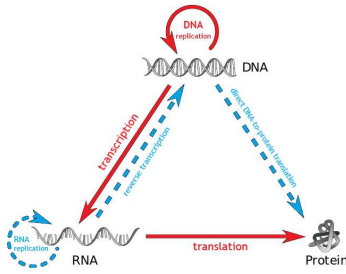


UNIT 12



TRANSLATION IN PROKARYOTES AND EUKARYOTES

Structure

12.1 Introduction	Termination
Expected Learning Outcomes	12.5 Translation in Eukaryotes
12.2 Major molecules in protein synthesis	Initiation
mRNA	Elongation
tRNA	Termination
Ribosomes,	12.6 Comparison of translation between prokaryotes and eukaryotic
Amino acid synthetases	12.7 Inhibitors of translation
12.3 Amino acid activation	12.8 Summary
12.4 Translation in Prokaryotes	12.9 Terminal Questions
Initiation	12.10 Answers
Shine-Dalgarno sequence	
Elongation	

12.1 INTRODUCTION

In this unit we will learn the translation process (protein synthesis) in prokaryotes and eukaryotes. If you look your hair, nails, skins etc are made up of cells and each cell has millions of proteins. Have you ever thought how these proteins are made up in cells? In fact, proteins are the building block of every organism on earth. Cells can't survive without proteins. Proteins are the molecular tool of the biological system. It involves in every biological process from energy extraction from food and to the cell division etc. From previous unit 9 and 10 you have learned that the transcription in prokaryotes and eukaryotes and then in unit 11 the genetic code. The mRNA and genetic code

are essential for the translation process. Genetic code is the secret code for protein synthesis. You now that the codons in mRNA are paired with the anticodons of tRNA. Each anticodon is specific for each 20 amino acids. The primary emphasis of our discussion in this unit is to learn the protein machinery used in translation process and the mechanism of synthesis of protein. We will first have learnt the translation process in the bacteria, as this mechanism is well studied and then focus on translation in eukaryotic cell.

Therefore, in this unit, you shall study the major molecules of translation process, amino acid activation, initiation, elongation and termination process of prokaryotes and eukaryotes. The comparison between prokaryotic and eukaryotic translation and inhibitors of protein synthesis are also discussed. In next block IV you will learn the gene regulation.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ define translation and connection with genetic code of mRNA.
- ❖ learn essential molecules needs to translation;
- ❖ explain the structure and role of ribosomes;
- ❖ differentiate between prokaryotic and eukaryotic ribosomes
- ❖ explain how ribosome structures facilitate the translation;
- ❖ discuss how amino acid activate learn the steps of translation process
- ❖ describe steps of eukaryotic protein synthesis;
- ❖ discuss the regulation of translation; and
- ❖ enlist inhibitors of protein synthesis.

12.2 MAJOR MOLECULES IN TRANSLATION

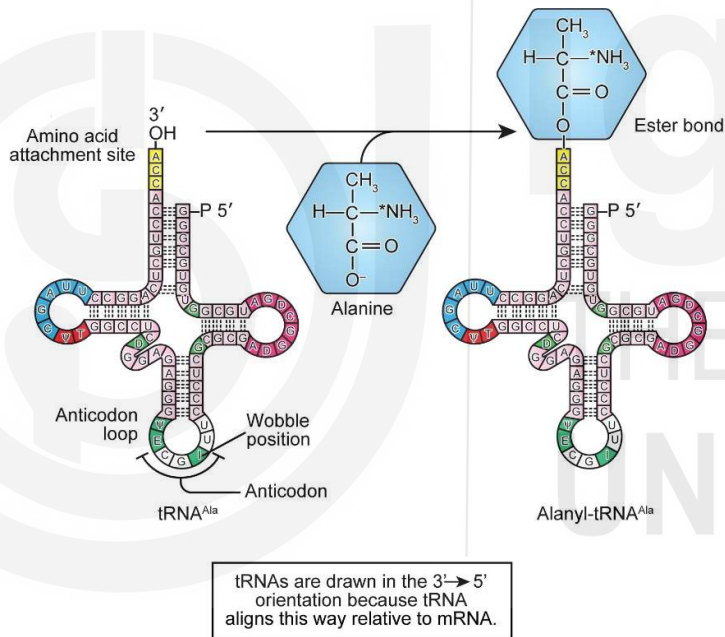
Protein biosynthesis is governed by genes, which carry coded instructions for each 20 amino acids. The information stored in DNA is transcribed into mRNA during transcription and then is translated into proteins by translation process. The three primary forms of RNA molecules that are transcribed from genes in DNA, each with a specific role to play in gene expression, are mRNA, rRNA, and tRNA. In Unit 10 and 11, you learned about the transcription process in prokaryotes and eukaryotes. Protein synthesis involves a complex interplay of many macromolecules. Protein synthesis takes place in the cytoplasm through a complicated process termed translation.

To complete translation within cells, there are three key participants needed:

- 1) **Messenger RNA (mRNA):** The mRNA is a specific form of ribonucleic acid that serves the purpose of transporting the coding segment of a gene for the process of protein synthesis. It comprises segments of non-coding and coding sequences. Prokaryotic mRNA is monocistronic (coding gene for one polypeptide chain) and eukaryotic polycistronic

mRNA (coding gene for multiple proteins). Eukaryotic mRNAs differ from prokaryotic mRNAs in that they have a 5' cap located at the beginning of the mRNA, and a poly-A tail at the end of the mRNA. The protein synthesis or translation mRNA begins with the start codon (AUG) and stop at termination codons (UAG, UAA and UGA). The basic structure of the mRNA of prokaryotes and eukaryotes is similar. The coding sequence organizes nucleotides into codons, which consist of three distinct nucleotides that correspond to a certain amino acid as dictated by the **genetic code**. Recall unit 11 where you have learned concept of genetic code their uses in gene expression. The genetic code is a particular sequence of bases in mRNA is essential for translation process.

- 2) **Transfer RNA (tRNA)**. The tRNA is the type of RNA which serves as adapter agents for carrying the amino acids to the ribosomal subunits for protein synthesis. Transfer RNAs convert the mRNA nucleotide code into the amino acid chain of a polypeptide. It ensures the accurate insertion of amino acids at each position in the polypeptide (Fig. 12.1).



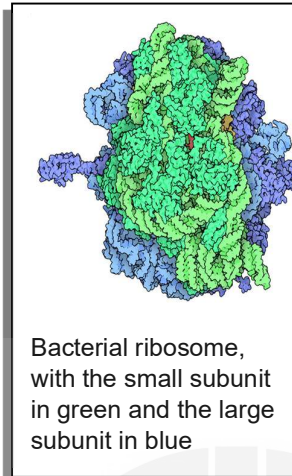
The 2009 Nobel Prize in Chemistry was granted to three structural biologist **Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath** who used X-ray crystallography to explain the structure and motions of the ribosome down to the level of individual atoms. They each received Nobel prize for this work.

Fig. 12.1: Secondary structure of tRNA, before and after amino acid attachment.

- 3) **Ribosomes**: Ribosomes are tiny and spherical molecular machinery that make proteins. They are available freely in cytosol or they attach on the surface of Rough endoplasmic reticulum (RER) and some ribosomes (70S) also occur in mitochondria as well as in chloroplast. **The term "ribosome"** is derived by combining the element "ribo" from ribonucleic acid with "soma," (in Latin word the body). Ribosomes consists of two subunits; **in prokaryotes** 30S (small subunit) and 50S (large subunit) forming **70S** ribosomes and **in eukaryotes** 40S and 60 S forming **80S** (where S=Svedberg units). The assembly of these subunits leads to the formation of functional ribosomes: 70S in prokaryotes and 80S in eukaryotes. However, they exhibit a remarkable degree of structural similarity across all living organism, regardless of the

differences in size observed between prokaryotic and eukaryotic cells. In addition, Ribosomes have a critical function in facilitating the catalysis of two vital biological processes. Peptidyl transfer and peptidyl hydrolysis. Hence, they act as an enzyme.

Fig. 12.2 shows the subunits of 50S (23S rRNA, 5SrRNA and 34 proteins) and 30S smaller subunits (16SrRNA and 21 proteins) of prokaryotes ribosomes(70S) that includes 16S rRNA, 23S and 5S rRNA



The A site receives incoming aminoacyl-tRNA, the P site retains the peptidyl-tRNA and the E site facilitates the removal of deacylated tRNA.

