UNIT 14 PROTEIN BIOSYNTHESIS

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14.1 INTRODUCTION

In Unit 12 you have studied biosynthetic pathways such as gluconeogenesis, glycogenesis and also about the synthesis of fats from carbohydrates. In this unit we will study various steps involved in the biosynthesis of proteins. Proteins are the most important components of living organisms, about which you have already studied in Blocks I and II. Proteins are not only required as a source of energy, they are more important as enzymes, catalysing hundreds of chemical reactions in a cell. You have already read about the various aspects of enzymes and regulation of enzyme activity in Units 9 and 10.

In the previous units you have studied that organisms maintain their internal environment in a dynamic steady state. In order to maintain the dynamic steady state, a cell regulates each and every activity taking place in it. In Units 11 and 12 you have studied various mechanisms involved in the control of synthesis and degradation of biomolecules releasing energy and precursors for the synthesis of other biomolecules. In this unit we will study mechanisms that regulate protein biosynthesis.

Objectives
After studying this unit you should be able to
- define and use in correct context the terms: initiation codon, termination codon, initiation factors
- describe various steps in the synthesis of a peptide chain
- explain the processing of a protein precursor molecule
- outline the various ways in which a cell regulates protein synthesis.

14.2 PROTEIN BIOSYNTHESIS

Protein biosynthesis is the most complex of biosynthetic mechanisms requiring a large number of enzymes and other specific biomolecules. In eukaryotic cells protein biosynthesis requires the participation of over 200 different enzymes and, 70 or more kinds of tRNA and rRNAs. Thus almost 300 different macromolecules must cooperate to synthesise polypeptides. You are already aware that proteins are made up of polypeptide chains, organised into a complex three dimensional structure. Chemists can make biologically active proteins from their constituent amino acids. It takes at least 24 hours to produce, a small polypeptide chain such as ribonuclease. However, however a cell synthesises proteins at a rate much higher than that in the laboratory. For example, about five seconds are required for an Escherichia coli to synthesise and make a complete polypeptide chain of 100 amino acid residues.
Protein biosynthesis is under the control of genes, which contain coded instructions for the synthesis of proteins. DNA makes RNA, in accordance with the sequence of bases. RNA, in turn, makes a sequence of amino acids. The main steps in protein biosynthesis are transcription and translation.

In transcription, the information contained in DNA is transcribed into mRNA. The sequences of deoxyribonucleotides in DNA provides the template for synthesis of a complementary sequence of ribonucleotides in the RNA. In translation, the information in mRNA is translated into proteins. Three major types of RNA molecules are transcribed from genes in DNA, each with a particular role to play in gene expression; these molecules are mRNA, rRNA and tRNA. All three kinds of RNAs interact in the process of translating the informational DNA into proteins.

You have already studied the process of transcription in Unit 13. Now we will study how genetic information from DNA, via mRNA, is decoded to synthesise a protein by a process called translation.

You already know that each code is composed of three nucleotide bases known as a triplet code or a codon, that specifically codes for a single amino acid. Any alteration in the sequence of bases results in the synthesis of a wrong amino acid in the polypeptide chain. Wrong amino acid in the polypeptide chain can cause serious disorders in the organism, such as sickle cell anaemia, haemophilia, thalassaemia etc. A collection of codons comprise the genetic code, about which you will study later in this section. The codons pair with anticodons of the tRNA molecule. Each anticodon consists of three free bases. This pairing follows the A-U and G-C combination. The codon GUC pairs with the anticodon CAG of tRNA. Thus the series of codons on the mRNA determine the series of anticodons of different tRNA molecules, and hence of the amino acids. You have studied in Unit 13 that the triplets of mRNA are complementary to the series of base sequence in DNA.

So you can see that the DNA molecule ultimately determines the structure of protein molecule.

The process of translation involves the following steps:
1) activation of amino acids,
2) transfer of the activated amino acids to tRNA,
3) initiation of polypeptide chain synthesis,
4) chain elongation,
5) chain termination and release of the polypeptide chain, and
6) Folding and processing of the polypeptide chain.

Before we study the process of initiation of protein biosynthesis, let us learn about the genetic code. The genetic code is a specific sequence of bases in mRNA that leads to the synthesis of a sequence of amino acids. The individual word in the code is composed of three nucleotide bases called the codon. The sequence of codons always leads from the 5' end to its 3' end.

The pioneering work of Nirenberg and Khorana in 1960 revealed the coding pattern of all the 64 triplets. Sixty-one out of the 64 triplets code for only 20 different amino acids. There are four nucleotide bases that produce a three-base codon and there are 64 different combinations of these bases. Since there is more than one code for single amino acid, it is assumed that most amino acids are coded for by more than one codon. Such a genetic code is called a degenerate code, although degeneracy does not mean malfunctioning. The genetic code is associated with specific punctuation signals such as initiation and termination codons for polypeptide synthesis. Table 14.1 summarises triplet codes for 20 amino acids and for the termination signal.

<table>
<thead>
<tr>
<th>5' OH terminal and First Base</th>
<th>Second Base</th>
<th>3' OH terminal</th>
<th>Third Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>C</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>UU = phe</td>
<td>UCU = ser</td>
<td>UAU = tyr</td>
<td>UGU = cys</td>
</tr>
<tr>
<td>UUC = phe</td>
<td>UCC = ser</td>
<td>UAC = tyr</td>
<td>UGC = cys</td>
</tr>
<tr>
<td>UUA = leu</td>
<td>UCA = ser</td>
<td>(UAA = STOP)</td>
<td>(UGA = STOP)</td>
</tr>
<tr>
<td>UUG = leu</td>
<td>UCG = ser</td>
<td>(UAG = STOP)</td>
<td>UGG = trp</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2' OH terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
</tr>
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</table>

Sickle cell anaemia is a severe anaemia in which sickle-shaped red blood cells appear in the blood.

The patients of haemophilia bleed severely from even a slight injury, because the blood fails to clot.

Thalassaemia is a group of inherited anaemias in which there is a defect in the alpha or beta chain of haemoglobin. It is fatal in children.

Table 14.1: The Genetic Code
The enzymes are also specific for the corresponding tRNA. However, some enzymes can activate more than one amino acid, for example, asparagine (asn) and arginine (arg). A high energy acyl bond is formed between the α-phosphate of ATP and the carboxyl group of the amino acid. The β-phosphates of ATP break away as inorganic pyrophosphates (PPi). The aminoacyl AMP remains bound to the activating enzyme.

