Cell Biology

Chapter (6): DNA replication and protein synthesis

- DNA replication
- DNA transcription
- mRNA translation and protein synthesis
- Mutations

DNA Replication

- **Before a cell divides into new daughter cells through mitosis division or into haploid cells through meiosis division, DNA must be replicated in order to ensure that each new cell receives the correct number of chromosomes.**

- **The process of DNA duplication is called DNA replication.**

- **DNA replication occurs in the S phase of interphase during the cell cycle, which is the biological process of producing two identical replicas of DNA from one original DNA molecule before cell division.**

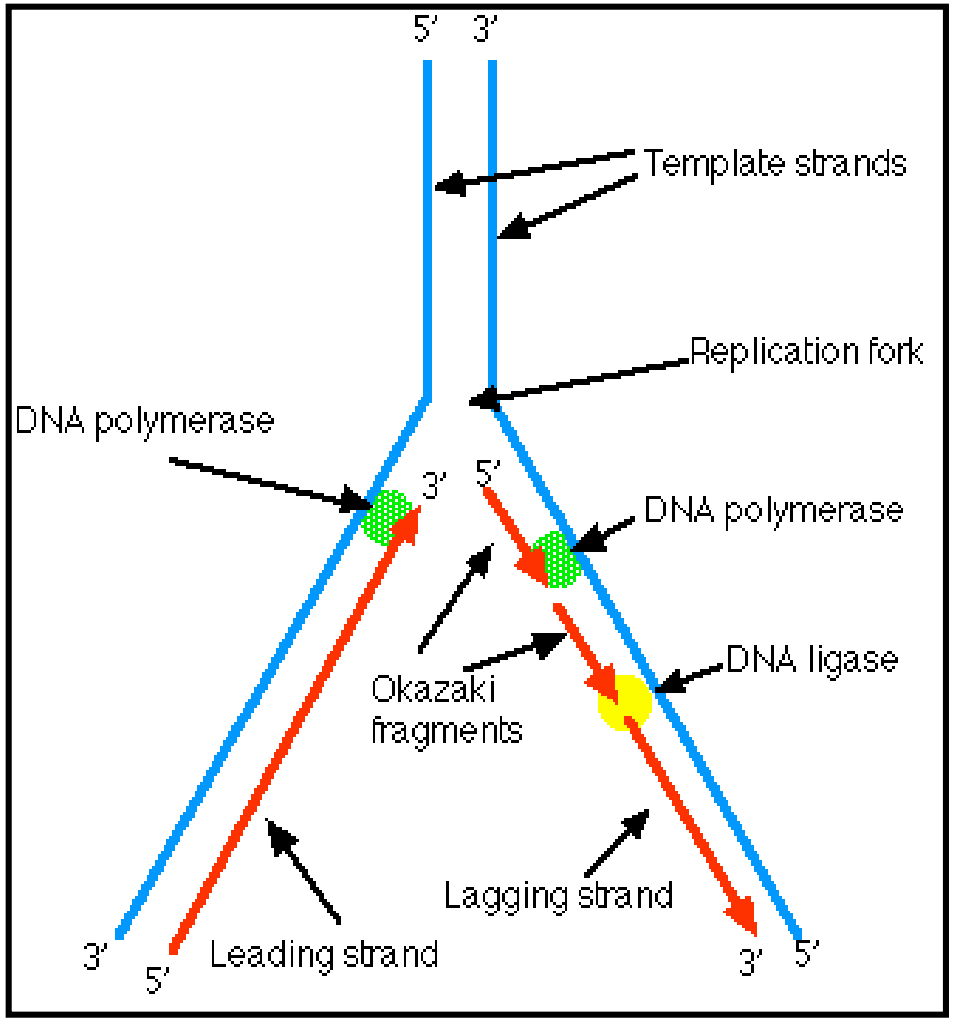
- **DNA is made up of a double helix of two complementary strands. These strands have two ends called 3’ and 5’ ends.**

- **3’ and 5’ numbers refer to the third and fifth carbon atoms in the deoxyribose sugar, respectively.**

- **The 3' end has a hydroxyl (OH) group, while the 5' end has a phosphate (P) group. This direction is important for DNA replication.**

DNA Replication

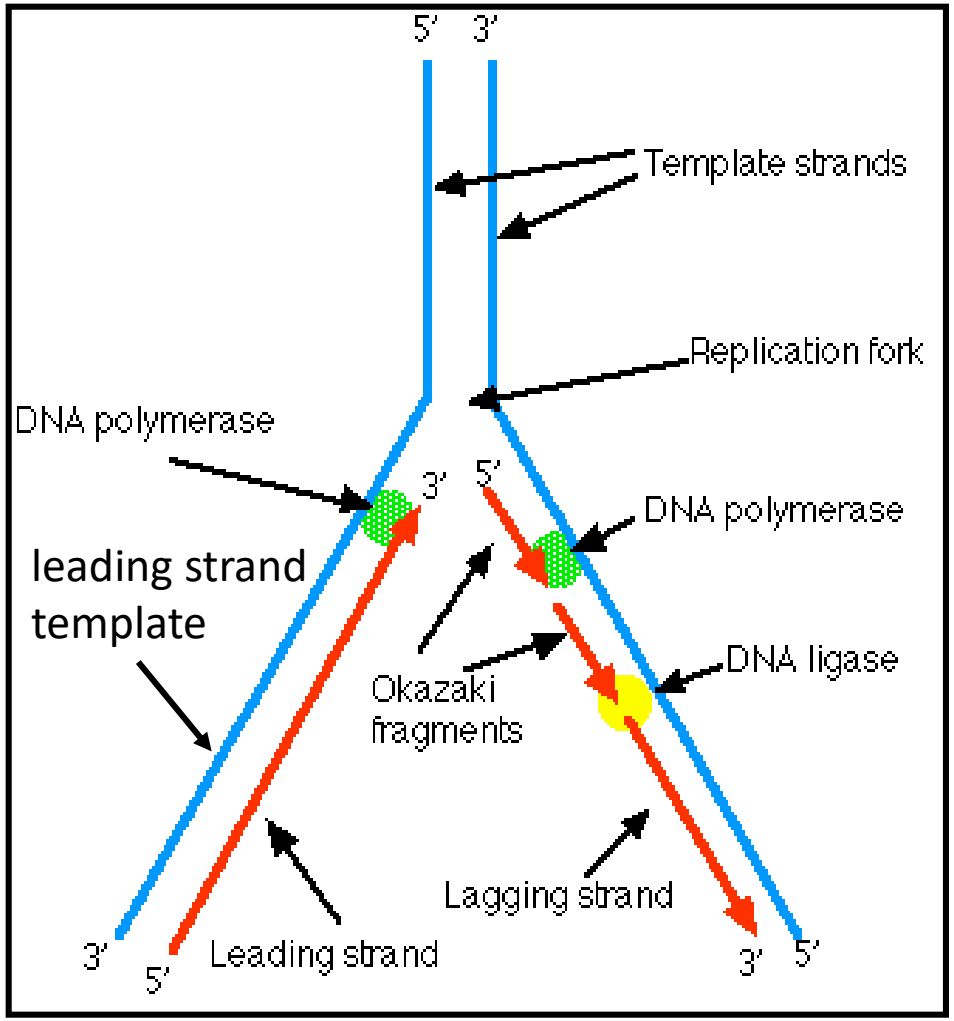
- During replication, these strands are separated and each strand of the original DNA molecule serves as a template for the production of its complementary strand.
- The separation process is performed by an enzyme known as DNA helicase.
- DNA helicase disrupts the hydrogen bonds between nitrogen base pairs to separate the strands into a Y shape known as the replication fork.
- Replication fork is a structure that forms during DNA replication by DNA helicase, where DNA double strands are separated into two branches.



