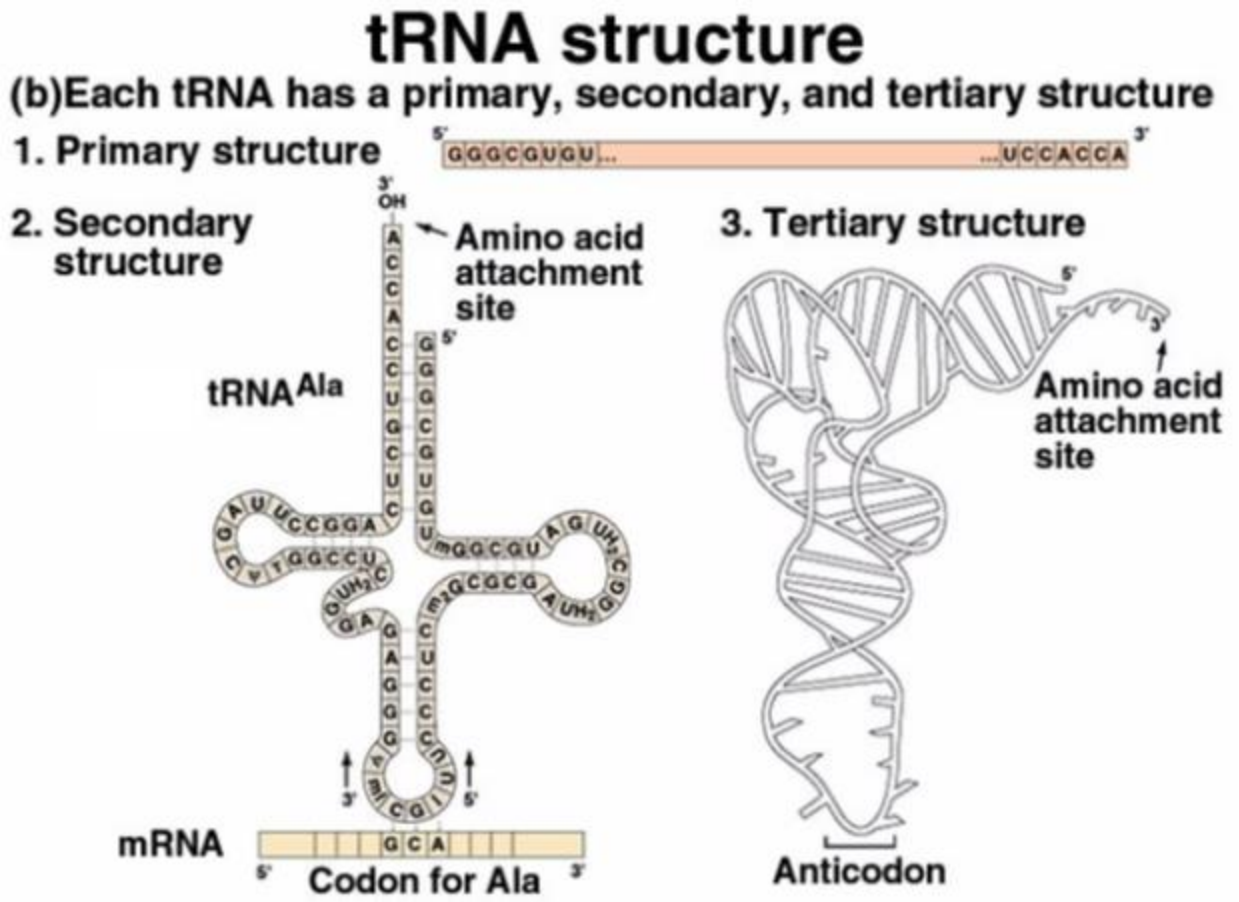


Figure 2: Structure of tRNA molecule

3.2.3 rRNA (Ribosomal RNA)

The ribosomal RNA's associate with proteins to form structures known as ribosomes, which are the protein synthesizing machines. The prokaryotic 70S ribosomes are composed of 30S and 50S ribosomal subunits. The 30S ribosomal subunits is further made up of 16SrRNA and 21 different proteins while the 50S ribosome is made up of 23SrRNA, 5SrRNA and 34 different proteins (Figure 3).

They have several important functions in the protein synthesis process which are as follows:

They act as sites of protein synthesis.

They recognize signal sequences that allow initiation of protein synthesis.

They ensure accuracy of protein synthesis process by stabilizing the tRNA - mRNA interaction.

They are involved in enzymatically linking the aminoacids in a polypeptide chain.

They move along the mRNA molecule as they read through the mRNA in 5'-3'.