### 14.2.1 Activation of Amino Acids

You are already aware that there are at least 20 different amino acids, and these different amino acids are first activated by specific activating enzymes called aminoacyl-tRNA synthetase and bind to ATP to form an amino acid AMP molecule. The enzymes are also specific for the corresponding tRNA. However, some enzymes can activate more than one amino acid, for example, isoleucine-tRNA synthetase, which activates isoleucine can also activate valine. A high energy acyl bond is formed between the α-phosphate of ATP and the carboxyl group of the amino acid. The β-phosphates of ATP break away as inorganic pyrophosphates (PPi). The aminoacyl AMP remains bound to the activating enzyme.

\[
\text{Enzyme} \quad \text{Amino acid} + \text{ATP} \rightarrow \text{Amino acyl-Pyrophosphate} \quad \text{Amino acid-tRNA adenylate} \quad \text{synthetase}
\]

### 14.2.2 Transfer of Amino Acid to tRNA

The next step, after activation of the amino acid, is the transfer of the activated amino acids to its specific tRNA. About 100 different types of tRNA are found in each cell. The tRNA is named after the amino acid for which it is specific. For example, the tRNA for the amino acid isoleucine and valine are designated as tRNA_{ile} and tRNA_{val} respectively.

Transfer RNAs serve as adaptors in translating the language of DNA (genes) into the language of proteins. It has already been mentioned that there is at least one tRNA for each amino acid and some amino acids have two or more specific tRNAs. At least 32 tRNAs are required to recognise all the amino acid codons. However, some cells possess many more than 32, for example, each bacterial cell probably contains about a hundred or more different types of tRNA.

Transfer RNAs are relatively small, single-stranded molecules. In bacteria and extra mitochondrial cytosol of eukaryotes, tRNAs consist of 73 to 93 nucleotide residues having molecular weights between 24,000 and 31,000 respectively. Mitochondrial tRNAs are smaller than the tRNA present in the cytosol, and have a distinct structure.

The complete nucleotide sequence of tRNA was first worked out by Holley and his colleagues in 1965 for yeast alanine tRNA. Since then, the sequence of about 75 different tRNAs, ranging from bacteria to mammals has been established. The different tRNAs are all minor variants of the same basic structure. Several models of the secondary structure of tRNA have been proposed, and of these the clover leaf model of Holley is the most widely accepted.

According to the clover leaf model, the single polynucleotide chain of tRNA is folded upon itself to form a five-armed structure. As a result of the folding the 3' and the 5'
The ends of the chain come close to each other. An arm consists of a stem and a loop. In the double helical stems there is internal Watson-Crick base pairing which follows the A-U and G-C combinations except for an occasional G-U base pair or a mismatch. There is no base pairing in the loops. One of the arms has a stem but not a loop and is called the adapter or acceptor arm. The other arms are called the D arm, the anticodon arm, the variable arm and the $\text{T}_\Psi\text{C}$ arm. The variable arm may or may not have a stem (Fig. 14.1).

The adapter or acceptor arm consists of seven base pairs and four unpaired nucleotide units. The unpaired nucleotide units include a constant 3' terminal — CCA sequence and a fourth nucleotide which is a variable purine (A or G). The amino acid molecule attaches to the — CCA sequence of 3' terminal called the amino acid binding site. The 5' end of tRNA is either guanine or cytosine.

The second arm is called the D arm. It consists of 15-18 nucleotides with 3-4 base pairs in the stem and 7-11 unpaired nucleotides in the loop. The loop of the D arm is called Loop I or dihydrouridine (DHU) loop or D loop. It contains two variable regions, $\alpha$ and $\beta$ on either side of two constant guanine residues. These regions consist of 1-3 nucleotides, mostly pyrimidines with a high proportion of DHU. The synthetase site, which recognises the amino acid activating enzyme, is located on the $\alpha$ part of the D loop and the $\omega$ part of the acceptor stem on the 5', side.

The third arm, or anticodon arm, consists of an anticodon stem of five base pairs and a loop, called Loop II or anticodon loop. This loop consists of seven unpaired nucleotides of which the middle three form the anticodon. The anticodon recognises the three complementary bases which constitute the codon of mRNA. On the 3' side of the anticodon is a hypermodified purine (H) while on the 5' side is U and a pyrimidine (y).

The variable arm (the milli loop, Loop III) is of two types. In one type, there is a loop containing 4-5 bases but no stem. In the other type, the arm consists of 13-21 nucleotide residues, and both the stem and the loop can be distinguished.

The $\text{T}_\Psi\text{C}$ arm consists of a stem having five base pairs and a loop of seven nucleotides. The outermost of the five pairs of the stem is C-G. The $\text{T}_\Psi\text{C}$ loop contains a constant $\text{T}_\Psi\text{C}$ sequence. All tRNAs have a ribosome recognition site on the $\text{T}_\Psi\text{C}$ loop consisting of the G-$\text{T}_\Psi\text{C}$-R sequence.

A study of different tRNAs shows that the structure of the acceptor stem, the anticodon arm and the $\text{T}_\Psi\text{C}$ arm are constant. The difference in the tRNAs lie in the D arm and the variable arm.

X-ray diffraction analysis has revealed that tRNA has a tertiary structure. This structure is due to hydrogen bonds between bases, between bases and the ribose-phosphate backbone and between the ribose phosphates. Fig. 14.2 illustrates the tertiary structure of the yeast phenylalanine tRNA.

After the structure of tRNA, let us now study the next step of protein biosynthesis, the transfer of the activated amino acid to tRNA.