Replication fork

DNA Replication

- These two branches serve as the template for the leading and lagging strands, so they are called leading strand template and the lagging strand template based on DNA strand direction.
- This means that the replication fork is bi-directional; one strand is oriented in the 3' to 5' direction (leading strand template), while the other is oriented 5' to 3' (lagging strand template).
- DNA is read in 3' to 5' direction, whereas the new strand is synthesized in the 5' to 3' direction.
- The two sides are therefore replicated with two different processes to accommodate the directional difference.



Replication fork

DNA Replication

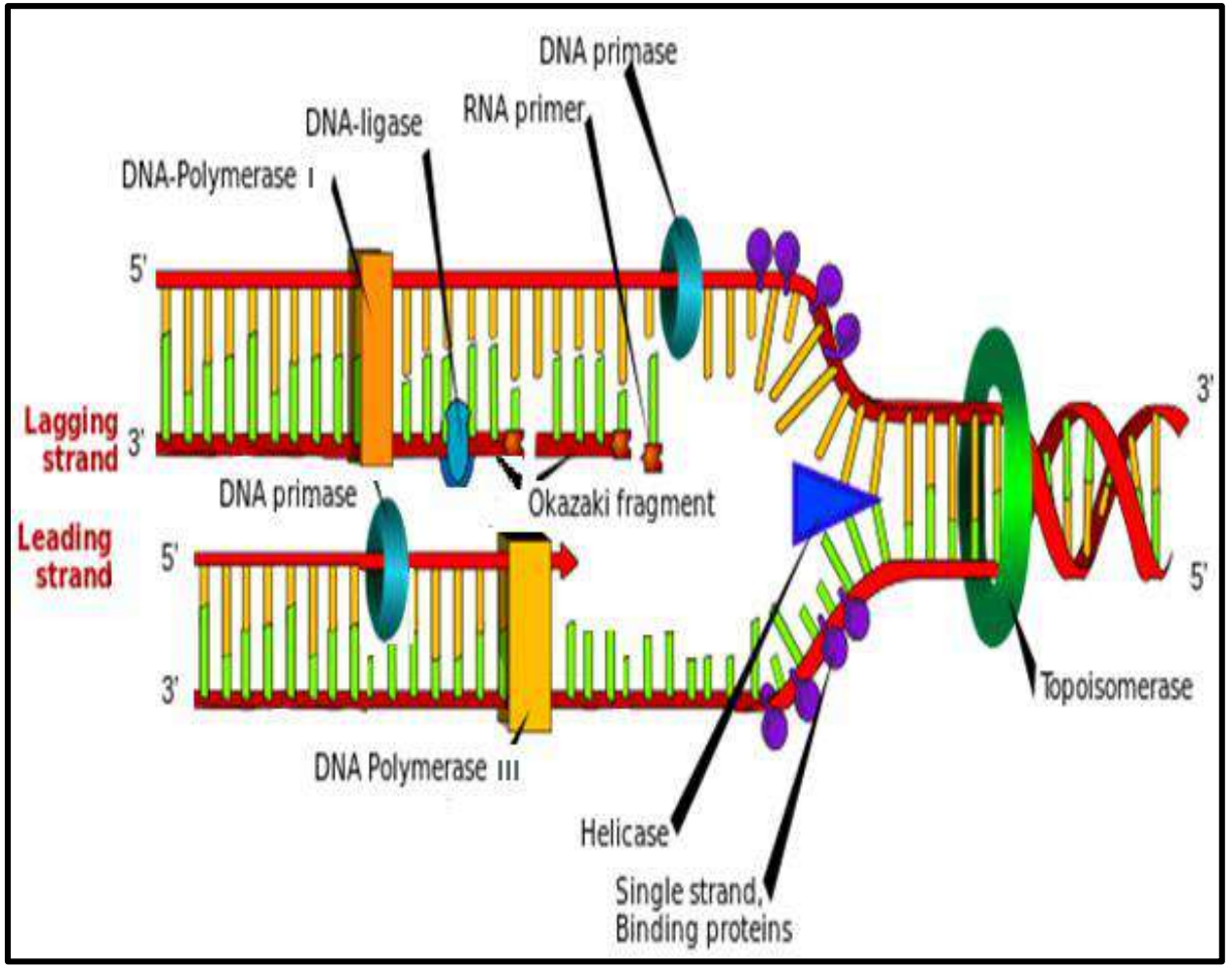
Steps of DNA replication

1- Opening the DNA double strands

- **The DNA double strands are separated into two branches by a specific enzyme called DNA helicase, which breaks down the hydrogen bonds between nitrogen bases forming the replication fork. Each single strand serves as a template for the leading and lagging strands.**

2- Stabilization of the single strand

- **This process takes place by specific proteins called single-strand binding (SSB) proteins which bind to each single strand of DNA to prevent them from rewinding (*preventing reformation of DNA double helix*).**



