

III B.Sc Biochemistry – Molecular Biology

SHRIMATI INDIRA GANDHI COLLEGE
(Nationally Accredited at “A” Grade (3rd cycle) By NAAC)
Tiruchirappalli -2

TUTORIAL MATERIAL
MOLECULAR BIOLOGY

By

DR.B.Varalakshmi, Associate Professor,

Department of Biochemistry

2017 -2018



DEPARTMENT OF BIOCHEMISTRY
SHRIMATI INDIRA GANDHI COLLEGE

**B.Sc BIOCHEMISTRY
MOLECULAR BIOLOGY**

S.NO	CONTENTS	PAGE NO
1.	PART A QUESTIONS	3
2.	MECHANISM OF DNA REPLICATION IN PROKARYOTES	19
3.	ENZYMES INVOLVED IN DNA REPLICATION	14
4.	TRANSCRIPTION	19
5.	DIFFERENCES BETWEEN DNA AND RNA	20
6.	POST TRANSCRIPTIONAL PROCESSING OF RNA	21
7.	MECHANISM OF SPLICING	23
8.	GENETIC CODE	25
9.	TRASLATION IN PROKARYOTES	26
10.	TRASLATION IN EUKARYOTES	37

MOLECULAR BIOLOGY

PART A QUESTIONS

STRUCTURE OF DNA

1. Compare and contrast Heterochromatin and Euchromatin.

Heterochromatin:

Transcriptionally inactive, methylated, deacetylated histones, dark-staining on electron microscopy.

Euchromatin:

Transcriptionally active, unmethylated DNA, acetylated histones, light-staining on electron microscopy

2. SRP (signal recognition peptide) is important for what cell function?

The SRP binds to the N-terminal amino acid signal during initial synthesis of proteins destined for the plasma membrane or the organelles of the endocytic or exocytic pathways. SRP binding to the newly synthesized N-terminal signal sequence arrests synthesis on free ribosomes to provide time for the complex to bind to an SRP receptor on the RER. Subsequently the SRP is released, which allows protein synthesis to continue simultaneously with proper insertion into the RER membrane or RER lumen

3. Which motor proteins are directed towards the (+) end of microtubules, i.e. those ends that are located towards the outside of the cell and are used in anterograde transport?

Kinesins are (+) directed motor proteins

4. Mention the types of nucleotides in DNA.

Deoxy adenosine monophosphate/ dAMP /d - Adenylic acid. Deoxy guanosine monophosphate/ dGMP /d - Guanylic acid. Deoxy Cytidine monophosphate/ dCMP /dCytidylic acid. Deoxy thymidine mono phosphate/ dTMP /d - Thymidylic acid

5. Name the pentose sugar present in DNA. Name the pentose sugar present in RNA.

Deoxyribose Ribose

6. Mention the name s of pyrimidines of DNA.

Cytosine and Thymine

7. Mention the name s of pyrimidine s of RNA

Uracil and Cytosine.

8. Mention the names of purines of DNA

III B.Sc Biochemistry – Molecular Biology

Adenine and Guanine

9. Mention the names of purines of RNA

Adenine and Guanine

10. What phase of the cell cycle do these errors in replication occur in?

Microsatellite instability can predispose to cancer. These result from errors in replication, which occur during S-phase.

11. What is a nucleoside? What is a nucleotide?

It is a combination of nitrogenous base pentose sugar. Nitrogenous base pentose sugar and phosphate.

12. Name the unstable RNA. Name the least occurring type of RNA..

Messenger RNA.

13. Name the smallest RNA. Name the RNA capable of carrying amino acids.

Transfer RNA.

14. Name the scientist who discovered mRNA

Volkin.

15. Name the most abundant RNA. Name the largest RNA

Ribosomal RNA. rRNA

16. Name the scientist who proposed the fine structure of gene

Seymour Benzer

17. Give reason – why DNA is acidic in nature?

Due to the presence of phosphoric acid or phosphate group

DNA REPAIR

18. What special repair of the replication errors that occur in the cell cycle S phase, happen in what subsequent phase?

Repair of replication errors occurs during G2 (specifically mismatch repair)

19. What are the 5 phases of the cell cycle? Which phase do inactive cells live most of their lives in? Which phase do active cells spend the most time in?

The five phases are G0, G1, S, G2, and M. Inactive cells spend most of their life cycles in G0 (e.g. neurons). Active cells spend most of their life cycles in G1 (e.g. intestinal epithelial cells).

III B.Sc Biochemistry – Molecular Biology

20. DNA repair of what error can take place in the S phase of the cell cycle? Which method of repair is employed?

Proofreading action of DNA polymerase III (prokaryotes) and DNA polymerase δ and ϵ (eukaryotes) removes incorrect bases with a 3' to 5' exonuclease activity.

21. DNA repair of what error can take place in the G2 phase of the cell cycle? Which method of repair is employed?

DNA mismatch base repair occurs here via hMLH1 and hMSH2 gene activity

22. How does methylation of certain sequences of DNA affect the transcription of those regions.

Methylation of DNA (particularly CG sequences) are typically associated with silencing certain gene regions

23. Name some of the most important genes associated with maintaining fidelity of replicating DNA, and whose loss of function is associated with the development of cancer.

G0 phase: XP (thymine dimer- bulky lesion-repair)

G1 phase (check point): Rb, TP53

G2 phase: MLH1, MSH2 (mismatch repair)

S phase: DNA polymerase proofreading during DNA synthesis.

24. What type of DNA damage is base excision repair used for?

Base excision repair recognizes and repairs individual bases damages by chemical modification (e.g. deamination of cytosine to uracil)

25. What is Photolyase and what is its job? What other cellular mechanisms perform the same function?

Photolyase is an enzyme that mediates the direct repair of ultraviolet (UV) radiation-induced pyrimidine dimers by breaking the abnormal covalent bonds between the adjacent pyrimidines. Global genomic nucleotide excision repair and Transcription-coupled nucleotide excision repair perform similar functions.

26. Xeroderma pigmentosum is due to defects in what type of cellular repair mechanism?

Deficiencies in nucleotide excision repair proteins lead to the development of Xeroderma pigmentosum.

DNA REPLICATION

27. What is the job of DNA Topoisomerase vs. DNA Helicase?

DNA Topoisomerase relieves the tension on the DNA upstream and downstream of the replication fork by cutting the DNA allowing it to unravel, and resealing the nick. DNA Helicase uses ATP energy to unwind the dsDNA at the replication fork.

28. Where are DNA polymerases located in the Nucleus or Nucleolus

DNA Polymerases are largely restricted to the nucleus, but not to the nucleolus, because it participates in DNA replication, rather than ribosome synthesis.

29. Which form of chromatin is loosely packed and transcriptionally active? Is this the form that DNA takes during mitosis

Euchromatin is seen as the loosely packed, "open", transcriptionally active form of chromatin. During mitosis Euchromatin becomes condensed to form Heterochromatin, in which the nucleic acid wraps tightly around histones.

30. Why DNA replication is called semi - conservative

Parental strands are conserved in daughter DNA molecule. Or

Daughter molecule has one parental strand and one new strand

TRANSCRIPTION

31. What type of RNA polymerase is confined to the Nucleolus of the cell?

The nucleolus is the structure of the cell that is required for ribosomal synthesis. While all RNA polymerases are located in the nucleus, only RNA Polymerase I is restricted to the nucleolus, because it is involved in the synthesis of 28S, 18S, and 5.8S rRNAs, which are employed for ribosome synthesis.

32. What is the function of RNA Polymerase II? Where is it found

RNA polymerase II is the primary polymerase that transcribes DNA to RNA. It is found all around the nucleus

33. What are the jobs of RNA Polymerase III?

RNA Polymerase III synthesizes tRNA, but also rRNAs and other small RNAs found in the cytosol and nucleus

34. RNA Polymerase III synthesizes tRNA, but also rRNAs and other small RNAs found in the cytosol and nucleus.

TYPES OF RNA AND ITS PROCESSING

35. Name the codon with double function.

AUG.

36. Why Chargaff's rule is not applicable for RNA?

Because RNA is single stranded.

37. Why the nucleotide ratio in RNA is not usually constant?

Due to the absence of complementary base pairing, RNA is single stranded.

38. Why is processed mRNA in eukaryotes is shorter than its gene?

Because the eukaryotic gene is split gene and the transcribed mRNA has intron portions.

39. What are introns?

The nucleotide sequence is found between the exons and do not code for amino acids

40. Name the cell organelle where protein synthesis takes place?

Ribosome.

41. Write the central dogma of life

DNA ----- Transcription RNA ---- Translation PROTEIN

42. When does Posttranscriptional regulation occurs?

Posttranscriptional regulation occurs after transcription but before translation.

43. What are some of the key sequences within a promoter that RNA polymerase binds to ?

“Pribnow box”, CAAT box, GC box

44. What are all type post-transcriptional modifications involved in the control of gene expression.?

mRNA processing, methylation, polyadenylation

TRANSLATION

45. Name the enzyme, which directs DNA synthesis by RNA. Name the pentose sugar present in RNA

Reverse transcriptase , Ribose

46. Name the pyrimidine, present in DNA, but not in RNA . Name the pyrimidine, present in RNA, but not in DNA.

Thymine, Uracil

III B.Sc Biochemistry – Molecular Biology

47. Why codons are redundant?

Codons are redundant because, single amino acid can be coded by two or three codons

48. Why codons are sensible?

Codons codes for a specific amino acid.

49. Why redundancy concept of genetic code does not apply to all amino acids

Some amino acids like tryptophan and methionine have one codon each.

50. During translation, if the codon is AUG, then, What is the anti codon present on the complimentary tRNA ?

UAC. During translation, if the codon is

AUG, then Name the amino acid carried by this tRNA Methionine

51. How many amino acids are present in a nascent polypeptide decoded from mRNA with the reading frame having 1002 nucleotides?

333 amino acids. Out of 334 amino acid, methionine being first amino acid, which will be removed off, when processing of polypeptide chain takes place

52. What are non – sense codons? Mention 2 of them.

These codons do not code for any amino acids. When these codons appear on mRNA termination of polypeptide chain takes place. UAA,UGA,UAG

53. Where are the codons and anticodons

Codons are present in mRNA and code for Amino acids during proteinsynthesis, Anticodons are present in tRNA and recognise codons on mRNA.

54. What are enhancer elements?

Enhancer elements are DNA sequences that associate with certain proteins that enhance transcription. DNA can fold upon itself to bring any enhancer element into close proximity to a promoter region.

55. Which evidence that primitive life forms lacked both DNA and enzymes.?

RNA can both code genetic information and act as a catalyst

REGULATION OF GENE EXPRESSION

56. Bacterial protein called catabolic activator protein (CAP) is an example of which type of regulation?

second type of positive control of gene expression

57. What is epigenetic gene regulation?

III B.Sc Biochemistry – Molecular Biology

DNA is regulated other than the sequence is called epigenetic regulation. DNA methylation and chromatin structure also involve in regulation of gene expression.

