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.

1. Requirement of the components
2. Activation of amino acids
3. Protein synthesis proper

1. 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.

a. Amino Acids

b. Ribosomes:

c. 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.

d. 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.

e. Energy Sources: Both ATP and GTP are required for the supply of energy in protein synthesis. Some of the reactions involve the breakdown of ATP or GTP, respectively, to AMP and GMP with the liberation of pyrophosphate. Each one of these reactions consumes two high energy phosphates (equivalent to 2 ATP).

f. 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.

2. 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. 4.16).

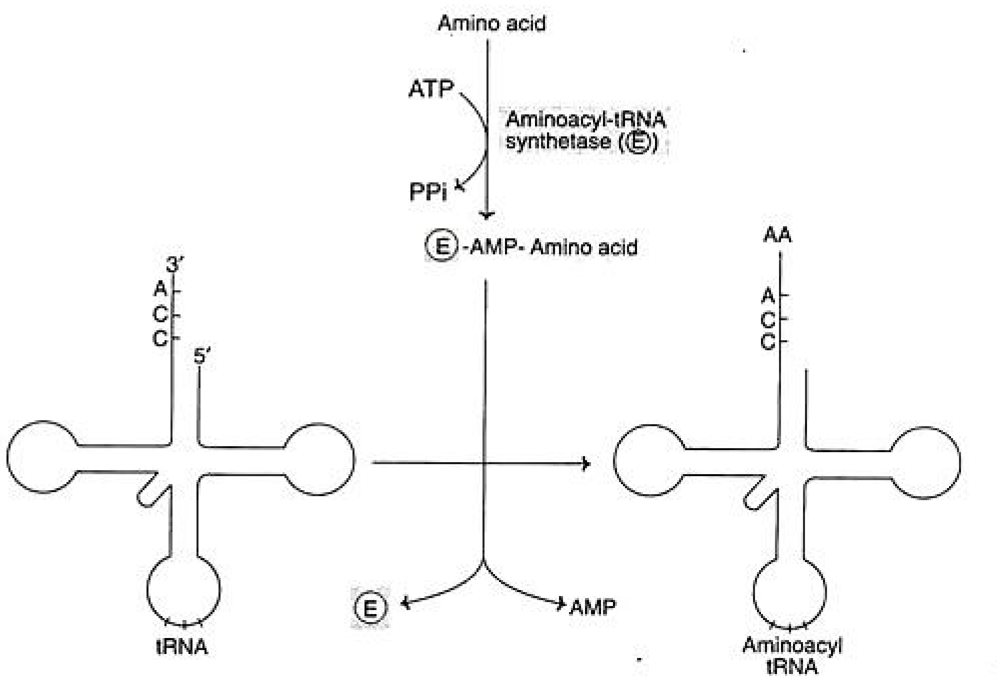

Fig. 4.16 : Formation of aminocacyl tRNA (AA–Amino acid; E–Enzyme).

3. Protein Synthesis Proper:

Initiation of Translation:

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

1. Ribosomal dissociation.
2. Recognition of initiation codon
3. Formation of 80S initiation complex.

1. Ribosomal dissociation:

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

2. 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 starting codon (Kozak consensus sequences). In case of prokaryotes the recognition sequence of initiation codon is referred to as Shine- Dalgarno sequence.

3. 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 elF-2). This step is facilitated by the involvement of elF-5. As the 80S complex is formed, the initiation factors bound to 48S initiation complex are released, and recycled. The activation of elF-2 requires elF-2B (also called as guanine nucleotide exchange factor) and GTP. The activated elF-2 (i.e. bound to GTP) requires elF-2C to form the ternary complex.

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. 4.18).

