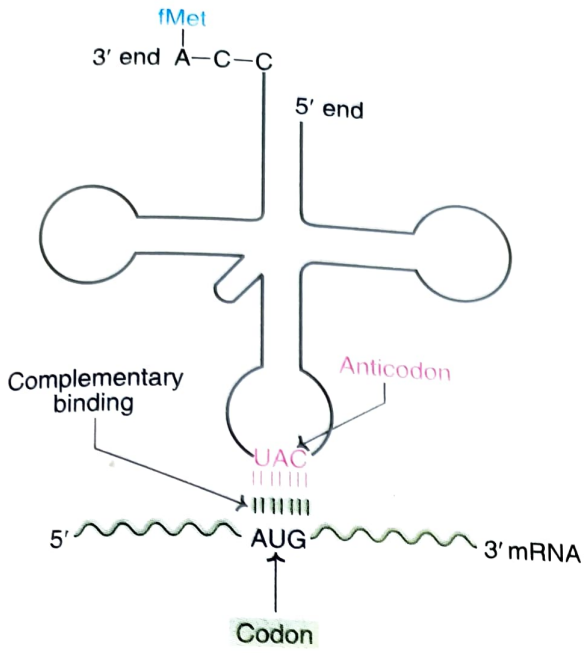

***Fig.25.10** : Complementary binding of codon (of mRNA) and anticodon (of tRNA).*

other in antiparallel direction (5′ → 3′ of mRNA with 3′ → 5′ of tRNA). The usual conventional complementary base pairing (A=U, C≡G) occurs between the first two bases of codon and the last two bases of anticodon. The third base of the codon is rather lenient or flexible with regard to the complementary base.

Wobble hypothesis

Wobble hypothesis, put forth by Crick, is the phenomenon in which a ***single tRNA can recognize more than one codon.*** This is due to the fact that the third base (3′-base) in the codon often fails to recognize the specific complementary base in the anticodon (5′-base). Wobbling is attributed to the difference in the spatial arrangement of the 5′-end of the anticodon. The possible pairing of 5′-end base of anticodon (of tRNA) with the 3′-end base of codon (mRNA) is given

Anticodon		Codon	
C	—	G	} Conventional base pairing
A	—	U	
U	—	G or A	} Non-conventional base (coloured) pairing
G	—	U or C	

Wobble hypothesis explains the degeneracy of the genetic code, i.e. existence of multiple codons for a single amino acid. Although there are 61 codons for amino acids, the number of tRNAs is far less (around 40) which is due to wobbling.

PROTEIN BIOSYNTHESIS

The ***protein synthesis*** which involves the translation of nucleotide base sequence of mRNA into the language of amino acid sequence may be divided into the ***following stages*** for the convenience of understanding.

I. Requirement of the components
II. Activation of amino acids
III. Protein synthesis proper
IV. Chaperones and protein folding
V. Post-translational modifications.

I. REQUIREMENT OF THE COMPONENTS

The protein synthesis may be considered as a biochemical factory operating on the ribosomes. As a factory is dependent on the supply of raw materials to give a final product, the protein synthesis also requires many components.

1. **Amino acids :** Proteins are polymers of amino acids. Of the ***20 amino acids*** found ***in protein structure***, half of them (10) can be synthesized by man. About ***10 essential amino acids*** have to be provided through the diet. Protein synthesis can occur only when all the amino acids needed for a particular protein are available.

As regards prokaryotes, there is no requirement of amino acids, since all the 20 are synthesized from the inorganic components.

2. **Ribosomes :** The functionally active ribosomes are the ***centres*** or ***factories for protein synthesis***. Ribosomes may also be considered as workbenches of translation. Ribosomes are huge complex structures (70S for prokaryotes and 80S for eukaryotes) of proteins and ribosomal RNAs. Each ribosome consists of two subunits—one big and one small. The functional ribosome has two sites—A site and P site. Each site covers both the subunits. ***A site*** is for binding of aminoacyl tRNA and ***P site*** is for binding peptidyl tRNA, during the course of translation. Some authors consider A site as acceptor site, and P site as donor site. In case of eukaryotes, there is another site called ***exit site*** or ***E site***. Thus,