58. In *E. coli*, the inability of the lac repressor to bind an inducer would result in
- (A) No substantial synthesis of β -galactosidase
 - (b) Constitutive synthesis of β -galactosidase
 - (c) Inducible synthesis of β -galactosidase
 - (d) Synthesis of inactive β -galactosidase
 - (e) Synthesis of β -galactosidase only in the absence of lactose
59. If the genetic code consisted of four bases per codon rather than three, the maximum number of unique amino acids that could be encoded would be
- (A) 16
 - (B) 64
 - (C) 128
 - (D) 256
60. In humans, the Barr body is an
- (A) Active X chromosome in females
 - (B) Active X chromosome in males
 - (C) Inactive Y chromosome in males
 - (D) Inactive Y chromosome in females
 - (E) Inactive X chromosome in females
61. Which of the following statements about retrotransposons is correct?
- (A) They transpose via an RNA intermediate.
 - (B) They contain genes for ribosomal proteins.
 - (C) They possess a gene for RNA-dependent RNA polymerase.
 - (D) They possess genes that encode proteins that integrate RNA into chromosomes.
 - (E) They are found only in bacteria.
62. A mutation deleting an upstream activating sequence for a single gene would be expected to be
- (A) Polar
 - (B) Trans -dominant
 - (C) Cis -dominant

(D) Silent

(E) Reversible

SHORT ANSWER AND ESSAYS

MECHANISM OF DNA REPLICATION IN PROKARYOTES

1.Explain the stages of DNA replication in prokaryotes.

The three main phases of DNA replication in prokaryotes. The phases are: 1. Initiation 2. Elongation 3. Termination.

Phase # 1. Replication Initiation:

Replication initiation involves the following events:

- (1) Recognition of origin,
- (2) DNA melting, i.e., separation of the two strands in the origin region,
- (3) Stabilization of the single strands,
- (4) Assembly of primosome at the two forks so produced, and finally, and
- (5) Start of synthesis of the two daughter strands.

Replication initiation in *E. coli* requires 6 proteins, viz., DnaA, DnaB, DnaC, HU, gyrase and SSBP (single strand binding proteins). First, 2-4 molecules of DnaA bind *oriC*; this results in the folding of the origin *Oric* DNA around DnaA aggregate. As a result, DnaA now induces melting at *OricC*. Now an aggregate having 6 molecules each of DnaB and DnaC binds to each of the three separate single-stranded regions produced by DnaA.

The aggregate eventually displaces DnaA, and DnaC loads the DnaB hexamer at the two forks produced by melting. DnaB functions as helicase and begins to unwind the DNA. Gyrase facilitates unwinding by helicase as it provides a swivel. SSBP bind to the single-stranded regions so produced and stabilize them. Initiation of replication generally requires ~ 60 bp of unwound DNA, and the process consumes ATP. One DnaB hexamer binds to each of the two forks produced by unwinding at the origin (Fig. 28.10).

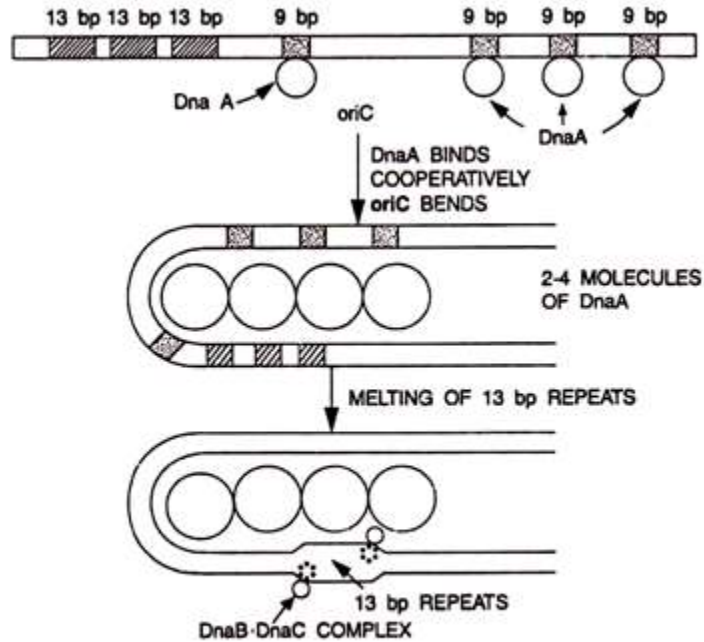


FIG. 28.10. In *E. coli*, replication initiation begins with binding of DnaA to *oriC*, which induces melting. DnaB (☆) then attaches to the potential forks and begins unwinding.

Once a replication fork is generated, primosome assembles at the origin, and initiates primer synthesis; this is called priming. Priming occurs only once and at the origin for the replication of the leading strand. But for replication of the lagging strand, priming occurs repeatedly at intervals of 1000 to 2000 bases.

Priming reaction at *oriC* is rather simple the primosome consists of a single protein, DnaG. DnaG needs to be activated by DnaB. DnaB also serves as helicase, while DnaG carries out primer synthesis; primers of 15-50 bases are normally synthesized.

The replication fork proceeds in the 5'→3' direction in relation to the lagging strand. The replication fork advances and generates a single-stranded region of the lagging strand bound to SSBP ahead of the primosome. The primosome moves along this single-stranded region. When the primosome reaches a site at which priming can occur, it synthesizes an RNA primer. This primer sponsors synthesis of a new Okazaki fragment (Fig. 28.11).

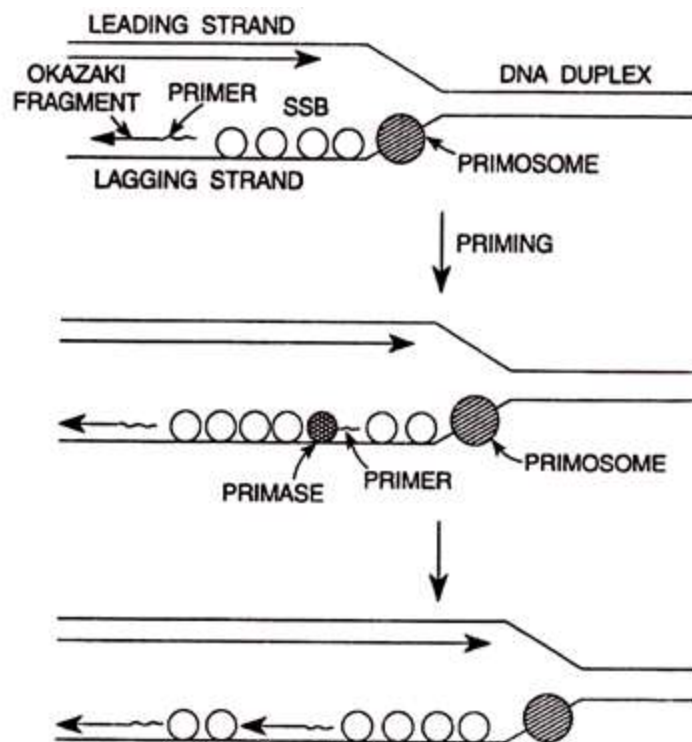


FIG. 28.11. A simplified schematic representation of the events involved in priming during replication of the lagging strand. Primosome may consist of simply DnaG at *oriC* or DnaG plus 5 other proteins in case of ϕ X type replicons.

Energy from ATP is required during:

- (1) Melting of DNA by DnaA,
- (2) Release of DnaB at the forks by DnaC,
- (3) Helicase action of DnaB,
- (4) Swivel action of DNA gyrase,
- (5) Activation of primase DnaG by DnaB, and
- (6) Activation of DNA polymerase III to begin replication.

Phase 2. Primer Elongation (DNA Replication):

Once the primer has been synthesized, DNA synthesis is taken up by replisome, which is a complex of proteins. In *E. coli*, DNA replication activity is provided by DNA polymerase III component of replisome.

Each *E. coli* cell has ~ 10 molecules of DNA polymerase III; most of these molecules are associated with replication forks. The complete enzyme, holoenzyme, molecule has the following subunits; α_2 , θ_2 , $\epsilon_2\gamma$, χ , ψ , δ , δ' , τ_2 , β_4 (Fig. 28.12).

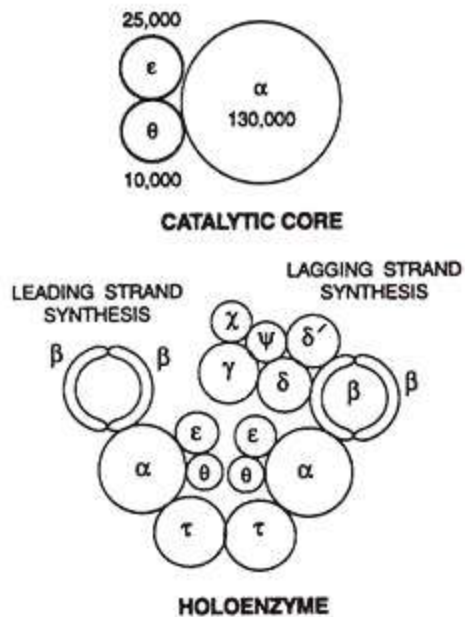


FIG. 28.12. A schematic representation of the organization of DNA polymerase III catalytic core and holoenzyme molecule.

The enzyme is assembled at the replication fork as follows:

1. First, the γ - δ complex (subunits γ δ δ' X ψ) or 'clamp loader' and a pair of β subunit (the 'clamp') recognize the primed-template and bind to it.
2. They now attach to a catalytic core (α θ ϵ subunits).
3. Subunit τ now joins the complex. It brings two more β subunits and another catalytic core to the complex. This generates a DNA polymerase III holoenzyme.

According to one model, a single holo-enzyme molecule functions at one replication fork. Each holoenzyme molecule has 2 catalytic cores; one catalytic core catalyzes the replication of leading strand, while the other catalyzes that of the lagging strand (Fig. 28.13).

In the case of leading strand, the catalytic core extends the primer one nucleotide at a time. DnaB progressively unwinds the duplex and the replication fork moves along.

Replication of the lagging strand will begin sometime later. When DnaB associated with the advancing fork reaches a site suitable for priming, it activates DnaG to synthesize a primer in the normal $5' \rightarrow 3'$ direction, i.e., moving from the fork toward the origin. When the primer become 10-14 bases long, the other catalytic core begins to elongate this primer in the $5' \rightarrow 3'$ direction.

The lagging strand, is in effect, pulled up by the replisome in the process of replication; it therefore, forms a progressively larger loop between the fork and the replisome (Fig. 28.13).

III B.Sc Biochemistry – Molecular Biology

When the replisome reaches the 5'-end of the primer of the previous Okazaki fragment, it stops replication and dissociates from the lagging strand. Meanwhile DNAB continues to move forward with the replication fork. When it reaches the appropriate site, it again induces primer synthesis by DnaG and the events described above take place again.

In eukaryotes, two different enzymes are used to replicate the leading and the lagging strands. Leading strand is replicated by DNA polymerase δ , while replication of the lagging strand is due to DNA polymerase ϵ . Primase activity is due to DNA polymerase α , which primes both the leading and the lagging strands. It also begins to synthesize DNA using this primer, but is soon replaced by DNA polymerase δ (in the case of leading strand) and ϵ (in the case of lagging strand).

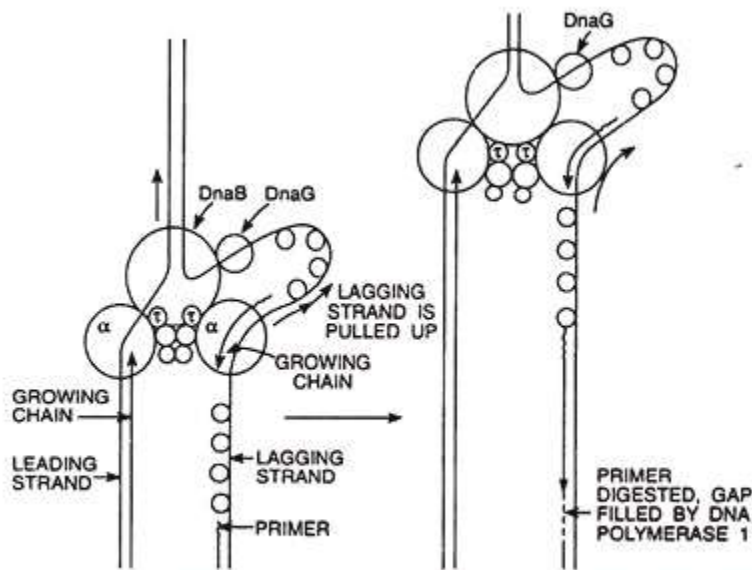


FIG. 28.13. Coordinated synthesis of leading and lagging strands by the same holoenzyme molecule of DNA polymerase III.