In this step, the aminoacyl group from the enzyme-bound aminoacyl adenylate is transferred to its corresponding specific tRNA, forming tRNA-aminoacyl complex. A high energy ester bond is formed between the carboxyl group of the amino acid and the 3'-hydroxyl group of the terminal adenosine of tRNA. You have studied above that during the activation of an amino acid, an inorganic pyrophosphate is formed. This inorganic pyrophosphate now undergoes hydrolysis to form orthophosphate by an enzyme Pyrophosphatase. Thus, two high energy phosphate bonds may ultimately be expended for each amino acid.

\[ \text{Aminoacyl adenylate} + \text{tRNA} \rightarrow \text{Aminoacyl-tRNA} + \text{Adenosine-} \]
\[ \text{monophosphate} + \text{Aminoacyl-} \]
\[ \text{tRNA synthetase (Enzyme).} \]

The transfer of the activated amino acid to its corresponding specific tRNA is also catalysed by amino acyl-tRNA synthetase. You have studied earlier that aminoacyl-tRNA synthetase catalyses the attachment of amino acids to their corresponding tRNAs. The enzyme aminoacyl synthetase has two active sites, one for recognising the specific amino acid and the other for recognising the specific tRNA molecule. Thus the enzyme aminoacyl-tRNA synthetase brings the amino acid.
molecule and its specific tRNA molecule together.

\[ \text{Amino acid} + \text{ATP} + \text{E} \rightarrow \text{E-[aminoacyl adenylate]} + \text{PPi} \]
\[ \text{E-[aminoacyl adenylate]} + \text{tRNA} \rightarrow \text{aminoacyl tRNA} + \text{E + adenylate}. \]

You are already aware that the aminoacyl-tRNA synthetases are specific for both tRNA and its corresponding amino acid. If a wrong amino acid is joined to a tRNA to form a mismatched aminoacyl-tRNA, an incorrect amino acid residue will be formed in the polypeptide chain. However, some of the aminoacyl-tRNA synthetases are capable of proof reading and correcting their own errors, like DNA polymerases about which you have studied in Unit 13.

### 14.2.3 Initiation of Polypeptide Chain Synthesis

Before studying the steps involved in the initiation of polypeptide chain synthesis, let us learn about ribosomes. You have already studied in Blocks I and II that ribosomes are cell organelles. The complex process of translation takes place on ribosomes. Therefore ribosomes are regarded as the site of protein synthesis. Several ribosomes are held along the length of a single mRNA. This structure is called a polyribosomes. Polyribosomes greatly enhance the efficiency of mRNA by rapid translation (Fig. 14.3).

A ribosome is a spherical particle of 23 nm and is composed of a large and small subunit. Eukaryotic ribosomes sediment in sucrose gradients with a sedimentation coefficient of 80S. In the absence of Mg$^{2+}$, these eukaryotic ribosomes dissociate reversibly into subunits of 40S and 60S. Prokaryotic ribosomes are smaller and sediment at 70S; these have subunits of 30S and 50S. The major constituents of ribosomes are RNA and proteins, present approximately in equal amounts. Prokaryotic ribosomes contain three RNA molecules: 16S RNA present in the small subunit, and 23S and 5S in the large subunit. In eukaryotes there are four rRNAs: 18S in the small subunit and 28S, 5.8S and 5S in the large subunit.

In prokaryotes ribosomes contain 21 proteins in the small subunit and 34 in the large. In eukaryotes there are 30 proteins in the small subunit and 40 in the large subunit. There is no functional difference between the prokaryotic ribosomes and eukaryotic ribosomes; they perform the same function, in the same set of chemical reactions. The genetic code is the same for all living organisms. Eukaryotic ribosomes are able to translate bacterial mRNAs efficiently, provided that a 'cap' (50S subunit: the smaller subunit and the larger subunit of the ribosomes are also known as cap and sphere respectively) is added enzymatically (Fig. 14.4).
After learning about the ribosomes, let us now study the initiation of the polypeptide chain. The initiation of a polypeptide chain requires,

1) the ribosomal subunit
2) mRNA coding for polypeptide
3) activated amino acid attached to its tRNA
4) an energy source (GTP and ATP) and
5) a set of proteins called initiation factors.

The initiation factors are called IF-1, IF-2 and IF-3 in prokaryotes and eIF-1, eIF-2, eIF-3, eIF-4A, eIF-4B, eIF-4C, eIF-4D, eIF-4E, eIF-5 and an extra ancillary protein factor (SP) in eukaryotes (e stands for eukaryotes).

The mRNA bearing the code AUG for polypeptide synthesis, binds to the smaller subunit of the ribosome, followed by the initiating amino acid attached to its tRNA, to form an initiation complex (70S-f-met-tRNA-mRNA in prokaryotes and 80S-met-tRNA-mRNA in eukaryotes).

The formation of the initiation complex takes place in three steps. In prokaryotes, at the first step, the 30S ribosomal subunit binds to the initiation factor-3 (IF-3), which prevents the 30S and 50S subunits from recombining. A segment of 16S rRNA in the 30S subunit provides a binding site for IF-3. 16S rRNA is also involved in mRNA binding. It positions the mRNA correctly at the initiation codon (AUG) for the initiation of translation.

In prokaryotes, at the second step of the initiation process, the complex of 30S subunit, IF-3 and mRNA bind with IF-2 and IF-1, and N-formylmethionine-tRNA. This step requires Mg^{2+} and GTP. IF-2 is required for the recognising and binding of N-formylmethionine-tRNA. IF-1 probably also participates, but IF-2 is the most important. You have studied that AUG is the initiation codon on mRNA. Therefore, the initiation tRNA (attached to N-formylmethionine, along with its UAC anticodon) binds to an AUG codon of mRNA. AUG and UUG rarely act as initiation codons of mRNA. (Fig. 14.5a).

In the third step, the complex of 30S subunit, IF-3, mRNA, IF-2 containing bound GTP and N-formylmethionyl-tRNA bind with the 50S ribosomal subunit. Simultaneously, the GTP molecule bound to IF-2 is hydrolysed to GDP and phosphate, and are set free. IF-3 and IF-2 are also released from the ribosome. Now the functional 70S ribosome is called initiation complex containing mRNA and the initiating N-formyl methionyl tRNA. The 70S ribosome has two binding sites for tRNA, the aminacyl or acceptor site (A-site) and the peptidyl or polymerisation site (P-site), also called the donor site. Each ribosome thus functionally accommodates two codons at a time. The P-site and the A-site are located in the 50S subunit of the ribosome. f-met – tRNA binds to the P-site. All other tRNAs first bind to the A-site and then shift to the P-site. The initiation complex is now ready for the steps of elongation (Fig. 14.5b).

In eukaryotes, the initiating amino acid for polypeptide chain synthesis is methionine. Methionine is transferred to its corresponding specific tRNA by an enzyme methionine aminoaeryl-tRNA synthetase, forming methionine tRNA (met-tRNA). This activated methionine bound to its tRNA in the presence of GTP and the initiation factor-2 (eIF-2), forms a stable complex called ternary complex, which binds to the 40S subunit of the ribosome.

\[
\text{Methionine + tRNA^{met} + ATP} \quad \stackrel{E}{\rightarrow} \quad \text{Methionyl-tRNA^{met} + AMP + PPI}
\]

The mRNA next binds to the initiation complex. Four different protein factors; eIF-1, eIF-4A, eIF-4B, eIF-4E are required for the formation of the initiation complex. Later, the 60S ribosomal subunit binds to 40S pre-initiation complex to form an 80S initiation complex. At this stage, all the initiation factors are released and recycled.