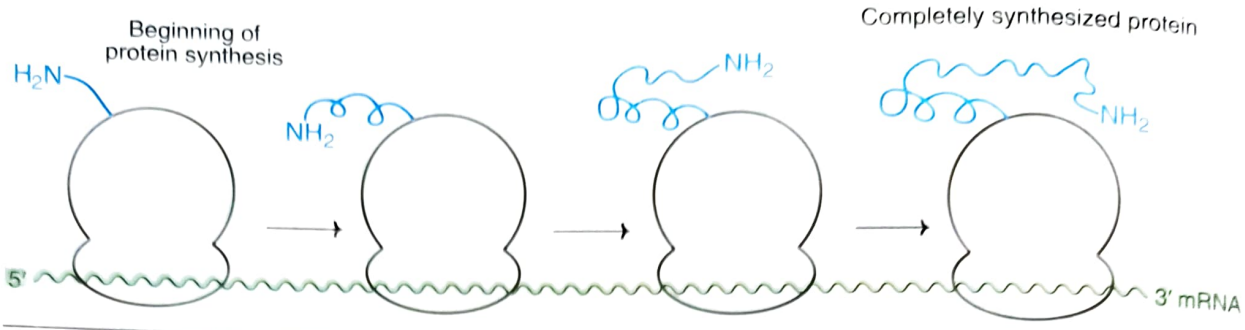

***Fig.25.11** : A polyribosome in protein synthesis.*

eukaryotes contain three sites (A, P and E) on the ribosomes.

The ribosomes are located in the cytosomal fraction of the cell. They are found in association with rough endoplasmic reticulum (RER) to form clusters RER—ribosomes, where the protein synthesis occurs. The term ***polyribosome*** (polysome) is used when several ribosomes simultaneously translate on a single mRNA (***Fig.25.11***).

3. **Messenger RNA (mRNA) :** The specific information required for the synthesis of a given protein is present on the mRNA. The DNA has passed on the genetic information in the form of ***codons*** to mRNA to translate into a protein sequence.

4. **Transfer RNAs (tRNAs) :** They carry the amino acids, and hand them over to the growing peptide chain. The amino acid is covalently bound to tRNA at the 3′-end. Each tRNA has a three nucleotide base sequence—the ***anticodon***, which is responsible to recognize the codon (complementary bases) of mRNA for protein synthesis.

In man, there are about 50 different tRNAs whereas in bacteria around 40 tRNAs are found. Some amino acids (particularly those with multiple codons) have more than one tRNA.

5. **Energy sources :** Both ***ATP*** and ***GTP*** are required for the supply of energy in protein synthesis.

6. **Protein factors :** The process of translation involves a number of protein factors. These are needed for initiation, elongation and termination of protein synthesis. The protein factors are more complex in eukaryotes compared to prokaryotes.

II. ACTIVATION OF AMINO ACIDS

Amino acids are activated and attached to tRNAs in a two step reaction. A group of enzymes—namely aminoacyl tRNA synthetases—are required for this process. These enzymes are highly specific for the amino acid and the corresponding tRNA.

The amino acid is first attached to the enzyme utilizing ATP to form enzyme-AMP-amino acid complex. The amino acid is then transferred to the 3′ end of the tRNA to form aminoacyl tRNA (***Fig.25.12***).

III. PROTEIN SYNTHESIS PROPER

The protein or polypeptide synthesis occurs on the ribosomes (rather polyribosomes). The ***mRNA is read in the 5′ → 3′ direction and the polypeptide synthesis proceeds from N-terminal end to C-terminal end.*** Translation is directional and collinear with mRNA.

The prokaryotic mRNAs are ***polycistronic,*** since a single mRNA has many coding regions that code for different polypeptides. In contrast, eukaryotic mRNA is ***monocistronic,*** since it codes for a single polypeptide.

In case of prokaryotes, translation commences before the transcription of the gene is completed. Thus, simultaneous transcription and translation are possible. This is not so in case of eukaryotic organisms since transcription occurs in the nucleus whereas translation takes place in the cytosol. Further, the primary transcript (hnRNA) formed from DNA has to undergo several modifications to generate functional mRNA.

Protein synthesis is comparatively simple in case of prokaryotes compared to eukaryotes. Further, many steps in eukaryotic translation were not understood for quite sometime. For these reasons, majority of the textbooks earlier used to describe translation in prokaryotes in detail, and give most