Phase # 3. Termination of DNA Replication:

In *E. coli*, termination is signalled by specific sequences called *ter* elements, which serve as a binding site for protein Tus. Tus protein binds to *ter* element and stops DnaB from unwinding DNA.

This stops the movement of the replication fork. The leading strand is replicated up to the *ter* element, while the lagging strand replication is stopped 50-100 bp before the *ter* element. It is significant that Tus protein is able to stop fork movement in only one direction.

ENZYMES INVOLVED IN DNA REPLICATION

2. Explain the role of various enzymes involved in DNA replication

III B.Sc Biochemistry – Molecular Biology

Both the prokaryotic and eukaryotic cells contain three types of nuclear enzymes that are essential for DNA replication. These enzymes are nucleases, polymerases and ligases.

(i) Nucleases:

The polynucleotide is held together by phosphodiester bonds. The nucleases hydrolyse the polynucleotide chain into the nucleotides. It attacks either at 3' OH end or 5' phosphate end of the chain. The nucleases are of two types (Fig. 5.17-B).

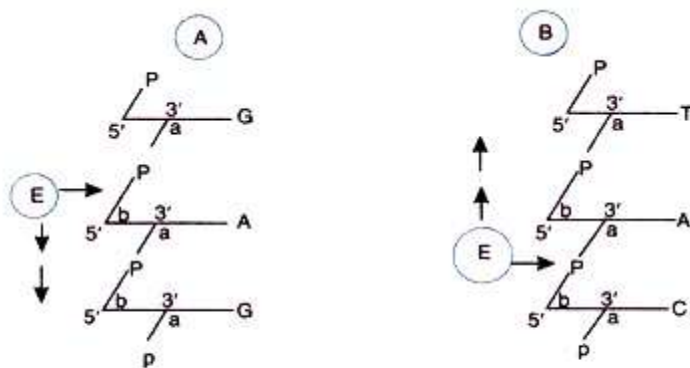


Fig. 5.17 : Exonuclease action on a polynucleotide chain, A, action in 5'→3' direction; B, action in 3'→5' direction; a 3' OH side of phosphodiester linkage; b, 5' side of phosphodiester linkage.

(a) Exonucleases:

The nuclease that attacks on outer free end of the polynucleotide chain is called exonuclease. It breaks phosphodiester bond either in direction (A) or in 3'→5' direction (B). The enzyme moves in either cases stepwise along the chain and removes nucleotides one by one. Thus, the whole chain is digested.

(b) Endonucleases:

The endonucleases attack within the inner portion of one or the double strands. Therefore, a nick is made on double stranded DNA molecule. However, if the polypeptide chain is single stranded (e.g. in DNA viruses), the attack of endonuclease will render the chain into two pieces.

On double stranded DNA the nick contains two free ends that in turn act as template for DNA replication. Apart from this, the nicked double helix is distorted due to rotation of free molecules around its intact strand.

3. Explain the role of DNA Polymerases:

DNA polymerases carry out the process of polymerization of nucleotides and formation of polynucleotide chain. This enzyme is called replicase when it replicates the DNA molecules and inherited by daughter cells. In 1959, for the first time A. Romberg discovered an enzyme in *E. coli* which polymerized the deoxyribonucleotide triphosphate on a DNA template and produced complementary strand of DNA.

This enzyme was called DNA polymerase. Later on it was named as Komberg polymerase or Romberg enzyme after the name of discoverer, for demonstrating in vitro polymerization of DNA. For the catalysis of polymerization, it requires the four deoxyribonucleotide

III B.Sc Biochemistry – Molecular Biology

triphosphates e.g. dATP, dGTP, dTTP and dCTP, a DNA template, a primer for initiation of catalytic activity and Mg^{++} (Fig. 5.18).

In prokaryotes, three types of DNA polymerases e.g. polymerase I (Poly-I), polymerase II (Pol II), and DNA polymerase III (Pol III) are found, whereas in eukaryotes three or four polymerases termed as α , β and γ polymerases and mitochondria (mt) DNA polymerase are present.

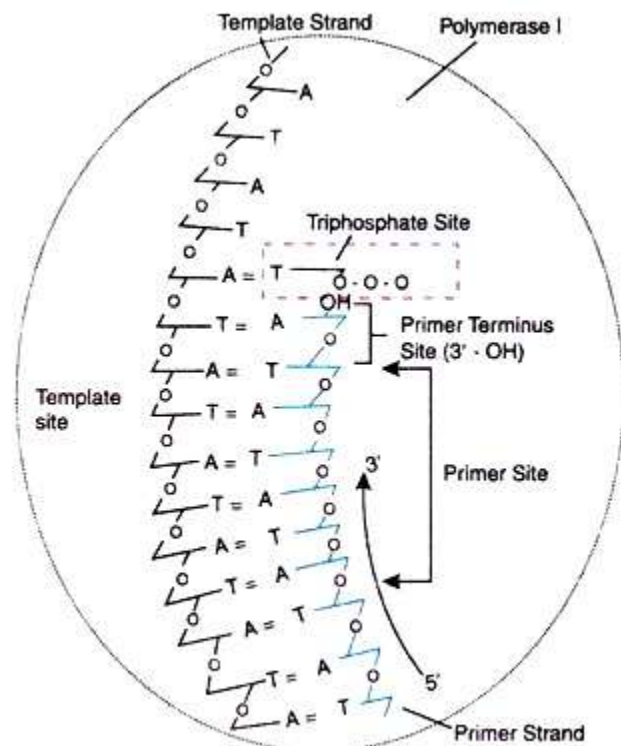


Fig. 5.18 : Diagram of DNA polymerase I of *E. coli*.

The molecular weight of α and γ polymerases are over 100,000 and that of β -polymerase is 30,000-50,000. The α and β polymerases are located in the nucleus. The β -polymerase copies a poly (A) or poly (C) template. The γ -polymerase copies many poly-ribonucleotides such as poly (A), poly (C), etc. The mtDNA polymerase is like γ -polymerase.

(a) Polymerase I (Pol I):

The Kornberg polymerase is known as Pol I. It is a single peptide chain with a molecular weight of 109,000 D. It is the largest single chain of globular protein known so far. One atom of zinc (Zn) per chain is present, therefore, it is metalloenzyme. In *E. coli*, approximately 400 molecules of Pol I are present.

Early experiments carried out by Kornberg revealed that when artificially synthesized DNA template strands alternating A and T i.e. poly d(AT) were incubated with polymerase and four radio- labelled nucleoside triphosphate, radioactive DNA containing alternating A and T was synthesized.

III B.Sc Biochemistry – Molecular Biology

Though sufficient amount of dGTP and dCTP was present in the solution but these were not synthesized into DNA because the DNA strand contained only poly dAT. This emphasizes that Pol I synthesizes only complimentary copy of the template.

Shape of Pol I has been studied through electron microscope. It is roughly spherical of about 65 Å diameters (Fig. 5.18) which gets attached regularly to the DNA chain.

Pol I possesses several attachment sites such as:

- (i) A template site for attachment to the DNA template,
- (ii) A primer site of about 100 nucleotides contemporary to a segment of RNA on which the growth of newly synthesized DNA occur,
- (iii) A primer terminus site containing a terminal 3'OH group at the tip, and
- (iv) A triphosphate site for matching the incoming nucleoside triphosphates according to complementary nucleotide of DNA template.

Function:

Pol I plays a significant role in polymerization (synthetic) as well as degradation (exonucleolytic) process of nucleotides, Pol I is broken by trypsin into two fragments, a large fragment (MW 75,000) and a small fragment (MW 36,000). The large fragment shows 3' → 5' exonuclease activity, and the small fragment shows 5' → 3' exonuclease activity. In E.coli the following three types of functions of Pol I have been found.

Polymerization:

Polymerization is a process of synthesis in 5' → 3' direction of short segments of DNA chain from deoxyribonucleoside triphosphate monomers to the 3' -OH end of a DNA strand. It is not the main polymerization enzyme because it cannot synthesize a long chain. It synthesizes only a small segment of DNA.

It binds only to a DNA and forms nick in dsDNA. Therefore, it takes part in repair synthesis. In E.coli Pol I polymerize the nucleotides at the rate of 1,000 nucleotides per minute at 37°C. The chief enzyme associated with polymerization is known as polymerase III.

Exonuclease activity:

3' → 5' exonuclease activity:

Pol I catalyses the breaking of one or two DNA strands in 3' → 5' direction into the nucleotide components i.e. the nucleotides are set free in 3' → 5' direction which is reverse to polymerization direction.

Therefore, it is called 3' → 5' exonuclease activity. Pol I correct the errors made during the polymerization, and edits the mismatching nucleotides at the primer terminus before the start of strand synthesis. Therefore, the function of Pol I is termed as repair synthesis.

5' → 3' exonuclease activity:

Pol I also breaks the polynucleotide chain in 5' → 3' direction with the removal of nucleotide residues. Upon exposure of DNA to the ultraviolet light two adjacent pyrimidines such as thymines are covalently linked forming pyrimidine dimers. These dimers block the replication of DNA. Therefore, removal of pyrimidine dimers e.g. thymine dimers (T=T) is necessary.

III B.Sc Biochemistry – Molecular Biology

Through $5' \rightarrow 3'$ exonuclease activity, Pol I removes pyrimidine dimers. Secondly, DNA synthesis occurs on RNA primer in the form Okazaki fragments. Through $5' \rightarrow 3'$ exonuclease activity Pol I removes RNA primer and seals the gap with deoxyribonucleotides. Its onward movement results in removal of ribonucleotides from the front portion followed by deoxyribonucleotides behind it.

(b) Polymerase II (Pol II):

For several years Pol I was considered to be responsible for replicating in E.coli. but the work done during 1970s made it clear that Pol I is associated only with repair synthesis and the other enzymes, Pol II and Pol III are involved in polymerization process. Pol II is a single polypeptide chain (MW 90,000) that shows polymerization in $5' \rightarrow 3'$ direction of a complementary chain.

It also shows exonuclease activity in $3' \rightarrow 5'$ direction but not in $5' \rightarrow 3'$ direction. The polymerization activity of Pol II is much less than Pol I in E.coli cells. About 50 nucleotides per minute are synthesized. E.coli cells contain about 40 Pol II molecules.

The $3' \rightarrow 5'$ exonuclease activity of Pol II shows that it also plays a role in repair synthesis or DNA damaged by U.V. light just like Pol I. In the absence of Pol I, it can elongate the Okazaki fragments. Therefore, Pol II is an alternative to Pol I.

(c) Polymerase III (Pol III):

DNA polymerase III is several times more active than Pol I and Pol II enzymes. It is the dimer of two polypeptide chains with molecular weight 1,40,000 and 40,000 D respectively. Pol III polymerises deoxyribonucleoside triphosphates in direction very efficiently. Therefore, Pol III is the main polymerization enzyme that can polymerize about 15,000 nucleotides per minutes in E. coli.