Additionally, the ribosome functions as an enzyme, catalysing the chemical reaction that joins amino acids together to form a polypeptide chain.

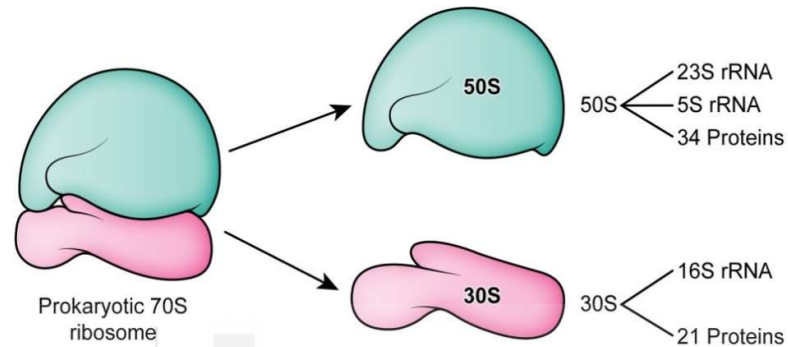


Fig. 12.2: Types of Ribosomal subunits in prokaryotes.

Therefore, ribosomes comprise of proteins and ribosomal RNA (rRNA) and serve as the sites where polypeptides (proteins) are synthesised. Ribosomal RNA (rRNA) is essential for the organization and operation of ribosomes. It serves as the framework for ribosomal proteins and facilitates the chemical processes required for protein synthesis. Ribosomal proteins bind to the ribosomal RNA (rRNA) to enhance the stability of the structure and contribute to several roles during the process of translation.

The large and small subunits of ribosome assemble around an mRNA molecule and facilitates the binding of transfer RNAs (tRNAs) to their corresponding codons on the mRNA template. To facilitates the delivery of amino acids to the growing polypeptide chain, ribosomes has specific binding sites are referred to as the A, P, and E sites. These are three primary functional sites on ribosomes where protein synthesis takes place: **the A (aminoacyl) site, the P (peptidyl) site, and the E (exit) site (Fig.12.3).**

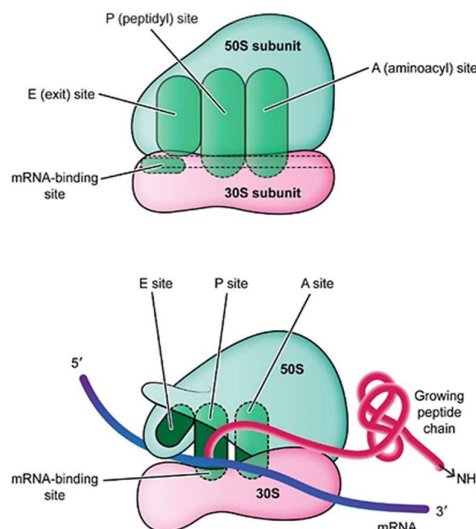


Fig. 12.3: Binding sites on a prokaryotic ribosome showing A (aminoacyl) site, P (peptidyl) site and an E (exit) site.

Eukaryotic ribosomes: Translation in eukaryotes differs in terms of ribosomal subunits as it has 80S ribosomes unlike to 70S in prokaryotes. Eukaryotic ribosomes, specifically the 80S type, are composed of two subunits: the 40S subunit and the 60S subunit. Its large subunit 60S contains the peptidyl transferase center which is responsible for aiding the peptide bonds formation during the process of protein synthesis. On the other hand, the smaller subunit contains the decoding center, which is responsible for the reading and interpretation of codon sequences in mRNA by charged tRNAs. Eukaryotic ribosomal subunits are bigger and include a higher number of proteins compared to prokaryotic ribosomal subunits. The eukaryotic ribosomes consist of the 28S, 5.8S, and 5S rRNAs, together with 46 proteins, which make up the big subunit (60S). Meanwhile, the small subunit (40S) consists of the 18S rRNA and 33 proteins (see Fig. 12.4). The large ribosomal subunit 60S also contain two tRNAs binding sites A and P except E site while three binding sites (A, P and E) present in prokaryotes.

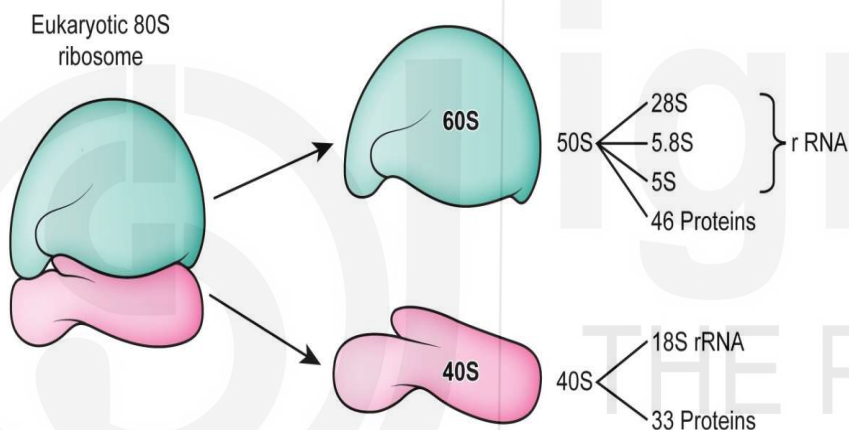


Fig. 12.4: Ribosomal subunits of eukaryotes (80S).

4. Aminoacyl-tRNA Synthetases:

Aminoacyl-tRNA synthetases (AARS) are a group of enzymes that play a crucial role in protein synthesis by ensuring the accurate pairing of amino acids with their corresponding transfer RNA (tRNA) molecules. This process, known as tRNA charging or aminoacylation, is a fundamental step in the translation of genetic information from mRNA to protein. Paul Schimmel and his colleagues were among the scientists who made significant contributions to understanding how these enzymes recognize specific amino acids and tRNAs. The specified tRNA is transferred to its matched tRNA, resulting in the formation of a tRNA-aminoacyl complex. Each of 20 amino acids recognized by its specific Aminoacyl-tRNA synthetases. It is possible for some synthetases to recognise more than one tRNA as an acceptor, which refers to distinct tRNAs that accept the same amino acid but have distinct anticodon sequences as well. The mechanism of amino acid activation is shown in Fig. 12.5.

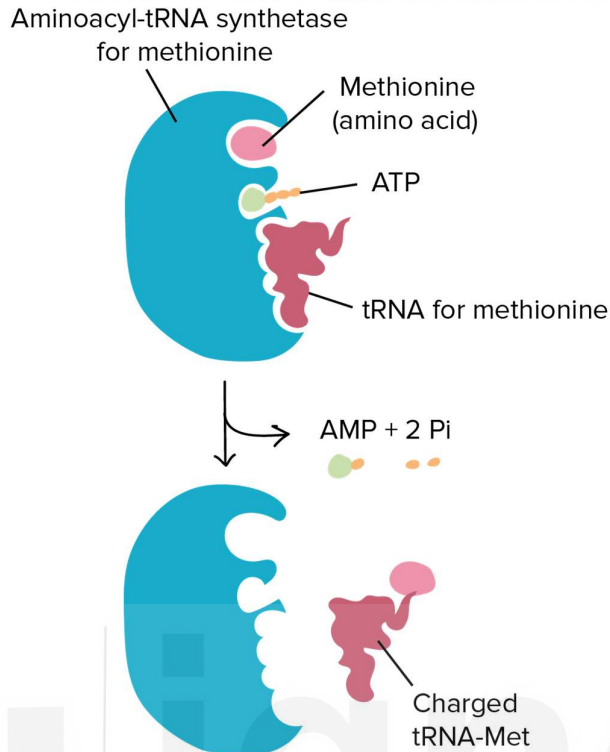


Fig.12.5: Charged tRNA. When the amino acid and its tRNA both bind to the enzyme, the enzyme catalyzes their linkage through a aminoacylation which is mediated by adenosine triphosphate (ATP). This image is under is licensed under a CC BY-SA 3.0.