### 14.2.4 Chain Elongation

After the initiation, the polypeptide chain is lengthened by covalant attachment of successive amino acid units, each carried to the ribosome and put into its proper position by its corresponding tRNA (aminoacyl-tRNA is base-paired to its corresponding codon in the mRNA). Elongation involves the relative movement of
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mRNA and the ribosome. The process of elongation consists of three steps and in prokaryotes the process requires,

1) initiation complex
2) the next amino acyl-tRNA, specified by the next coding triplet of mRNA,
3) a set of three soluble proteins of the cytosol called elongation factors, EF-TU, EF-TS, and EF-G and
4) GTP.

You are already aware that in prokaryotes, formyl methionine is the first amino acid, which binds to its corresponding specific tRNA. In the first step of the elongation process the next aminoacyl-tRNA binds to the A-site of the ribosome. This step requires GTP and elongation factors; EF-TU and EF-Ts. EF-TU is required to bring amino acyl-tRNAs to the A-site of the ribosome, whereas EF-Ts is needed to recycle EF-TU. In the presence of GTP, EF-Ts is released and then EF-TU. GTP then interacts with aminoacyl tRNA to form a ternary complex, which binds to ribosome.

\[
\text{EF-TS} \\
\text{Amino acid-tRNA} + \text{EF-TU} + \text{GTP} \rightarrow (\text{aa-tRNA} - \text{EF-TU} \text{GTP})
\]

\[
\text{Ternary complex} \\
\text{Amino acid-tRNA} - \text{EF-TU} (\text{GTP}) + \text{Ribosome} \rightarrow (\text{aa-tRNA} - \text{Ribosome}) \\
+ (\text{EF-TU-GDP}) + \text{Pi}.
\]

At this stage you will find that the first aminoacyl-tRNAhet gets attached at the P-site of the ribosome and the rest of the incoming aminoacyl-tRNA attaches at the A-site.

In the second step of chain elongation, a peptide bond is formed between the amino acids whose tRNAs are located at the A-site and P-site of the ribosome. This step occurs by the transfer of the initiating N-formyl methionine acyl group from its tRNA to the amino group of the new amino acid that has just entered the A site. This step is catalysed by peptidyl transferase and does not require any external source of energy or other factors. Now the tRNA present at the A-site contains two amino acids: the first amino acid (N-formyl methionine in prokaryotes and methionine in eukaryotes) and the second amino acid. Therefore it is called dipeptidyl tRNA. The empty tRNA at the P-site, whose amino acid is transferred to the tRNA at the P-site is now discharged from the P-site of the ribosome into the cytosol for recycling (Fig. 14.5c).

In the third step, after the bond formation, the ribosome moves along the mRNA strand, one codon at a time, from the initiation site (which is at or near the 5' end of mRNA) to the termination codon (which is located near the 3' end). The movement of the ribosome relative to mRNA is in the 5' ——> 3' direction, and is called transllocation. As a result of the first transllocation, the dipeptidyl tRNA present at the A-site shifts to the P-site. The A-site, therefore, becomes vacant to allow in the aminoacyl-tRNA. Further, at every step of transllocation the new aminoacyl-tRNA occupies the A-site and forms a peptide bond with the amino acids at the P-site forming a polypeptide chain. The emptied tRNAs are discharged for recycling. The process is repeated, and elongation of the polypeptide chain is thus brought about by step-by-step addition of amino acids. Energy for transllocation is supplied in the form of GTP.

In prokaryotes, transllocation is brought about by elongation factor G (EF-G) (also called translacase), EF-G rapidly hydrolysates GTP to GDP + Pi. For each amino acid added to the chain, two GTPs are hydrolysed to GDP and Pi.

In eukaryotes, elongation factor, EF-2 corresponding to EF-G of prokaryotes, catalyses the transfer of peptidyl tRNA from the A-site to the P-site. GTP is hydrolysed to provide energy for transllocation. When the ribosome moves about 80 nucleotides i.e. towards the 3' terminal of the mRNA, the same mRNA strand is now utilised by the second ribosome to synthesise an identical polypeptide chain.

In this way a series of ribosomes may associate with the mRNA to form a polyribosome. The number of ribosomes in a polyribosome depends upon the molecular weight of the polypeptide chain synthesised. For example, the polyribosome synthesising a haemoglobin subunit consists of about 150 amino acids and contains four to six ribosomes. The subunits of myosin are synthesised by a polyribosome of about 60 ribosomes. In eukaryotes, a mRNA strand functions simultaneously on several ribosomes. Therefore the number of mRNA required is much fewer than the number of polypeptide chains synthesised (Fig. 14.5 d.e.).
Protein Biosynthesis

Streptomycin binds to the ribosome and distorts its structure, causing dissociation of mRNA from the ribosome.

Initiation factors aid in the formation of the 30S initiation complex.

Tetracyclines interact with small ribosomal subunits, and prevent aminoacyl-tRNA anticodons from recognizing their corresponding codons.

Elongation factors direct the binding of the appropriate tRNA to the anticodon in the empty A-site.

The ribosome moves a distance of three nucleotides along the mRNA in the 5'-3' direction.

Diphtheria toxin inactivates the eukaryotic elongation factor, eEF-2, thus preventing translocation.

A termination codon is recognized by a release factor (RF) which activates the release of the newly synthesized peptide, and causes dissolution of the synthesizing complex.

Fig. 14.5: Steps of Protein Synthesis
14.2.5 Termination of Polypeptide Chain

Till now you have studied about the synthesis of a polypeptide chain. In this section, we will study how the polypeptide chain synthesis is terminated.

Elongation of a polypeptide chain continues till the ribosome adds the last amino acid to the chain, coded by the mRNA. The termination of the polypeptide chain occurs when the 70s ribosome (in prokaryotes) carrying the peptidyl-tRNA, reaches the termination codon located at the end of the mRNA. The termination codon may be UAA, UAG or UGA. These are special stop signals that do not code for any amino acid and have been called 'nonsense codons'. Ordinarily no tRNA anticodons pair with them. The termination codon need not be the last codon on the mRNA. In polygenic mRNAs, which are common in bacteria and bacteriophages, a number of initiation and termination codons are present. Each segment of the mRNA between the initiation and termination codons directs the synthesis of a separate polypeptide chain.