Like Pol II, it cannot polymerize efficiently if the template DNA is too long but can do when ATP and certain protein factors are present. Synthesis of a long template also occurs when an auxiliary protein DNA (co-polymerase II) is linked with Pol III and produced Pol III-co Pol II complex. In addition Pol III also shows $3' \rightarrow 5'$ exonuclease activity like Pol II.

The $5' \rightarrow 3'$ exonuclease activity is absent. All the polymerases e.g. Pol I, Pol II and Pol III show $3' \rightarrow 5'$ exonuclease activity, whereas besides Pol I, the other two polymerases (Pol II and Pol III) lack $5' \rightarrow 3'$ exonuclease activity. However, some workers have shown both $3' \rightarrow 5'$ and $5' \rightarrow 3'$ exonuclease activity in Pol III.

(iii) DNA Ligases:

The DNA ligases seal single strand nicks in DNA which has $5' \rightarrow 3'$ termini. It catalyses the formation of phosphodiester bonds between $3'$ -OH and $5'$ - PO_4 group of a nick, and turns into an intact DNA. There are two types of DNA ligases: E. coli DNA ligase and T4 DNA ligase. The E. coli DNA ligase requires nicotinamide adenine dinucleotide (NAD^+) as cofactor,

whereas T4 DNA ligase uses ATP as cofactor for joining reaction of the nick (Fig 5.19).

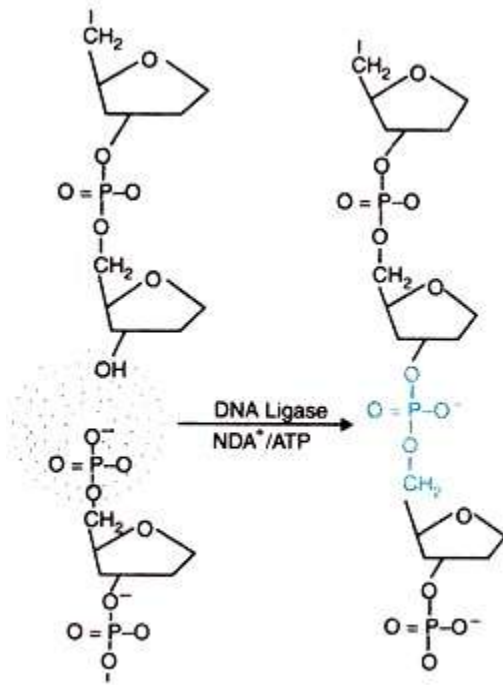


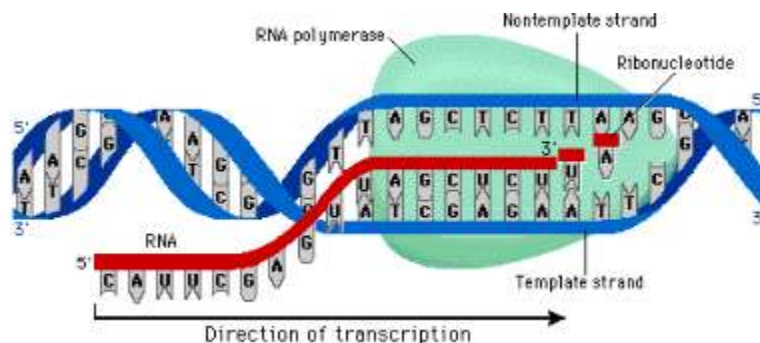
Fig. 5.19 : Action of DNA ligase in the presence of NAD⁺/ATP.

TRANSCRIPTION

4. Explain the process of Transcription with the help of a labelled diagram

Genetic DNA is confined to the nucleus. Since it is a macro molecule the nucleus membrane is impermeable. Hence DNA acts as the template for the synthesis of mRNA chain. Unwinding of the chain takes place by the enzyme unwindase. One of the chains becomes template for the synthesis of mRNA chain. This strand is called anti sense strand.

The strand complementary to this strand is called sense strand. mRNA synthesis takes place on the sense strand . The nucleotide sequences of DNA are coded on the m RNA is called transcription. mRNA synthesised is complimentary to DNA transcribed. Enzyme RNA polymerase polymerise s RNA nucleotides. Rewinding of DNA strands takes place by windase



5. Write a note on Inhibitors of Transcription

• Rifampicin- binds with Beta subunit of prokaryotic RNA polymerase, • It is an inhibitor of prokaryotic transcription initiation. • It binds only to bacterial RNA polymerase but not to eukaryotic RNA polymerases. • Therefore, Rifampicin is a powerful drug for treatment of bacterial infections. • Used for the treatment of tuberculosis and leprosy

6. Write the Mechanism of action of Actinomycin

Mechanism of action of Actinomycin D • Actinomycin D- Intercalates with DNA strands • Actinomycins inhibit both DNA synthesis and RNA synthesis by blocking chain elongation. • They interact with G·C base pairs as they require the 2-amino group of guanine for binding. • Actinomycins are used as anticancer drugs

Mitomycin • Mitomycin- Intercalates with DNA strands • blocks transcription, • used as anticancer drug

Alpha amanitin • Alpha amanitin is a molecule made from the “death cap” mushroom and is a known potent inhibitor RNA polymerase. • One single mushroom could very easily lead to a fast death in 10 days. • The mechanism of action is that alpha amanitin inhibits RNA polymerase –II at both the initiation and elongation states of transcription.

DIFFERENCES BETWEEN DNA AND RNA

7. List five differences between DNA and RNA

DNA	RNA
• Mostly double stranded	Single stranded, except in some viruses
• Nucleotides are AGCT	Nucleotides are AGCU

Pentose sugar DIFFERENCES BETWEEN DNA AND RNA

• is deoxy ribose	Pentose sugar is ribose
• It acts as the template for Transcription.	It involves in protein synthesis
• Types of DNA co - exist in a DNA molecule	There are three types mRNA, tRNA, rRNA
• It is hereditary material	Only in RNA viruses it is genetic material
• It is self replicating	RNA synthesis takes on DNA template

- It directs protein synthesis Directly produced proteins .
- It can produce RNA It generally does not produce DNA

POST TRANSCRIPTIONAL PROCESSING OF RNA

8. Explain Modifications of primary transcript in prokaryotes

- In prokaryotic organisms, the primary transcripts of mRNA-encoding genes begin to serve as translation templates even before their transcription has been completed.
- This is because the site of transcription is not compartmentalized into a nucleus as it is in eukaryotic organisms.
- Consequently, prokaryotic mRNAs are subjected to little processing prior to carrying out their intended function in protein synthesis
- Transcription and translation are coupled in prokaryotic cells.

9. Describe Post Transcriptional modifications in Eukaryotes

- All eukaryotic RNA primary transcripts undergo extensive processing whether it be as mRNA or as a component of the translation machinery such as rRNA, 5S RNA, or tRNA or RNA processing machinery, snRNAs.
- Processing occurs primarily within the nucleus and includes nucleolytic cleavage to smaller molecules and coupled nucleolytic and ligation reactions (splicing of exons).
- The processes of transcription, RNA processing, and even RNA transport from the nucleus are highly coordinated.

10. Briefly explain Processes involved in the post transcriptional modifications

- Some of the processes involved in the post transcriptional modifications of primary transcript of major RNAs are as follows- A) Ribosomal RNA • In mammalian cells, the three rRNA molecules are transcribed as part of a single large precursor molecules called Pre ribosomal RNAs

11. Describe Post Transcriptional modifications of Ribosomal RNA(r- RNA)

- The precursor is subsequently processed in the nucleolus to provide the RNA component for the ribosome subunits found in the cytoplasm.
- The 23S,16S, and 5S ribosomal RNAs of prokaryotes are produced from a single RNA precursor molecule as

are the 28S, 18S and 5.8S r RNAs of eukaryotes. • Eukaryotic 5S rRNA in eukaryotes is synthesized by RNA polymerase III and modified separately.

The 23S,16S, and 5S ribosomal RNAs of prokaryotes are produced from a single RNA precursor molecule • Cleavage and trimming are the mechanisms involved, • Similar modifications are observed in the processing of eukaryotic r-RNA.

12. Describe Post Transcriptional modifications of Transfer RNA (t- RNA)

The tRNA molecules serve as adapter molecules for the translation of mRNA into protein sequences. • Both eukaryotic and prokaryotic transfer RNAs are made from longer precursor molecules that must be modified. • The basic mechanisms involved are as follows. Splicing- An intron must be removed from the anticodon loop • Trimming- The sequences at both the 5' and 3' ends of the molecule are trimmed • Base modifications- The tRNAs contain many modifications of the standard bases A, U, G, and C, including methylation, reduction, deamination, and rearranged glycosidic bonds. Further modification of the tRNA molecules includes nucleotide alkylations.

CCA attachment • The attachment of the characteristic C_pC_pA-OH terminal at the 3' end of the molecule by the enzyme nucleotidyl transferase is the most important modification. • The 3' OH of the A ribose is the point of attachment for the specific amino acid that is to enter into the polymerization reaction of protein synthesis.

The extra nucleotides at both 5' and 3' ends of t- RNA are removed, an intron from the anticodon arm is removed, bases are modified and CCA arm is attached to form the mature functional t RNA.

13. Explain Post Transcriptional modifications of pre m- RNA

• In prokaryotic organisms, the primary transcripts of mRNA-encoding genes begin to serve as translation templates even before their transcription has been completed. • In all eukaryotes the primary transcripts of mRNA-encoding genes undergo extensive processing before they are converted to mature functional forms

5' Capping • Mammalian mRNA molecules contain a 7- methylguanosine cap structure at their 5' terminal. • The cap structure is added to the 5' end of the newly transcribed mRNA precursor in the nucleus prior to transport of the mRNA molecule to the cytoplasm. • The 5' cap of the RNA transcript is required both for efficient translation initiation and protection of the 5' end of

mRNA from attack by 5'-3' exonucleases. • Eukaryotic mRNAs lacking the cap are not efficiently translated.

- The addition of the Guanosine triphosphate (part of the cap) is catalyzed by the nuclear enzyme guanylyl transferase.
- Methylation of the terminal guanine occurs in the cytoplasm and is catalyzed by guanine-7- methyl transferase.
- S-Adenosyl methionine is the methyl group donor.
- Additional methylation steps may occur. The secondary methylations of mRNA molecules, those on the 2'-hydroxy and the N6 of adenylyl residues, occur after the mRNA molecule has appeared in the cytoplasm.

Addition of poly A tail • Poly(A) tails are added to the 3' end of mRNA molecules in a posttranscriptional processing step. • The mRNA is first cleaved about 20 nucleotides downstream from an AAUAA recognition sequence • Another enzyme, poly(A) polymerase, adds a poly(A) tail which is subsequently extended to as many as 200 A residues. • The poly (A) tail appears to protect the 3' end of mRNA from 3' 5' exonuclease attack. • Histone and interferon's mRNAs lack poly A tail. • After the mRNA enters the cytosol, the poly A tail is gradually shortened.

MECHANISM OF SPLICING

14. Explain the mechanism of Splicing

- Introns or intervening sequences are the RNA sequences which do not code for the proteins. • These introns are removed from the primary transcript in the nucleus, exons (coding sequences) are ligated to form the mRNA molecule, and the mRNA molecule is transported to the cytoplasm.

Role of small nuclear RNA (sn RNA) and Spliceosome

- The molecular machine that accomplishes the task of splicing is known as the spliceosome. Spliceosomes consist of the primary transcript, five small nuclear RNAs (U1, U2, U4, U5, and U6) and more than 60 proteins.

- Collectively, these form a small ribonucleoprotein (snRNP) complex, sometimes called a "snurp" (snRNPs)

Snurps are thought to position the RNA segments for the necessary splicing reactions.