DNA Replication

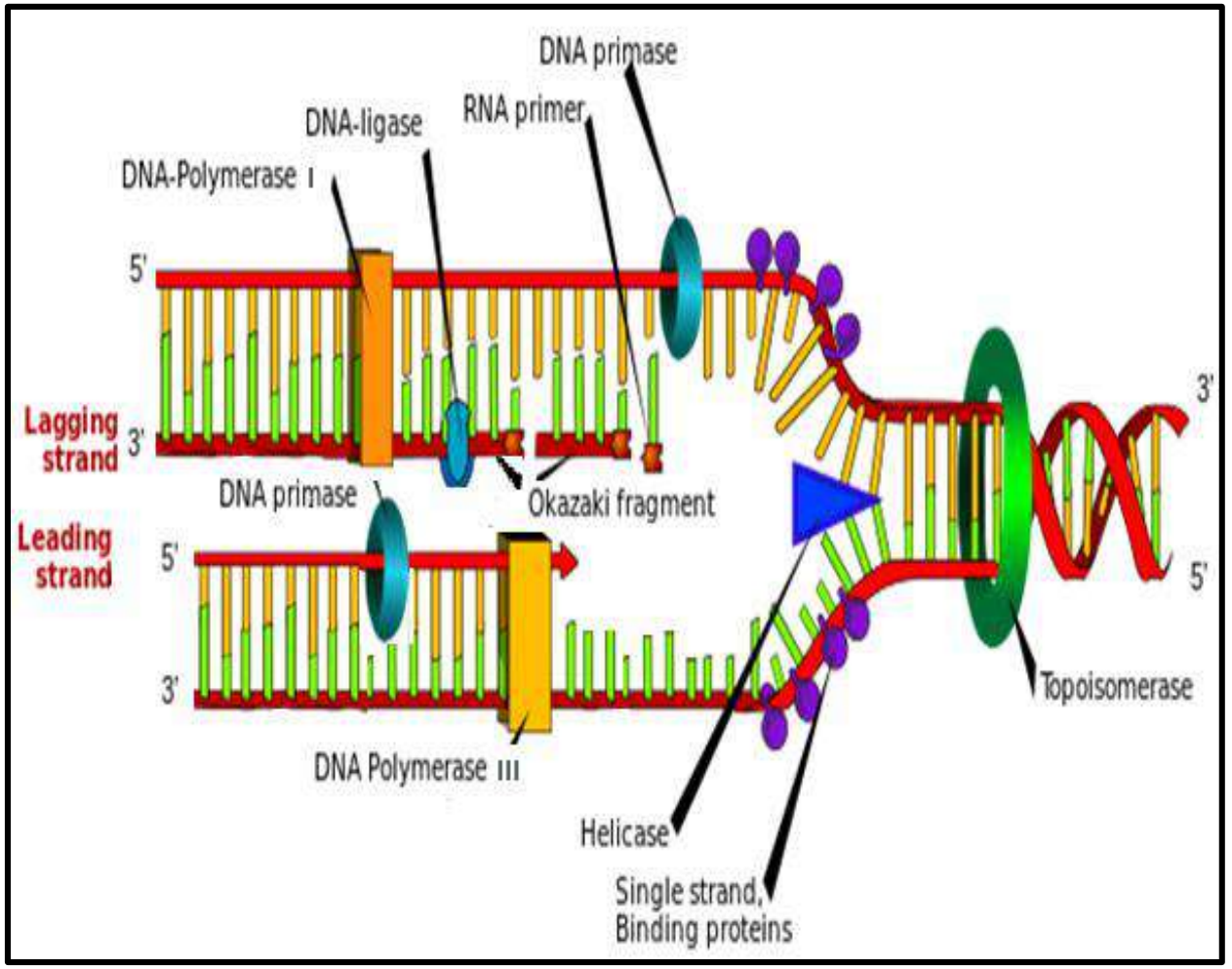
Steps of DNA replication

3- Building the primer

- **Elongation process is the main step of DNA replication, which is addition of the complementary nucleotides to the 3' end of each replication fork.**

- **DNA polymerase III is the enzyme that carries out the elongation process.**

- **But the problem is that the DNA polymerase III cannot begin the synthesis of a new DNA strand.**