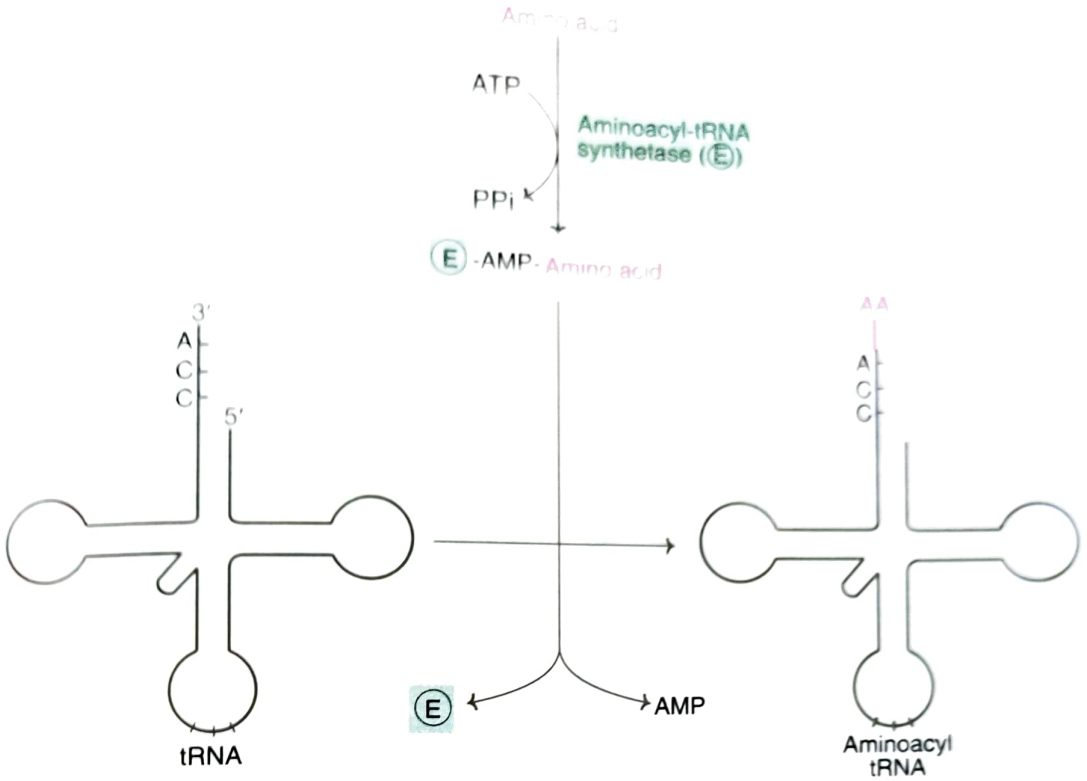

Fig.25.12 : *Formation of aminocacyl tRNA (AA–Amino acid; E–Enzyme).*

important and relevant information for eukaryotic translation. With the advances in molecular biology, the process of protein biosynthesis in eukaryotes is better understood now.

Translation in eukaryotes is briefly ***described here***, along with some relevant features of prokaryotic protein biosynthesis. Translation proper is divided into three stages—initiation, elongation and termination (as it is done for transcription).

INITIATION OF TRANSLATION

The initiation of translation ***in eukaryotes*** is complex, involving at least ***ten eukaryotic initiation factors (eIFs)***. Some of the eIFs contain multiple (3-8) subunits. The process of translation initiation can be divided into four steps (***Fig.25.13***).

1. Ribosomal dissociation.
2. Formation of 43S preinitiation complex.
3. Formation of 48S initiation complex.
4. Formation of 80S initiation complex.

Ribosomal dissociation

The 80S ribosome dissociates to form 40S and 60S subunits. Two initiating factors namely eIF-3 and eIF-1A bind to the newly formed 40S subunit, and thereby block its reassociation with 60S subunit. For this reason, some workers name eIF-3 as ***anti-association factor***.

Formation of 43S preinitiation complex

A ternary complex containing met-tRNA$_i$ and eIF-2 bound to GTP attaches to 40S ribosomal subunit to form 43S preinitiation complex. The presence of eIF-3 and eIF-1A stabilizes this complex (***Note :*** Met-tRNA is specifically involved in binding to the initiation condon AUGs; hence the superscript$_i$ is used in met-tRNA$_i$).

Formation of 48S initiation complex

The binding of mRNA to 43S preinitiation complex results in the formation of 48S initiation complex through the intermediate 43S initiation complex. This, however, involves certain interactions between some of the eIFs and activation of mRNA.