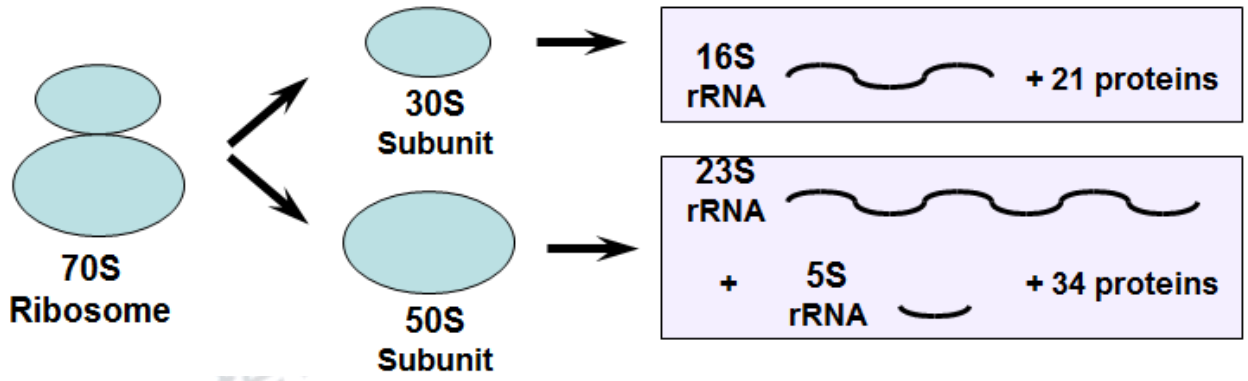


Figure 3: Components of prokaryotic ribosomes

3.2.4 Enzymes:

Two enzymes required for the protein synthesis process are as follows-

1. **Aminoacyl tRNA synthetase** that catalyzes attachment of tRNA molecule to its corresponding aminoacid. 20 different aminoacyl tRNA synthetases each specific for one aminoacid molecule are required for the catalytic reaction. The attachment of aminoacid charges the tRNA molecule as it attaches the aminoacid to the carboxy terminal end of the amino acid molecule.

2. **Peptidyl transferase catalyzes** peptide bond formation between the amino acids bonded by their corresponding tRNAs to the ribosomal P site and A site, thus allowing sequential transfer of amino acids to the growing chain.

3.2.5 Proteins or soluble factors:

They required for proper initiation, elongation and termination steps of protein synthesis. They are discussed in more details in the explanation about different steps of protein synthesis.

3.3 Genetic code:

Gene is defined as a series of bases that code for a functional protein molecule. It defines the correlation between the nucleotide sequence of the gene and the corresponding aminoacid sequence of the polypeptide molecule as guided by the gene sequence. In other word a set of 3 bases form one codon or a genetic codon and specify for one of the 20 aminoacid. At least 3 bases are required to encode for each aminoacid. The 4 nucleotides of DNA (A, T,G and C) in a group of 2 can make 4^2 = 16 different combination which are insufficient to code for a total of 20 amino acids. However if the nucleotides form a group of 3 then it can generate 4^3=64 different combinations which are more than the number of amino acids. Thus each codon is made up of a sequence of 3 nucleotides. Some of the

important properties of genetic code are explained below.

	Amino Acid	Abbreviation	mRNA Codons		Total no. of codon(s)
			Common bases	Complete codon(s)	
1.	Alanine	Ala	GC–	GCU, GCC, GCA, GCG	4
2.	Arginine	Arg	CG–	CGU, CGC, CGA, CGG	6
			AG–	AGA, AGG	
3.	Asparagine	Asn	AA–	AAU, AAC	2
4.	Aspartic acid	Asp	GA–	GAU, GAC	2
5.	Cysteine	Cys	UG–	UGU, UGC	2
6.	Glutamic acid	Glu	GA–	GAA, GAG	2
7.	Glutamine	Gln	CA–	CAA, CAG	2
8.	Glycine	Gly	GG–	GGU, GGC, GGA, GGG	4
9.	Histidine	His	CA–	CAU, CAC	2
10.	Isoleucine	Ile	AU–	AUU, AUC, AUA	3
11.	Leucine	Leu	UU–	UUA, UUG	6
			CU–	CUU, CUC, CUA, CUG	
12.	Lysine	Lys	AA–	AAA, AAG	2
13.	Methionine	Met	AU–	AUG	1
14.	Phenylalanine	Phe	UU–	UUU, UUC	2
15.	Proline	Pro	CC–	CCU, CCC, CCA, CCG	4
16.	Serine	Ser	UC–	UCU, UCC, UCA, UCG	6
			AG–	AGU, AGC	
17.	Threonine	Thr	AC–	ACU, ACC, ACA, ACG	4
18.	Tryptophan	Trp	UG–	UGG	1
19.	Tyrosine	Tyr	UA–	UAU, UAC	2
20.	Valine	Val	GU–	GUU, GUC, GUA, GUG	4
Terminator triplets		**Trm**	UA–	UAA, UAG	3
			UG–	UGA	
				Total	**64**

Figure 4: Aminoacids and their mRNA codons

3.3.1 Genetic code is a triplet:

As explained above singlet and doublet codons are not sufficient to code for all 20 aminoacids, there fore genetic code needs to be atleast a triplet. The triplet nature of genetic code is the most suitable as represented in Figure 4.