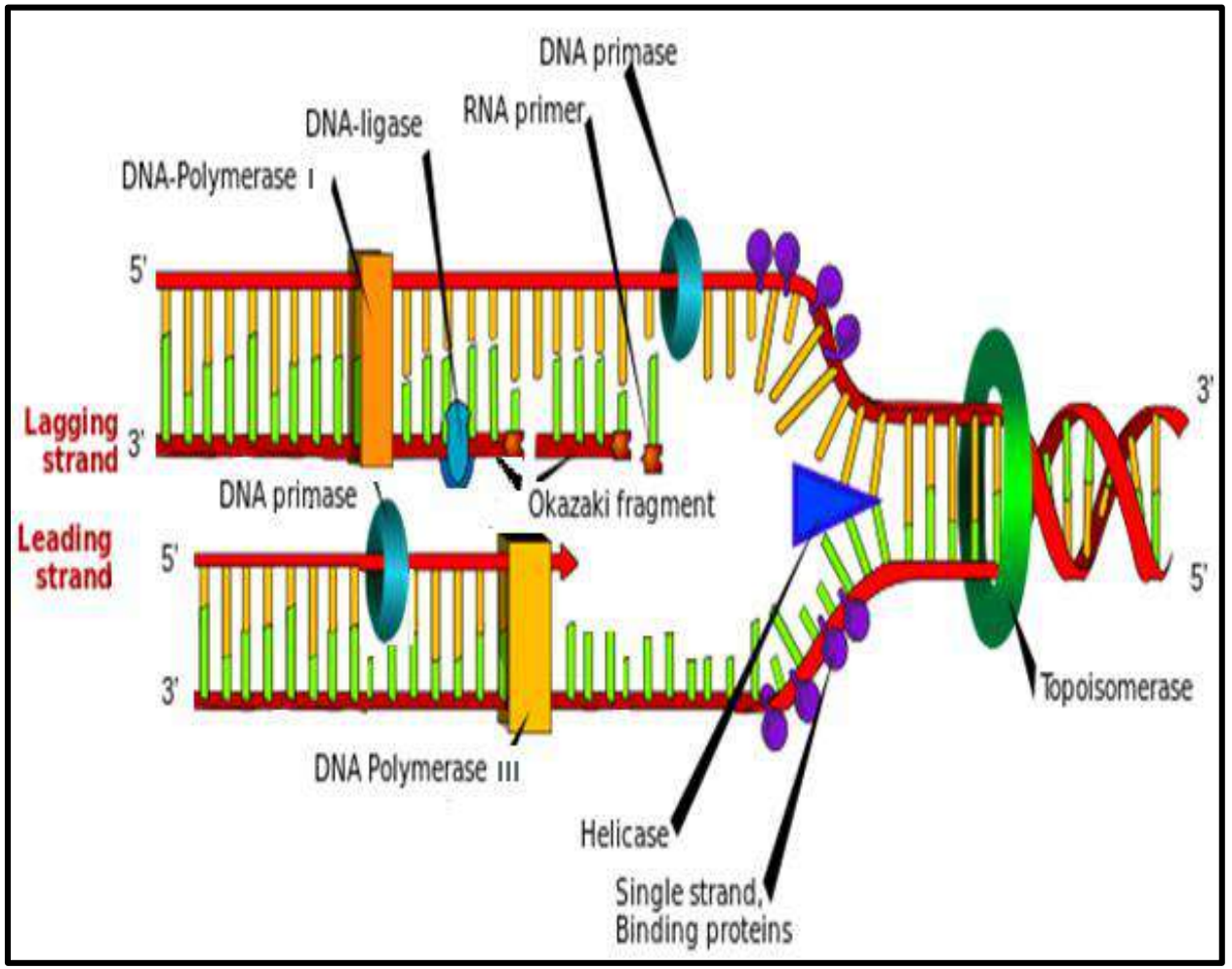
DNA Replication

Steps of DNA replication

3- Building the primer

- So to begin addition of the complementary nucleotides, a short fragment called a primer must be generated and bound to the 3' end direction of DNA templates by an enzyme called primase (one type of RNA polymerase).

- Primer is a short sequence of nucleotides (10 nucleotides) which acts as the starting point for DNA polymerase III action to begin synthesis of the new DNA strand.



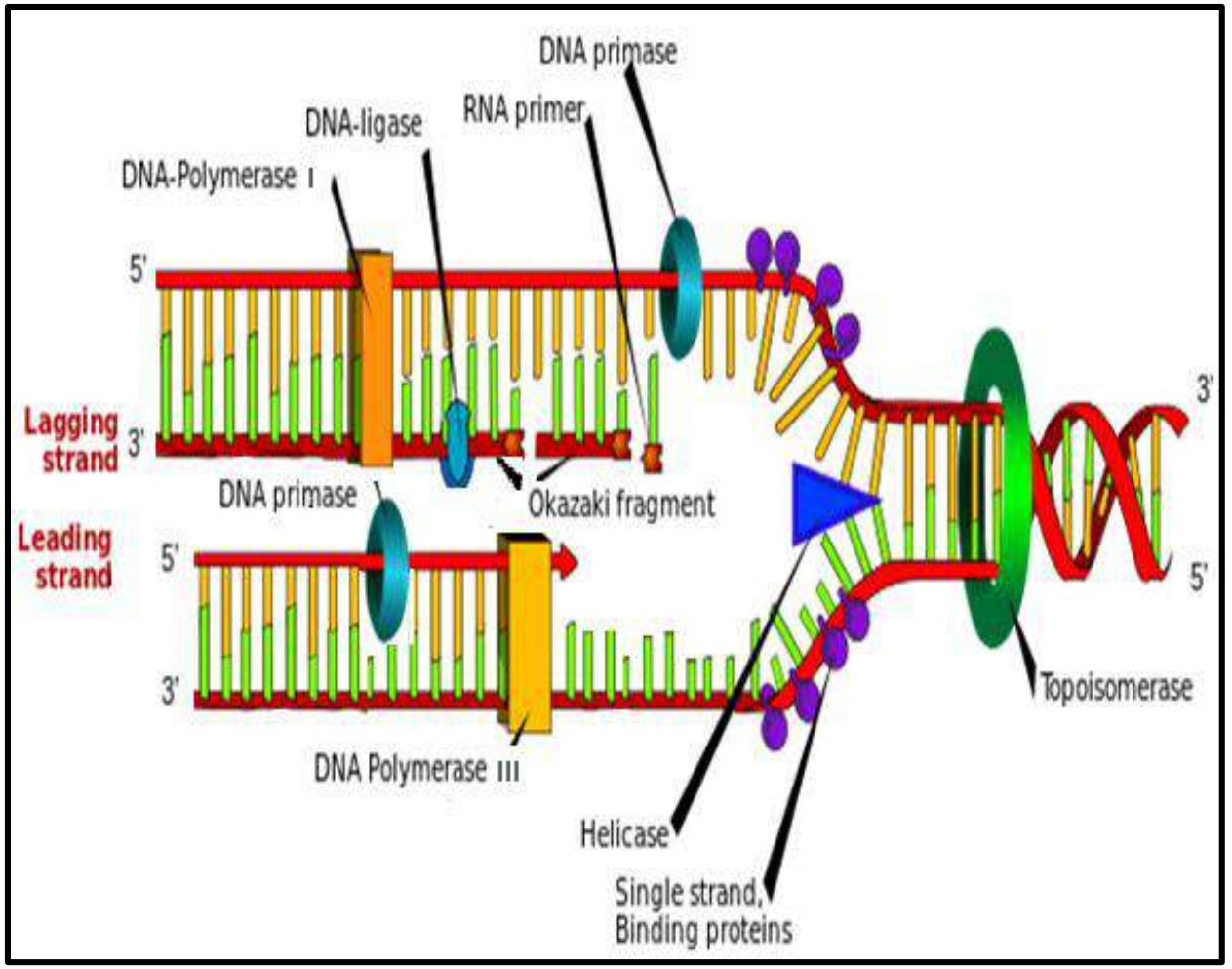
DNA Replication

Steps of DNA replication

4- Elongation process

- **Elongation process is formation of the complementary strands by DNA polymerase III through addition of the complementary nucleotides to the 3' end of each replication fork.**

- **Because the result of opening up the DNA double strands is formation two template strands, DNA polymerase III will form two complementary strands called leading strand and lagging strand according to the direction of synthesis (3'- 5' direction or 5'- 3' direction) as the following:**