- These facilitate the splicing of exon segments by forming base pairs with the consensus sequence at each end of the intron.

Splicing of m-RNA • The newly- feed 3'OH of the upstream exon 1 then forms a phosphodiester bond with the 5'end of the downstream exon 2. • The excised intron is released as a "lariat" structure, which is degraded • After removal of all the introns, the mature m RNA molecules leave the nucleus by passing in to the cytosol through pores in to the nuclear membrane.

15. Give the Clinical significance of Splicing

Antibodies against snRNPs In systemic Lupus Erythematosus (SLE), an auto immune disease, the antibodies are produced against host proteins, including sn RNPs.

Clinical significance of Splicing 2) Mutations at the splice site • Mutations at the splice site can lead to improper splicing and the production of aberrant proteins . • For example some cases of Beta thalassemia are as a result of incorrect splicing of beta globin m-RNA due to mutation at the splice site.

16. Explain Alternative Splicing

• Alternative patterns of RNA splicing is adapted for the synthesis of tissue-specific proteins. • The pre-m RNA molecules from some genes can be spliced in two or more alternative ways in different tissues. • This produces multiple variations of the m RNA and thus diverse set of proteins can be synthesized from a given set of genes.

Tissue specific tropomyosins are produced from the same primary transcript by alternative splicing. • Alternative splicing and processing results in the formation of seven unique α - tropomyosin mRNAs in seven different tissues

Biological significance of Splicing • Tissue specific proteins are produced from the same primary transcript by alternative splicing

17. Explain the Biological significance of Splicing

• Similarly, the use of alternative termination- cleavage-polyadenylation sites also results in mRNA variability. • Alternative polyadenylation sites in the immunoglobulin heavy chain primary transcript result in mRNAs that are either 2700 bases long (m) or 2400 bases long (s). • This results in a different carboxyl terminal region of the encoded proteins such that the m protein remains attached to the membrane of the B lymphocyte and the s immunoglobulin is secreted.

Biological significance of Splicing • By means of alternative poly A sites variability in mRNA can be produced and thus different proteins can be synthesized from a given set of genes

GENETIC CODE & TRANSLATION

18. Explain the Characteristics of genetic code.

Triplet code; the genetic code is a triplet code.

It means that three nucleotides of DNA code for one amino acid. eg : AUG.

The genetic code is universal; It means that a particular mRNA codon codes for the specific amino acid in all living organisms.

Genetic code is degenerate or redundant; some of the amino acids are coded by two or more codons

These redundant codons code for the same amino acids and are called degenerate codons.

eg : Valine has four codons GUG, GUU, GUC, GUA

Genetic code is non overlapping; In this property the base of the one codon is not shared by the neighbouring codon

Genetic code is comma less ;

Genetic code has no punctuation mark inside the message.

AUG is the initiator codon.

UAA UGA UAG is terminator codons

19. Write a short note on repetitive DNA.

(A) Repetitive DNA is associated with the centromeres and telomeres in higher eukaryotes. (B) Repetitive DNA is restricted to nontranscribed regions of the genome. (C) Repetitive DNA sequences are often found in tandem clusters throughout the genome. (D) Repetitive DNA was first detected because of its rapid reassociation kinetics. (E) Transposable elements can contribute to the repetitive DNA fraction.

17. Give the characteristic of a eukaryotic enhancer element?

(A) Its activity is independent of its orientation (i.e., the sequence can be inverted without effect).

- (B) Its activity is dependent on its distance from the start site of transcription.
- (C) It may be found as far as 1 to 2 kilobases from the promoter.
- (D) It may be positioned at the 5' end or the 3' end of the gene.
- (E) It increases the level of transcription of genes under its control.

TRANSLATION OR PROTEIN SYNTHESIS

20. Explain the components of translation in prokaryotes

Proteins are giant molecules formed by polypeptide chains of hundreds to thousands of amino acids. These polypeptide chains are formed by about twenty kinds of amino acids. An amino acid consists of a basic amino group (-NH₂) and an acidic carboxyl group (-COOH). Different arrangement of amino acids in a polypeptide chain makes each protein unique.

Proteins are fundamental constituents of protoplasm and building material of the cell.

They take part in the structural and functional organization of the cell. Functional proteins like enzymes and hormones control the metabolism, biosynthesis, energy production, growth regulation, sensory and reproductive functions of the cell. Enzymes are catalysts in most of the biochemical reactions. Even the gene expression is controlled by enzymes. The replication of DNA and transcription of RNA is controlled by the proteinous enzymes.

Components of Protein Synthesis:

Protein synthesis is governed by the genetic information carried in the genes on DNA of the chromosomes.

The main components of the protein synthesis are:

1. DNA
2. Three types of RNAs
3. Amino acids
4. Ribosomes
5. Enzymes.

DNA is the master molecule which possesses the genetic information about the sequence of amino acids in a polypeptide chain. Structure and properties of DNA regulate and control the synthesis of proteins.

DNA present in the nucleus sends out information in the form of messenger RNA into the cytoplasm, which is the site of the protein synthesis in eukaryotes. The messenger RNA carries

the information regarding the sequence of amino acids of the polypeptide chain to be synthesized. This message or information is in the form of a genetic code. This genetic code specifies the language of amino acids to be assembled in a polypeptide.

The genetic code is deciphered or translated into a sequence of amino acids.

21. Composition of Genetic Code:

DNA molecule has three components. They are sugar, phosphates and nitrogen bases. Only nitrogen base sequence varies in different DNA molecules. Thus, the sequence of nitrogen bases or nucleotides in a DNA segment is the code or language in which the DNA sends out the message in the form of messenger RNA (mRNA).

The mRNA carries the genetic message (genetic code) in the form of nucleotide sequence. It has been found that there is colinearity between nucleotide sequence of mRNA and amino acid sequence of the polypeptide chain synthesized.

The genetic code is the language of nitrogen bases. There are four kinds of nitrogen bases and twenty kinds of amino acids. Therefore four-letter language of nitrogen bases specifies the twenty letter language of amino acids.

22. Describe Mechanisms of Protein Synthesis:

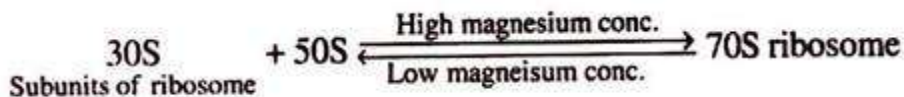
In prokaryotes, the RNA synthesis (transcription) and protein synthesis (translation) take place in the same compartment as there is no separate nucleus. But in eukaryotes, the RNA synthesis takes place in the nucleus while the protein synthesis takes place in the cytoplasm. The mRNA synthesized in the nucleus is exported to cytoplasm through nucleopores.

First, Francis Crick in 1955 suggested and later Zamecnik proved that prior to their incorporation into polypeptides, the amino acids attach to a special adaptor molecule called tRNA. This tRNA has a three nucleotide long anticodon which recognizes three nucleotide long codon on mRNA.

Role of Ribosomes in Protein synthesis:

Ribosome is a macromolecular structure that directs the synthesis of proteins. A ribosome is a multicomponent, compact, ribonucleoprotein particle which contains rRNA, many proteins and enzymes needed for protein synthesis. Ribosome brings together a single mRNA molecule and tRNAs charged with amino acids in a proper orientation so that the base sequence of mRNA molecule is translated into amino acid sequence of polypeptides.

Ribosome is a nucleoprotein particle having two subunits. These two subunits lie separately but come together for the synthesis of polypeptide chain. In *E. coli* ribosome is a 70S particle having two subunits of 30S and 50S. Their association and dissociation depends upon the concentration of magnesium.



Small subunit of ribosome contains the decoding centre in which charged tRNAs decode o the codons of mRNA. Large subunit contains peptidyl transferase centre, which forms the peptide bonds between successive amino acids of the newly synthesized peptide chain.

Both 30S and 50S subunits consist of ribosomal RNA (rRNA) and proteins.

The mRNA binds to the 16S rRNA of smaller subunit. Near its 5'-end mRNA binds to the 3'-end of 16S rRNA.

The main role of ribosome is the formation of peptide bond between successive amino acids of the newly synthesized polypeptide chain. The ribosome has two channels in it. The linear mRNA enters and escapes through one channel, which has the decoding centre. This channel is accessible to the charged tRNAs. The newly synthesized polypeptide chain escapes through the other channel.

Direction of Translation:

Each protein molecule has an $-\text{NH}_2$ end and $-\text{COOH}$ end. Synthesis begins at amino end and ends at carboxyl end. The mRNA is translated in $5' \rightarrow 3'$ direction from amino to carboxyl end. Synthesis of mRNA from DNA transcription also occurs in $5' \rightarrow 3'$ direction.

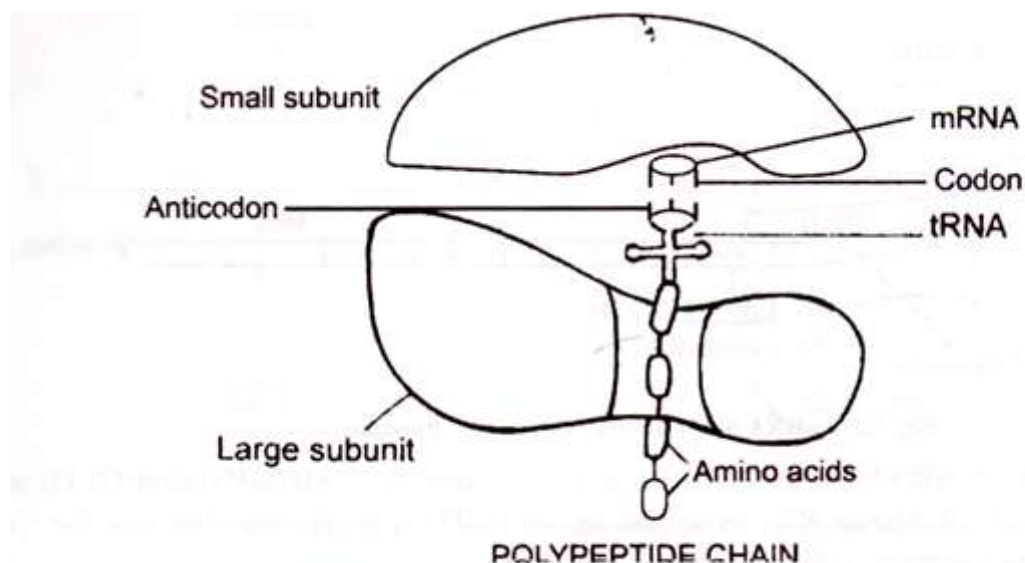


Fig. 12.1. Ribosome showing two subunits and position of mRNA and tRNA. The nascent polypeptide chain passes through a channel.

Initiation of Protein Synthesis:

Formation of Initiation Complex:

First of all 30S subunit of the 70S ribosome starts initiation process. The 30S subunit, mRNA and charged tRNA combine to form pre-initiation complex. Formation of pre-initiation complex involves three initiation factors IF1, IF2 and IF3 along with GTP (guanosine triphosphate). Later 50S subunit of ribosome joins 30S subunit to form 70S initiation complex.

Information for protein synthesis is present in the form of three nucleotide codons on mRNA. Protein coding regions on mRNA consist of continuous, non-overlapping triplet codons. The protein coding region on mRNA is called open reading frame which has a start codon 5'-AUG-3' and a stop codon in the end. Each open reading frame specifies a single protein. Prokaryote mRNA has many open reading frames, therefore encode multiple polypeptides and are called polycistronic mRNAs.