3.3.2 Genetic code is degenerate:

This means that a particular aminoacid can be coded by more than one triplet codon. The degeneracy of genetic code is varying with some aminoacids like methionine encoded by only one codon (AUG), aminoacids lysine having two fold degeneracy (encoded by codons AAA, AAG) and amino acid proline, has four fold degeneracy (encoded by codons, CCU,CCC, CCA and CCG).

3.3.3 Genetic code is non overlapping

Non overlapping means that the same base is not shared between two adjacent codons. Figure 5 shows an illustration of overlapping codons. Because of overlapping codons, three codons are formed from seven bases and four codons are formed from six bases. However this is never the case and only two codons can be formed out of six bases which means that the genetic code is non overlapping.

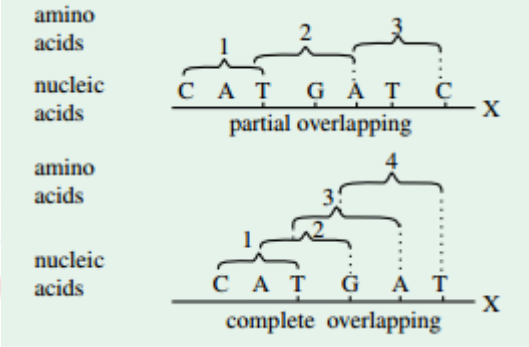


Figure 5. Overlapping of codons due to one letter or two letters.

3.3.4 Genetic codon is commaless.

Genetic code is commaless or non punctuating which means that no bases are dedicated to indicate the end of one codon and the beginning of the next codon. In other words, after one aminoacid is code by first three bases in a mRNA sequence, the next amino acid is automatically encoded by next three bases on the mRNA. Below is an illustration of nonpunctuating and a punctuating codon (Figure 6).

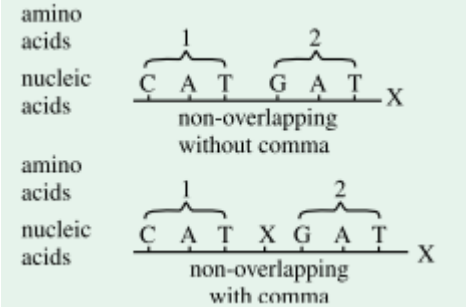


Figure 6: Representation of punctuating and non-punctuating (comma less) codons.

3.3.5 Genetic code is non ambiguous

In ambiguous code the same codon codes for more than one amino acid. But this is never the case and one codon always codes for a single amino acid or it is non ambiguous. For example codon AUG always codes for aminoacid methionine.

3.3.6 Genetic code is universal.

Universality of genetic code means that in all living systems a particular genetic code will always code for the same amino acid. For example- codon AAG codes for lysine in plant, animals as well as microbes.

3.3.7 Polarity

The genetic code has polarity and it is always read in 5′ → 3′ direction. If the code would have been read in both directions that it would mean that 2 different proteins would be synthesized because the codons will be read in both directions 5′ → 3′ and 3′ → 5′.

3.3.8 Chain initiation codons

The triplet codon 5'AUG 3' encodes for the initiation codon from where a new polypeptide synthesis begins. Also the initiating AUG codon codes for a formylated methionine residue while all the other internal AUG codon code for unformylated methionine. There are also separate tRNAs that act as carriers for initiating formylated methionine and the internal methionine residues.

3.3.9 Chain termination codons

The termination codons direct the release of the nascent polypeptide chain synthesized by the ribosomes. There are three codons UGA, UAA and UAG that function as termination codons.

3.4 Wobble Hypothesis: Multiple recognition of codons by tRNA's.

Wobbles hypothesis suggest that whenever there is codon anticodon base pairing during translation process the first two bases of anticodon are strictly standard and they pair tightly with the corresponding bases of the codons. The third base of the codon is not so specific in pairing and may be loose or wobble in its corresponding pairing with the base of the anticodon. Hence each tRNA molecule recognizes several codons for its aminoacid. Hence the total number of tRNAs required for the translation process are significantly less than the total number of codons. Based on the examinations of these codon anticodon base pairing Crick proposed a set of four relationships known as wobble hypothesis.