SAQ 1

Fill in the blanks:

- i) 70S ribosomal unit consists of.....and 80S
- ii) Which RNA serves as an adapter agent in translation?
- iii) The attachment of amino acid to the tRNA reaction is catalyzed by
- iv) Prokaryotic ribosome contains specific three sites: A for site, P for..... site and an E site.
- v) In prokaryotes, the A, P and E sites are located inribosomal subunit.

Let us first understand amino acid activation followed by translation process

12.3 AMINO ACID ACTIVATION

Protein synthesis requires amino acid activation, also known as aminoacylation or amino acid charging. The aminoacyl-tRNA, formed by connecting the right amino acid to its transfer RNA (tRNA), is the building block for protein synthesis during elongation. The scientist Mahlon Hoagland

first recognised the process of amino acid activation, whereby certain enzymes catalyse the formation of an intermediate molecule called aminoacyl adenylate from amino acids. These enzymes were subsequently recognised as aminoacyl t-RNA synthetases. The synthetases have a vital function in facilitating the bonding of an amino acid to a short RNA molecule. In eukaryotes, the initial amino acid used in protein synthesis is methionine, but in prokaryotes it is N-formyl methionine. In bacteria, there are two different tRNA namely methionyl tRNA ($tRNA^{met}$) and N-formyl methionyl tRNA ($tRNA^{fmet}$) for methionine triple codon (AUG). The amino group of methionine ($tRNA^{met}$) is modified by the addition of formyl group (donated by tetrahydrofolate) to form the formyl methionine tRNA ($tRNA^{fMet}$). This reaction is catalyzed by formylase enzyme, Keep in mind that there is two AUG codon- in mRNA, one for binding the $tRNA^{fmet}$ as an **initiator tRNA** and another one codes for methione in an internal position of growing amino chain.

The first step is for the matching aminoacyl-tRNA synthetase enzyme to specifically recognise the correct amino acid.

Binding of Aminoacyl-tRNA Synthetase:

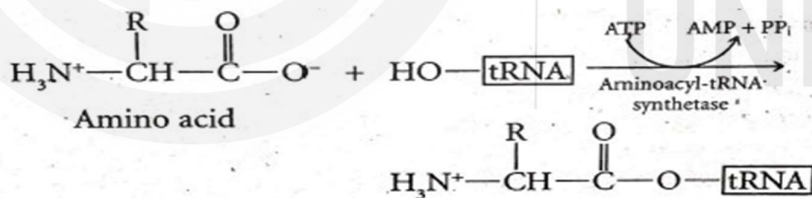
The enzyme aminoacyl-tRNA synthetase has binding site for both the amino acid as well as the tRNA. There are distinct binding sites on the enzyme that are dedicated to the amino acid and the tRNA.

Amino Acids activation:

Step I: This activation step is catalyzed by amino acyl tRNA synthetase. In which methionine is lined to the $tRNA^{fmet}$.

The activation of an amino acid involves the attachment of the amino acid to the 3' end of the relevant tRNA molecule. This process needs adenosine triphosphate (ATP) which is hydrolyzed into adenosine monophosphate (AMP) and inorganic pyrophosphate (PPi).

The activating enzyme is attached to the amino acyl AMP.



In the next Step II, AMP is released when the aminoacyl adenylate intermediate is nucleophilically attacked, adding an aminoacyl group to the tRNA at the 3'-OH.

The inorganic pyrophosphate is hydrolyzed by the enzyme Pyrophosphatase to produce orthophosphate. Therefore, it is possible that two high energy phosphate bonds are used for each amino acid.

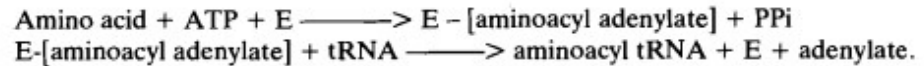
Aminoacyl adenylate + tRNA \longrightarrow Aminoacyl-tRNA + AMP (Adenosine-monophosphate + Aminoacyl-tRNA synthetase (Enzyme))

Formation of aminoacyl-tRNA:

Following the activation of the amino acid, the subsequent stage involves the transfer of the activated amino acids to their corresponding tRNA molecules. The transfer of the activated amino acid to its corresponding particular tRNA is

facilitated by aminoacyl-tRNA synthetase. The aminoacyl synthetase contains two distinct active sites, one dedicated to the recognition of the specific amino acid and the other to the recognition of the specific tRNA molecule. The enzyme aminoacyl-tRNA synthetase facilitates the transportation of the amino acid.

The net reaction is



After being charged with the appropriate amino acid, the tRNA functions as an intermediary molecule throughout the process of translation. It identifies the codons present in mRNA and transports the relevant amino acid to the ribosome for the process of protein synthesis. By learning the above section, you try to solve the SAQ given below:

SAQ 2

Fill in the blanks:

- i) The first charged tRNA during the initiation is known as
 - ii) Aminoacyl adenylate + tRNA \longrightarrow Aminoacyl-tRNA + AMP+
.....Aminoacyl-tRNA synthetase (Enzyme).
 - iii) In eukaryotes, the initial amino acid used in protein synthesis is
....., but in prokaryotes it is
 - iv) The transfer of the activated amino acid to its corresponding tRNA is
facilitated by
 - v) An enzyme that contains two distinct active sites, one dedicated to the
recognition of the specific amino acid and the other to the recognition of
the specific tRNA molecule is
-

Now you have understood the process of amino acid activation. Let us now turn our focus to the prokaryotic translation process.

12.4 TRANSLATION PROCESS IN PROKARYOTES

In this section, we will discuss the translation process in prokaryotic cell. The translation of genetic information and correct linking of amino acids are the heart of the translation. Following the process of DNA transcription to mRNA, the subsequent step is translation, which involves the interpretation of these mRNA molecules to synthesize proteins. The process of mRNA translation results in the production of polypeptides in a sequential manner, starting from the N-terminal (mRNA 5' end) and progressing towards the C-terminal (mRNA 3' end).

The translation process consists of three stages: **initiation, elongation, and termination (Fig.12.6)**. In the initiation stage, mRNA binds to the ribosome and positions itself for translation. During the elongation stage, amino acids

are joined together in a sequential manner through peptide bonds, following the arrangement of codons in mRNA. Finally, in the termination stage, both the mRNA and the newly formed polypeptide chains are released from the ribosome.

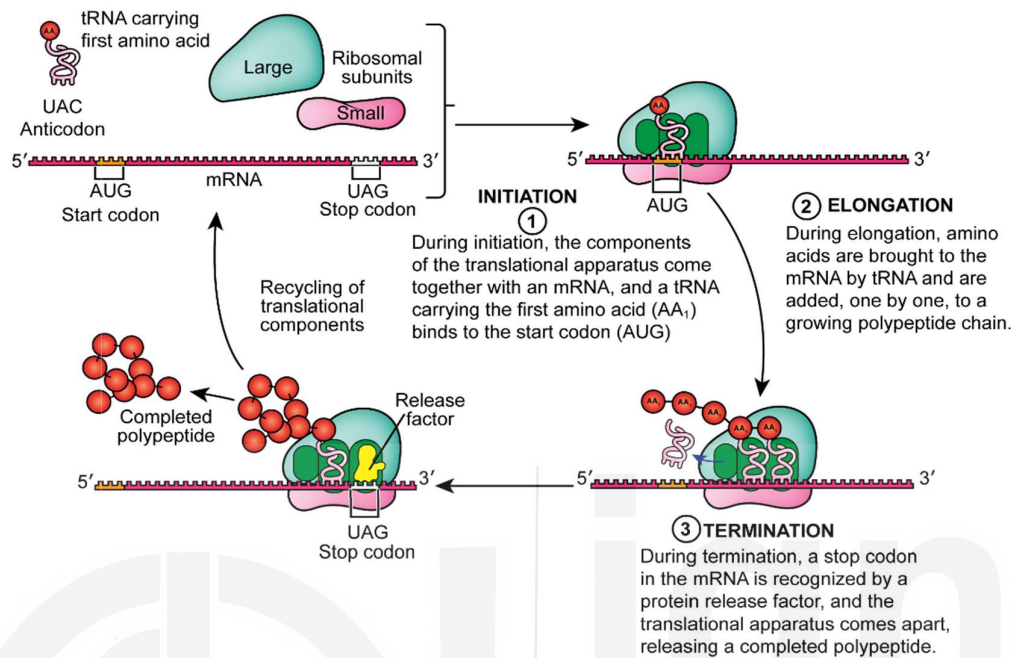


Fig. 12.6: Overview of Translation process include three steps: Initiation, Elongation and Termination.