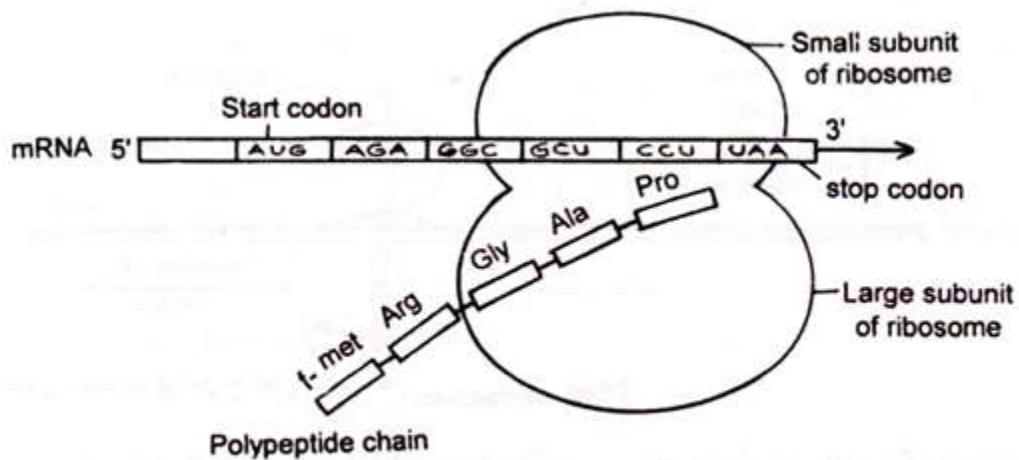


Fig. 12.2. A ribosome translates an mRNA molecule.

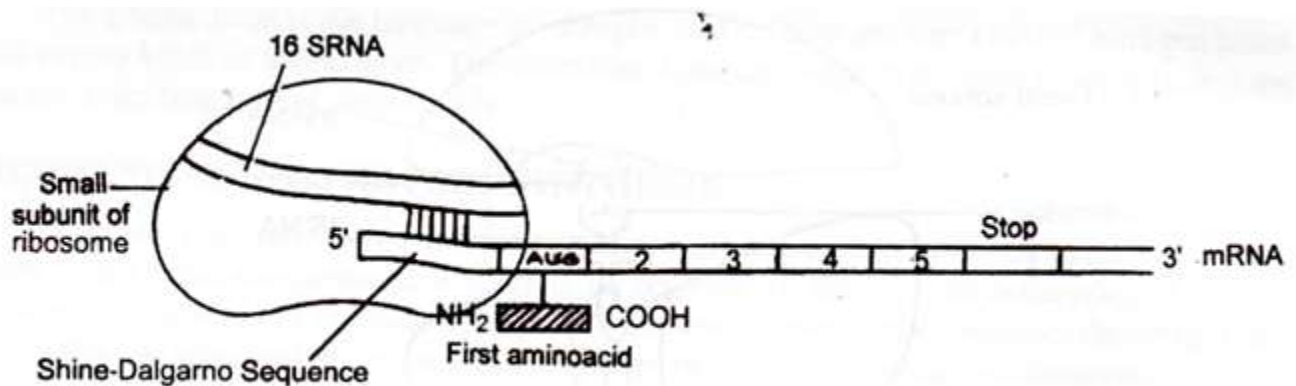


Fig. 12.3. mRNA binds to small subunit of ribosome.

III B.Sc Biochemistry – Molecular Biology

Near the 5'-end of mRNA lies the start codon which is mostly 5'-AUG-3' (rarely GUG) in both prokaryotes and eukaryotes. Ribosome binding site (RBS) in prokaryotes lies near the 5'- end of mRNA ahead (upstream) of AUG codon.

Between 5'-end and AUG codon there is a sequence of 20-30 bases. Of these, there is a sequence 5'-AGGAGGU-3'. This purine rich sequence is called Shine-Dalgarno sequence and lies 4-7 bases ahead (upstream) of AUG codon.

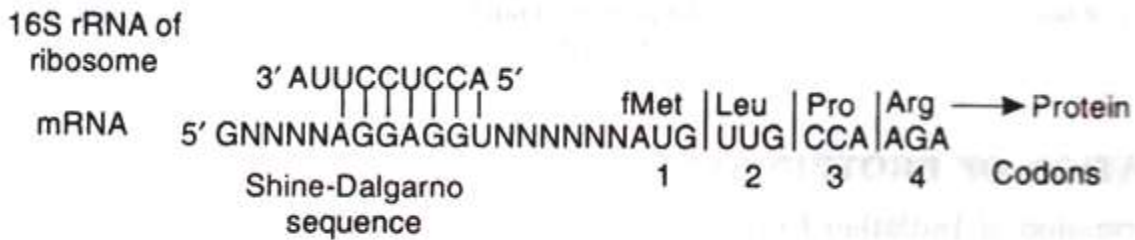


Fig. 12.4.

The 3'-end region of 16S rRNA in 30S subunit has a complementary sequence 3'-AUUCCUCCA-5'. This sequence forms base pairs with Shine-Dalgarno sequence for binding of mRNA to ribosome. Shine-Dalgarno sequence is the ribosome binding site (RBS). It positions the ribosome correctly with respect to the start codon.

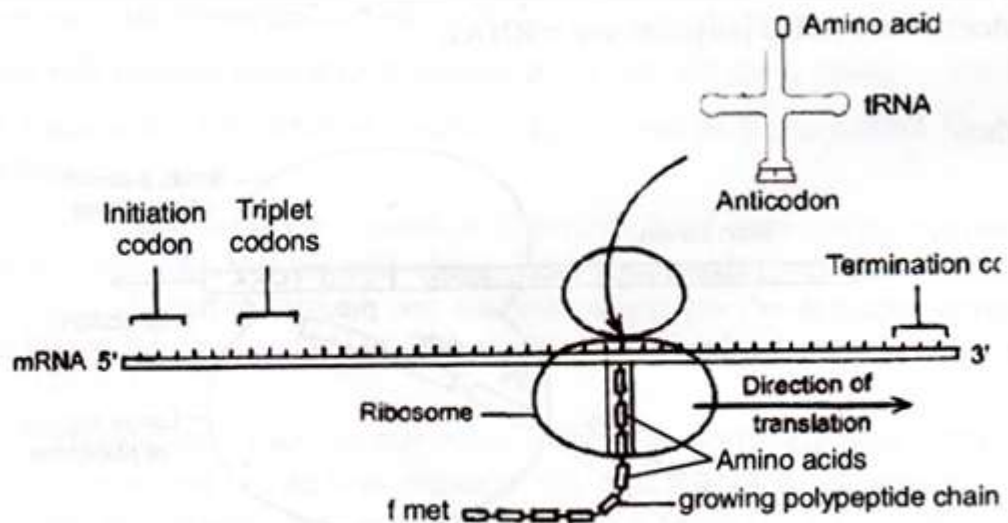


Fig. 12.5. Components of protein synthesis, ribosome translates mRNA in 5' → 3' direction Codons on mRNA are recognized by tRNA which acts as an adaptor molecule bringing in amino acids for polypeptide chain.

There are two tRNA binding sites on ribosome covering 30S and 50S subunits. The first site is called "P" site or peptidyl site. The second site is called "A" site or aminoacyl site. Only the initiator tRNA enters the "P" site. All other tRNAs enter the "A" site.

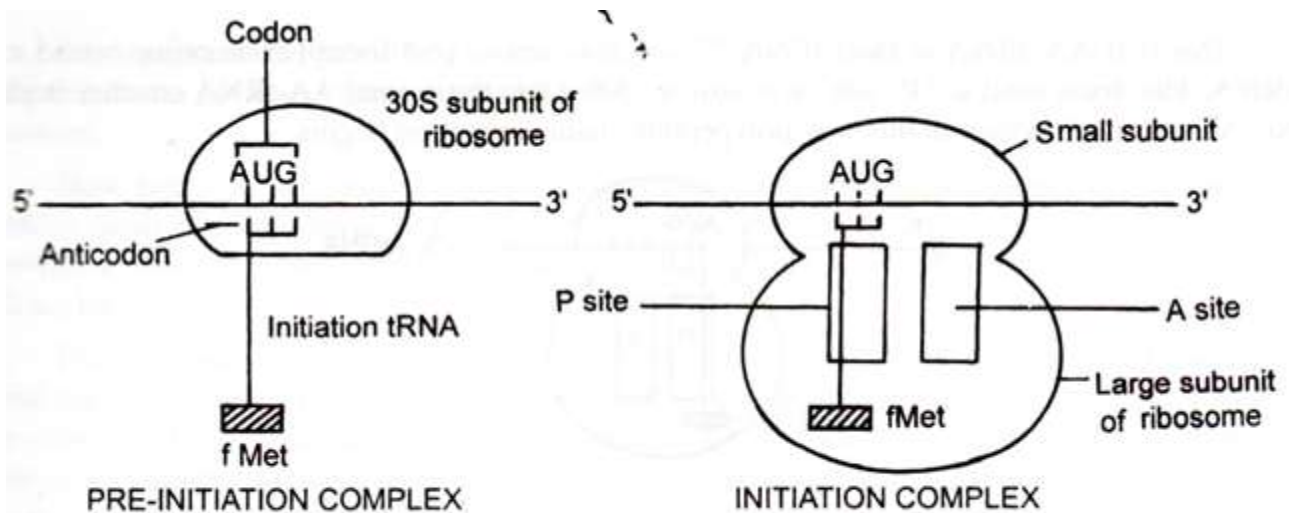


Fig. 12.6. (a) Pre-initiation complex, (b) Initiation complex.

For every amino acid, there is a separate tRNA. The identity of a tRNA is indicated by superscript, such as $tRNA^{Arg}$ (specific for amino acid Arginine). When this tRNA is charged with amino acid Arginine, it is written as Arginine- $tRNA^{Arg}$ or Arg- $tRNA^{Arg}$. Charged tRNA is called aminoacylated tRNA.

In bacteria, the first amino acid starting the protein is always formyl methionine (fMet). When AUG appears as the start codon on mRNA only fMet is incorporated. The tRNA molecule carrying formyl methionine is called $tRNA^{fMet}$. Therefore the first initiator charged aminoacyl tRNA is always fMet- $tRNA^{fMet}$. When AUG codon is encountered in the internal location (other than the start codon), methionine is not formylated and tRNA carrying this methionine is $tRNA_m^{Met}$.

First of all the charged initiator tRNA called $tMet-tRNA^{fMet}$ occupies the “P” site on ribosome. This position brings its anticodon and start codon AUG of mRNA together in such a way that the anticodon of charged tRNA and codon of mRNA form base pair with each other. Thus reading or translation of mRNA begins.

The “A” site is available to the second incoming charged tRNA whose anticodon forms base pairs with the second codon on mRNA.

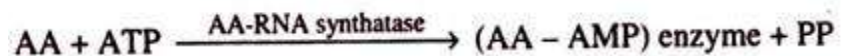
Charging of tRNA:

Attachment of amino acids to tRNAs is called charging of tRNA. All tRNAs at their 3'-terminus have a sequence 5'-CCA-3'. At this site amino acids bind with the help of enzyme aminoacyl tRNA synthetase. Charging of tRNA occurs in two steps.

1. Activation of amino acids:

III B.Sc Biochemistry – Molecular Biology

Energy molecule ATP activates the amino acids. This step is catalysed by specific activating enzymes called aminoacyl tRNA synthetases. Every amino acid has a separate enzyme AA-RNA synthetase enzyme.



2. Transfer of amino acids to tRNA:

AA-AMP enzyme complex reacts with a specific tRNA and transfers the amino acid to tRNA, as a result of which AMP and enzyme are set free.