1. The first two bases of the mRNA codon forms a strong Watson and Crick base pairing with the corresponding base of the anticodon and are very specific.
2. The first base of the anticodon read in 5′ → 3′ determines the number of codons recognized by the tRNA. When the first base of the anticodon is C or A the pairing is more specific and the tRNA recognizes only one codon, while when the first base is U or G the pairing is less specific and it may read two different codons. When inosine (I) is the first of the wobble base then it recognize 3 different codons.
3. When an amino acid is specified by several different codons, the codons that differ in either of the two bases require different tRNAs.
4. A minimum of 32 tRNA are required to translate all 61 codons (31 to encode for aminoacids and one for initiations).

*Anticodon base** *(first base)*	*Codon base (third base)*
U	A, G
C	G only
A	U only
G	U, C
I (Inosine, resembles G)	U, C, A

Figure 7: Wobble base pairing hypothesis

3.5 Steps of prokaryotic translation: Prokaryotic translation process can be divided into 5 stages as follows. The details of each stage are described in the following section.

1. Activation of amino acids

2. Initiation

3. Elongation

4. Termination and release

5. Post translation modifications

3.5.1 Activation of amino acids

Components required for the activation of aminoacids step include 20 aminoacids, 20 different aminoacyl tRNA synthetases, corresponding t- RNA's as carriers of aminoacids, ATP as energy source and Mg^{2+}.

During the activation of aminoacids the enzyme aminoacyl tRNA synthetase catalyzes attachment of the aminoacid to corresponding tRNA. Each enzyme is dedicated to one of the aminoacid molecule and for one or more corresponding tRNA molecules, thus there are 20 different aminoacyl t RNA synthetases.

The reaction takes place in the cytosol and occurs in reaction requiring ATP and Mg^{2+}. The attachment of the aminoacid takes place at its carboxy terminal end. (NH2-CH2-CO-3'tRNA5')

$$\text{Amino acid} + \text{tRNA} + \text{ATP} \xrightleftharpoons{Mg^{2+}} \text{aminoacyl-tRNA} + \text{AMP} + \text{PPi}$$

3.5.2 Initiation stage: It is the second stage of translation.

3.5.2.1 Initiating amino acid-

Protein synthesis process involves step wise addition of aminoacids to the carboxy terminal end of the growing polypeptide chain that begins with an initiating aminoacid residue. The initiating aminoacid is a modified methionine residue encoded by initiation codon – (5')AUG. Even though there is only on codon for methionine all organisms have 2 different t RNA for methionine amino acid- one for the initiating methionine residue (called $tRNA^{fMet}$) and the other for the internal methionine residues ($tRNA^{Met}$). The N formyl methionine is incorporated in response to the initiating (5')AUG codon while a methionine is incorporated in response to all other internal (5')AUG codons. Formation of N

formyl methionine t RNA ^{fMet}(fMet-tRNA^{fMet}) occurs in 2 steps. In the first step the amino acid methionine is attached to tRNA^{fMet} in a reaction catalyzed by Met-tRNA synthetase. In *E.coli* the same enzyme is involved in aminoacylation of both tRNA^{fMet} and tRNA^{met}.

$$\text{Methionine} + \text{tRNA}^{fMet} + \text{ATP} \longrightarrow \text{Met-tRNA}^{fMet} + \text{AMP} + \text{PPi}$$

In the second step a transformylase enzyme formylates the amino group of the methionine residue using *N*10-formyltetrahydrofolate as a formyl group donor. The enzyme transformylase in selective in formylating methioning attached to tRNA^{fMet}, possibly by recognition its unique structural feature. This modification also allows fMet-tRNAfMet to bind to 30 S ribosomal P site which does not accept Met-tRNAMet or any other aminoacyl-tRNA.

$$N\text{10-Formyltetrahydrofolate} + \text{Met-tRNA}^{fMet} \longrightarrow \text{tetrahydrofolate} + \text{fMet-tRNA}^{fMet}$$

3.5.2.2 Steps of Initiation

Components required for prokaryotic initiation process includes-30S ribosomal subunit, the coding mRNA sequence, the initiating fMet-tRNA^{fMet}, three initiation factors (IF-1, IF-2, and IF-3), GTP (energy source), the 50S ribosomal subunit, and Mg^{2+}. The different steps of initiation are represented in figure 8.

During the first step of initiation process the 30 S ribosomal subunit binds to the two iniitiation factors IF1 and IF3. IF3 prevents premature association of 30S and 50 S ribosomal subunits. The m RNA then binds precisely to the 30S ribosomal subunit such that the 5'AUG codon is placed at its P site. The intitiating AUG is directed to the appropriate location because of its close proximity to **Shine-Dalgarno sequence.** This consensus sequence consists of 4 to 9 purine bases, placed approximately 8 to 13 bp before of the 5'end of the initiation codon. The 16S rRNA of the 30S ribosomal subunit contains a pyrimidine rich series of bases near its 3' end which is complementary and binds specifically to the Shine Dalgarno sequence allowing correct positioning of initiating 5' AUG codon. The Shine Dalgarno sequence forms complementary base pairing with the pyrimidine rich series of bases towards the 3'end of the 16S ribosomal RNA of the 30S ribosomal subunit.