Let us move to learn the translation steps one by one:

12.4.1 Initiation

In bacteria, translation begins with a sequence of events that culminate in the development of a functioning ribosome-mRNA complex that allows the synthesis of a polypeptide chain. In prokaryotes, the initiation factors are known as IF-1, IF-2, and IF-3 while in eukaryotes, they are known as eIF-1, eIF-2, eIF-3, eIF4A, eIF4B, eIF4C, eIF4D, eIF4E7, eIF-5, and an additional ancillary 9 protein factor (SP). This process necessitates the use of Mg^{2+} and GTP. IF-2 is essential for N-formylmethionine-tRNA recognition and binding. IF-1 is certainly involved as well, although IF-2 is the most crucial. AUG is the mRNA start codon. As a result, the initiation tRNA (attached to N-formylmethionine together with its UAC anticodon) attaches to an mRNA AUG codon in prokaryotes. AUG and UUG are infrequently used as mRNA start codons.

Protein synthesis begins with the formation of initiation complex. The mRNA bearing the AUG codon for polypeptide synthesis attaches to the smaller subunit (30S) of the ribosome, followed by the starting amino acid connected to its tRNA to form an initiation complex (70s-f met-tRNA-mRNA in prokaryotes and 80s-met-tRNA-mRNA in eukaryotes),

The key steps involved in the initiation of translation in bacteria is divided in three stages:

- 1) In prokaryotes, the 30s ribosomal subunit attaches first to the initiation factor-3 (IF-3) in the first step. This factor prevents the 30s from the rejoining the 50s subunits. The 16s rRNA molecule also participates in the process of binding to mRNA. It accurately aligns the mRNA at the initiation codon (AUG) to initiate translation. The 30s subunit serves as a binding site for IF-3, and the 16s rRNA is also involved in mRNA binding. It accurately puts the mRNA at the initiation codon (AUG) for translation to begin. IF-3 helps in stabilizing the 30S subunit.

Recognition of mRNA: A small subunit of the ribosome called 30S binds to the mRNA's **Shine-Dalgarno (SD)** sequence (Fig.12.7). The SD sequence is a short, preserved, purine-rich sequence (AGGAGG) that starts a few bases before the AUG start codon. This sequence binds with complementary base pair UCCUCC located at the 3' end of 16S rRNA (part of 30S subunit of ribosome). It anchors the 30S subunits of the ribosome to the correct position of mRNA.

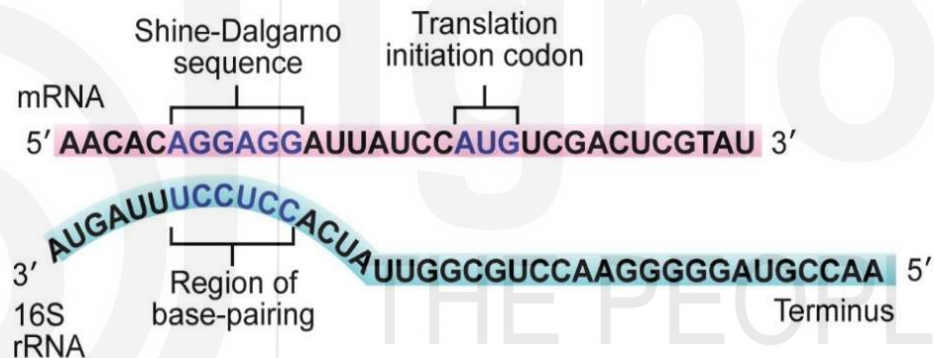


Fig.12.7: Shine-Dalgarno consensus sequence in mRNA required for attachment of small sub unit of ribosome.

Step II:

During initiation, the special initiator tRNA bearing N-formyl methionine ($\text{tRNA}^{\text{Metf}}$) is linked to the P site of the 30S ribosomal subunit with the help of initiation factor IF2, which forms a complex with GTP. The IF3 is released as the formyl methionine-tRNA-mRNA pairs with the start codon AUG in the mRNA.

At this stage the 30S ribosomal subunit, IF1, IF2-GTP, and f met-tRNA-mRNA formed the **initiation complex (Fig.12.8)**. This complex is able to differentiate the initiator tRNA from other tRNAs.

Step III Finally large subunit 50S binds to the 30S initiation complex. This attachment is mediated by GTP hydrolysis and all three initiation factors are released.

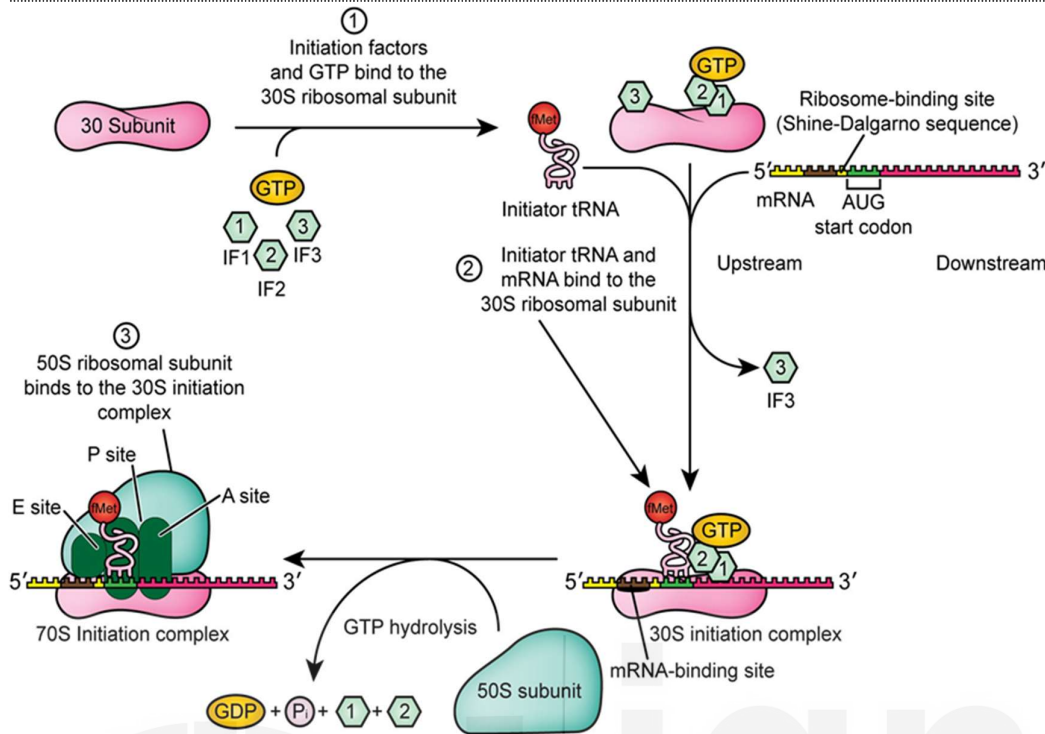


Fig. 12.8: Steps involved in Initiation of translation in bacteria:(1) 30S subunit binds with the initiation factors (IF1,IF2 and IF3) and GTP(2) The initiator aminoacyl tRNA and mRNA are attached on shine Shine-Dalgarno (SD) sequence. (3) The 70S fMet-tRNA^{fMet} initiation complex is formed in the ribosome's P site upon hydrolysis of GTP and releasing the all three initiator factor. Large ribosomal subunit joins the 30S initiation complex.

12.4.2 Elongation

After initiation, the next step is the elongation in which amino acid chain becomes longer. The elongation requires (i) initiation complex as described above, (ii) aminoacyl tRNA (iii) Elongation factors EF-Tu and EF-Ts, (iv) GTP. The 50S ribosomal subunit contains three tRNA binding sites. An aminoacyl site, also known as the **A site**, is a specific location on a site where an aminoacyl-tRNA molecule binds during protein synthesis. The **P site**, also known as the Peptidyl Site, is responsible for holding the peptidyl-tRNA molecule. This molecule carries the polypeptide chain that is currently being synthesized. **The E site**, also known as the Exit Site, is the location within the ribosome where the deacylated tRNA, which is the tRNA without its amino acid, escapes after its involvement in the synthesis of the expanding polypeptide chain.