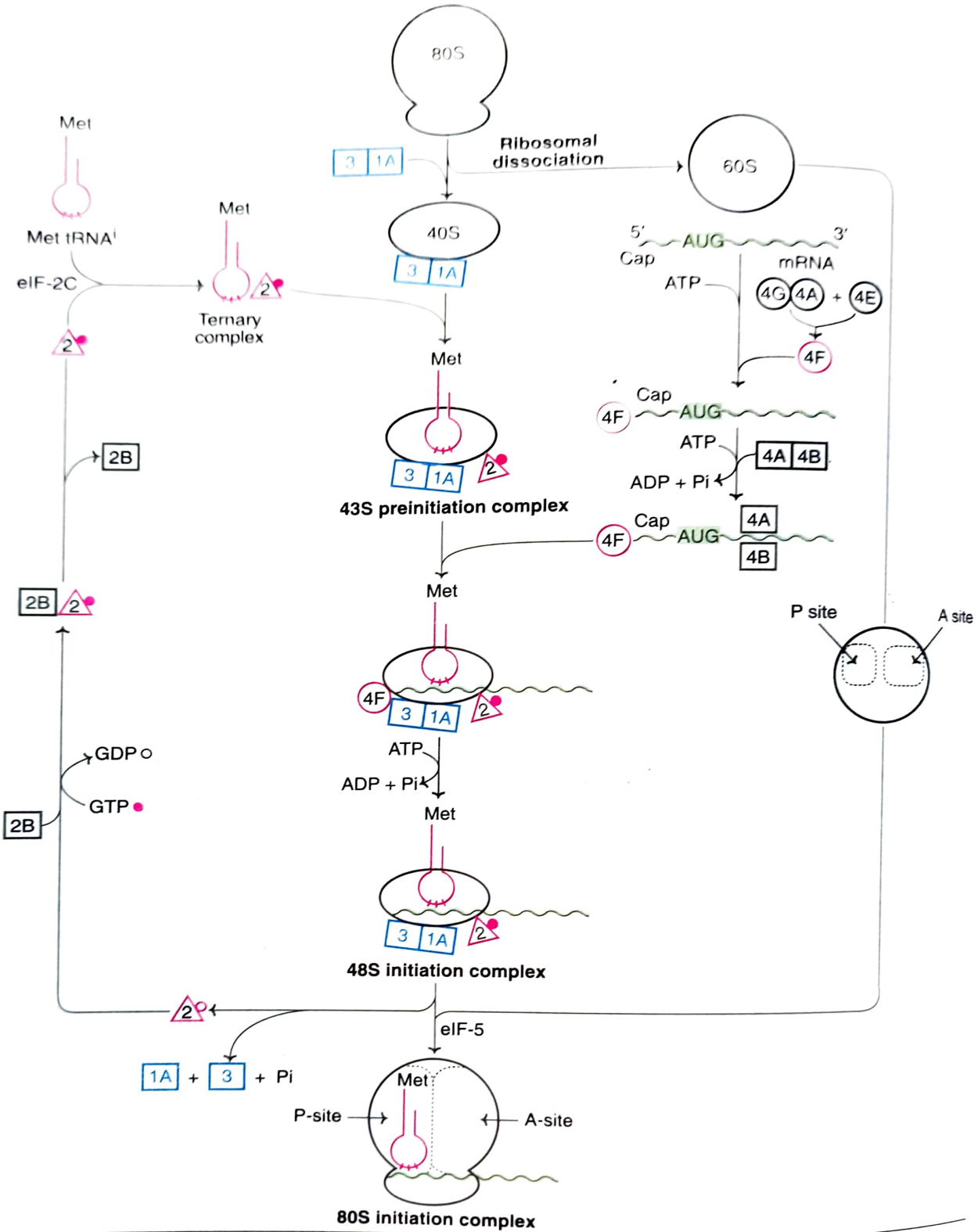

Fig.25.13 : *A diagrammatic representation of initiation of protein biosynthesis (translation) in eukaryotic cells (The eukaryotic initiation factors are represented by symbols □, △, and ○. By prefixing with eIF, the full names of the factors are obtained e.g. [3] represents eIF–3).*

eIF-4F complex is formed by the association of eIF-4G, eIF-4A with eIF-4E. The so formed eIF-4F (referred to as cap binding protein) binds to the cap of mRNA. Then eIF-4A and eIF-4B bind to mRNA and reduce its complex structure. This mRNA is then transferred to 43S complex. For the appropriate association of 43S preinitiation complex with mRNA, energy has to be supplied by ATP.

Recognition of initiation codon : The ribosomal initiation complex scans the mRNA for the identification of appropriate initiation codon. 5'-AUG is the initiation codon and its recognition is facilitated by a specific sequence of nucleotides surrounding it. This marker sequence for the identification of AUG is called as ***Kozak consensus sequences***. In case of ***prokaryotes*** the recognition sequence of initiation codon is referred to as ***Shine-Dalgarno sequence***.

Formation of 80S initiation complex

48S initiation complex binds to 60S ribosomal subunit to form 80S initiation complex. The binding involves the hydrolysis of GTP (bound to eIF-2). This step is facilitated by the involvement of eIF-5.

As the 80S complex is formed, the initiation factors bound to 48S initiation complex are released, and recycled. The activation of eIF-2 requires eIF-2B (also called as guanine nucleotide exchange factor) and GTP. The activated eIF-2 (i.e. bound to GTP) requires eIF-2C to form the ternary complex.