This first AA-tRNA is fMet-tRNA^{fmet} which is amino acid formyl methionine bound to tRNA. This fixes itself to “P” site on ribosome. After this the second AA-tRNA attaches itself to “A” site on ribosome. In this way polypeptide chain elongation begins.

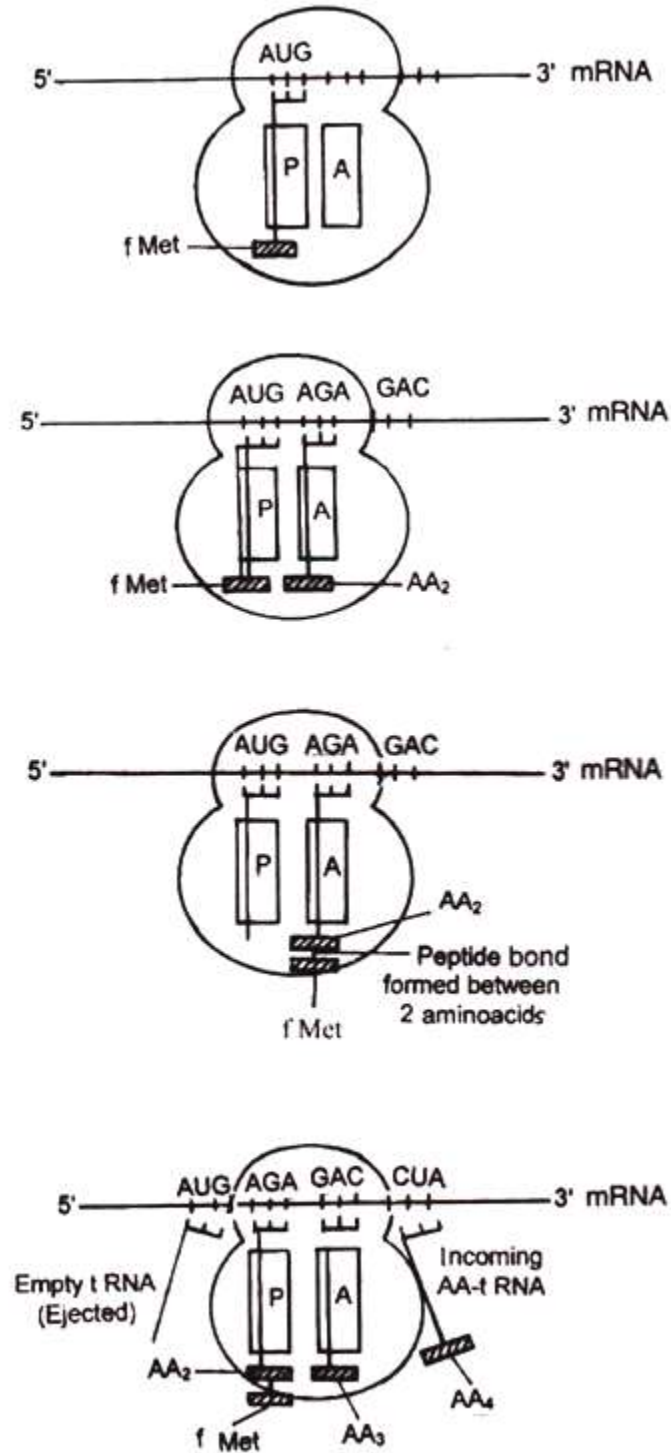


Fig. 12.7. Polypeptide chain formation, binding of charged tRNA, Peptide bond formation, and translocation.

Polypeptide Chain Elongation:

Polypeptide chain elongation requires some elongation factors. These elongation factors are Tu and G.

EF-Tu forms a complex with AA₂-tRNA and GTP and brings it to the “A” site of ribosome. Once the AA₂-tRNA is in place at “A” site, the GTP is hydrolysed to GDP and EF- Tu is released from the ribosome. EF-Tu-GTP complex is regenerated with the help of another factor Ts.

Formation of Peptide Bond:

The main role of ribosome is to catalyse the formation of peptide bonds between successive amino acids. In this way amino acids are incorporated into protein.

Now both “P” site and “A” site on ribosome are occupied by charged tRNAs having amino acids. Peptide bond is formed between two successive amino acids at “A” site. It involves cleavage of bond between f-Met and tRNA. This is catalysed by the enzyme tRNA deacylase.

Peptide bond is formed between the free carboxyl group (-COOH) of the first amino acid and the free amino group (-NH₂) of the second amino acid at the “A” site. The enzyme involved in this reaction is peptidyl transferase. After the formation of peptide bond, between two amino acids, the tRNA at “P” site becomes uncharged or deacylated and tRNA at “A” site now carries a – ill protein chain having two amino acids. This occurs in 50S subunit of ribosome.

The peptidyltransferase which catalyzes the peptide bond formation between successive amino acids consists of several proteins and molecule of 23S rRNA in the ribosome. This 23S rRNA is a ribozyme.

Translocation:

The peptidyl tRNA carrying two amino acids present at “A” site is now translocated to “P” site. This movement is called translocation. Elongation factor called EF-G control translocation. This factor G is called translocase. Hydrolysis of GTP provides energy for translocation and release of deacylated tRNA (free of amino acid).

Translocation also involves movement of ribosome along mRNA towards its 3'-end by a distance of one codon from first to second codon. This movement shifts the dipeptidyl tRNA (carrying two amino acids) from “A” to “P” site.

In addition to these two sites P and A, a third site “E” (exit site) on 50 S ribosome is present. Deacylated tRNA (deprived of amino acid) moves for “P” site to “E” site from where it is ejected out.

Then the third amino acid (next amino acid) charged on tRNA comes to lie in now empty site “A”. Then dipeptidyl chain having two amino acids present on P site form peptide bond with the

third amino acid at “A” site. Then the three amino acid chain is translocated to “P” site. Now the polypeptide chain has three amino acids. This elongation process goes on and on. At each step a new amino acid is added to the polypeptide chain. After each elongation, ribosome moves by one codon in 5' → 3' direction.

Chain Termination:

The presence of termination codons or stop codons on mRNA causes the polypeptide chain to be terminated. Synthesis stops when elongation chain comes across stop codons on “A” site. The stop codons are UAA, UGA and UAG. There is no tRNA which can bind these codons.

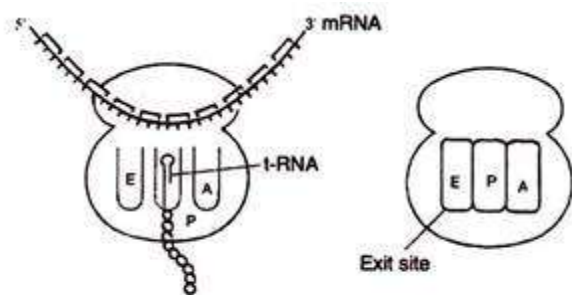


Fig. 12.8.

There are three release factors in prokaryotes, which help in chain termination. They are RF1, RF2 and RF3.

23. Write a short note on Polyribosome or Polysome:

A single mRNA molecule can be read simultaneously by several ribosomes. A polyribosome or polysome consists of several ribosomes attached to the same RNA. The number of ribosomes in a polysome depends upon the length of mRNA.

A fully active mRNA has one ribosome after every 80 nucleotides. There may be about 50 ribosomes in a polycistronic mRNA of prokaryotes. Ribosomes move along mRNA in 5' 3' direction. There is a gradual increase in the size of polypeptide chain as the ribosomes move along mRNA towards its 3'-end. Polypeptide chain starts near the 5'-end and is completed near the 3'-end.

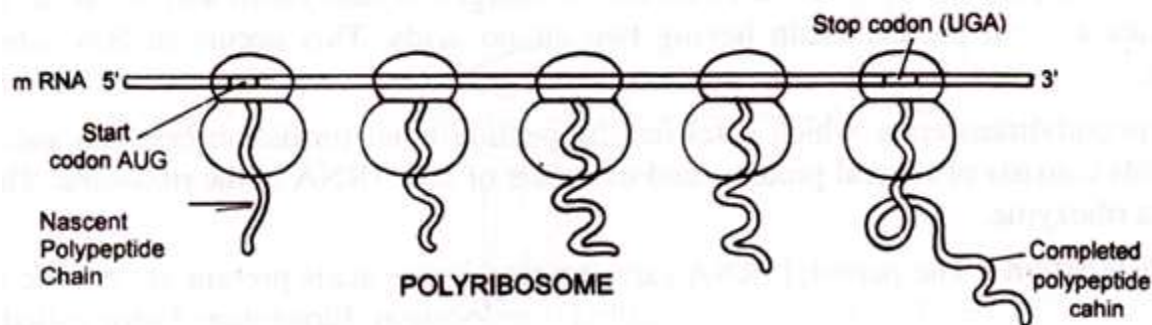


Fig. 12.9. Polyribosome

The ribosomes closest to the 5'-end of mRNA have the smallest polypeptide chain, while ribosomes nearest to the 3'-end have longest chain. Polysome increases the rate of protein synthesis tremendously. In bacteria protein is synthesized at the rate of about 20 amino acids per second.

Simultaneous Transcription and Translation in Prokaryotes:

In prokaryotes, all components of transcription and translation are present in the same compartment. The mRNA molecule is synthesized in 5' → 3' direction and protein synthesis also occurs in 5' → 3' direction. In this way mRNA molecule while still under synthesis has a free 5'-end whose other end is still under synthesis.

Ribosomes bind at free 5'-end and start protein synthesis. In this way the free end (5'-end) of mRNA starts the process of protein synthesis while still attached to DNA. This is called Coupled Transcription and Translation. This increases the speed of protein synthesis. After the protein synthesis is completed, the degradation of mRNA molecule by nucleases also starts at 5'-end and proceeds in 5' → 3' direction.

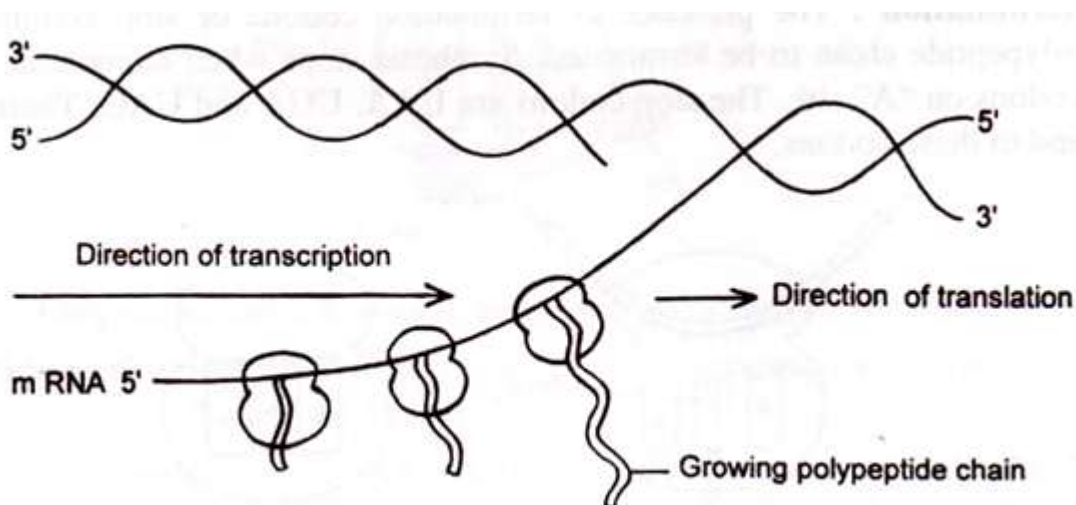


Fig. 12.10. Coupled transcription and translation.