The bacterial ribosomes contain three binding sites for aminoacyl-tRNA, the the A site P site and E site that binds new incoming aminoacyl tRNA (expection- the initiating aminoacyl tRNA binds to Psite), peptidyl tRNA and deacylated tRNA respectively. The initiating 5'AUG positions itself correctly to the ribosomal P site which binds with the fMettRNA^{fMet}. The subsequent incoming aminoacyl-tRNA of

the translation process binds first to the A site. The E site or the exit site is the site from where the uncharged tRNA leaves following peptide bond formation during elongation.

In the next step of initiation a complex consisting of GTP bound IF2 and fMet-tRNA^{fMet} associate with the complex of ribosomal 30S subunit, IF1, IF3 and mRNA. The anticodon of the tRNA pairs correctly with the initiating 5'AUG positions at the ribosomal P site.

In the third step a the ribosomal 50S subunit associates with the complex which is accompanied with the GTP hydrolysis into GDP and Pi along with the release of the 3 initiating factors. This results in a functional 70S initiation complex associated with the mRNA and the initiating fMettRNA^{fMet}. This is now ready to continue with the elongation steps.

3 different interaction ensure proper binding of intiating fMet-tRNA^{fMet} to ribosomal P site-

1) Codon anticodon interaction between the fMettRNA^{fMet} and intiating 5'AUG codon.
2) Interaction between the Shine –Dalgarno sequence and the 16SrRNA ribosomal sequence.
3) Binding interactions between the fMettRNA^{fMet} and the ribosomal P-site.

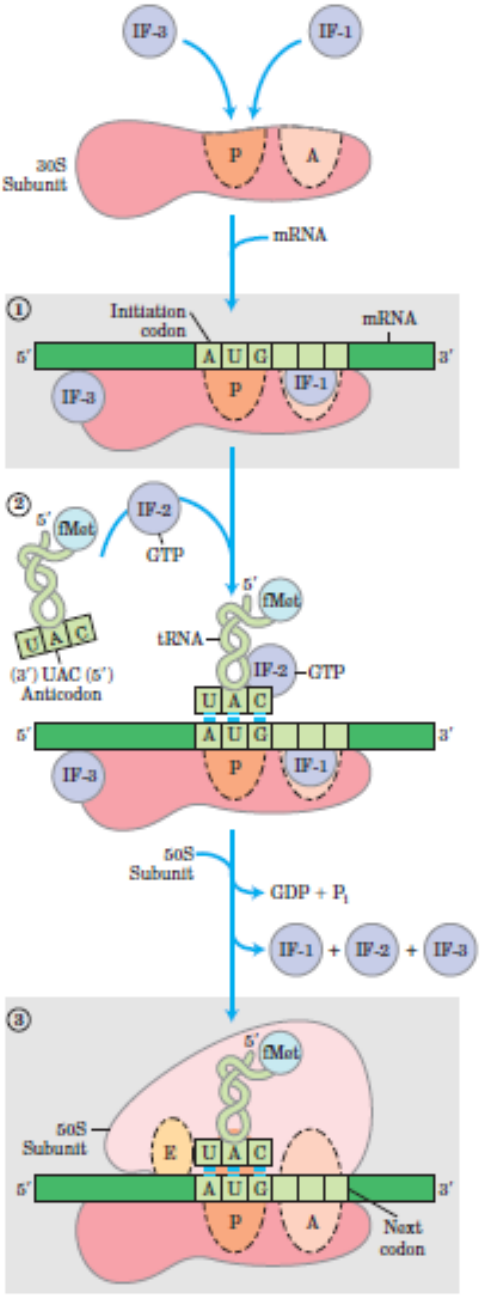


Figure 8 : Formation of initiation complex in bacteria

3.5.2.3 Elongation:

It represents the third stage of protein synthesis process. The components required for prokaryotic elongation process includes, the initiation complex (explained above), aminoacyl-tRNAs, there **elongation factors** (EF-Tu, EF-Ts, and EF-G in bacteria), and energy from GTP hydrolysis.

During the first step of elongation cycle (Figure 9A) the subsequent aminoacyl-tRNA binds to the A site of ribosomes. The appropriate aminoacyl-tRNA associates with a GTP-EF-Tu complex resulting in formation of aminoacyltRNA–EF-Tu–GTP complex. It binds to the ribosomal A site with simultaneous hydrolyzed of GTP and an EF-Tu–GDP complex is released from the 70S ribosome. The EF-Tu–GTP complex is then reformed in a reaction involving EF-Ts and GTP.