Charged aminoacyl tRNAs bind to the A site. charged tRNAs carrying amino acids that have made peptide bonds with the developing polypeptide chain but have not yet dissociated from their tRNA bind to the P (peptidyl) site.

Elongation completes in three stages (Fig.12.8):

- **Aminoacyl-tRNA binding,**
- **Formation of peptide bond,** and
- **Translocation**

1. Aminoacyl-tRNA binding:

The first formyl methionine tRNA (initiator tRNA) binds to the P site while other tRNA binds to the A site and shifts to P site during the initiation of prokaryotic translation. In this first step of elongation process, the second aminoacyl tRNA binds to the EF-Tu-GTP and this elongation complex (aminoacyl tRNA –EF-Tu-GTP) now binds to the ribosomal A site (70S initiation complex). In prokaryotes, aminoacyl tRNA binds to the ribosome requires EF-Tu and EF-Ts. The mechanism involves EF-Tu creating a ternary complex with aminoacyl tRNA and GTP. The A site on the ribosome is bound by this ternary complex of EF-Tu, aminoacyl tRNA, and GTP. GTP is hydrolysed into GDP and EF-Tu-GDP complex is released. This process leads to conformational modifications that initiate ribosomal movements, causing it to migrate forward by a codon in the 3' direction.

2. Formation of peptide bond:

In this second step, peptide bond formation occurs between the two amino acids that are located on the A and P sites of ribosomes. The peptidyl transferase enzyme catalyzes the peptide bond formation without needed energy. This process occurs when the first amino acid (N-formyl-methionine group in prokaryotes and methionine in eukaryotes) of its tRNA transfers (P site- peptidyl donor site) to the second amino group of amino acid residue-tRNA which is located at the A site. At this step, two amino acids are linked together via peptide bond and the dipeptidyl-tRNA is produced in A site and deacylated tRNA^{Met} (uncharged tRNA) is still bound to the P site (Fig. 12.9). During the creation of peptide bonds, a water molecule is released when the α -amino group of one amino acid makes a covalent bond with the α -carboxyl group of the next amino acid. This whole process takes place in the peptidyl transferase center (PTC), where the ribosome principally functions as a "entropic trap" for the polypeptide synthesis. This center lies in the 23S rRNA (50S) in prokaryotes and 28S rRNA of 60S ribosomal subunit in eukaryotes. Now, tRNA present at the A site contains two amino acids and dipeptidyl-tRNA. The amino acid that is connected to the P-site tRNA becomes part of the elongating polypeptide chain.

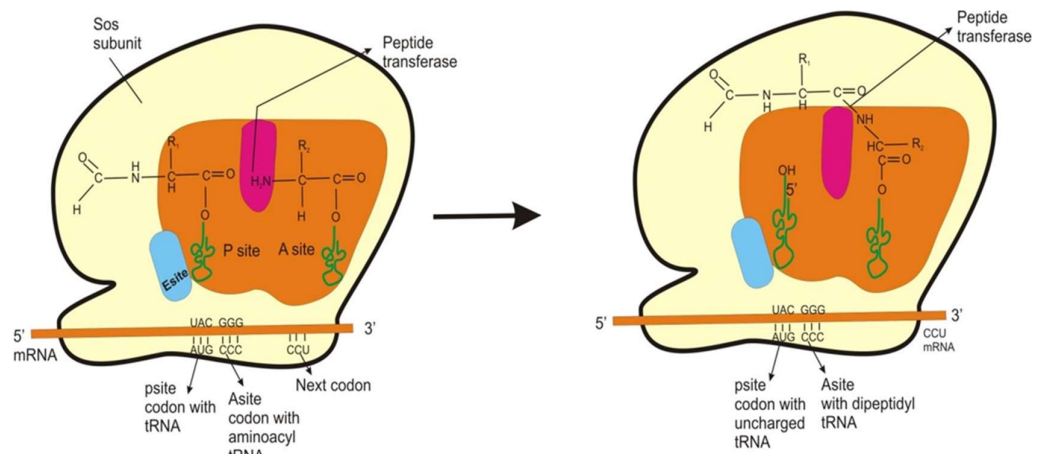


Fig. 12.9: Mechanism of Peptide bond Formation.

- 3. Translocation:** This is the last step of elongation cycle in which the ribosome moves along the mRNA a codon at a time toward mRNA 3' end. This movement forward in the 5' → 3' direction and called translocation. Due to this ribosomal movement, the anticodon of dipeptidyl-tRNA moves from the A site to the P-site and the deacylated tRNA is transferred from P to the E site (exit site) and the finally discharged tRNA (empty tRNA) is released into the cytoplasm for recycling. This translocation requires EF-G and GTP-to-GDP hydrolysis for the movement of ribosomes and align the ribosome with the next codon. Two GTP are required for each amino acids for growing amino acid chain as ribosomes moves from the codon to codon along the mRNA. These elongation factors directs the binding of specific amino acylated tRNA to the anticodon on the empty A site. Now, the A site reopens for third incoming charged tRNA and so on for the next round of elongation cycle. The third codon lies in the A site and the second codon in the P site.

In a nutshell, there are three reaction involved during the translocation
 (a) The transfer of acylated tRNA from the A site to the P site, requiring EF-G and GTP.

(b) The alignment of mRNA with the ribosome, a step that also necessitates the participation of EF-G and GTP.

(c) The act of discharging or expelling deacylated tRNA from the P site of the ribosome. GTP and EF-G help this process.

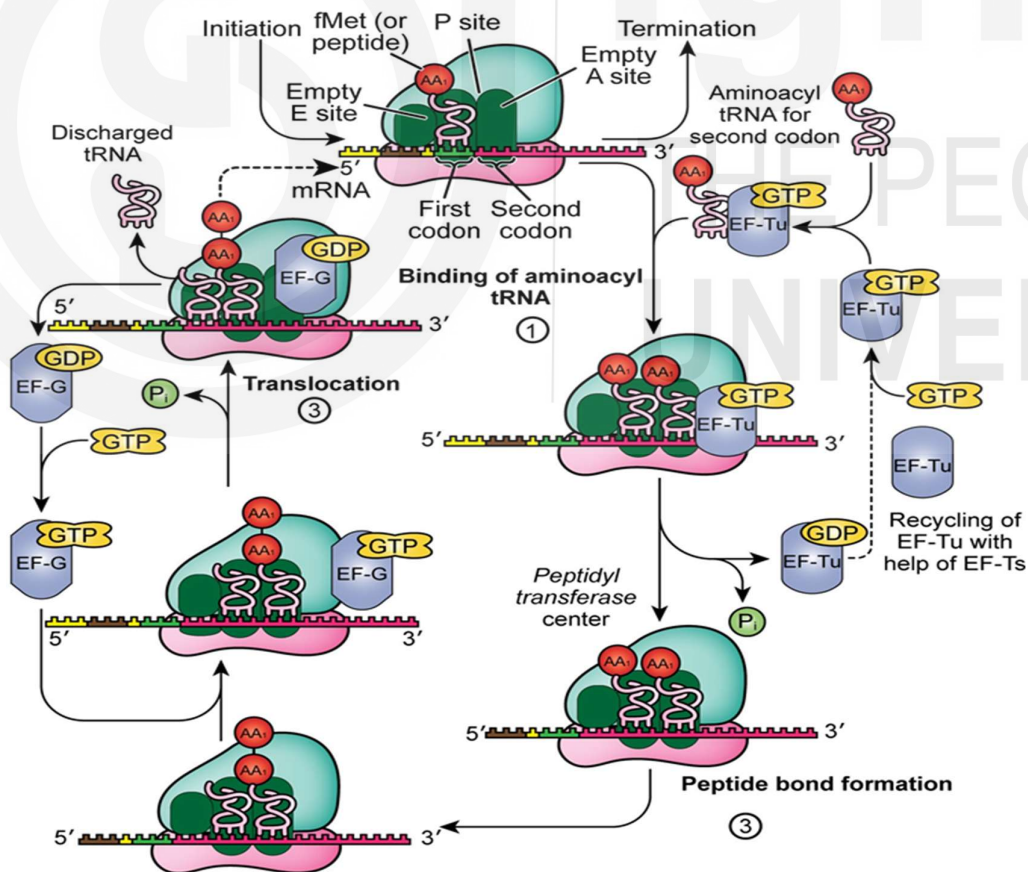


Fig. 12.10: Three stages of Polypeptide Chain Elongation in Bacteria: 1. Binding of An aminoacyl tRNA to the A site. 2). A trans peptidation at the P site occurs between the –COOH group of fMet and the newly arrived amino acid at the A-site. 3). Translocation- The peptidyl tRNA moves from the A site to the P site.