Initiation of translation in prokaryotes

The formation of translation initiation complex in prokaryotes is less complicated compared to eukaryotes. The 30S ribosomal subunit is bound to initiation factor 3 (IF-3) and attached to ternary complex of IF-2, formyl met-tRNA and GTP. Another initiation factor namely IF-1 also participates in the formation of preinitiation complex. The recognition of ***initiation codon AUG*** is done through Shine-Dalgarno sequence. A 50S ribosome unit is now bound with the 30S unit to produce 70S initiation complex in prokaryotes.

ELONGATION OF TRANSLATION

Ribosomes elongate the polypeptide chain by a sequential addition of amino acids. The amino acid sequence is determined by the order of the codons in the specific mRNA. Elongation, a cyclic process involving certain elongation factors (EFs), may be divided into three steps (***Fig.25.14***).

1. Binding of aminoacyl t-RNA to A-site.
2. Peptide bond formation.
3. Translocation.

Binding of aminoacyl—tRNA to A-site

The 80S initiation complex contains met-tRNA$_i$ in the P-site, and the A-site is free. Another aminoacyl-tRNA is placed in the A-site. This requires proper codon recognition on the mRNA and the involvement of elongation factor 1a (EF-Ia) and supply of energy by GTP. As the aminoacyl-tRNA is placed in the A-site, EF-1α and GDP are recycled to bring another aminoacyl-tRNA.

Peptide bond formation

The enzyme ***peptidyltransferase*** catalyses the formation of peptide bond (***Fig.25.15***). The activity of this enzyme lies on 28S RNA of 60S ribosomal subunit. It is therefore the ***rRNA*** (and not protein) referred to as ***ribozyme*** that catalyses the peptide bond formation.

Translocation

As the peptide bond formation occurs, the ribosome moves to the next codon of the mRNA (towards 3'-end). This process called translocation, basically involves the movement of growing peptide chain from A-site to P-site. Translocation requires EF-2 and GTP. GTP gets hydrolysed and supplies energy to move mRNA. EF-2 and GTP complex recycles for translocation.

In recent years, another site namely ***exit site*** (E-site) has been identified in eukaryotes. The deacylated tRNA moves into the E-site, from where it leaves the ribosome.

In case of prokaryotes, the elongation factors are different, and they are EF-Tu, EF-Ts (in place of of EF-1a) and EF-G (instead of EF-2).

Incorporation of amino acids

It is estimated that about ***six amino acids per second*** are incorporated during the course of elongation of translation ***in eukaryotes***. In case of prokaryotes, as many as 20 amino acids can be incorporated per second. Thus the process of protein/polypeptide synthesis in translation occurs with ***great speed*** and ***accuracy***.