In the second step of the elongation cycle (Figure 9B) peptide bond formation takes place among the amino acids attached to the ribosomal P and A site by their respective t- RNA molecules. During this step the amino group of aminoacid at the ribosomal A site makes a nucleophilic attack and displaces the tRNA in the Psite resulting in peptide bond formation between the 2 aminoacids. This results in formation of dipeptidyl t-RNA at the A site of ribosomes and a deacylated tRNA at the P site of ribosomes. The reaction is catalyzed by enzyme peptidyl transferase which is now known to be catalyzed by the ribosomal 23srRNA subunit of ribosome.

Translocation is the third step of elongation (Figure 9C) during which the ribosome moves by a distance of one codon towards the 3’ direction of the mRNA. This results in repositioning of the dipeptidyl tRNA to the P site of ribosomes and the deacylated tRNA to the E site of ribosomes, from where the tRNA is released into the cytosol. The reaction is catalyzed by enzyme translocase or EF-G in a reaction requiring energy from GTP hydrolysis. The 3 rd codon is now at ribosomal A site and the ribosomes are ready for the next elongation cycle. For each amino acid molecule added to the growing polypeptide chain 2 GTP molecules are hydrolyzed into GDP and Pi.

A. B.

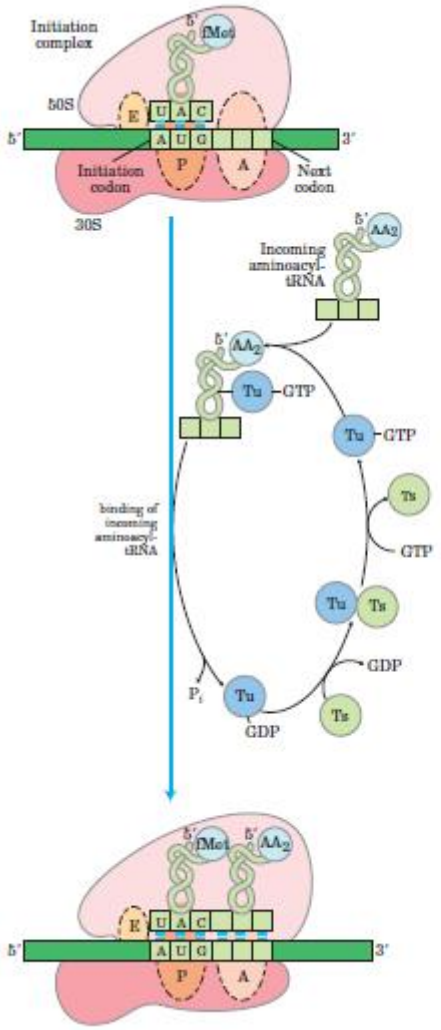


Figure 9A First elongation step in bacteria: binding of the second aminoacyl-tRNA.

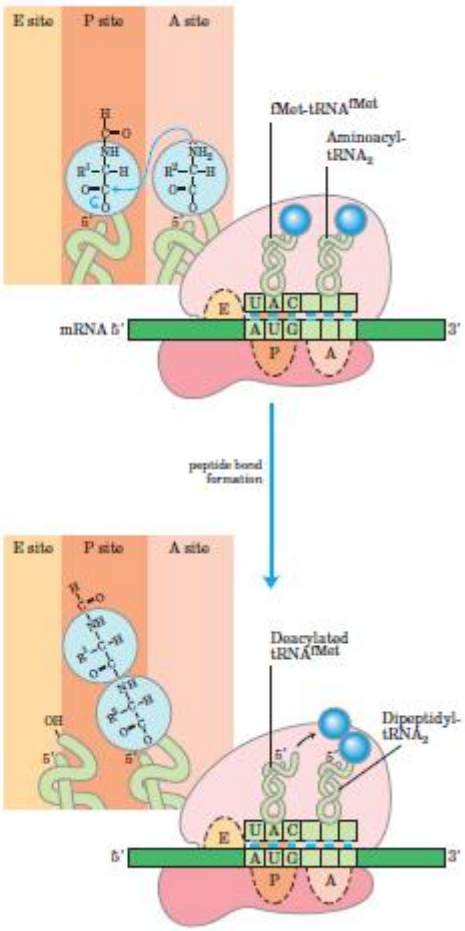


Figure 9B Second elongation step in bacteria: formation of the first peptide bond.

