TRANSLATION IN PROKARYOTES

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The genetic information contained within the order of nucleotides in messenger RNA (mRNA) is interpreted to generate linear sequences of amino acids in the proteins. This process is known as translation.

translation. Thus, translation is the key step in the production of protein molecules, involving decoding of language from the nucleotide base sequence of an mRNA molecule and translating it to the amino acid sequence of a polypeptide chain. During this process, there are three major players, 1) a sequence of mRNA nucleotides, read as triplet codons, which specifies the order in which the amino acids are added to a growing polypeptide chain, 2) Ribosomes which serve as intracellular sites for translation, and 3) tRNA molecules which are the adaptor agents that ensure insertion of correct amino acids at each position in the polypeptide. Our discussion in this unit will mainly

In the previous unit you studied about genetic code that stores information in the form of triplet codons in DNA, and that this information is initially expressed through the process of transcription. However, the final product of gene expression, in most of the instances, is a polypeptide chain which consists of a linear series of amino acids whose sequence has been specified by the genetic code. We will first start surveying the cell's cast of characters for performing translation, and then we will examine the mechanism of each step in detail.

The cellular machinery involved in translating mRNAs into polypeptides includes five major components: 1) Ribosomes, 2) tRNA molecules, 3) *aminoacyl tRNA synthetases*, 4) mRNA molecule, and 5) Protein factors.

1) Ribosomes

- Ribosomes are macromolecular machines that direct the synthesis of proteins. Ribosomes, therefore, serve as protein factories and play crucial role in orienting the mRNA and amino acid- carrying tRNAs in such a manner that the genetic code can be read accurately. They also help in catalyzing peptide bond formation that links the amino acids into a polypeptide.

- Ribosomes are particles made of ribosomal RNA (rRNA) and ribosomal protein and are present in the cytoplasm. The ribosomes of prokaryotes (archaea and bacteria) are smaller than those of eukaryotes, although many of the ribosomal proteins, translation factors, and tRNAs used by archaea resemble their eukaryotic counterparts more closely than do the comparable components of bacteria.

- Ribosomes are made up of two dissociable subunits called the large and small subunits. The bacterial ribosome has a sedimentation coefficient of about 70S and consists of a 30S small subunit and a 50S large subunit. The larger ribosomal subunit of prokaryotes consists of 23S rRNA, and a 5S rRNA molecule along with 34 proteins. On the other hand, the smaller subunit has a 16S rRNA component and 21 proteins (see Figure 13.1).

- The rRNA component of ribosome performs all the important catalytic functions associated with translation while ribosomal proteins are thought to promote binding of various molecules involved in translation and, in general, to fine-tune the process.

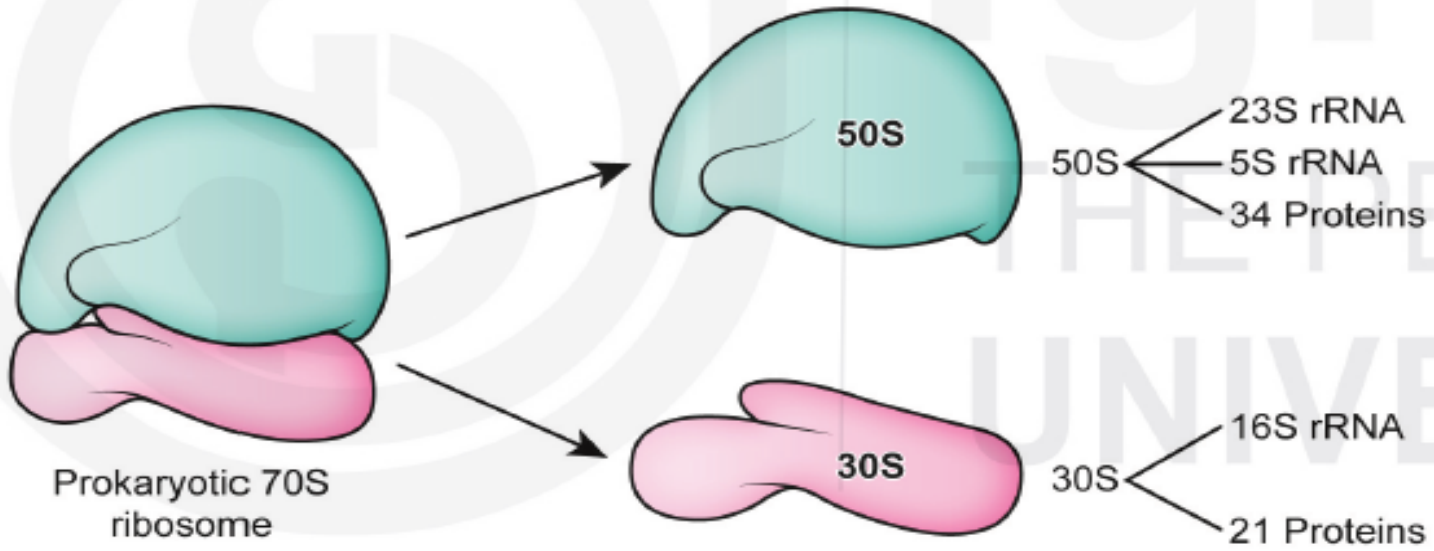
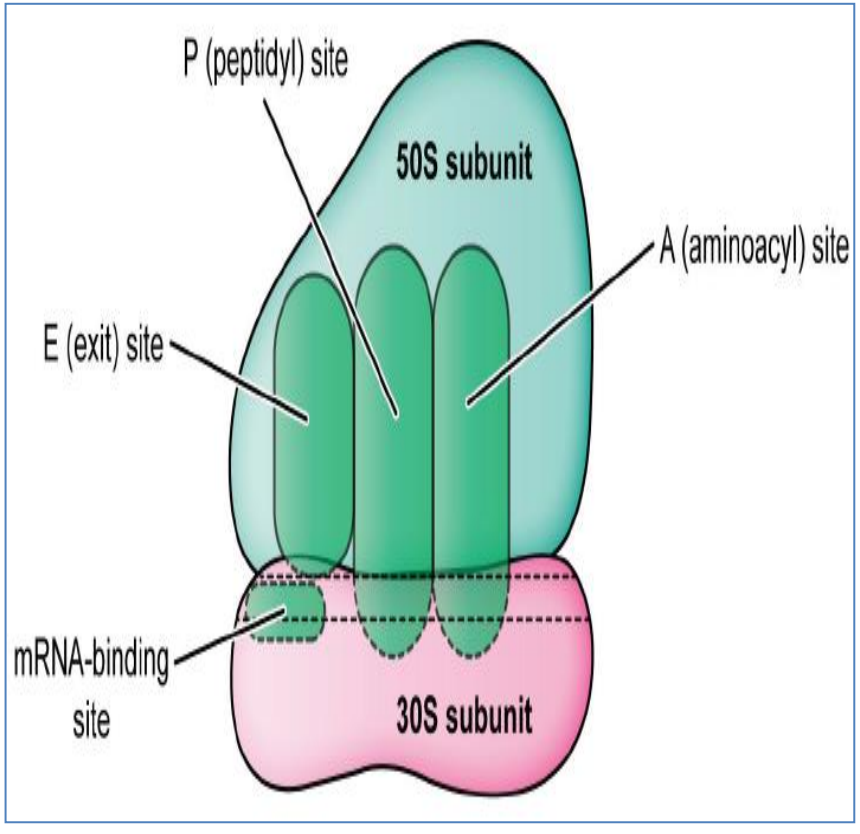


Fig. 13.1: Components of prokaryotic ribosome showing large and small subunits which contain both ribosomal proteins and rRNAs.