Chain termination leads to the release of the free polypeptide and tRNA and to the dissociation of the 70S ribosome into 50S and 30S subunits. These dissociated subunits are reutilised for the formation of new initiation complex. When a ribosome comes across a termination codon, a termination factor called 'release factor' (RF) causes hydrolysis of the peptide bond between the completed polypeptide chain and the tRNA at the P-site. The residual tRNA is discharged from the P-site.

In prokaryotes, two different release factors (RF-1 and RF-2) with two different codon specificities have been reported. RF-1 is specific for UAA and UAG whereas RF-2 is specific for UAA and UGA termination codons. In addition to RF-1 and RF-2, another factor called RF-3 stimulates the activity of RF-1 and RF-2.

In eukaryotes, a single release factor (RF) recognises all the termination codons (UAA, UAG and UGA). The first step in chain termination is the binding of the RF to the A site containing a termination codon. This step requires GTP and activates the peptidyl transferase system. Hydrolysis of peptidyl-tRNA at the P-site results in the release of the polypeptide chain from the ribosome. GTP hydrolysis results in the dissociation of the RF from the ribosome and the released RF is recycled (Fig. 14.5 f.g).

As the synthesis of a polypeptide chain continues, it undergoes folding. Secondary bonds are formed between the folded regions. Thus the tertiary structure of the protein may be attained and the final native shape formed even before the terminal amino acids have been added. Nevertheless, proteins are known to undergo many post-translational modifications. In the next section (14.3) we will study how polypeptide chains are modified to form specific proteins.

14.2.6 Rate of Protein Synthesis

You have read in the previous section that protein synthesis is a complex process. Chemists can synthesise biologically active proteins in the laboratory, but the rate of synthesis is extremely slow with a low yield. However, in living cells proteins are synthesised at a much faster rate. For example in E.coli, mRNA for the enzymes controlling tryptophan biosynthesis is translated at the rate of about seven amino acids per second. Messenger RNA for haemoglobin synthesis consists of 670 nucleotides. It is translated in 35 seconds at the rate of about 4 amino acids per second.

It is evident from the above examples that the rate of protein biosynthesis varies from one individual to another. With the help of various techniques, the rate of protein synthesis can be determined; when cells are cultured without essential amino acids, glucose, or serum, there is a marked inhibition of the rate of polypeptide chain initiation due to inactivation of initiation factor IF-2. In immature red blood cells, activity of IF-2 is known to be reduced in a controlled way by phosphorylation of one of its three protein subunits. This suggests that the rate of eukaryote protein synthesis is controlled in part by specific protein kinases, by inhibiting the initiation of polypeptide chain synthesis.

The initiation factors required for protein synthesis are more numerous and complex in eukaryotes than in prokaryotes, even though they perform the same basic functions. In eukaryotes there are many regulatory proteins that control protein synthesis.
14.3 PROCESSING OF PROTEIN MOLECULE

In the previous section (14.2) you have studied how a polypeptide chain is synthesised in organisms. You have also read that a protein is not biologically active until it is in its specific three dimensional native form. The three dimensional structure of a protein is ultimately determined by its amino acid sequence. A polypeptide chain assumes its three dimensional structure either during synthesis or after synthesis. However, the newly formed polypeptide chain often does not attain its final biologically active conformation until it has been subjected to processing or covalent modifications, known as post-translational modifications. Several post-translational modifications are known to take place, depending upon the type of protein. These are:

1) phosphorylation
2) methylation
3) nucleotidation
4) attachment of carbohydrate side chain,
5) addition of prosthetic group, and
6) formation of disulphide bonds.

The modifications listed above are catalysed by specific enzymes. For example, phosphorylation of proteins is catalysed by a group of enzymes called “protein kinases”. These enzymes transfer terminal phosphate of ATP to amino acid residues of the proteins. As many as 22 enzymes are known to get phosphorylated and as a result their activities are altered. In addition many structural proteins are also phosphorylated.

There is another process known as proteolysis in which some inactive enzymes are activated due to removal of a peptide fragment(s). For example, inactive trypsinogen is converted into active trypsin by removal of a peptide fragment. The other examples are:
Prothrombin → thrombin
Proalbumin → albumin, and
Proinsulin → insulin.

This proteolytic fragmentation leads to production of the final three-dimensional structure of the active protein.

Another similar example is the synthesis of an inactive form of collagen that undergoes post-translational modification to become a functional collagen molecule.

The collagens are a family of highly specific, fibrous proteins, found in all multicellular organisms. They are the most abundant proteins in mammals, constituting 25% of their total protein. Collagens are the major fibrous structured elements of cartilage, tendons, skin, bone, lung and blood vessels. These are synthesised by fibroblasts, located in the interstitial spaces of the cells. The collagens combine with hydrated polysaccharides to form glycosaminoglycans that constitute the extracellular matrix in the multicellular organisms. Collagen fibres are embedded in the glycosaminoglycans providing strength and help to organise the matrix.

Rope-like organisation of the collagens in tendons gives enormous tensile strength. Tendons consist almost entirely of collagen fibres. They usually attach muscles to the bone. In Block I and II you have already studied about the types of collagen and their properties.

In the previous sections you have studied how proteins are synthesised in the cytosol of the organisms. It is interesting to know that all the proteins are not synthesised in the cytosol. The secretory proteins are synthesised in the rough endoplasmic reticulum. The polypeptide chain formed is released into the lumen of the endoplasmic reticulum after their synthesis. From here, secretory proteins are transported to the Golgi complex and lysosomes. They are then secreted from the cell by a process known as exocytosis.

Blobel and his colleagues in 1975 proposed a hypothesis known as “signal hypothesis”, regarding the synthesis of proteins in the endoplasmic reticulum. This hypothesis was later experimentally investigated in the synthesis of immunoglobins (antibodies). It is discovered that the mRNA coding for secretory proteins contains a group of codons known as signal codons, located at the 3' side of the initiation codon, AUG. These signal codons are translated into signal peptides. These are located at the extreme amino terminus of the growing immunoglobin polypeptide. The membrane of endoplasmic reticulum has receptors that recognise proteins containing signal peptides. As a result the ribosomes containing signal peptides are attracted towards the endoplasmic reticulum. The cells also contain a protein called signal recognition protein, present in the cytosol. This protein binds to the ribosome shortly after it has synthesised the signal peptide and halts further protein synthesis by the ribosome until it has become linked to the rough endoplasmic reticulum. The signal peptide interacts with the receptor proteins of the endoplasmic reticulum forming a tunnel in the membrane, which coincides with the ribosomal tunnel. After entering the tunnel of the endoplasmic reticulum, the signal peptide is cleaved from the

![Rough endoplasmic reticulum](image)

Fig. 14.6 Schematic view of the signal peptide hypothesis.
growing polypeptide by an enzyme called signal peptidase. After the completion of polypeptide chain synthesis, it is released into the space of the endoplasmic reticulum. The ribosome detaches from the endoplasmic reticulum and the ribosome receptor proteins diffuse into the plane of the membrane closing the tunnel (Fig. 14.6).