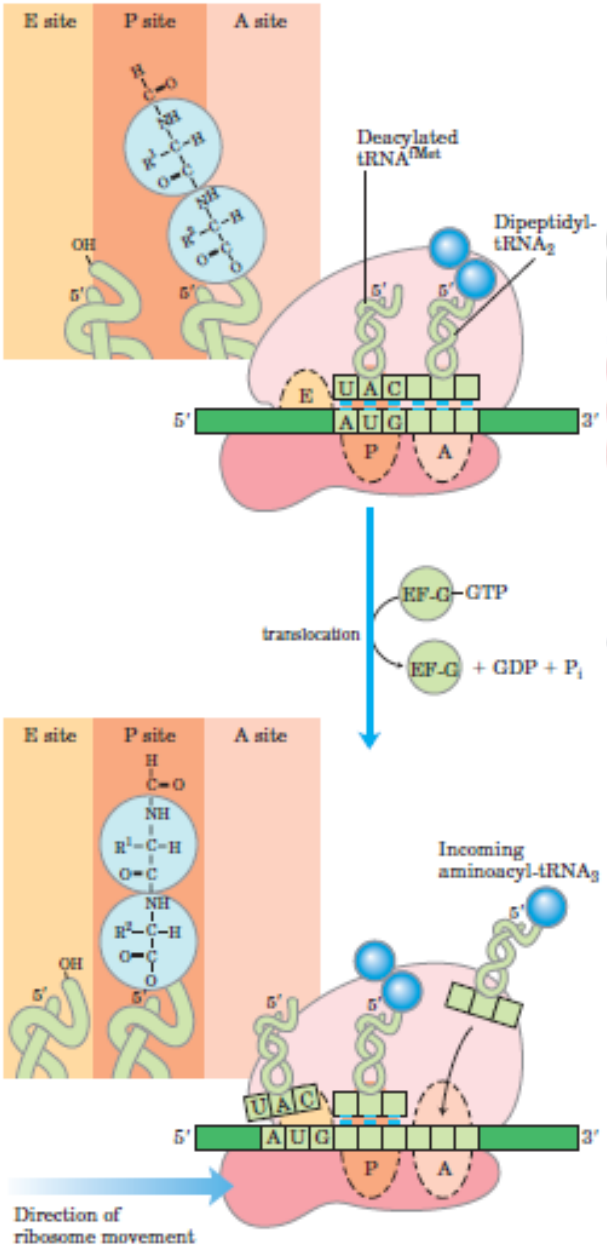


Figure 9C Third elongation step in bacteria: translocation.

3.5.2.4 Termination

The Elongation process continues until the ribosomes encounters a termination codon on the m RNA molecule. There are three codons that signal translation termination, these are UAG, UAA, and UGA. Presence of one of these codons after the final coded amino acid acts as a translation termination signal. In bacteria, when the termination codon are positioned at the A site of the ribosomes the **termination factors,** or **release factors (**RF-1, RF-2, and RF-3) carry out breakdown of the bond between polypeptide and the tRNA molecule, releasing free polypeptide and tRNA molecule (Figure 10). The 70S ribosomal subunit then dissociate into 30S and 50S subunits to begin with a new protein synthesis cycle. Termination codons UAG and UAA, are recognized by RF-1 and UGA and UAA are recognized by RF-2. Depending on the codon present appropriate release factor binds it, and brings about hydrolysis of bond between the polypeptide and tRNA molecule. The specific function of RF-3 is not yet known.

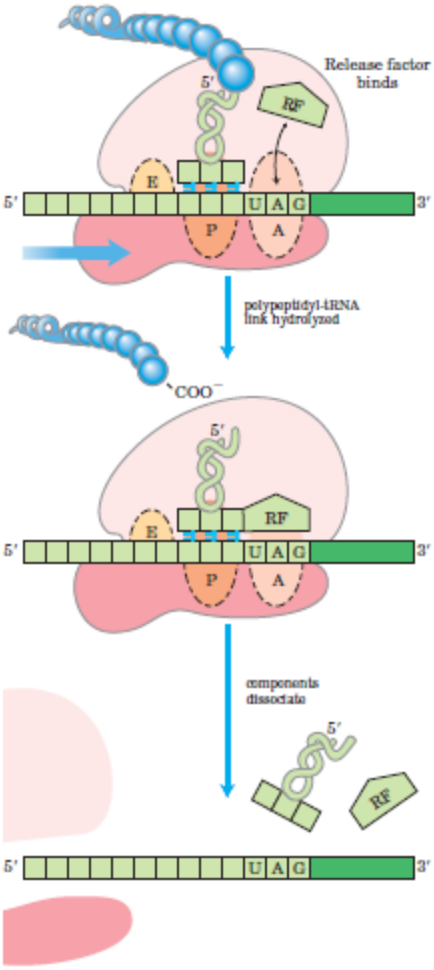


Figure 10 Translation termination in bacteria

3.5.2.5 Post Translational modifications: Folding and processing of the newly synthesized polypeptide chain.

After synthesis the newly synthesized polypeptide chain assumes its native conformation that is guided by a number of interactions like hydrogen bonds, vander waals, ionic, and hydrophobic interactions. Some of the polypeptides however under goes processing or post translational modifications. Some of these post translational modifications are discussed below.