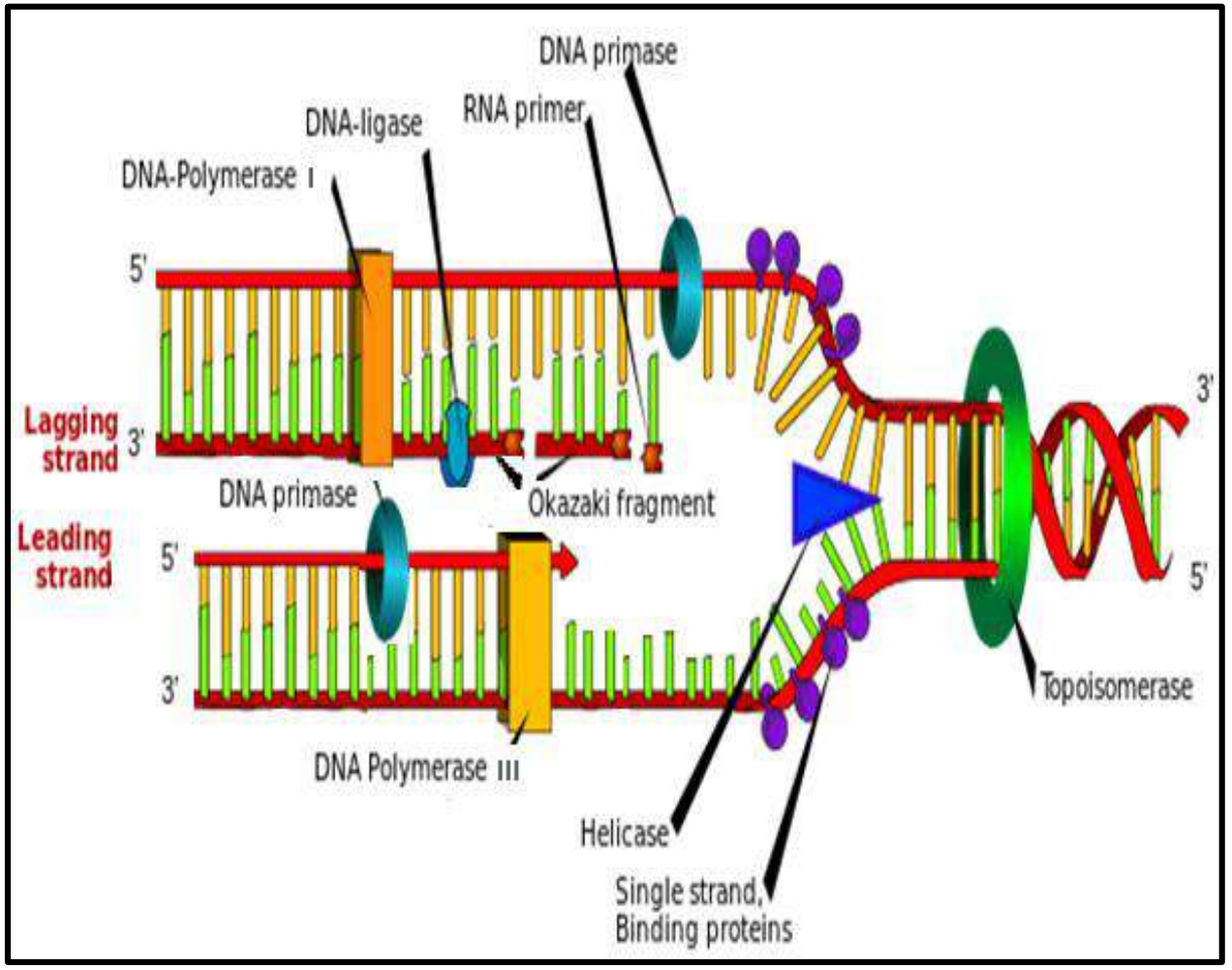
DNA Replication

Steps of DNA replication

4- Elongation process

a- Leading strand synthesis

- **It is the complementary strand for the 3' template strand of DNA replication fork.**
- **This strand forms when DNA polymerase III binds with the primer at 3- OH, reads the leading strand template, and then adds the complementary nucleotides to it continuously.**
- **So the direction of reading is 3'- 5‘ direction, while the direction of synthesis is 5‘- 3' direction.**