The signal theory also applies to the secretory proteins produced by plant cells, such as zein from corn, chymotrypsin inhibitor from tomato leaves and seed reserve protein from the common beans.

Collagens are a good example of secretory proteins. They are also synthesised in the endoplasmic reticulum. After synthesis they are released into the lumen of the endoplasmic reticulum as large precursors, called Pro-α-Chains. These precursors have not only the ‘signal peptide’ sequence as in other secretory proteins, but also other extra amino acids, called extension peptides, present at both amino and carboxyl ends of the polypeptide chain. In the lumen of the endoplasmic reticulum the pro-α-chains undergo post-translational modifications. Now let us study what post-translational changes a precursor collagen undergoes. At the first post-translational change, both the proline and lysine residues of the polypeptide are hydroxylated. Soon after the hydroxylation, three pro-α-chains combine to form a triple-stranded helical molecule. Each pro-α-chain combines with the other by hydrogen bonding. It has been found that the hydroxyl group of hydroxyproline forms interchain hydrogen bonds that help to stabilise the triple helix.

The second post-translational modification is the glycosylation of pro-collagen molecule. At this stage, oligosaccharide side chains of the pro-collagen molecules are covalently attached to the hydroxyl group of the hydroxylysine. The hydroxylysine residues are important as they are useful not only for glycosylation, but also for the extensive cross linking of collagen molecules that occurs in the extracellular space after its secretion.

The third post-translational modification occurs during the process of secretion. At this stage, the extension peptides of the pro-collagen molecules are removed by specific proteolytic enzymes called pro-collagen peptidases. This converts the pro-collagen molecule to collagen molecules. As stated earlier, this step of post-translational modification, i.e. cleavage of the extension peptides occurs after the molecules are secreted into the interstitial spaces. In the interstitial space, the collagen molecules undergo polymerisation to form collagen fibrils.

Fibroblasts modify the properties of the extracellular matrix both by synthesising different types of collagen peptides and by differentially modifying the chains to influence the type and strength of packing. Fig. 14.7 illustrates various intercellular and extracellular events involved in the formation of a collagen fibril.

SAQ 2
Fill in the blanks with suitable words.

a) i) Signal hypothesis was proposed by .............................................
   ii) Signal codons are located at the .................. end of the initiation codon (AUG) on the mRNA coding for secretory proteins.

b) What are the several kinds of post-translational modifications that enable a protein to achieve its final biologically active conformation?
   i) ........................................................
   ii) ........................................................
   iii) ........................................................
   iv) ........................................................
   v) ........................................................
   vi) ........................................................

c) What is the function of the enzyme signal peptidase? Answer briefly in the space given below:
In the previous section you have studied how proteins are synthesised in the organisms. You have learnt that DNA determines the primary structure of the proteins. In a DNA molecule there are varying numbers of nucleotide sequences, each of them determines a polypeptide chain. A sequence of nucleotides that specifies the amino sequence of a polypeptide chain is known as a gene. E.coli contains 2000-4000 genes that code for 3000 different proteins in different amounts.

There are two types of genes present in a DNA molecule. They are constitutive genes and regulated genes. A constitutive gene is expressed continuously, so that its product is invariably found in the cell at roughly the same concentration. Regulated genes on the other hand, do not function at all the times. Their expression is switched on and switched off as per the need of their products in the cellular or extracellular environment. The products of the constitutive genes are the enzymes involved in glucose metabolism.

Glucose metabolism is an important process for the survival of the organism. Therefore this should be continuously functional. Products of regulated genes are enzymes that metabolise less common sugars such as lactose, arabinose and galactose, and also enzymes for the biosynthesis of amino acids, purines, pyrimidines and so on. Since these enzymes are not needed all the time, as the enzymes of glycolysis are synthesised according to the demand for them. The expression of regulated genes is regulated at various levels. It is regulated at the transcriptional level, the translation level and also at the post-translational level. Now we will study each of these regulatory mechanisms in detail.

14.4.1 At Transcriptional Level

You have studied in Unit 13 that synthesis of mRNA is known as transcription. The messenger RNA transcribed, is complementary to the DNA and it contains the DNA message for the synthesis of proteins. The process of transcription is under the control of the messenger RNA, which is transcribed only when required. Regulation of gene activity is better understood in prokaryotes than in eukaryotes.

In prokaryotes, biosynthesis of proteins is regulated at the transcriptional level by specific controlling factors. You have studied in Unit 10 that an operon contains a regulator gene, a promoter gene, an opeator gene and structural genes. The promoter gene and the operator gene are the controlling factors. These genes respond to the environmental signals in either allow or disallow the synthesis of mRNA from the structural genes.

RNA polymerase, the enzyme that catalyses synthesis of mRNA, binds to the promoter and initiates transcription of the structural genes. Operator is the site for the binding of a regulatory protein. You have studied in Unit 10 that to stop transcription of structural genes, the regulator gene transcribes a mRNA, which is translated to form a regulatory protein. The regulatory protein binds at the operator site, blocking the movement of RNA polymerase, thereby, preventing transcription of structural genes.

The transcription which is stopped by the binding of a regulatory protein at the operator site, starts with the activation of molecules known as inducer or effector molecules. The effector molecules are basically the substrate of the enzymes to be coded by the structural genes. These effector molecules bind with regulator proteins thereby changing their ability to form a complex with the operator. Here, once again, transcription of structural genes begins.

One of the most intensively studied enzyme system is that of lactose metabolism. Studies have shown that in the lactose operon (Lac Operon), the substrate lactose does not function directly as the effector molecule. In the cytoplasm, lactose is acted upon by the enzyme β-galactosidase, transforming lactose into allolactose. Allolactose is the actual effector molecule. It binds to the repressor protein at the allosteric site, Allolactose interacts directly with the regulator protein allowing transcription of lac genes.