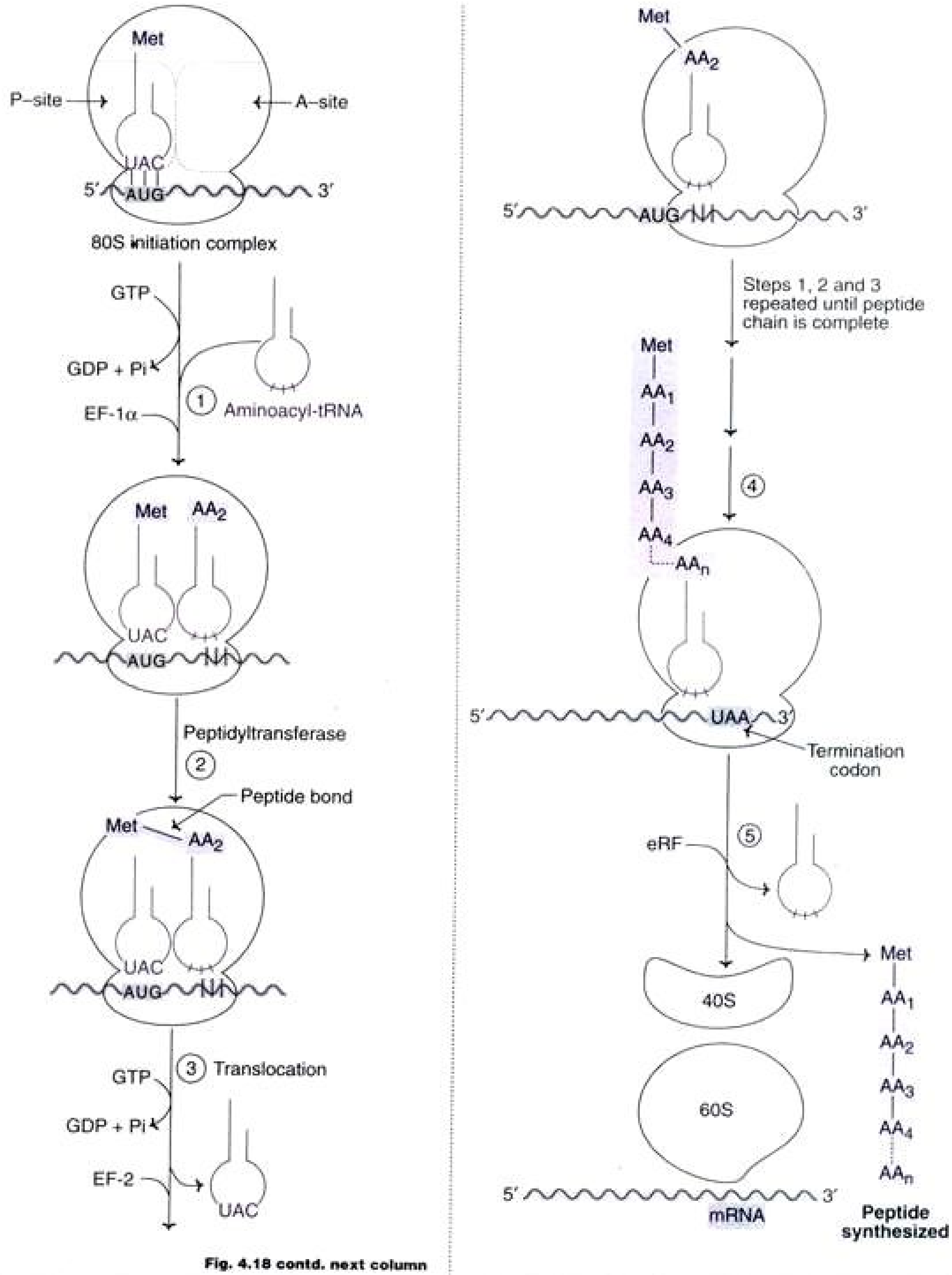

Fig. 4.18 : Protein biosynthesis — Elongation and termination (for initiation refer Fig. 4.17). Met-Methionine; P-site — Peptidyl tRNA binding site; A-site — Aminoacyl tRNA binding site. AA-Amino acid; EF-Elongation factor; RF-Releasing factor.

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[1] 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-la) and supply of energy by GTP. As the aminoacyl-tRNA is placed in the A-site, EF-1a and GDP are recycled to bring another aminoacyl- tRNA.

Peptide bond formation:

The enzyme peptidyltransferase catalyses the formation of peptide bond (Fig. 4.19). 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. As the amino acid in the aminoacyl-tRNA is already activated, no additional energy is required for peptide bond formation.

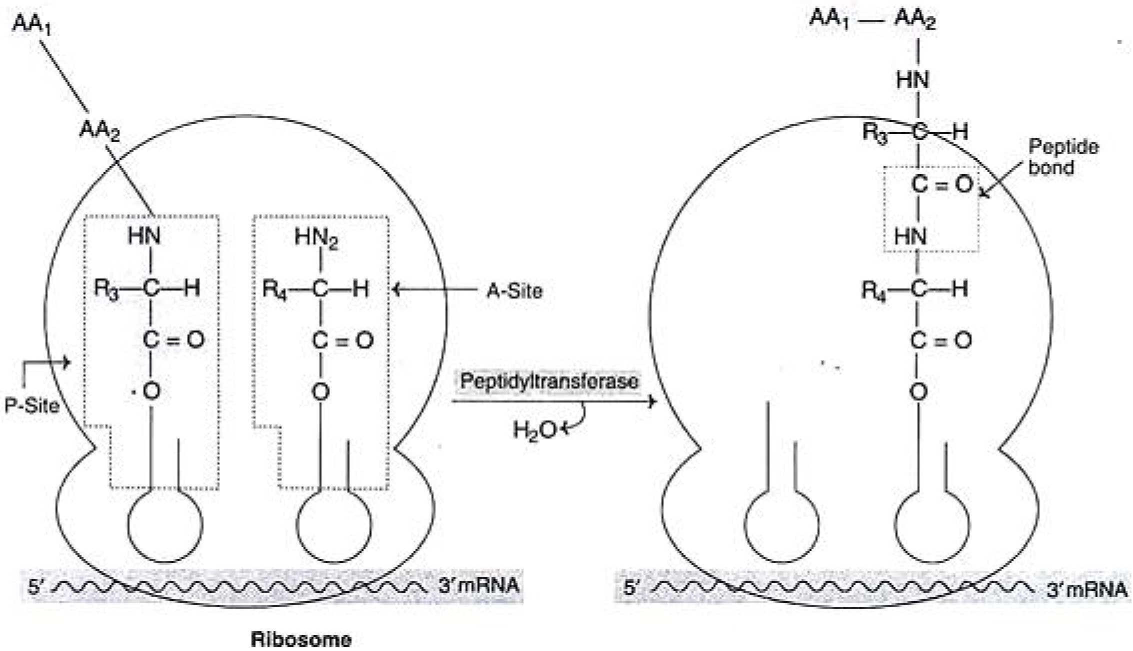

Fig. 4.19 : Formation of peptide bond in translation (P-site — Peptidyl tRNA site; A-site — Aminoacyl tRNA site).

The net result of peptide bond formation is the attachment of the growing peptide chain to the tRNA in the A-site.