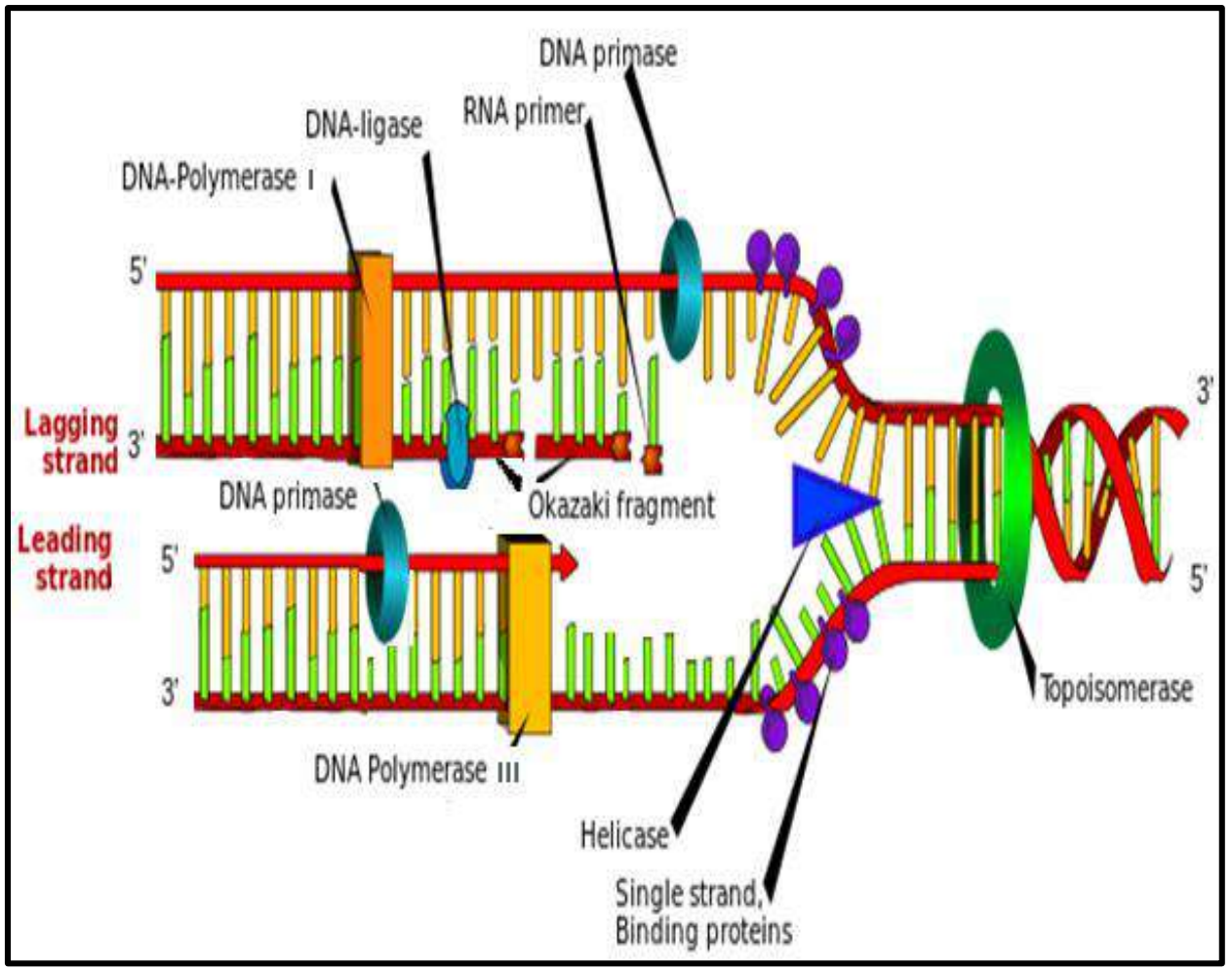
DNA Replication

Steps of DNA replication

4- Elongation process

b- Lagging strand synthesis

- **It is the complementary strand for the 5' template strand of DNA replication fork.**
- **Because of its direction is opposite to the working direction of DNA polymerase III, formation of the lagging strand is more complicated than the leading strand.**
- **This strand forms when primase reads the lagging strand template and adds the complementary primers to it in 3'- 5‘ direction as short and separated segments.**



DNA Replication

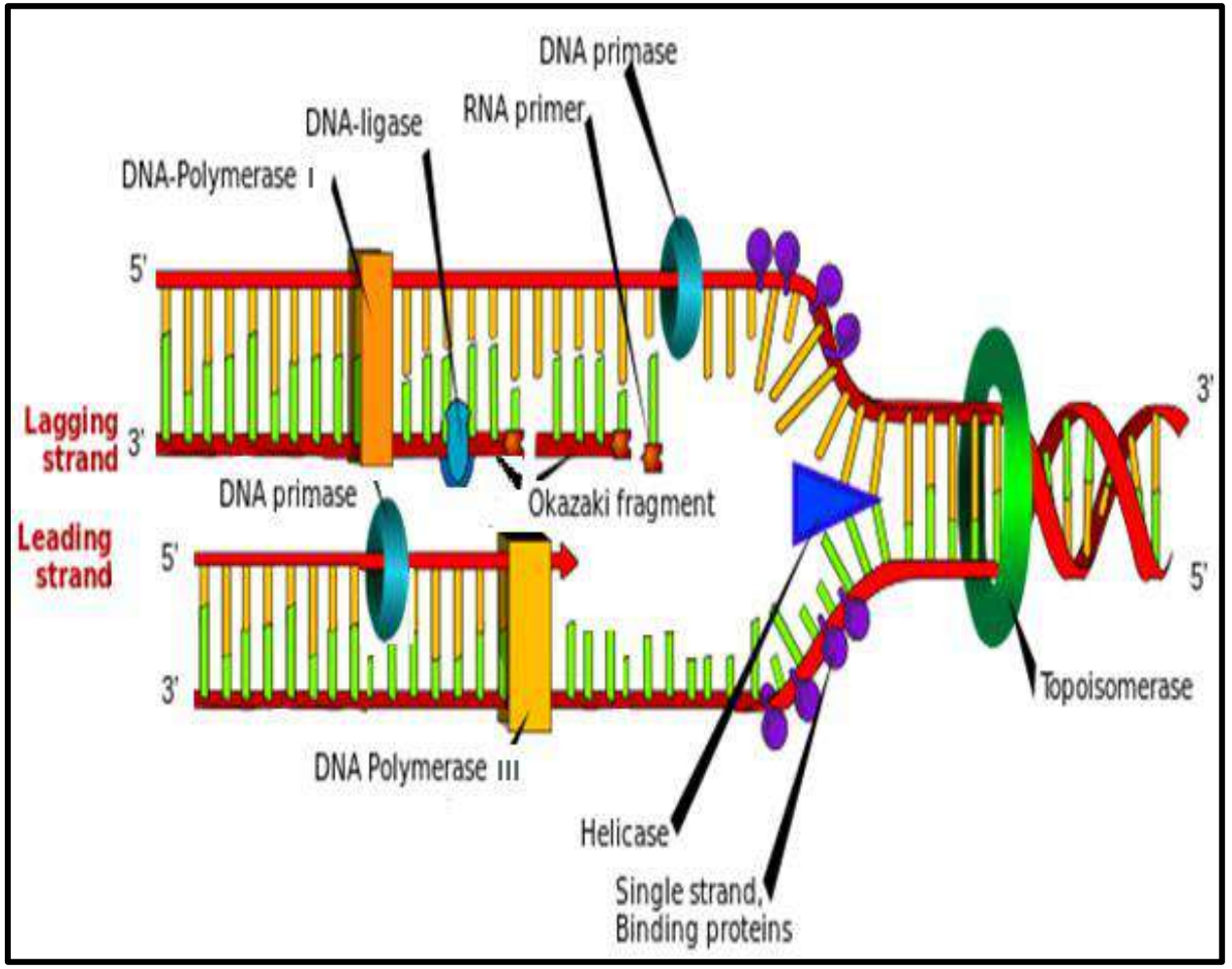
Steps of DNA replication

4- Elongation process

b- Lagging strand synthesis

- **After that DNA polymerase III binds with the primers and begins reading and adding the complementary nucleotides to it discontinuously forming a separated segments called okazaki fragments.**

- **Okazaki fragments are complementary segments to lagging strand template formed by DNA polymerase III and separated by primers.**