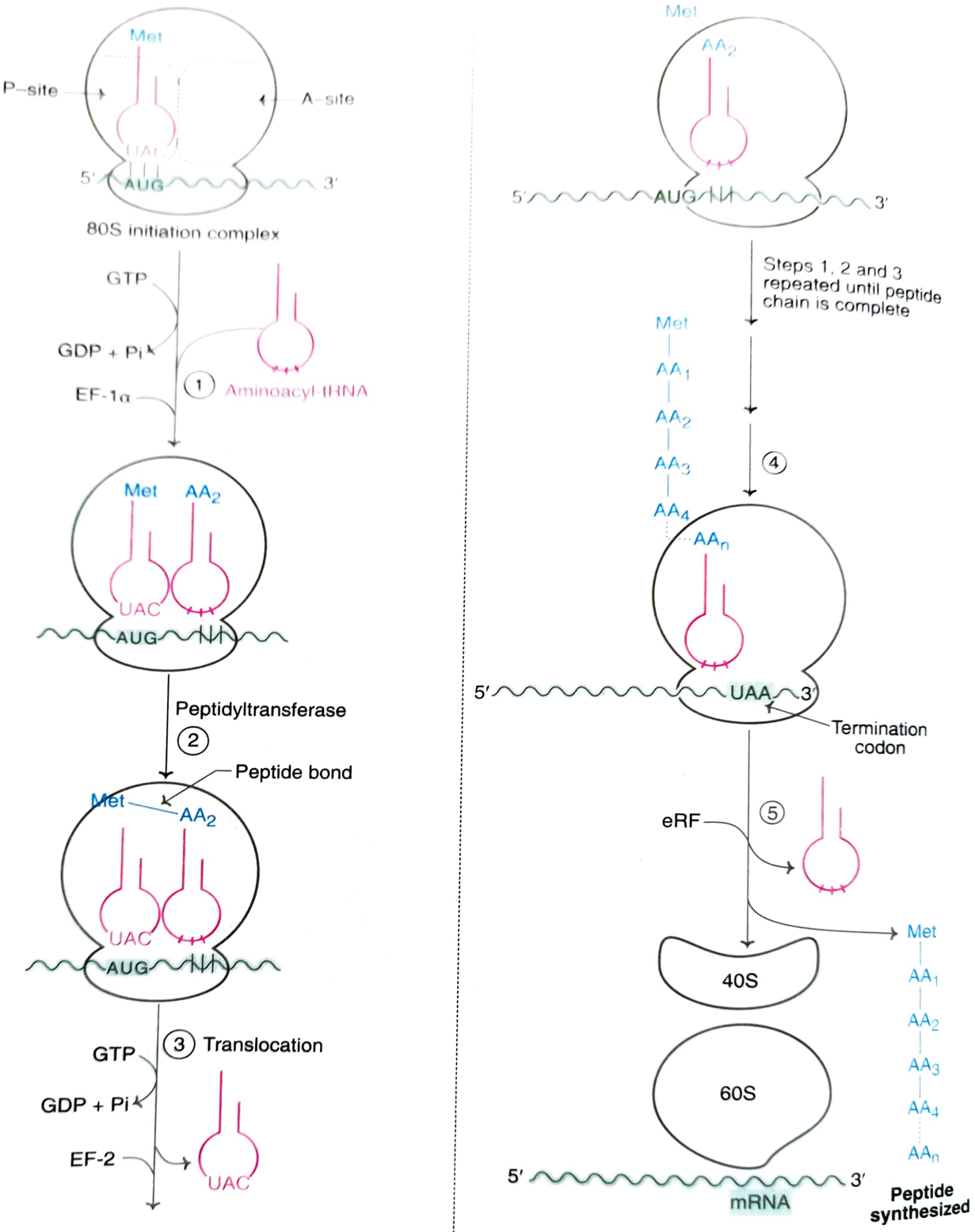

Fig. contd. next column

Fig.25.14 : *Protein biosynthesis – Elongation and termination (for initiation* ***See Fig. 25.13****). Met-Methionine; P-site – Peptidyl tRNA binding site; A-site – Aminoacyl tRNA binding site. AA-Amino acid; EF-Elongation factor; RF-Releasing factor.*

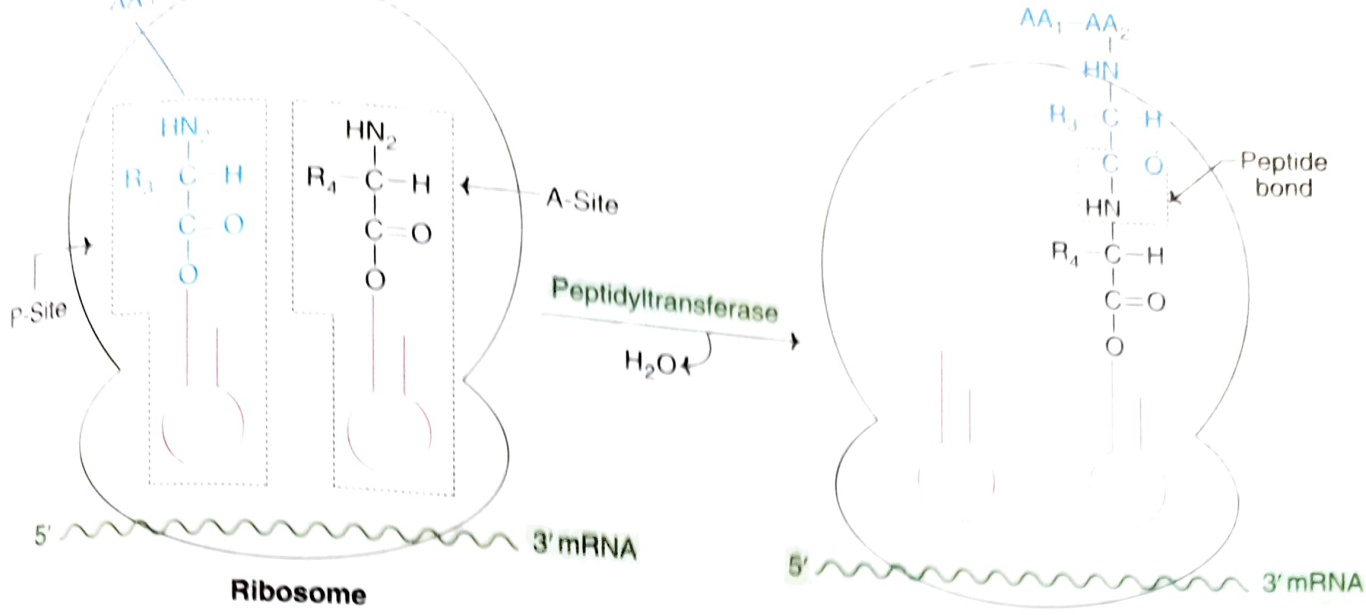

Fig.25.15 : Formation of peptide bond in translation (P–site—Peptidyl tRNA site; A–site—Aminoacyl tRNA site).