- Ribosomes have four important sites which are particularly important for protein synthesis (see Figure 13.2). In addition to an **mRNA binding site** there are three sites where tRNA can bind. The three tRNA binding sites are: an **A (aminoacyl) site,** that binds each newly arriving tRNA with its attached amino acid, **a P (peptidyl) site,** where the tRNA carrying the growing/elongating polypeptide chain resides, and an **E (exit) site**, from where tRNA , after discharging its amino acids, exits the ribosomes.



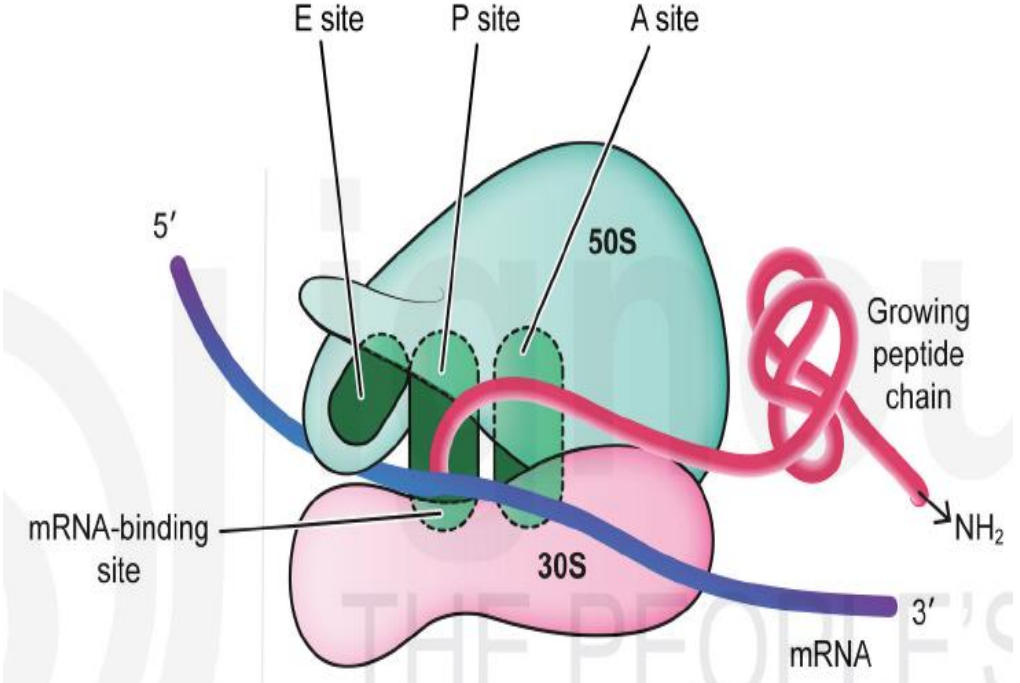


Fig. 13.2: Binding sites on a ribosome showing A (aminoacyl) site, P (peptidyl) site and an E (exit) site. In addition, there is an mRNA- binding site which binds a specific nucleotide sequence near the 5′ end of mRNA.

2. tRNA molecules

You have already studied the structure and function of tRNAs in previous unit 12. Here you are going to learn how tRNA molecules serve as adaptors, enabling sequence of codons in mRNA to ultimately determine the amino acid sequence of polypeptide chain.

- Francis Crick, postulated in early 1957 that as the amino acids cannot directly recognize nucleotide base sequences, a hypothetical ''adapter'' must be present that can mediate between amino acids and mRNA. In the year following Crick's adaptor hypothesis, Mahlon Hogland, while investigating protein synthesis in cell free system, discovered a family of adaptor molecules that were named as transfer RNAs (tRNAs).

- Transfer RNA plays a vital role as an intermediary between mRNA and amino acids. Each tRNA has two levels of specificity. On one hand, and as specified by genetic code, each tRNA binds to one specific amino acid. On the other hand, each tRNA recognizes one or more mRNA codons specifying that amino acid.