12.4.3 Termination

Termination of translation in prokaryotes involves the recognition of a stop codon on the mRNA by a release factor, leading to the release of the newly synthesized polypeptide chain from the ribosome (Fig. 12.11)

The termination process initiates when the ribosome meets any of the three stop codons: UAA, UAG, or UGA and halt the peptide formation during translation process. The first step of chain termination involves the binding of release factor (RF) to the A site of the ribosome. This process requires GTP. The peptidyl transferase enzyme hydrolyzes peptidyl tRNA at the P site and frees the polypeptide chain from the ribosome. Prokaryotes possess release factors, specifically RF1 and RF2. RF1 is responsible for recognizing the UAA and UAG codons, while RF2 identifies the UGA and UAA codons.

The fully synthesized polypeptide is removed from the ribosome which is then followed by the separation of the tRNA, mRNA, ribosomal subunits, and release factors. The free constituents, such as the ribosomal subunits, are now free to participate in the next rounds of translation. The termination codon does not necessarily have to be the final codon on mRNA. The newly made polypeptide will further undergo posttranslational modification such protein folding and glycosylation.

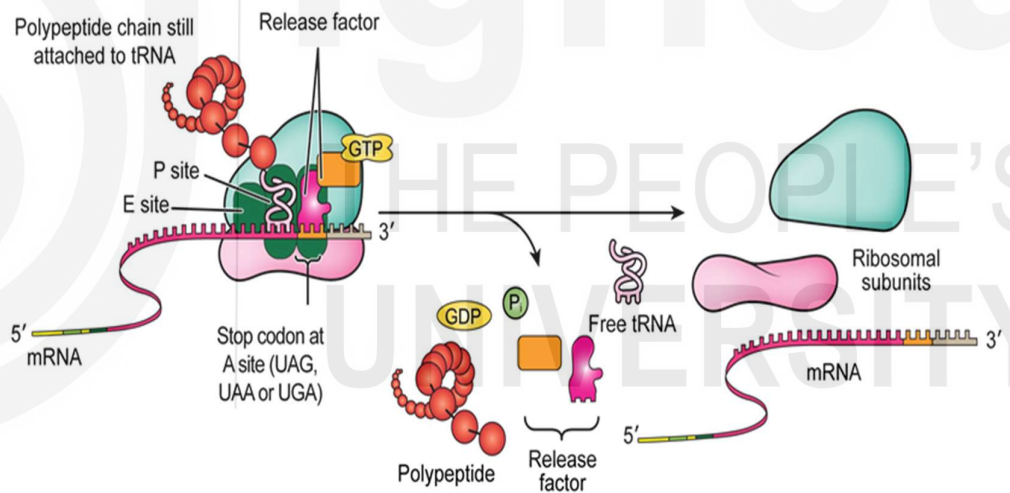


Fig. 12.11: Termination of translation. Protein release factors associated with GTP identify and attach to a stop codon (UAA, UAG, or UGA) when it reaches the A site. The hydrolysis of GTP is accompanied by the liberation of the fully formed polypeptide, which is then followed by the separation of the tRNA, mRNA, ribosomal subunits, and release factors.

Speed of translation

This process occurs sequentially many times till the new codons are read and new charged tRNAs are added to the growing chain similarly like second amino acid is added. The translation machinery of *E. coli* demonstrates remarkable efficiency, accomplishing the incorporation of each amino acid in a little 0.05 seconds. Therefore, a protein consisting of 200 amino acids can be synthesized in about 10 seconds (0.05 seconds to add each amino acids).

SAQ 2

Fill in the blanks:

Which component joins the initiation complex last during the initiation of translation?.....

- i) IF-2 is essential for recognition and binding.
- ii) The start codon is..... is the initiation codon for translation.
- iii) The purine-rich sequence that anchors both ribosomes and mRNA in their correct position (AGGAGG) is called.....
- iv) During the initiation of translation, the first amino acids bound to its tRNA binds site of ribosomes while other tRNA binds toand then shifts tosites.
- v) The transfer of acylated tRNA from the A site to the P site is needed requiring EF-G and GTP.
- vi) Ribosomal movement takes place at.....stage
- vii) The dipeptidyl-tRNA shifts from the to the where as deacylated tRNA is removed from of the ribosome.
- viii) Release factor binds to to halt the protein synthesis.
- ix) The peptide bond formation is catalyzed by
- x) Which ribosomal subunits acts as an enzyme?
- xi) Elongation sequence of tRNAs is:
cytoplasm → _____ → P site → _____ → cytoplasm.

After learning the mechanism of protein synthesis in prokaryotes now we would learn translation process of eukaryotic

12.5 TRANSLATION IN EUKARYOTES

The eukaryotic translation as like in prokaryotes with differences in but the initiation process in eukaryotic cells is more complicated than in prokaryotes. Eukaryotic translation also contains three steps: initiation, elongation and termination.

We will focus to understand the initiation process which is the complex process in eukaryotes. In eukaryotes, the initiator tRNA is the methylated (tRNA^{Met}) binds to the initiation codon rather than N-formyl methionine (tRNA^{Met}) in bacteria. The AUG within mRNA is coded for methionine amino acids for an internal amino acid chain during polypeptide synthesis. In eukaryotes, the AUG codon is often located downstream of the cap structure, which is situated at the 5' end of the mRNA.

In order to identification of initiation codon (AUG on mRNA), the small subunit of the ribosomes binds to the 5' end mRNA and scan the initiation codon along the mRNA rather than binding directly to the initiation codon within mRNA. It recognises the correct AUG triplet when it finds the short consensus sequence 5'-ACCAUGG-3' which is called **kozak sequence**.

The pre-initiation complex is required for the initiation of eukaryotic translation. It comprises of:

- The small subunits of the ribosomes
- A ternary complex (eIF2-tRNA^{met}-GTP) composed of eIF2 and initiator tRNA and GTP.
- Initiator factors eIF-1, eEF-1A and eEF-3

12.5.1 Initiation

Translation initiation in eukaryotes begins with three components/complexes that are shown near the top of Fig. 12.2. The components involved in the initiation of translation are: **1)** the 40S ribosomal subunit, which has the eIF1, eIF1A, and eIF3 initiation factors attached to it; **2)** the eIF2.GTP + Met-tRNA^{Met} ternary complex; and **3)** a circular mRNA formed by the binding of the eIF4 cap-binding complex at the 5' end of the mRNA to poly(A) binding protein (PABP) associated with the 3' end of the mRNA. This assembly interacts with the 40S subunit alongside eIF5. Subsequently, the mRNA molecule associates to the 40S subunit through the coordinated action of eIF4E (binding to the 5' cap of the mRNA), eIF4G (acting as a bridge between eIF4E and PABP at the 3' poly-A tail), eIF4A, and eIF4B. The ribosome then scans the mRNA until it recognizes the initiation AUG start codon, a process fueled by ATP hydrolysis. Upon identifying the initiating AUG codon, eIF5 catalyzes the hydrolysis of GTP bound to eIF2, leading to the release of eIF2-GTP and other initiation components. Finally, the 60S ribosomal subunit joins the 40S complex, facilitated by eIF5B and leads to the release of all initiation factors, therefore initiating the elongation phase of translation.