TERMINATION OF TRANSLATION

Termination is a simple process when compared to initiation and elongation. After several cycles of elongation, incorporating amino acids and the formation of the specific protein/polypeptide molecule, one of the ***stop or termination signals (UAA, UAG and UCA)*** terminates the growing polypeptide. The termination codons which act as stop signals do not have specific tRNAs to bind. As the termination codon occupies the ribosomal A-site, the release factor namely eRF recognizes the stop signal. eRF-GTP complex, in association with the enzyme peptidyltransferase, cleaves the peptide bond between the polypeptide and the tRNA occupying P-site. In this reaction, a water molecule, instead of an amino acid is added. This hydrolysis releases the protein and tRNA from the P-site. The 80S ribosome dissociates to form 40S and 60S subunits which are recycled. The mRNA is also released.

INHIBITORS OF PROTEIN SYNTHESIS

Translation is a complex process and it has become a favourite target for inhibition by antibiotics. Antibiotics are the substances produced by bacteria or fungi which inhibit the growth of other organisms. Majority of the antibiotics interfere with the bacterial protein synthesis and are harmless to higher organisms. This is due to the fact that the process of translation sufficiently differs between prokaryotes and eukaryotes.

Streptomycin, tetracycline, puromycin, chloramphenicol, erythromycin and diphtheria toxin are among the commonly used inhibitors of protein synthesis.

IV. CHAPERONES AND PROTEIN FOLDING

The three dimensional conformation of proteins is important for their biological functions. Some of the proteins can spontaneously generate the correct functionally active conformation e.g. denatured pancreatic ribonuclease. However, a vast majority of proteins can attain correct conformation, only through the assistance of certain proteins referred to as chaperones. Chaperones are ***heat shock proteins*** (originally discovered in response to heat shock). They ***facilitate*** and favour the interactions on the polypeptide surfaces to finally give the ***specific conformation of a protein***. Chaperones can reversibly bind to hydrophobic regions of unfolded proteins and folding intermediates. They can stabilize intermediates, prevent formation of incorrect intermediates, and also prevent undesirable interactions with other proteins. All these activities of chaperones help the protein to attain compact and biologically active conformation.

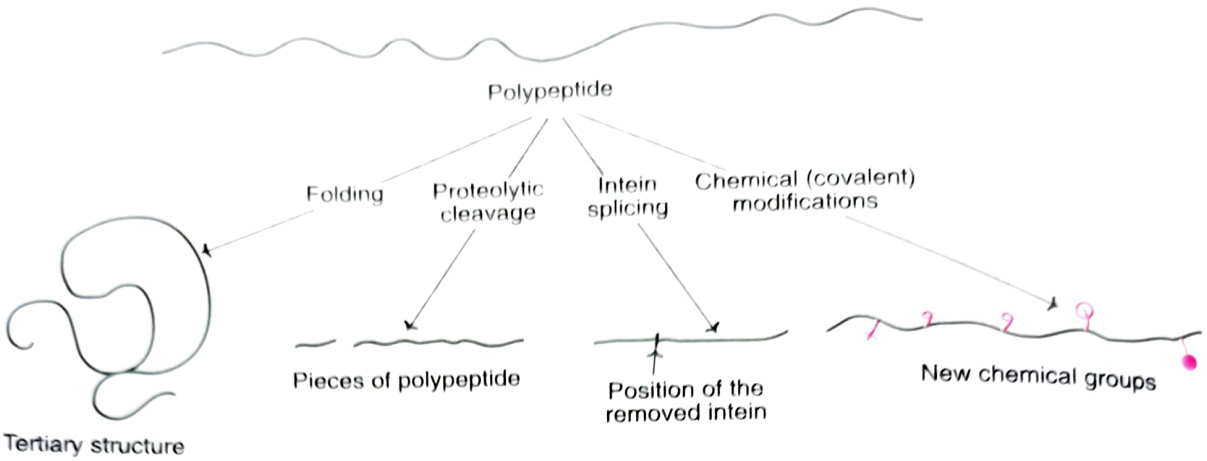

***Fig.25.16** : An outline of post-translational modifications of proteins.*

Protein misfolding and diseases

The failure of a protein to fold properly generally leads to its rapid degradation. ***Cystic fibrosis*** (CF) is a common autosomal recessive disease. Some cases of CF with mutations that result in altered protein (cystic fibrosis transmembrane conductance regulator or in short CFTR) have been reported. Mutated CFTR cannot fold properly, besides not being able to get glycosylated or transported. Therefore, CFTR gets degraded.