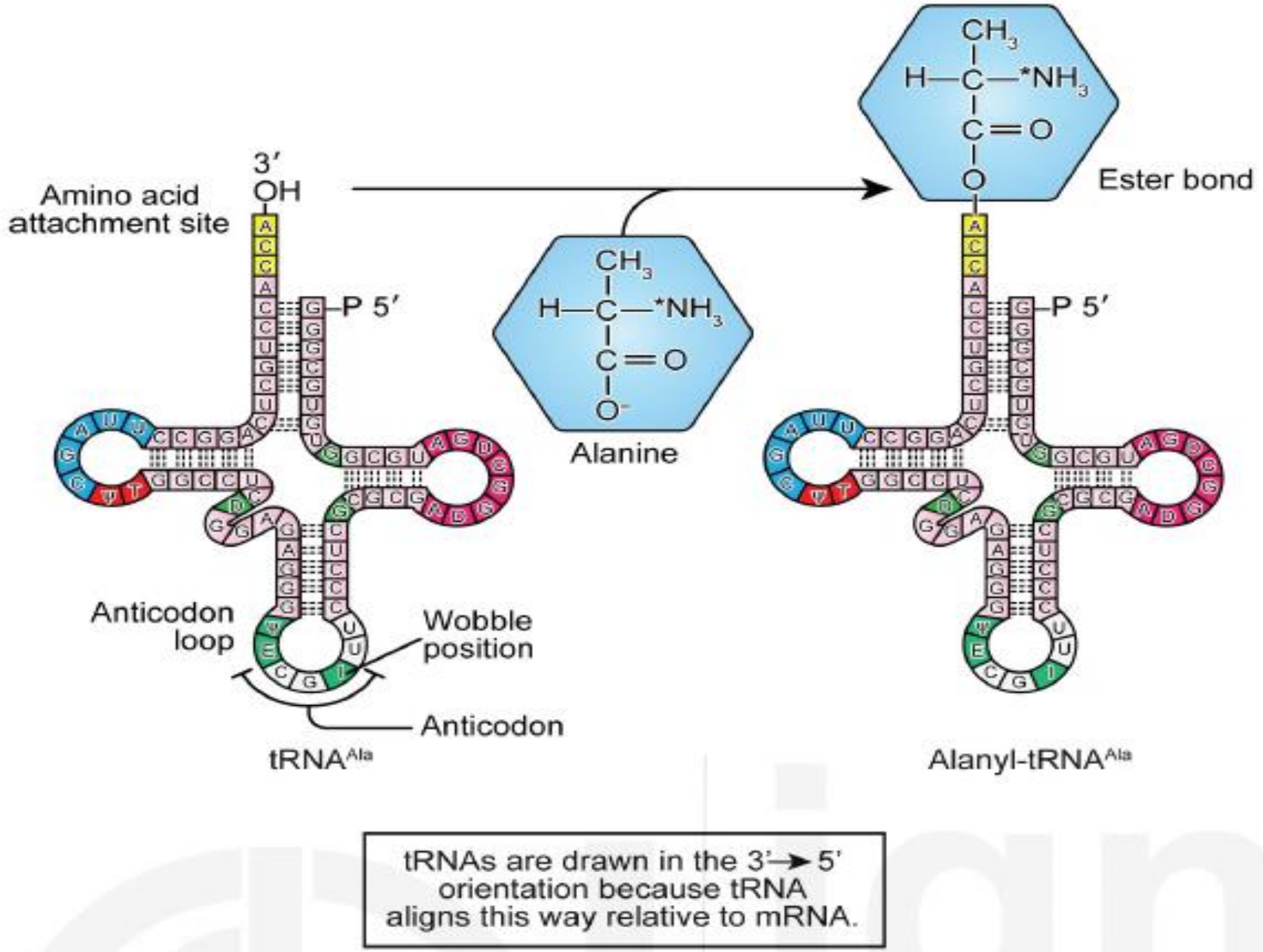


Fig. 13.3: Secondary structure of tRNA, before and after amino acid attachment.

- The tRNAs are linked to their corresponding amino acids by an ester bond. This bond joins an amino acid to the 2' - or 3´ – hydroxyl group of the adenine (A) nucleotide located at 3´ end of all tRNA molecules (see Figure 13.3). The enzymes that catalyze the formation of ester bond also assist in choosing the correct amino acid for each tRNA.
- The name of the amino acid that attaches to given tRNA is indicated by superscript. For example, tRNA molecules specific for amino acid alanine are designated as $tRNA^{Ala}$. After attachment, the tRNA is now called an **aminoacyl tRNA** (e.g., alanyl $tRNA^{Ala}$). The tRNA is now said to be in its charged form, and the amino acid is said to be activated.
- Each tRNA possesses an **anticodon**, which is a special trinucleotide sequence located within one of the loops of the tRNA molecule. The tRNA molecules can recognize codons in mRNA .The anticodon of each tRNA are complementary to one or more mRNA codons that specify the amino acid being carried by that tRNA. The codons in mRNA are represented in the 5´ → 3´ direction, whereas anticodons in tRNA are usually written in the 3´ → 5´ orientation. Thus, if one of the codons for alanine is 5´ –GCC -3´, the corresponding anticodon in tRNA is 3´ – CGG – 5´.

5´ G G C 3´ **mRNA** Codon

tRNA Anticodon 3´ C C G 5´

3. *Aminoacyl-tRNA Synthetases*

- *Aminoacyl-tRNA synthetases* are the enzymes responsible for linking amino acids to their corresponding tRNAs. Cells normally have 20 different *aminoacyl-tRNA synthetases*, one for each 20 amino acids commonly used in protein synthesis.

- Number of *aminoacyl-tRNA synthetases* in some cells may be less than 20. In such cases, the same *aminoacyl-tRNA synthetase* may catalyze the attachment of two different amino acids to their corresponding tRNAs, or it may attach an incorrect amino acid to a tRNA molecule. These latter 'errors' are corrected by a second enzyme that alters the incorrect amino acid after it has been attached to the tRNA.

- *Aminoacyl-tRNA synthetases* catalyze the attachment of amino acids to their corresponding tRNA through an ester bond. This is accompanied by the hydrolysis of ATP to AMP and pyrophosphate (Figure 13.4).

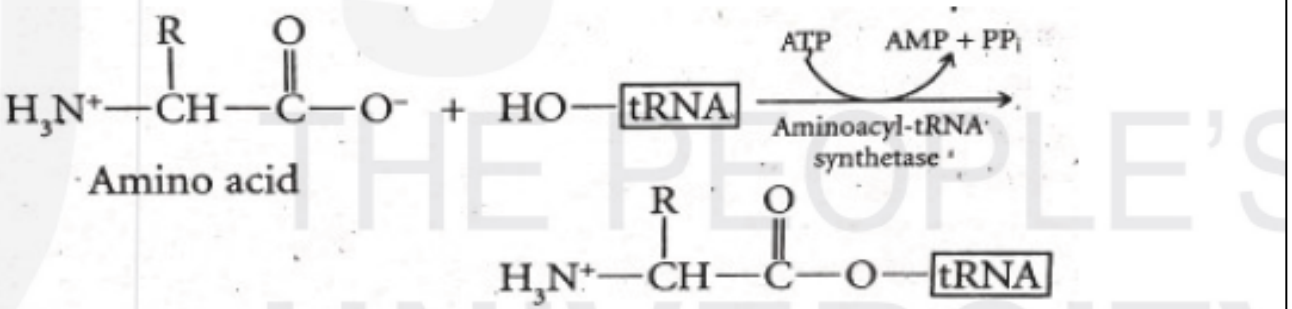


Fig. 13.4: Attachment of amino acid to tRNA via ester bond catalyzed by *Aminoacyl-tRNA synthetase*.

This enzyme catalyzes the formation of an ester bond between the carboxyl group of an amino acid and 3´ OH of the appropriate tRNA, thereby generating an aminoacyl tRNA. This ester bond is thought to be a '' high energy bond'' as the hydrolysis of this bond releases sufficient energy to drive the formation of the peptide bond that will eventually join the amino acids to a growing polypeptide chain. This process of aminoacylation of tRNA is called amino acid activation (see Figure 13.5).