DNA Replication

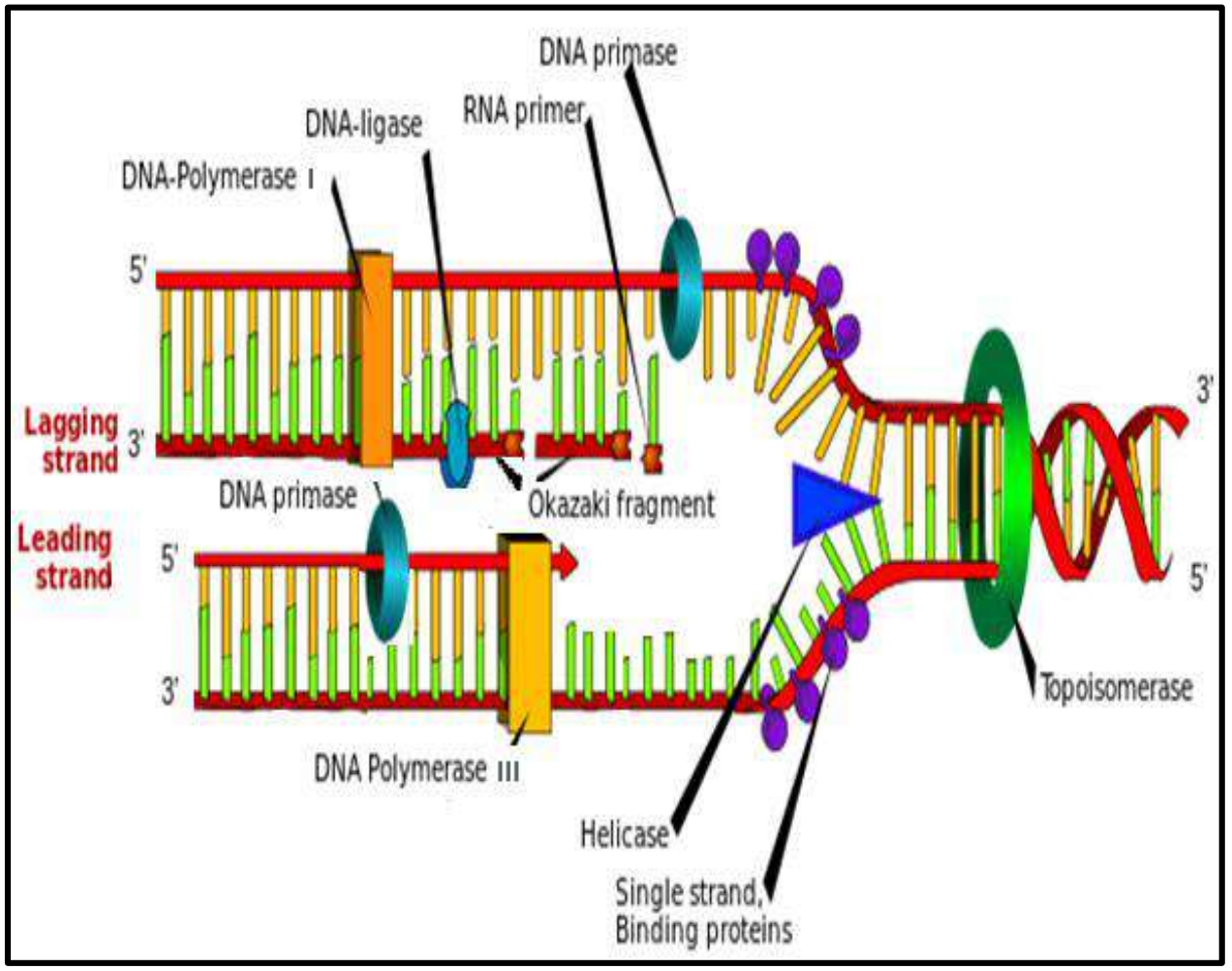
Steps of DNA replication

5- Removing the primers

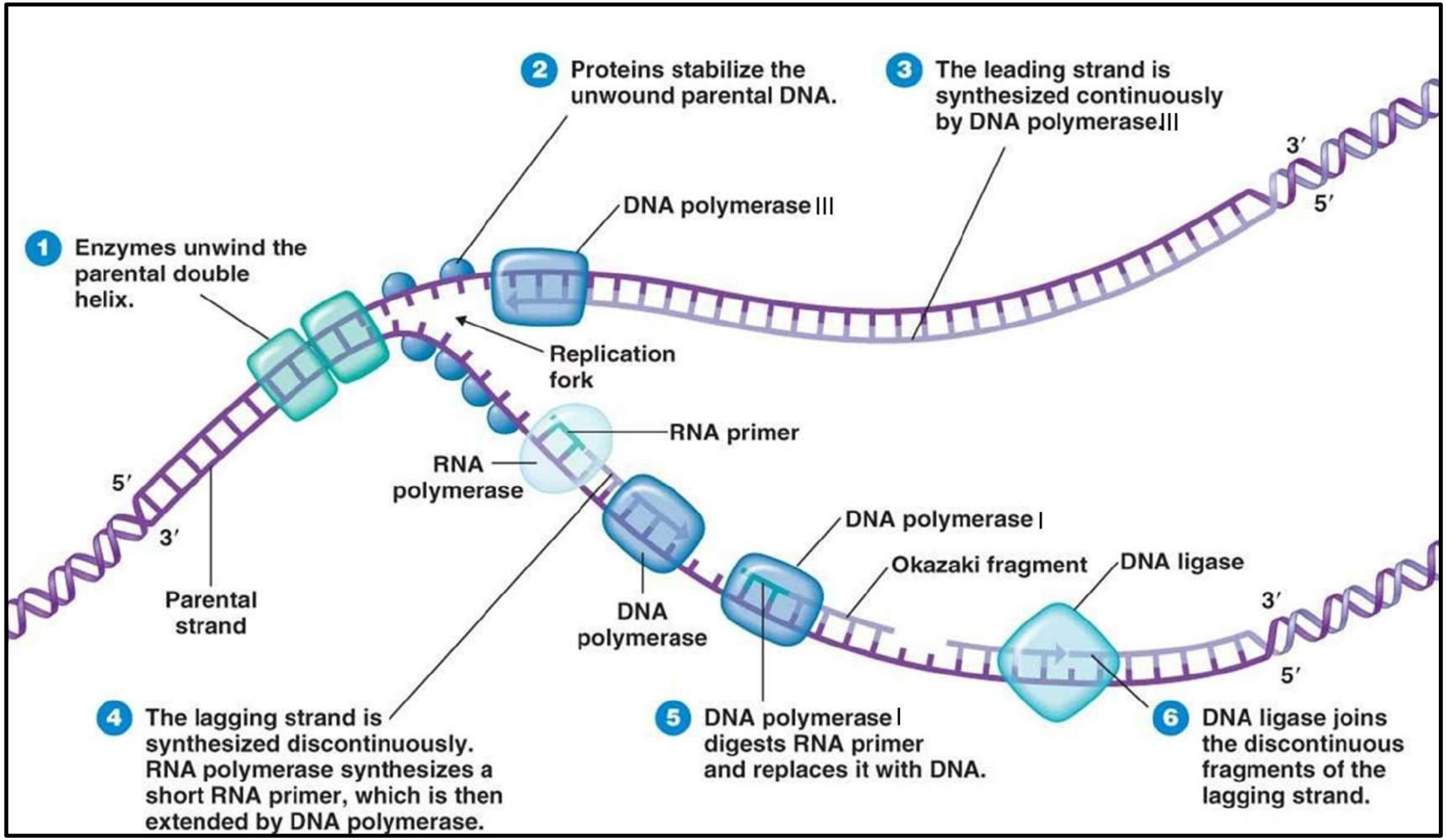
- **After finishing the elongation process for the two single strands template, specific enzyme called DNA polymerase I removes the primers and fills their gaps by new DNA segments.**