12.5.2 Elongation

Elongation process is same in both prokaryotes and eukaryotes. In eukaryotes, **elongation process** requires the elongation factors (EF1 α , eEF2 and eEF1A). Eukaryotic EF1A (eEF1A) binds with aminoacyl-tRNAs and GTP to create ternary complexes. The ternary complexes enter the vacant A site of the ribosome. If a suitable codon-anticodon contact occurs between the entering aminoacyl-tRNA and the codon at the A site, GTP will be broken down and eEF1A will be liberated. Currently, the peptidyl-transferase site of the ribosome facilitates the synthesis of peptide bonds. This occurs when the amino group of the incoming aminoacyl-tRNA assaults the ester bond that connects the developing polypeptide to the tRNA at the ribosomal P site. The tRNA that is no longer charged and is located in the P site of the ribosome goes to the E (exit) site and exits the ribosome. The polypeptide chain, which was previously located in the P site, is extended by one amino acid as it moves to the aminoacyl-tRNA at the A site. The peptidyl-tRNA situated in the A site is subsequently relocated back to the P site with the assistance of eEF2

and GTP. The A site is now vacant, and the entire process is iterated continuously as the ribosome progresses down the mRNA (Fig. 12.12).

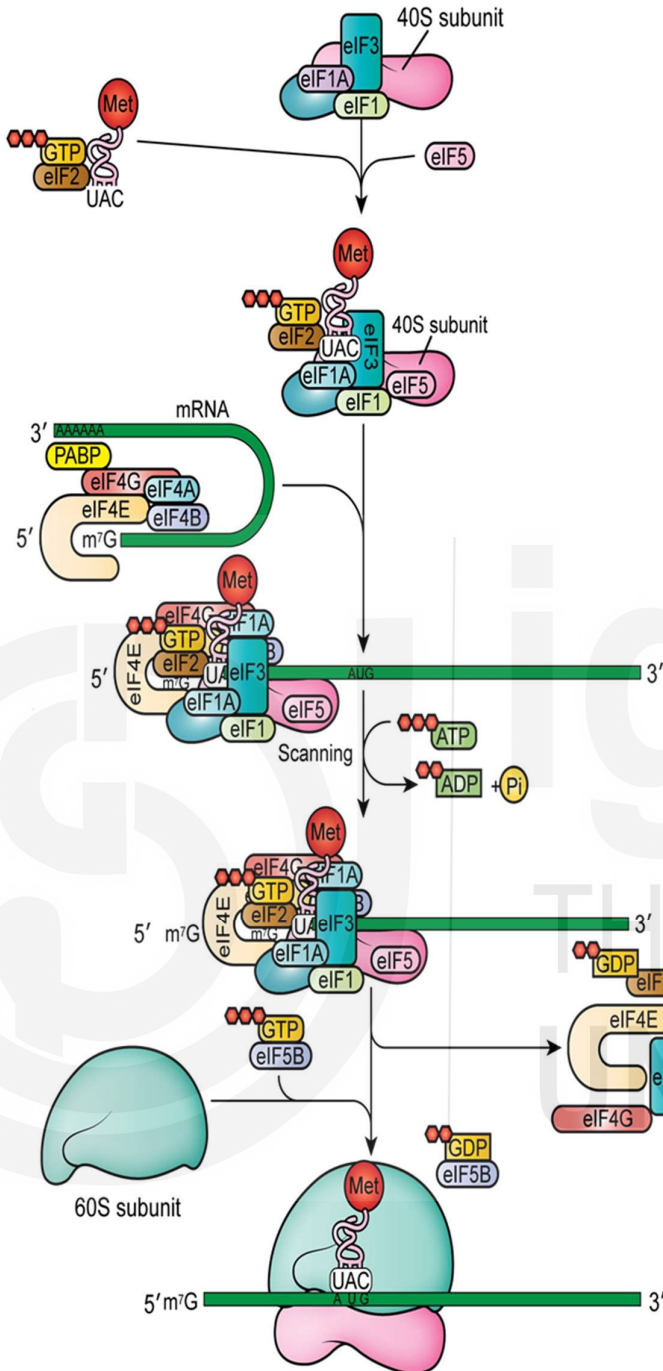


Fig. 12.12: The initiation of translation in eukaryotic cells involves several distinct factors.

12.5.3 Termination

The process of translation termination in eukaryotic cells is similar to that in bacteria, but with a significant distinction involving the presence of two release factors. Eukaryotic translation termination specifically involves two primary release factors: eRF1 and eRF2.

First and foremost, eRF1 plays a crucial function in identifying and detecting all three termination codons (UAA, UAG, and UGA). eRF1 associates to the A site of the ribosome when it encounters a termination codon along the mRNA and initiating the termination process of polypeptide chain.

Regulation of translation:

There are various internal and external factors involved in regulation of eukaryotic and prokaryotic translation process. However, the gene expression generally occurs in transcription level and also initiation level of protein synthesis. The control of prokaryotic translation is an intricate process that involves the interaction of various components, such as cis-acting elements present in mRNA molecules and trans-acting regulatory proteins. However, bacteria have the ability to directly control translation by attaching regulatory proteins to certain regions in the mRNA. In next block IV Gene regulation, you will study about regulation of gene expression in prokaryotes and eukaryotes in detail.

SAQ 3

a) State whether the following statements about eukaryotic translation are true (T) or false (F).

- i) The eukaryotic initiator tRNA is the methylated (tRNA^{Met}) []
 - ii) the small subunit of the ribosomes binds directly to the initiation codon on the mRNA. []
 - iii) A small specific sequence called kozak contain initiation codon for binding the ribosomal unit. []
 - iv) GTP is not required in elongation process of translation. []
 - v) The eRF1 associates to the P site of the ribosome for termination. []
 - vi) GTP hydrolysis is required for identification of initiation codon along the mRNA. []
 - vii) The initiator factor eIF2 recognises the initiator methionyl tRNA (charged tRNA) []
-

Now you have understood the mechanism of prokaryotic and eukaryotic translation. You may also identify the differences between the three steps involved in the tr.

12.6 COMPARISON BETWEEN PROKARYOTIC AND EUKARYOTIC TRANSLATION

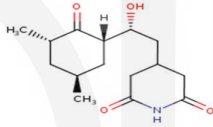
Components	Prokaryotic translation	Eukaryotic translation
Ribosomes	70S	80S
	30S (small subunit) with 16S rRNA subunit 50S (large subunit) with 5S and 23S rRNA subunits	40S (small subunit) with 18S rRNA subunit 60S (large subunit) with 5S, 5.8S, and 28S rRNA subunits
mRNA	Polycistronic with no 5' cap	Monocistronic with 5' cap
Initiator tRNA (carrier of Amino acid)	fMet-tRNA ^{fmet} (N-formyl methionine)	Met-tRNA ^{Met} (Methionine)
Shine-Dalgarno sequence in mRNA	Present	Absent
kozak sequence	Absent	Present
Simultaneous transcription and translation	Yes	YES
initiation factors -	IF-1, IF-2 and IF-3	eIF1A eIF2, eIF3, eIF5B eIF1, eIF4 complex
Elongation factors	EF-Tu EF-Ts EF-G	eEF1A eEF1B eEF2
Release factors	RF-1 RF-2 RF-3	eRF-1 eRF-3

12.7 INHIBITORS OF TRANSLATION

Protein synthesis inhibitors are chemicals that impede or decelerate the process of translation, which is the production of proteins. Typically, these inhibitors exert their effects at the ribosome level. Ribosomes serve as the location for protein synthesis in both prokaryotes and eukaryotes. These inhibitors can function at several phases of translation, including initiation, elongation, and termination. The translation inhibitors are summarized in the table 12.2

Table 12.2: Some of prokaryotic and eukaryotic protein synthesis inhibitors

Acting only on prokaryotes		
Inhibitors	Nature and source	Inhibition mechanism
Tetracycline	semisynthetic antibiotic	Prevents the attachment of aminoacyl tRNA to the A site of the ribosome (30S).