The *aminoacyl-tRNA synthetases* recognize nucleotides located at least two different regions of tRNA molecules where they identify and pick up the correct tRNA that is to become linked to a particular amino acid. Changes in the base sequence of either the anticodon template or the 3´ end of a tRNA molecule will result in a change in the amino acid that will get attached to a tRNA. The *aminoacyl-tRNA synthetases* also perform proofreading function to ensure that the correct amino acid has been incorporated. A site on the *aminoacyl-tRNA synthetase* molecule recognizes incorrect amino acids and releases them by hydrolyzing the bond that links the amino acid to the tRNA. The appropriate codon in mRNA is recognized by tRNA itself once the correct amino acid has

been joined to its tRNA. The specificity of the *aminoacyl-tRNA synthetase* reaction is crucial to the accuracy of gene expression because it ensures that the proper amino acid is linked to each tRNA.

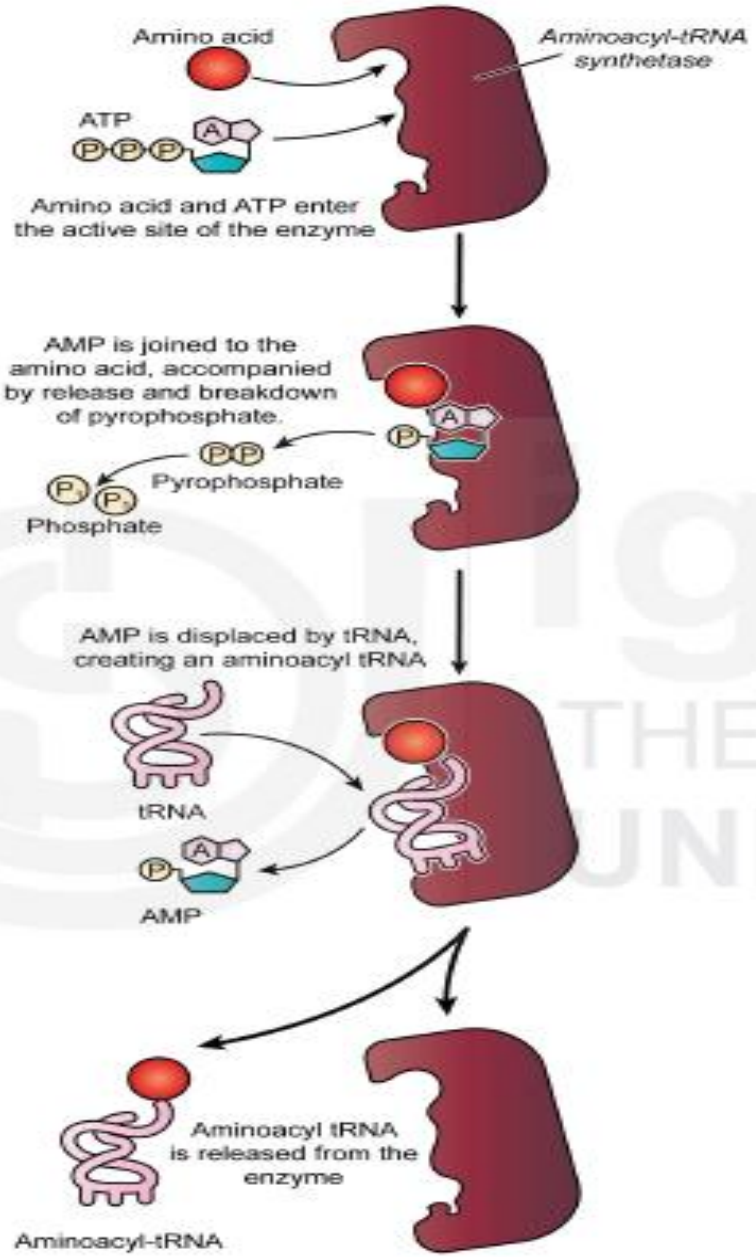


Fig. 13.5: Amino acid activation by *Aminoacyl-tRNA synthetase*.

4. mRNA template

The genetic information is encoded onto mRNA which acts as a template for polypeptide synthesis. Prokaryotes have polycistronic mRNAs with multiple translation start sites (Fig.13.6).

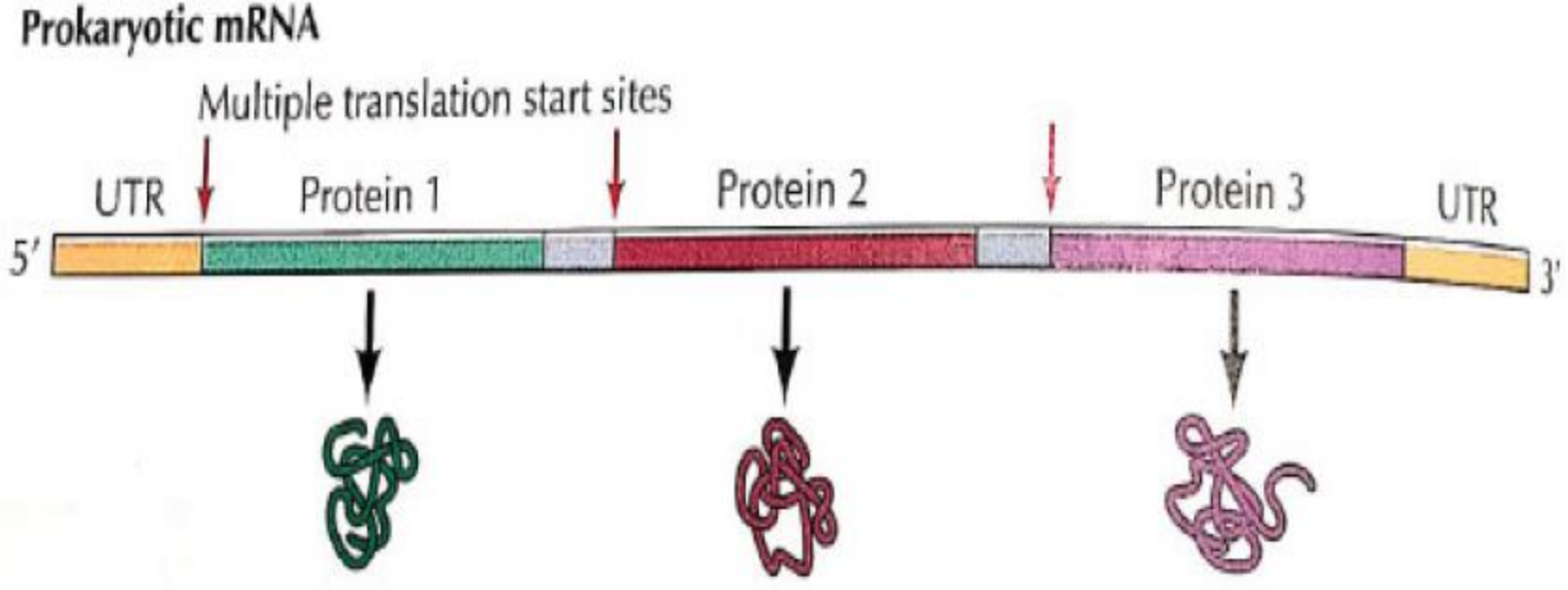


Fig. 13.6: A portion of prokaryotic polycistronic mRNAs encodes multiple proteins, each of which is translated from an independent start point.