There are two types of operons found in prokaryotes: i) inducible operons, ii) repressive operons. In inducible operons transcription of structural genes do not
occur unless there are effector molecules in the cell. Operons of lactose, galactose and arabinose represent inducible operons. Their effector molecules are lactose, galactose and arabinose respectively.

On the other hand in repressible operons, full levels of transcription occur until such time as their effectors reach a critical concentration, at which point transcription is inhibited. Operons of tryptophan, histidine and arginine represent repressive operons. Their effector molecules are tryptophan, histidine and arginine respectively.

Till now you were studying the control of protein biosynthesis in prokaryotes. In eukaryotes, regulation of transcription takes place by a different mechanism. In Block I and II you have studied that in eukaryotic chromosomes, DNA is associated with proteins, called histones, and non-histone chromosome proteins. These proteins are known to regulate the activity. Several models have been proposed to explain gene regulation by chromosomal proteins. According to the Frenster's model (1965), histones stabilise the DNA double helix by interacting with negative phosphate groups of DNA. This prevents separation of the strands and, consequently transcription. Thus histones act as general repressors of protein synthesis. According to this model, there are gene-specific derepressor DNA which interact with one of the non-transcribing DNA strands at a particular locus permitting the other strand to transcribe mRNA. Fig. 14.8 clearly illustrates the function of derepressor RNA.

Paul and co-workers (1971) are of the opinion that non-histone chromosomal proteins probably act as non-specific repressors of genetic functions. They recognise certain sites of DNA and bind to it, pulling off the histone facilitating transcriptions (Fig. 14.9).

![Fig. 14.9: Paul and co-worker's model of gene regulation by removal of histones.](image)

You know that eukaryotes are multicellular organisms. They have special signalling cells that detect changes in the external as well as internal environment and send signals for the alteration of protein biosynthesis. The “signalling cells” are the endocrine cells. These endocrine cells secrete hormones, and they regulate gene activity. In mammals, steroid hormones enter their target cells and bind with specific receptor proteins. Binding of the receptor to the steroid changes the shape of the receptor. The steroid-receptor complex enters the nucleus and stimulates transcription of specific genes.

In eukaryotes, steroids act as effectors and their receptor proteins as regulator molecules, becoming active only when bound to the effector. In insects ecdysone (a steroid hormone) switches on the transcription of particular genes. Experiments have shown that the DNA of larval chromosomes uncoil within the minutes of ecdysone treatment, facilitating transcription. The uncoiled parts of the chromosome look like puffs and are known as chromosomal puffs or Balbiani rings (Fig. 14.10).

### 14.4.2 At Translational Level

In the previous section, control of protein biosynthesis at transcriptional level was discussed. Biosynthesis of proteins is also regulated at translational level.
In prokaryotes, translational control is of lesser importance than transcriptional control, because their mRNAs are unstable. The life of prokaryotic mRNA is only about two minutes, therefore there is hardly any time for regulating the activity. In eukaryotes, the process of translation takes place in the cytoplasm. The mRNA transcribed are processed in the nucleus before their message is translated. Processing of mRNA is also one of the methods of regulation of gene activity. During mRNA processing, genes which are not of importance and which are not of use at the moment are spliced. You have already studied the processing of mRNA in Unit 13.

In prokaryotes genes that are transcribed last appear to be translated at a lower rate than the genes transcribed first. For example the three structural genes of the lac operon are transcribed roughly in ratios of 10:5:2. This is due to the polarity of the translation process, i.e. translation of the genetic code moves in the 5' to 3' direction. Therefore, genes located at the beginning of the operon will be available for translation before the genes at the end.

Translational control is also exerted by the efficiency with which the mRNA is bound to the ribosome. Different sequences in mRNA have different efficiencies of binding with ribosomes, and will be translated at different rates. The genetic code also plays a part in the translational control of some proteins, since different tRNAs occur in the cell in different quantities. Genes that cause the production of a large amount of protein products may have codons that specify the more common tRNAs. Genes that code for proteins not needed in abundance, have several codons specifying the rarer tRNAs, which would slow down the process of translation of these genes.

In eukaryotes translation of mRNA is regulated in the following three ways:

1. Control of the life period of the mRNA;
2. Control at the initiation of translation; and
3. By regulating the rate of overall protein synthesis.

1. An average eukaryotic mRNA molecule has a life of about three hours, before it is degraded. However, some mRNA survive for several days during which each mRNA molecule is translated repeatedly to yield large amounts of proteins.

Production of a large amount of a single type of protein by prolonging the life of an mRNA is common in highly differentiated cells. For example, cells of the chick oviduct, which make ovalbumin (egg white) and fibroin mRNA of silk glands of the silk worm, which synthesises a single type of protein, the silk fibroin. This mode of protein production is called gene amplification or translational amplification.

2. In some eukaryotes translation is regulated by controlling its initiation. For example, unfertilised sea urchin eggs store large quantities of mRNA. The mRNA remain inert, without being translated. Translation begins immediately after fertilisation.

3. Translation of mRNA is also kept under control by regulating the overall rate of protein synthesis. For example production of haemoglobin is regulated in this way. Haemoglobin is synthesised in cells called reticulocytes. Reticulocytes are the penultimate cell type in the line of differentiation leading to red blood cells. In the reticulocytes, there is one molecule of hemin per globin subunit. But for optimal efficiency, globin molecule should be synthesised at twice the rate of hemin synthesis. To maintain this ratio, a two phase regulatory system operates in the cells.
i) Hemin represses its own synthesis, and
ii) Hemin inactivates an inhibitor of globin synthesis.

With this system, if there is more hemin than globin, hemin synthesis is shut off and globin synthesis is stimulated. On the other hand, if there is not enough hemin, globin synthesis is repressed and hemin synthesis is switched on. In this way, the amount of hemin and globin are kept in balance.

In plants, gene expression is also regulated by light. The cells of many higher plants lose their chlorophyll resulting in the loss of their green colour, when they are kept in darkness for several days. This is due to the loss of production of enzymes that catalyse chlorophyll synthesis. When such plants are exposed to light again, within a few hours synthesis of photosynthetic enzymes and chlorophyll starts.

14.4.3 At Post-translational Level

In the previous sections you have studied that synthesis of proteins is regulated at transcription and at translational levels. A cell not only has an ability to stop transcription, and hence translation, it can also prevent the enzymes already translated. Control at post-translational levels is necessary to regulate the functioning of the enzymes in order to maintain the internal environment of the organisms in a dynamic steady state. In Unit 10 you have already studied various mechanisms, by which the activities of the enzymes are regulated.