Streptomycin	an aminoglycoside and originates from soil bacterium <i>Streptomyces griseus</i>	Binds to smaller subunit (30S) of the bacterial ribosome
Chloromphenicol	antibiotic and derived from <i>Streptomyces venezuelae</i> .	Blocking the recruitment of aa-tRNA into the A site of the 50S ribosomal subunit and interfere with peptidyl transferase activity
Erythromycin	derived from <i>Saccharopolyspora erythraea</i>	prevents elongation by binding to the 23S rRNA molecules found in the 50S subunit of the bacterial ribosome (inhibit peptide exit group)
Rifamycin	Antibiotics (ant tuberculosis drug)	Inhibit bacterial DNA-dependent RNA polymerase (RNAP) , and prevents mRNAtranscription
Inhibitors acting only eukaryotes		
Cycloheximide 	antibiotic produced by the bacterium <i>Streptomyces griseus</i> . Translation inhibitor used in research	Inhibit the movement of two tRNA molecules and mRNA in relation to the ribosome) and blocking eukaryotic translational elongation.
α -Amanitin	cyclic peptide	inhibits RNA polymerase II only at transcription level
Anisomycin	antibiotic produced by <i>Streptomyces griseolus</i>	inhibiting peptidyl transferase activity (80S ribosome system)
Diphtheria toxin	toxins	Binds and inactivates elongation factor (EF-2) required for protein synthesis
Ricin		inhibits translation through its ability to remove/depurinate a specific adenine base of the universally conserved SR
Acting on prokaryotes and Eukaryotes		
Puromycin	antibiotic obtained from <i>Streptomyces alboniger</i>	Inhibit the ribosomal A site (30S) and stop the peptide bond formation (block 23rRNA)
Actinomycin D		binds to DNA and leads to reduce the movement of RNA polymerase. Inhibitsboth transcription and translation

SAQ 5

Match some of the inhibitors (Column I) of protein synthesis with their functions (Column II)

Column I	Column II
i. Tetracycline	a). inhibits peptidyl transferase activity in eukaryotes
ii. Chloromphenicol	b). Blocks the 23rRNA
iii. Cycloheximide	c). Inhibit the (30S) of the bacterial ribosome
iv. Puromycin	d). Block the movement of two tRNA molecules and mRNA in relation to the ribosome) and inhibit eukaryotic translational elongation.
v. Anisomycin	e). blocks the recruitment of aa-tRNA into the A site of the large ribosomal subunit (50S)

12.8 SUMMARY

So far you have studied that:

- Protein synthesis is the process of converting the nucleotide base sequence of an mRNA molecule into the amino acid sequence of a polypeptide chain. The sequence of mRNA nucleotides determines the order of amino acids in the developing polypeptide chain.
- Both prokaryotes and eukaryotes have ribosomes in their cytoplasm and endoplasmic reticulum. Ribosomes translate mRNA when their big and tiny subunits combine. The tiny subunit binds the mRNA template, whereas the big subunit progressively binds tRNAs, which add amino acids to the polypeptide chain. Many ribosomes translate each mRNA molecule and synthesize protein in the same way.
- During the process of translation, the ribosomal RNA (rRNA) component plays a crucial role in facilitating the synthesis of peptide bonds and placing the messenger RNA (mRNA). Amino acids are linked to tRNA molecules through aminoacyl-tRNA synthetases by the amino acid activation process, which conveys them to the ribosome.
- Translation proceeds through three distinct phases: initiation, elongation, and termination in both bacterial and eukaryotic cells. It begins with the formation of an initiation complex. In bacteria, this complex contains the 30S ribosome, the mRNA template, three initiation factors, and a special

initiator tRNA. The initiation codon AUG in prokaryotes encodes the N-formyl methionine tRNA, which is a modified initiator tRNA. In contrast, eukaryotes utilise the initiation codon AUG to encode unformylated methionine Met-tRNA. In eukaryotes, ribosomes attach to the 5' cap and travel down eukaryotic mRNA to the first AUG, the methionine codon.

- The Shine-Dalgarno sequence on mRNA is the ribosomal binding site located near to the initiation codon in prokaryotic cells, assists in aligning the mRNA with the ribosome by forming base pairs with the 16S rRNA. Initiation factors (IFs 1, 2, and 3) play a crucial role in the process of bacterial translation initiation. In contrast, in eukaryotes, the majority of initiation codons are detected by scanning the mRNA from its 5' end, where the recognition of its 7-methylguanosine cap takes place.
- Prokaryotic ribosomes contain three specific sites A for aminoacylation, P peptide formation and E for exit. Charged tRNAs carrying specific aminoacids bind to the A site. The ribosome catalyzes each three-site activity by shifting one codon at a time. With each step, a charged tRNA enters the complex, the polypeptide grows one amino acid, and an uncharged tRNA leaves. The energy for each amino acid provided by GTP hydrolysis.
- Peptide bond formation occurs when the amino group of the amino acid in the A-site combines with the carboxyl group of the amino acid in the C-terminal of the tRNA in the P-site. Thus, the peptide chain is relocated from the transfer RNA (tRNA) in the P-site to the tRNA in the A-site through the formation of a peptide bond connecting the peptide chain and the incoming amino acid.
- Polypeptide chain elongation involves a series of cycles that include the binding of aminoacyl tRNA, the creation of peptide bonds, and translocation. The activity of elongation factors orchestrates these cycles.
- Polypeptide chain is terminated by the release factors (RF1, RF2, and RF3) when they find the stop codons (UAA, UGA and UAG) along the mRNA. The function of many initiation, elongation, and release factors relies on the binding and hydrolysis of GTP.
- Protein synthesis inhibitors, such as tetracycline, chloramphenicol, and aminoglycosides, are a prominent group of antibacterials that are extensively utilised as antibiotic medications.

12.9 TERMINAL QUESTIONS

1. What is Translation?
2. Describe the role of ribosomes and tRNA in translation process.
3. Explain the amino acid activation process
4. Describe the initiation mechanism of prokaryotic and eukaryotic translation.

5. Enlist the role of initiation factors in prokaryotes and eukaryotes
6. Write the three steps involved during chain elongation of both prokaryotes and eukaryotes.
7. Differentiate between prokaryotic and eukaryotic translation.
8. Outline the mode of action of bacterial protein inhibitors.

12.10 ANSWERS

Self-Assessment Questions

1.
 - i) 70S ribosomal unit consists of 50S and 30S.
 - ii) **tRNA**
 - iii) The attachment of amino acid to the tRNA reaction is catalyzed by aminoacyl synthetase.
 - iii) A for (aminoacyl) site, P (peptidyl) site and an E (exit) site.
 - v) In prokaryotes, the A, P and E sites are located in large subunit 50S subunit in prokaryotes and 60S in eukaryotes.
2.
 - i) methionine , N-formylmethionine-tRNA
 - ii) AUG
 - iii) **Shine-Dalgarno** sequence
 - iv) P, A, P
 - v) EF-G and GTP
 - vi) Elongation
 - vii) A site to the P-site from P site of the ribosome
 - viii) ribosomal subunit
 - ix) The peptide bond formation is catalyzed by peptidyl transferase (16SrRNA-50S subunits)
 - x) 50S subunits
 - xi) 50S subunits
3.
 - i) T
 - ii) T
 - iii) T
 - iv) T
 - v) T
 - vi) F
 - vii) T

4. i) a
 ii) c
 iii) d
 iv) e
 v) b

5.

i. c ii. a iii. d. iv. b v. a

Terminal Questions

1. Translation is the biological mechanism by which ribosomes decode the genetic information stored in messenger RNA (mRNA) to produce proteins. Please refer to section 12.1
2. Please refer to section 12.2
3. Please refer to section 12.3
4. Please refer to subsections 12.4.1 and 12.5.1
- 5.

Initiation factors in prokaryotes and eukaryotes. Please refer to subsections 12.4.1 and 12.5.1

Factors	Function
Bacteria	
IF-1	Stabilizing the binding of the initiator tRNA (charged with formyl-methionine) to the ribosomal P site and prevent pre-mature attachment of 50S ribosomal subunits
IF-2	Recognition of the start codon (AUG) in the mRNA sequence and GTP hydrolysis
IF-3	Proper assembly of the translation initiation complex of small and large subunits (30S+50S)
Eukaryotes	
eIF1	Major role in recognition of the start codon (AUG) and ensuring proper start codon selection
eIF1A	binding of the initiator tRNA (charged with methionine) to the small ribosomal subunit (40S)

eIF2	Binds to the initiator tRNA ^{Met} and promoting the formation of the 43S pre-initiation complex
eIF3	recruitment of the 40S ribosomal subunit to the mRNA and the assembly of the 43S pre-initiation complex
eIF4B	Acts as helicase that breaks base pairing in the mRNA
eIF5	Promoting the hydrolysis of GTP bound to eIF2-GTP
eIF5B	Bring the large ribosomal subunit (60S) to the initiation complex.

6. Please refer to subsections 12.4.2 and 12.5.2
7. Please refer to section 12.6 (Table 12.1)
8. Please refer to section 12.7 (Table 12.2)