5. Protein factors

In addition to *aminoacyl-tRNA synthetases* and protein components of ribosome, the process of translation requires participation of several other kinds of protein molecules. These protein factors participate in the initiation, elongation, and termination of polypeptide chain. The precise role played by these factors will now be discussed in the mechanism of each of the events occurring during translation.

Process of Translation

Translation of mRNA leads to the synthesis of polypeptides in the N-terminal to C-terminal direction. The complete translation process is subdivided into three stages; i) **Initiation stage**, where mRNA gets bound to the ribosome and positions itself for proper translation, ii) **Elongation stage**, in which amino acids are sequentially joined together through peptide bonds according to the arrangement of codons in mRNA, and iii) **Termination stage**, where both mRNA and the newly formed polypeptide chains are released from the ribosome.

An overview of translation has been shown in Figure 13.7.

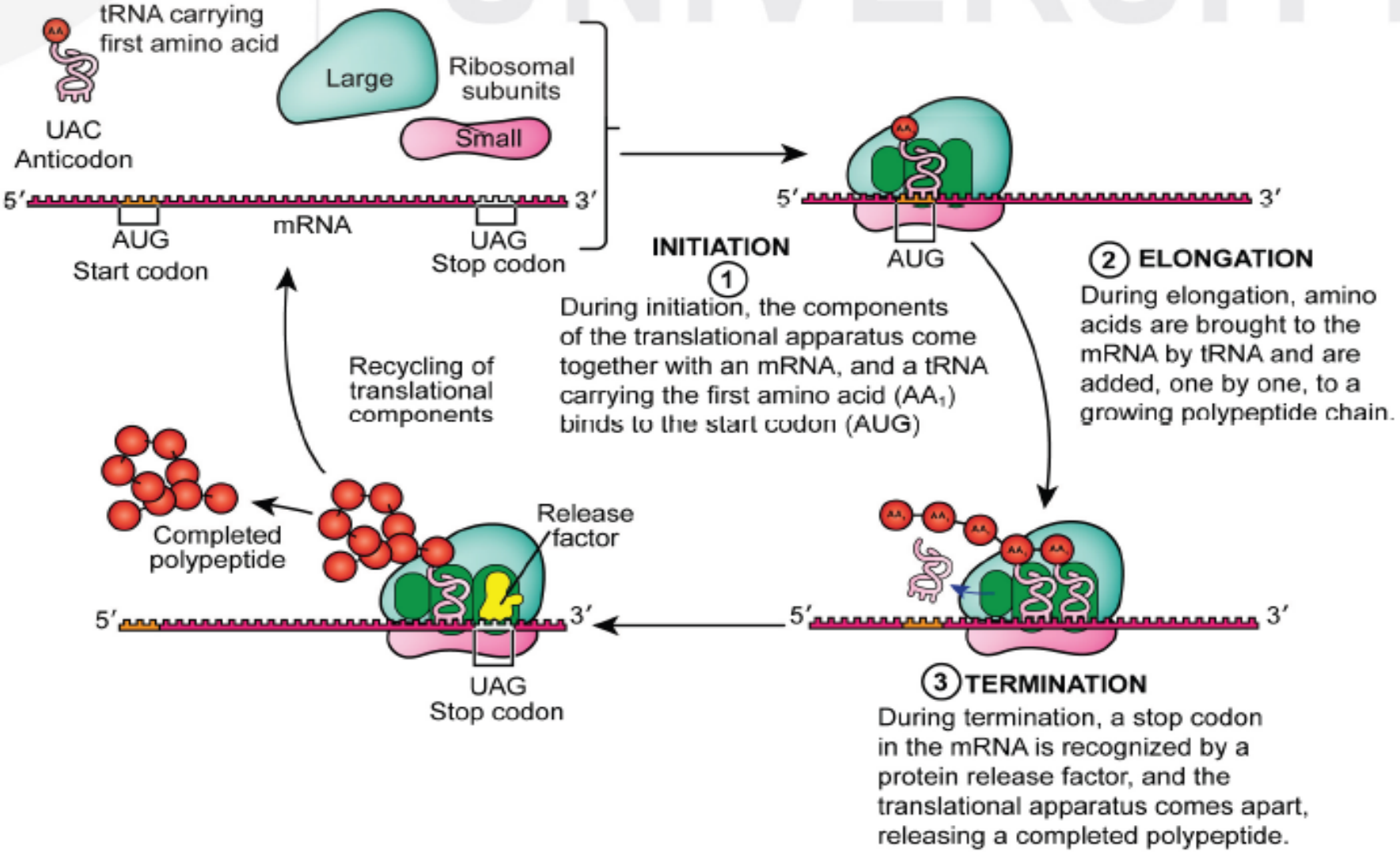


Fig. 13.7: An overview of translation showing three stages: Initiation, Elongation, and Termination.