14.4.4 Chemical Inhibitors of Protein Synthesis

In the previous sections you have read about regulation of protein biosynthesis at transcription, translation and at the post-translational levels. All these mechanisms are endogenous, operating in the cell itself. Protein biosynthesis can also be regulated exogenously by using certain drugs/chemicals.

Many antibiotics inhibit prokaryotic protein synthesis. This results in growth arrest or death of the bacterium. Most antibiotics do not interact with the specific proteins of eukaryotes and thus are not toxic to eukaryotes. Antibiotics are hence exploited for clinical purposes. Puromycin, an antibiotic is a structural analog of tyrosinyl—tRNA. It is incorporated in bacterial protein synthesis via the A-site on the ribosome, into the carboxyl terminal position of a peptide causing premature release of the polypeptide.

Many antibiotics are not clinically useful but have helped elucidate the role of protein synthesis in the regulation of metabolic processes, particularly enzyme induction by hormones. Table 14.2 and Fig. 14.5 summarise a few antibiotics that inhibit translation of protein biosynthesis in prokaryotes.

<table>
<thead>
<tr>
<th>Table 14.2: Antibiotic Inhibitors of Translation</th>
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<tbody>
<tr>
<td>Eukaryotes (Cytoplasm)</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Initiation</strong></td>
</tr>
<tr>
<td>Aurintricarboxylic acid</td>
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<tr>
<td>Elongation</td>
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<tr>
<td>Amicetin</td>
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<tr>
<td>Anisomycin</td>
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<tr>
<td>Chloramphenicol</td>
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<tr>
<td>Cycloheximide</td>
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<tr>
<td>Fusidic acid</td>
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<tr>
<td>Lincomycin</td>
</tr>
<tr>
<td>Puromycin</td>
</tr>
<tr>
<td>Sparsomycin</td>
</tr>
<tr>
<td>Tetracyclines</td>
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<tr>
<td><strong>Termination</strong></td>
</tr>
<tr>
<td>Anisomycin</td>
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<tr>
<td>Amicetin</td>
</tr>
<tr>
<td>Erythromycin</td>
</tr>
<tr>
<td>Lincomycin</td>
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<tr>
<td>Sparsomycin</td>
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<tr>
<td>Streptomycin</td>
</tr>
</tbody>
</table>

* = inhibition; - = no inhibition; * = stimulation; ? = unknown.
In this unit you have studied the various steps involved in the biosynthesis of proteins in prokaryotes and in eukaryotes. You have also studied the processing of the precursor proteins in eukaryotes and also about various methods that regulate synthesis of proteins in the organisms. We now summarise what we have learnt in this unit.

- Protein synthesis is a very complex process, involving participation of a large number of enzymes and other specific biomolecules.
- The mRNA has a large number of triplet bases, each triplet forming a codon. The codons pair with anticodons of the tRNA. This pairing follows the A-U, G-C combination.
- The translation process consists of activation of amino acids, transfer of activated amino acids to tRNA, initiation of polypeptide chain synthesis, chain elongation and chain termination.
- The rate of protein synthesis varies from one individual to another. In eukaryotes specific protein kinases control the rate of protein synthesis by inhibiting the initiation of polypeptide chain synthesis.

### 14.5 SUMMARY

In this unit you have studied the various steps involved in the biosynthesis of proteins in prokaryotes and in eukaryotes. You have also studied the processing of the precursor proteins in eukaryotes and also about various methods that regulate synthesis of proteins in the organisms. We now summarise what we have learnt in this unit.

- Protein synthesis is a very complex process, involving participation of a large number of enzymes and other specific biomolecules.
- The mRNA has a large number of triplet bases, each triplet forming a codon. The codons pair with anticodons of the tRNA. This pairing follows the A-U, G-C combination.
- The translation process consists of activation of amino acids, transfer of activated amino acids to tRNA, initiation of polypeptide chain synthesis, chain elongation and chain termination.
- The rate of protein synthesis varies from one individual to another. In eukaryotes specific protein kinases control the rate of protein synthesis by inhibiting the initiation of polypeptide chain synthesis.
Most of the proteins synthesised are not biologically active. They undergo various covalent modifications to attain biologically active forms.

Biosynthesis of proteins is regulated at the transcriptional level, translational level and even after translation. There are some antibiotics, which inhibit prokaryotic protein synthesis.

14.6 TERMINAL QUESTIONS

1 What constitute an initiation complex in prokaryotes and in eukaryotes? Discuss briefly in the space given below.

2 Explain the function of Release Factors RF-1, RF-2 and RF-3 in prokaryotes.

3 Briefly explain the processing of the precursor polypeptides of the collagen.

4 Explain briefly the role of histones in regulation of gene expression.

5 List a few antibiotics that inhibit translation of protein biosynthesis in bacteria.
   1) ............................................
   2) ............................................
   3) ............................................

14.7 ANSWERS

SAQs

1. A. i) amino acid-tRNA-synthetase  ii) 50S
      iii) P, A, P  iv) Translocation

B. For answer please see Section 4.2.1.

C. IF-3 prevents recombining of 30S and 50S subunits of the ribosomes.
2 A. i) Blobel and his colleagues in 1975
   ii) 5'

   B. i) Phosphorylation
       ii) Methylation
       iii) Nucleotidation
       iv) Attachment of carbohydrate side chain.
       v) Addition of prosthetic group
       vi) Formation of disulphide cross links.

   C. Signal peptidase cleaves the signal peptide from the growing polypeptide chain after it enters the tunnel of the endoplasmic reticulum.

3 A

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<td>1)</td>
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<td>3)</td>
<td>i)</td>
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B i) Ecdysone, Balbiani Rings
    ii) growth arrest, death

C Refer to page 96 for the answer.

**Terminal Questions**

1 In prokaryotes, the initiation complex consists of 70S ribosome — f methionine — tRNA — mRNA. In eukaryotes it consists of 80S ribosome — methionine — tRNA — mRNA.

2 Release factors (RF) cause hydrolysis of the peptide bond between the completed polypeptide chains and tRNA. As a result, the polypeptide chain is released. There are three types of release factors; RF-1, RF-2 and RF-3. RF-1 is specific for the termination codon UAA and UAG. RF-2 is specific for UAA and UGA. RF-3 stimulates the activity of RF-1 and RF-2.

3 Refer to page 93 for the answer.
4 Refer to page 95 for the answer.
5 Refer to Table 14.2 for the answer.