Modifications of Amino-Terminal and Carboxyl-Terminal amino acids

In bacteria the first amino acid in all polypeptide chains is a N-formylmethionine residue. The formyl group and the initiating methionine and sometimes further amino terminal and carboxy terminal aminoacids are enzymatically removed from the final protein structure.

Loss of Signal Sequences
Signal sequences range from 15 -30 residues in length and play an crucial role in targeting of the protein to their location in the cell. Once the protein reaches its final location the signal sequences are enzymatically cleaved and are not the part of the final functional protein.

Modification of individual aminoacids: Bacteria modify several amino acid side chains of proteins by addition and removal of phosphate groups by enzymes names kinases and phosphatases respectively. In bacteria kinases often phosphorylate histidine and aspartate residues which are important modification in bacteria two component regulatory systems. A classic bacteria two component system comprises of a sensor protein which contains a histidine kinase domain that autophosphorylates the histidine aminoacid in response to a signal. The kinase then transfers the acquired phosphate residue to the aspartate residue of the second component which is known as the response regulator. The phosphorylated regulator then controls the transcription of several downstream genes thus operating a regulatory cascade (Figure 11).

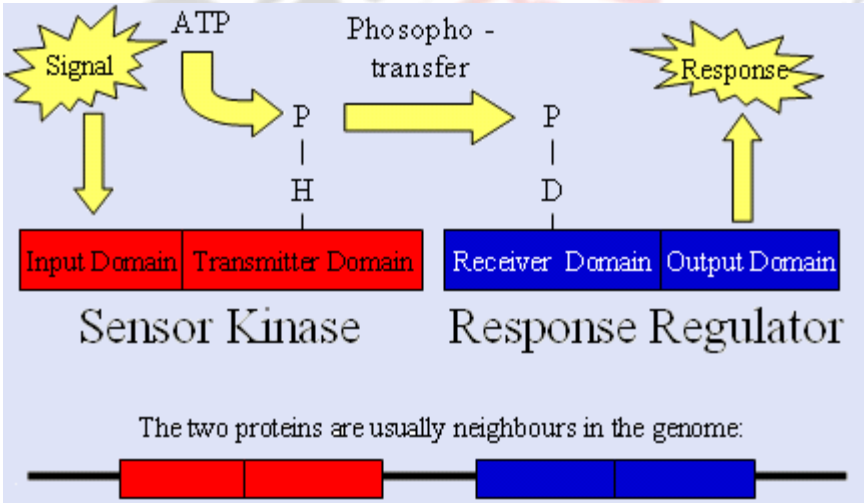


Figure 11: Modification of individual aminoacids by phosphorylation resulting in protein activation.

Protein glycosylation in bacteria:
It is a commonly post translational modification in bacteria. Many surface appendage proteins like pilin of pili and flagellin of flagella contains glycosylated residues. The process has been described in both gram positive and gram negative bacteria and may play an important role in adhesion, stabilization of proteins against proteolysis and evasion of host immune system. The commonly

glycosylated amino acid residues includes serine and threonine residues which are O glycosylated and asparagine residue which is N glycosylayted.

Addition of Prosthetic Groups Prosthetic group is a non-protein component of some proteins that is required for the their activity. Prosthetic group may be organic or inorganic in nature but are never made up of amino acids. These are tightly bound to the protein component through covalently bound. For example – Ferrodoxins are a family of bacterial proteins containing 2, 4 or 8 atoms of iron and additional inorganic sulphate. In bacteria ferrodoxins are components of electron transport chain during processes such as nitrate, nitrite and sulphate reduction.

Proteolytic Processing Many proteins are produced as large inactive forms of proteins known as precursor. Later during the post translational modifications these are proteolytically cleaved into smaller active form of the protein. Many bacterial toxins achieve their high potency by delivery of the catalytially active polypeptide fragment of the toxin to the eukaryotic cell cytosol. Activation occurs by proteolytic cleavage of the polypeptide at the defined site. Examples include Diptheria toxin, anthrax toxin, etc.

Formation of Disulfide Cross-Links
Post translationally many protein fold into its native conformation many proteins from intrachain or interchain disulfide bonds between their cysteine residues. These bonds are sometimes important in formation of the final functional active protein molecule. Alkaline phosphatase enzyme from E.coli is a homodimer where in two intramolecular disulfide bonds are involved in formation dimeric protein with full enzymatic activity.

4. Summary

In this lecture we learnt about:

- Genetic code and its properties
- Concept of wobble hypothesis
- Steps in prokaryotic translation process
- Different types of prokaryotic post translational modifications