Initiation of Translation

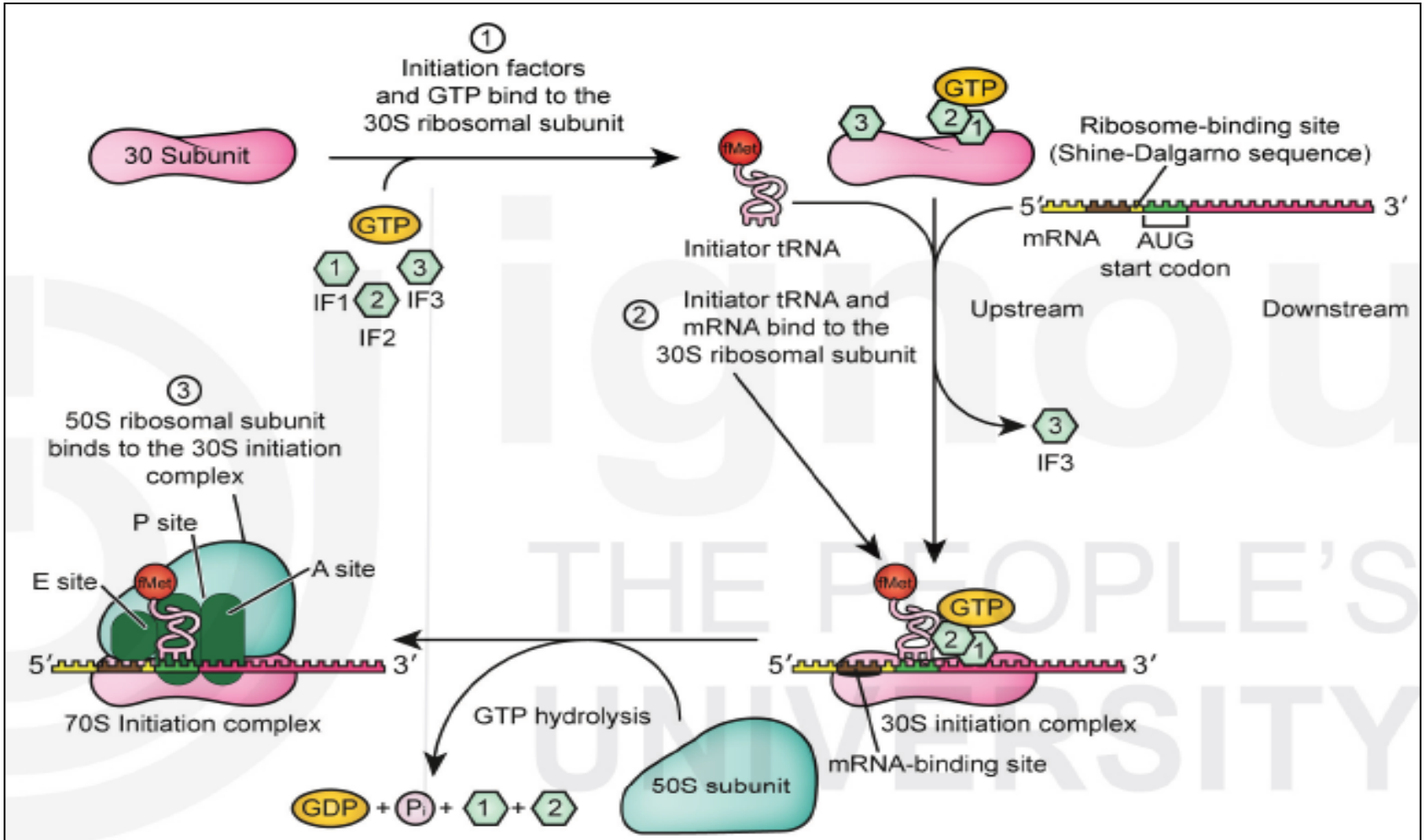


Fig.13.9: Steps involved in Initiation of translation in bacteria. Assembly of 70S initiation complex occurs in three steps. (1) Three initiation factors (IF1, IF2 and IF3) plus GTP bind to the small ribosomal subunit. (2) The initiator aminoacyl tRNA and mRNA are attached. (3) The large ribosomal subunit joins the complex. The resulting 70S initiation complex has fMet-tRNA^{fMet} residing in the ribosome's P site.

The functional ribosome of bacteria exists as two subunits, the small 30S subunit and large 50S subunit. An mRNA molecule can bind to the small ribosome subunit only when the subunits are separate. The initiation process of translation in bacteria can be subdivided into three distinct steps:

Step 1: The three initiation factors *IF1, IF2* and *IF3* first bind to the small (30S) ribosomal subunit, with GTP attaching to *IF2*. The presence of IF3 at this early stage prevents the 30S subunit from prematurely associating with the 50S subunit. The sequences on mRNA necessary for ribosome binding have been identified. The sequence covered by ribosome during initiation is from 30 to 40 nucleotides long and includes the AUG initiation codon. Within the ribosome binding site, there exists a special nucleotide sequence called the **Shine–Dalgarno consensus sequence** (Fig.13.8) .This consensus sequence AGGAGG is located seven nucleotides upstream to the initiation codon AUG and is complementary to a sequence of nucleotides UCCUCC located at the 3´ end of 16S rRNA (part of 30S subunit of ribosome).The nucleotides of Shine-Dalgarno sequence pair with their complementary nucleotides in the 16S rRNA during initiation.

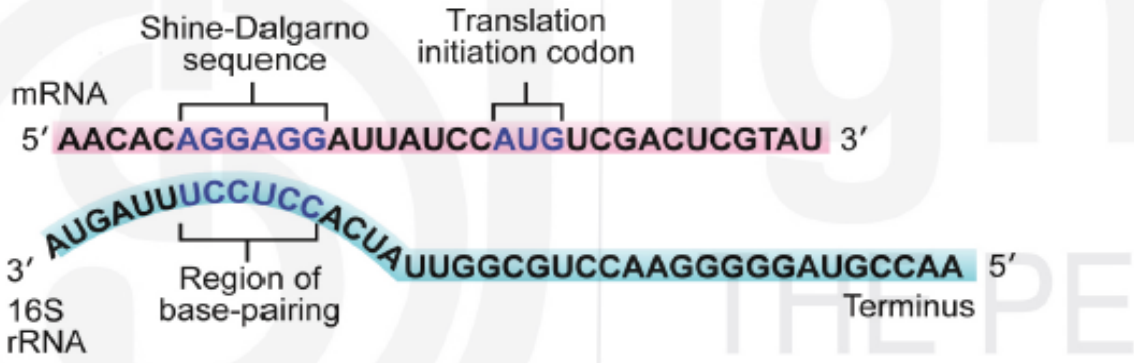


Fig. 13.8: Shine-Dalgarno consensus sequence in mRNA required for attachment of small subunit of ribosome.

Step 2: The binding of mRNA to the mRNA binding site of the small ribosomal subunit places the mRNA's AUG start codon at the ribosome's P site, where it can bind to the anticodon of the appropriate tRNA carrying the first amino acid It was discovered that methionine is the first amino acid, and the bacterial cells contain two different methionine-specific tRNAs. One, designated $tRNA^{Met}$ carries a normal methionine destined for insertion into the internal regions of polypeptide chain. The other, called $tRNA^{fMet}$, carries a methionine that is converted to the derivative *N-formyl methionine* (*fMet*) after linkage to the tRNA. In *N-formyl methionine*, the amino group of methionine is blocked by addition of a formyl group and so cannot form a peptide bond with another amino acid and only the carboxyl group is available for bonding to another amino acid. Hence N-formyl methionine can be situated only at the N-terminal end of polypeptide chain- suggesting that $tRNA^{fMet}$ functions as an **initiator tRNA** which starts the process of translation.

During initiation, the initiator tRNA with its attached N-formyl methionine is bound to the P site of the 30S ribosomal subunit by the action of initiation factor IF2 which forms a complex with GTP, which can distinguish initiator

$tRNA^{fMet}$ from other kind of tRNA. This quality of IF2 helps us to explain as to why AUG start codons bind to the initiator $tRNA^{fMet}$, whereas AUG codons located elsewhere in mRNA bind to the non-initiation $tRNA^{fMet}$. Once the $tRNA^{fMet}$ enters the P site, its anticodon becomes base-paired with the AUG start codon in the mRNA, and IF3 is released. At this point the 30S subunit with its associated IF1, IF2-GTP, mRNA and N-formyl methionyl $tRNA^{fMet}$ is referred to as the **30S initiation complex**.