Certain neurological diseases which are due to cellular accumulation of aggregates of misfolded proteins or their partially degraded products have been identified. The term ***prions*** (***pr***oteinous ***in***fectious agents) is used to collectively represent them (***Refer Chapter 22***).

V. POST-TRANSLATIONAL MODIFICATIONS OF PROTEINS

The proteins synthesized in translation are, as such, not functional. Many changes take place in the polypeptides after the initiation of their synthesis or, most frequently, ***after the protein synthesis is completed***. These ***modifications*** include protein folding (described already), trimming by proteolytic degradation, intein splicing and covalent changes which are collectively known as post-translational modifications (***Fig.25.16***).

Proteolytic degradation

Many proteins are synthesized as the precursors which are much bigger in size than the functional proteins. Some portions of precursor molecules are

BIOMEDICAL / CLINICAL CONCEPTS

- *Retroviruses (RNA is the genetic material) are oncogenic i.e. cause cancers.*
- *Several antibiotics selectively block bacterial translation, and thus inhibit their growth e.g. streptomycin, tetracycline, puromycin.*
- *Protein misfolding often results in the formation of prions (proteinous infectious agents) which have been implicated in many diseases e.g. mad cow disese, Alzheimer's disease.*
- *Lebers' hereditary optic neuropathy is caused by mutation in mtDNA in males. The victims become blind due to loss of central vision as a result of neuroretinal degeneration.*

removed by proteolysis to liberate active proteins. This process is commonly referred to as trimming. The formation of insulin from preproinsulin, conversion of ***zymogens*** (inactive digestive enzymes e.g. trypsinogen) to the active enzymes are some examples of trimming.

Intein splicing

Inteins are ***intervening sequences in certain proteins***. These are comparable to introns in mRNAs. ***Inteins*** have to be ***removed***, and ***exteins ligated*** in the appropriate order for the protein to become active.

Covalent modifications

The proteins synthesized in translation are subjected to many covalent changes. By these modifications in the amino acids, the proteins may be converted to active form or inactive form. Selected examples of covalent modifications are described below.

1. **Phosphorylation :** The hydroxyl group containing amino acids of proteins, namely serine, threonine and tyrosine are subjected to phosphorylation. The phosphorylation may either increase or decrease the activity of the proteins. A group of enzymes called ***protein kinases*** catalyse phosphorylation while protein phosphatases are responsible for dephosphorylation (removal of phosphate group). Many enzymes that undergo phosphorylation or dephosphorylation are known in metabolisms (e.g. glycogen synthase).

2. **Hydroxylation :** During the formation of collagen, the amino acids proline and lysine are respectively converted to hydroxyproline and hydroxylysine. This hydroxylation occurs in the endoplasmic reticulum and requires ***vitamin C***.

3. **Glycosylation :** The attachment of carbohydrate moiety is essential for some proteins to perform their functions. The complex carbohydrate moiety is attached to the amino acids, serine and threonine (O-linked) or to asparagine (N-linked), leading to the synthesis of glycoproteins.

Vitamin K dependent ***carboxylation*** of glutamic acid residues in certain clotting factors is also a post-translational modification.

MITOCHONDRIAL DNA, TRANSCRIPTION AND TRANSLATION

The mitochondrial DNA (***mtDNA***) has structural and functional resemblances with prokaryotic DNA. This fact supports the view that mitochondria are derivatives of prokaryotes. mtDNA is circular in nature and contains about 16,000 nucleotide bases.

A vast majority of structural and functional proteins of the mitochondria are synthesized in the cytosol, under the influence of nuclear DNA. However, certain proteins (around 13), most of them being the components of electron transport chain, are synthesized in the mitochondria (e.g. cytochrome b of complex III, two subunits of ATP synthase). Transcription takes place in the mitochondria leading to the synthesis of mRNAs, tRNAs and rRNAs. Two types of rRNA and about 22 species of tRNA have been so far identified. Transcription is followed by translation resulting in protein synthesis.

The mitochondria of the sperm cell do not enter the ovum during fertilization, therefore, ***mtDNA is inherited from the mother.*** Mitochondrial DNA is subjected to high rate of mutations (about 10 times more than nuclear DNA) that causes inherited defects in oxidative phosphorylation. The best known among them are certain mitochondrial myopathies and Leber's hereditary optic neuropathy. The latter is mostly found in males and is characterized by blindness due to loss of central vision as a result of neuroretinal degeneration. ***Leber's hereditary optic neuropathy*** is a consequence of single base mutation in mtDNA. Due to this, the amino acid histidine, in place of arginine, is incorporated into the enzyme NADH coenzyme Q reductase.