PROTEIN SYNTHESIS IN EUKARYOTES:

24. Describe the mechanism of translation in Eukaryotes

Protein synthesis in eukaryotes is basically similar to that of prokaryotes except some differences.

The ribosomes in eukaryotes are of 80S having 40S and 60S subunits. In eukaryotes the initiating amino acid is methionine and not f-methionine as in the case of prokaryotes. A special tRNA binds methionine to start codon AUG. This tRNA is called $tRNA^{Met}$. This is distinct from $tRNA^{Met}$ which binds amino acid methionine to any other internal position in the polypeptide.

There is no Shine-Dalgarno sequence in eukaryotic mRNA to function as ribosome binding site. Between 5'-end and AUG codon of mRNA there is a sequence of bases called cap. Small subunit of ribosome scans the mRNA in 5' → 3' direction until it comes across 5'- AUG-3' codon. This process is called scanning. Initiation factors also closely associated with 3'-end of mRNA through its poly-A tail. Initiation factors circularize mRNA by its poly-A tail. In this way poly-A tail also contributes to the translation of mRNA. Eukaryotic mRNAs are monocistronic and encode a single polypeptide, therefore have a single open reading frame.

There are ten initiation factors in eukaryotes. They are eIF (eukaryotic initiation factors) are eIF1, eIF2, eIF3, eIF4A, eIF4B, eIF4C, eIF4D, eIF4F, eIF5, eIF6.

There are two elongation factors in eukaryotes like prokaryotes. They are eEF1 (similar to EF-Tu) and eEF2 (similar to EF-G).

Eukaryotes have only one release factor eRF which requires GTP termination of protein synthesis. It recognizes all the three stop codons.

In eukaryotes the mRNA is synthesized in the nucleus, then processed, modified and passed on into the cytoplasm through nucleopores. The protein synthesis takes place in the cytoplasm. The mRNA in prokaryotes is very unstable and its life span is of a few minutes only. The mRNA of eukaryotes is quite stable and has a longer life span extending upto several days.

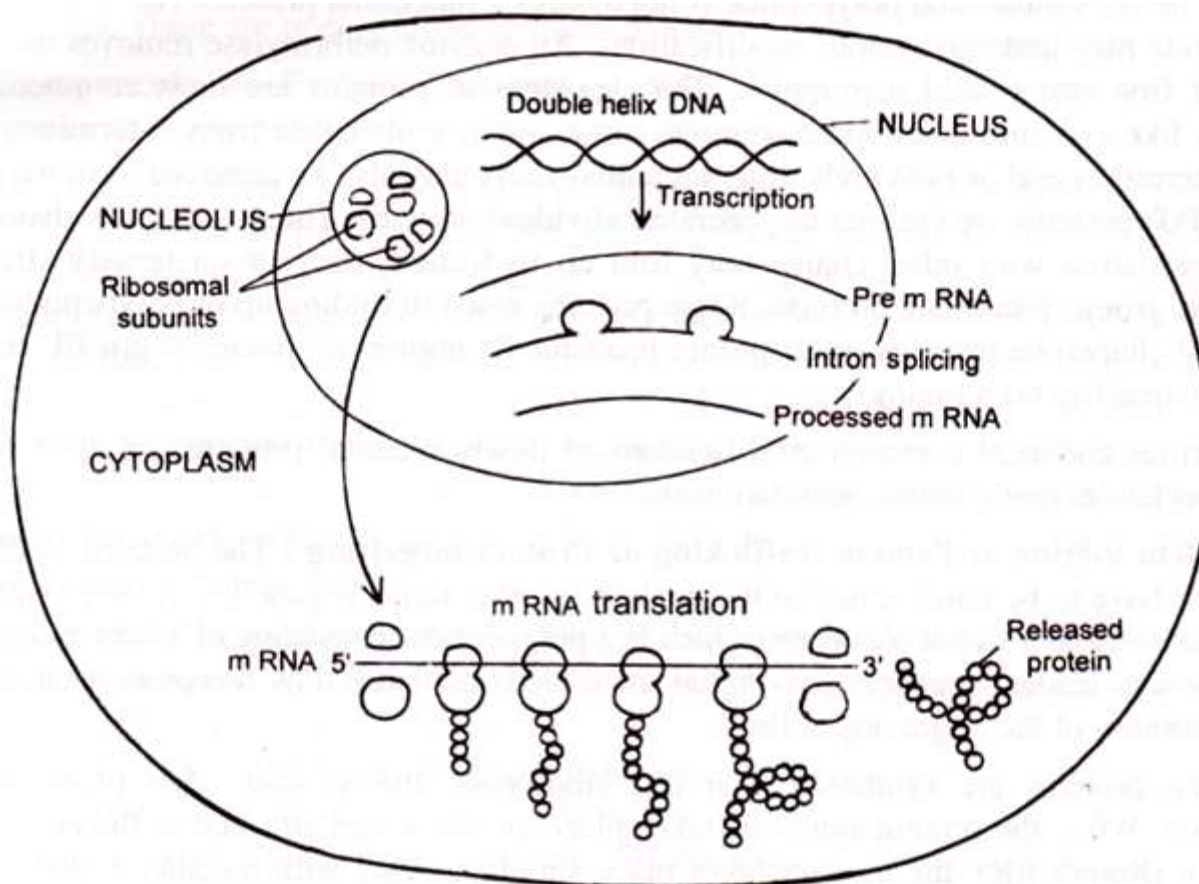


Fig. 12.11. Transcription, mRNA processing and mRNA translation.

Protein Synthesis on Bound Ribosomes:

Ribosomes occur in free state in the cytoplasm as well as bound to the outer surface of endoplasmic reticulum called rough endoplasmic reticulum (RER). The attachment of ribosomes to ER occurs after the protein synthesis starts. Whether the ribosomes synthesize protein on free or attached state depends upon the type of proteins to be synthesized by ribosomes. Most of the proteins which remain in free state in the cytoplasm are synthesized by free ribosomes.

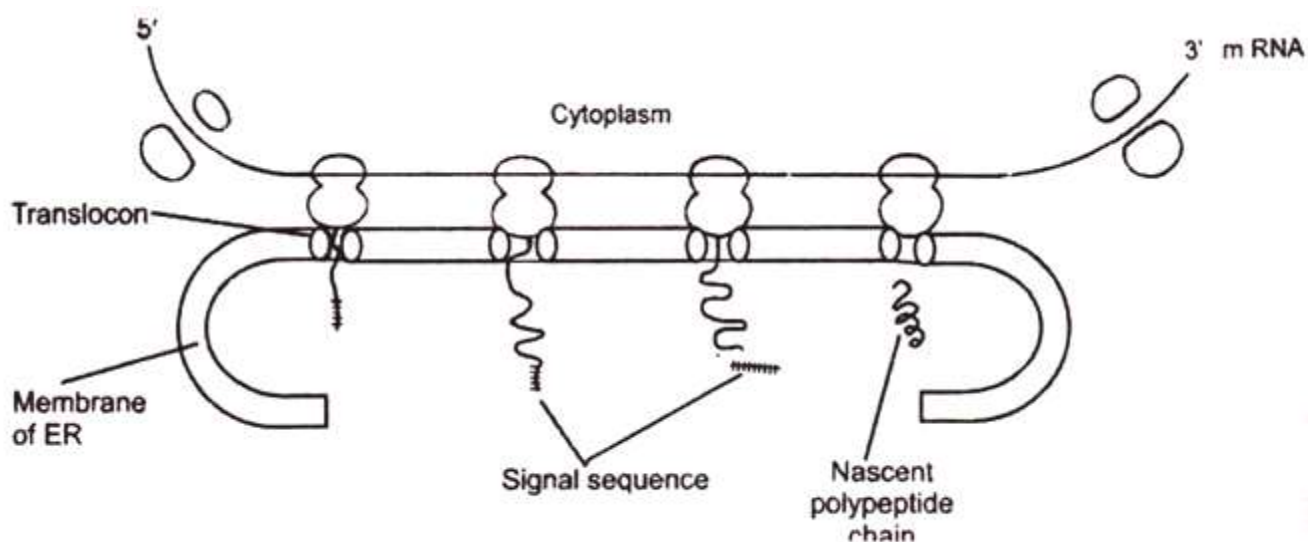


Fig. 12.12. Protein synthesis on ribosome bound on ER (RER).

Proteins synthesized by ribosomes on ER enter into the lumen of cisternae of ER from where they may enter into golgi apparatus where they are glycosylated and form secretory granules and many of them enter lysosomes.

Modification of Folding of Released Polypeptides:

DNA molecule specifies only the primary structure while folding and other modifications controlled by proteins themselves.

The newly synthesized polypeptide is not always a functional protein. The newly released polypeptide may undergo various modifications. An enzyme deformylase removes the formyl group of first amino acid methionine. The cleavages of proteins are most common. Some enzymes like exo-amino-peptidases remove some amino acids either from N-terminus end or from C-terminus end or both ends.

Internal amino acids may also be removed as in the case of insulin. Polyproteins are cleaved to generate individual proteins. The polypeptide chain singly or in association with other chains may fold up to form tertiary or quaternary structures. Prosthetic groups join many proteins. Some proteins assist in folding up of polypeptides. They are called chaperone proteins or chaperonin proteins. Examples are Bacterial gro EL (E. coli), mitochondrial hsp 60 mitonin.

Various chemical common modifications of newly released proteins are glycosylation, phosphorylation, methylation, acetylation etc.

Protein Sorting or Protein Trafficking or Protein Targeting:

The proteins synthesized in the cell have to be translocated to the nucleus or other target organelles. Newly synthesized polypeptides have a signal sequence (which is a polypeptide)

consisting of 13-36 amino acids. It is known as leader sequence. This signal sequence is recognized by receptors located within the membranes of the target organelles.

When proteins are synthesized on free ribosomes, the transfer takes place after the translation. When the protein synthesis takes place on ribosomes attached to the endoplasmic reticulum (Rough ER), the transfer takes place simultaneously with translation and is called co-translational transfer. The proteins which enter into the lumen of rough ER may enter into golgi apparatus, from where they may enter secretory lysosomes. The signal sequence is degraded by protease enzymes.

Once all these proteins are assembled into their proper place, they provide the proper biochemical machinery, which keeps the cell feeding, locomoting, multiplying and alive.

25. Describe the Inhibitors of Protein Synthesis:

There are many chemicals, both synthetic as well as those obtained from different sources like fungi, which bind to the components of translation machinery and arrest the translation process. Most of them are antibacterial agents or antibiotics that act exclusively on bacteria and are thus powerful tools in the hands of man to combat various infectious diseases. Most of antibiotics are inhibitors of translation machinery.

Puramycin:

It binds at “A” site on ribosome. This causes pre-mature termination of polypeptide chain.

Kirromycin:

It inhibits the elongation factor EF-Tu.

Fusidic acid:

It inhibits the elongation factor EF-G.

Tetracycline:

It attacks “A” site on ribosome and prevents the binding of aminoacyl- tRNA.

Chloramphenicol: It blocks the peptidyl transfer reaction.

Erythromycin:

It binds the polypeptide exit channel of ribosome, therefore blocks the exit of growing polypeptide chain, thus stops the translation process.

Streptomycin and Neomycin:

III B.Sc Biochemistry – Molecular Biology

These inhibit the binding of tRNA^{fMet} to the “P” site.

Inhibitors in Eukaryotes:

Diphtheria toxin is a toxin produced by corynebacterium diphtheriae. This causes modification of eukarotic elongation factor