Step 3: The 30S initiation complex can now bind to a free 50S ribosomal subunit once IF3 has been released, generating the **70S initiation complex**. The 50S subunit then promotes hydrolysis of the IF-bound GTP, leading to the release of IF2 and IF1. At this stage all the three initiation factors have been released (Fig. 13.9).

Chain Elongation

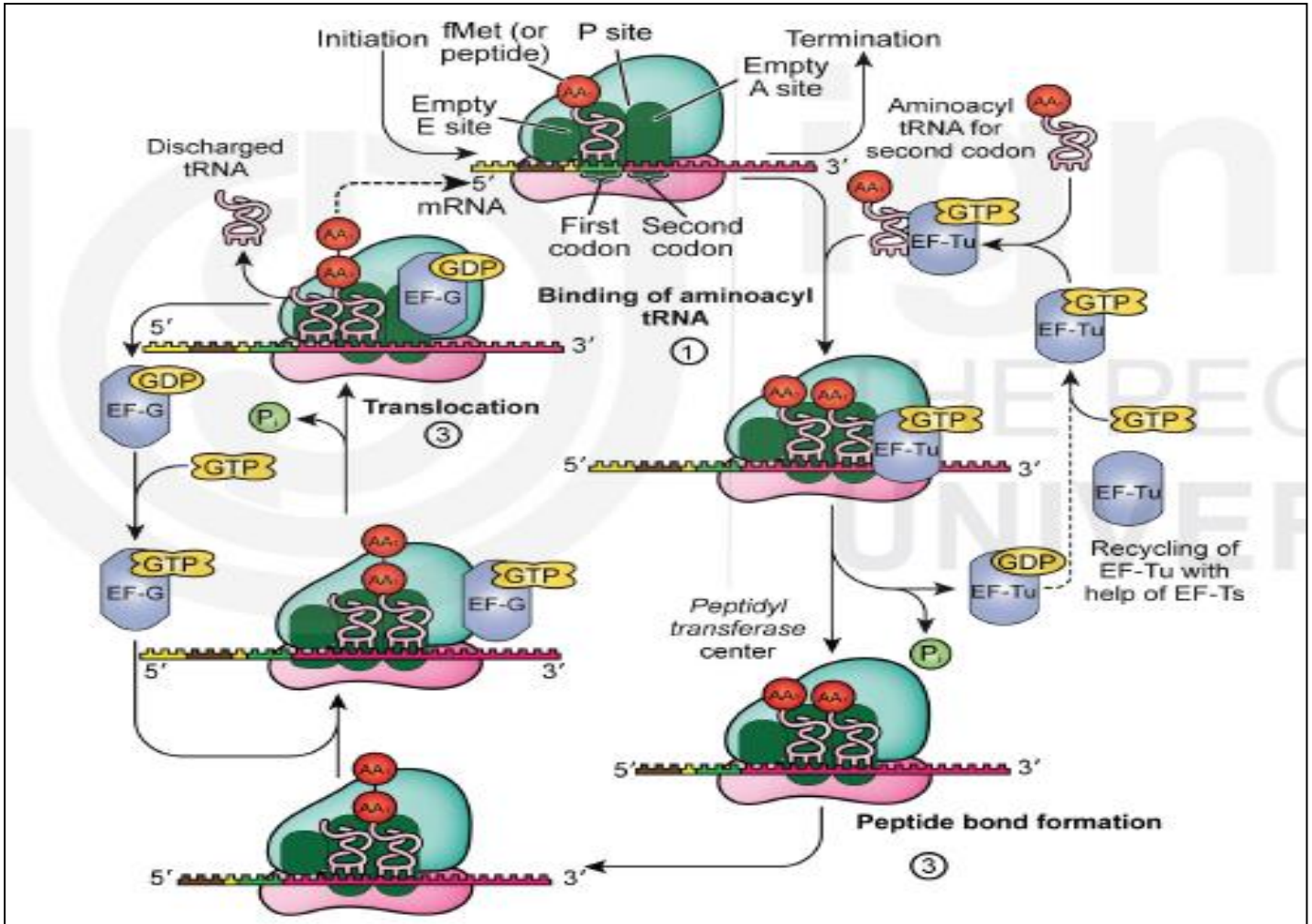


Fig. 13.10: Three stages of Polypeptide Chain Elongation in Bacteria: 1. An aminoacyl tRNA binds to the A site with the help of GTP-bound EF-Tu. During binding of tRNA, GTP gets hydrolyzed and EF-Tu is released. The recycling is supported by EF-Tu. 2. A peptide bond is formed at the P site between the –COOH group of fMet (and COOH of terminal amino acid in later cycles) and the newly arrived amino acid at the A-site. 3. The mRNA advances by three nucleotides. The peptidyl tRNA moves from the A site to the P site. Also the empty tRNA moves from the P site to the E site. During the process, GTP bound to EF-G gets hydrolysed.

At the onset of elongation stage, the AUG start codon in the mRNA is located at the ribosomal P site and the second codon(the codon immediately downstream of the start codon) is located at the A site. Elongation takes place in three steps. In the first step an aminoacyl tRNA (a charged tRNA) whose anticodon is complementary to the second codon binds to the ribosomal A site. The binding of this new aminoacyl tRNA to the codon in the A site requires two protein **elongation factors**, EF-Tu and EF-Ts, and is driven by hydrolysis of GTP. From now on, every incoming aminoacyl tRNA will bind first to the A (aminoacyl) site-hence the site's name.

The EF-Tu-GTP complex promotes the binding of all aminoacyl tRNAs to the ribosome except the initiator tRNA, thus ensuring that AUG codons located downstream from the start codon do not mistakenly bind an initiator tRNA to the ribosome. As the aminoacyl tRNA gets transferred to the ribosome, the GTP is hydrolyzed and the EF-Tu-GTP complex is released. The role of EF-Ts is to generate EF-Tu-GTP from EF-Tu-GDP for the next round of elongation cycle.

After the appropriate amino acyl tRNA has been bound to the ribosomal A site, the second step of elongation is formation of peptide bond between the amino group of amino acid bound at A site and the carboxyl group that links the initiating amino acid (or growing polypeptide chain) to the tRNA at P site. The source of energy during peptide bond formation is provided by cleavage of high energy bond (Box 13.1). The formation of this peptide bond causes the growing polypeptide chain to be transferred from the tRNA located at P site to the tRNA located at A site (Fig. 13.10).

The third step in elongation is translocation. After a peptide bond has been formed, the P site is occupied by an empty tRNA while the A site contains a peptidyl tRNA (the tRNA to which the growing polypeptide chain is attached). The movement of the ribosome down the mRNA in the 5′ → 3′ direction is called translocation. This step positions the ribosome over the next codon and requires elongation factor G (EF-G) and the hydrolysis of GTP to GDP. Because the tRNAs in the P and A sites are still attached to the mRNA through codon-anticodon pairing, they do not move with the ribosome as it translocates further. Consequently, the ribosome shifts so that the tRNA that previously occupied the P site now occupies the E site (exit site), from which it moves into the cytoplasm where it can be recharged with another amino acid. Translocation also causes the tRNA that occupied the A site (which is attached to the growing polypeptide chain) now to be relocated in the P site, leaving the A site open. Thus, the progress of each tRNA through the ribosome during elongation can be summarized as : cytoplasm → A site → P site → E site → cytoplasm.

The net effect of translocation is to bring the next mRNA codon into the S site, so the ribosome is now set to receive the next aminoacyl tRNA and repeat the elongation cycle. The only difference between the succeeding elongation cycles and the first elongation cycle is that an initiator tRNA occupies the P site at the beginning of the first elongation cycle, and the peptidyl tRNA occupies the P site at the beginning of all subsequent cycles. As each successive amino acid is added, the mRNA is progressively read in the 5′ → 3′ direction. The amino terminal of the growing polypeptide passes out of the ribosome through an exit tunnel in the 50S subunit. Immediately after its exit the polypeptide chain is folded and modified into its proper three-dimensional shape. Polypeptide synthesis is a very rapid process and a polypeptide of 400 amino acids can be made within 10 seconds in a growing *E. coli* cell.

Termination of Polypeptide Synthesis

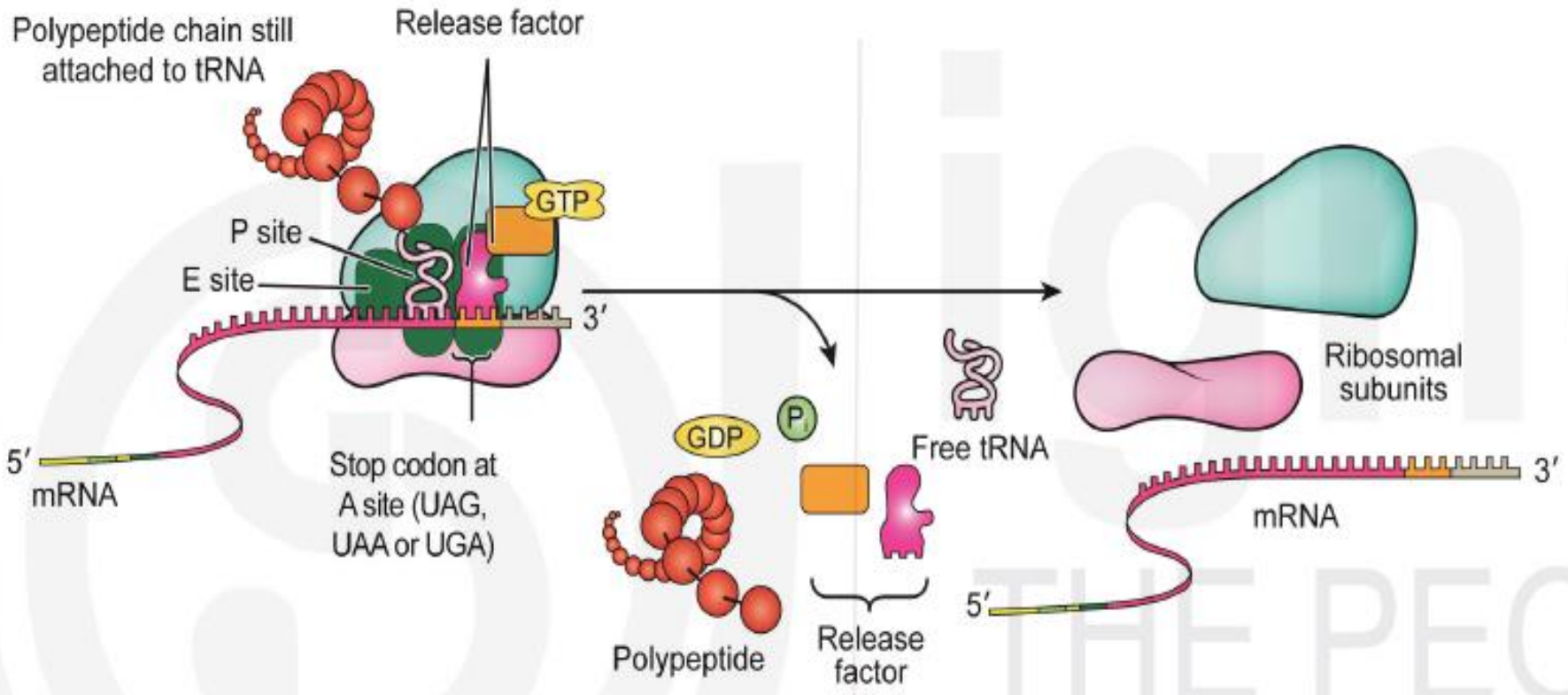


Fig. 13.11: A schematic diagram showing the mechanism of termination of translation: when a stop codon – UAA, UAG or UGA arrives at A site, it is recognized and bound by protein release factors associated with GTP. Hydrolysis of GTP is accompanied by release of the completed polypeptide, followed by dissociation of the tRNA, mRNA, ribosomal subunits, and release factors.

The elongation process continues, reading one codon after another and adding successive amino acids in polypeptide chain, until any one of the three chain-termination (stop) codons (UAG, UAA, or UGA) in the mRNA arrives and enters the ribosome's A site. Since there are no tRNAs with anticodons complementary to the termination codon, no tRNA enters the A site of the ribosome when a termination codon is encountered. Instead, the stop codons are recognized by proteins called **release factors** which possess special regions ('peptide anticodons') that bind to mRNA stop codons present at ribosomal A site. *E. coli* has three release factors – RF_1, RF_2 and RF_3. Release factor1 recognizes and binds to the termination codons UAA and UAG, while RF_2 binds to UGA and UAA. The binding of RF_1 or RF_2 to the A site of the ribosome promotes the cleavage of the tRNA in the P site from the polypeptide chain and the release of polypeptide. The release factor 3 binds to the ribosome and forms a complex with GTP. This binding brings about conformational change in the ribosome, releasing RF1 or RF2 from the A site and causing the tRNA in the P site to move to the E site. In this process GTP is hydrolyzed to GDP (Figure 13.11). Additional factors help bring about the release of the tRNA from the P site, the release of the mRNA from the ribosome, and the dissociation of the ribosome