6- Joining the okazaki fragments:

- **After all gaps between okazaki fragments are filled by DNA segments, DNA ligase enzyme joins the okazaki fragments together by forming phosphodiester bonds between nucleotides resulting in a complete lagging strand.**



DNA Replication



DNA Replication

Mutations

- **Mutation is a change in the nucleotide sequence or number of a section of DNA.**
- **Mutagenesis is the process of producing mutations.**
- **Mutagen is an agent that causes mutations.**
- **Mutant is an organism whose nucleotide sequence of DNA differs from the wild type (normal organism).**
- **In general, mutations may occur in somatic cells (aren't passed to offspring) or may occur in gametes (eggs & sperms) which can pass to offspring.**

DNA Replication

Types of mutation

1. Gene mutations

- **Mutation in one or more nucleotides in a gene sequence, so it could be Point mutation or Frameshift mutation.**

- **Point mutation occurs as a result of replacement of one nucleotide by other in specific nucleotide sequence of gene.**

- **Point mutations are two types based on the base pair substitution**

a. Translation

- **It occurs by substitution of one purine nucleotide (A, G) by another purine nucleotide or one pyrimidine nucleotide (T, C) by another pyrimidine nucleotide.**

b. Transversion

- **It occurs by substitution of purine nucleotide by pyrimidine nucleotide and vice versa.**

DNA Replication

Types of mutation

1. Gene mutations

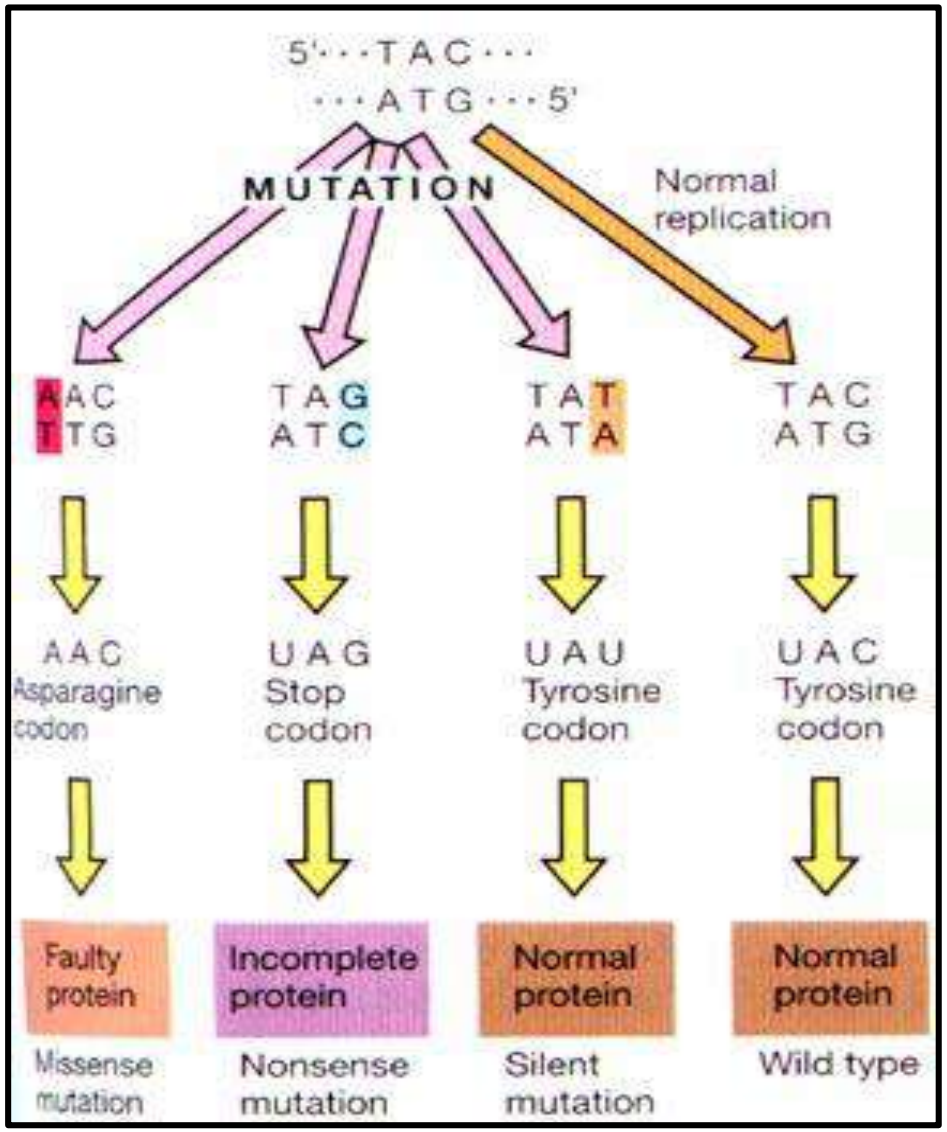
- **Based on transcription, point mutation are of three types**

  a. **Silent mutation**

  - **It is also known as neutral mutation.**
  - **It is the mutation in which mutated codon leads to the same amino acid as the original one. So, it does not effect the structure and composition of protein.**

  b. **Missense mutation**

- **In this mutation, mutated codon leads to different amino acid (other than original). So, the protein formed from it is also altered. Such protein can be less active or completely inactive.**



DNA Replication

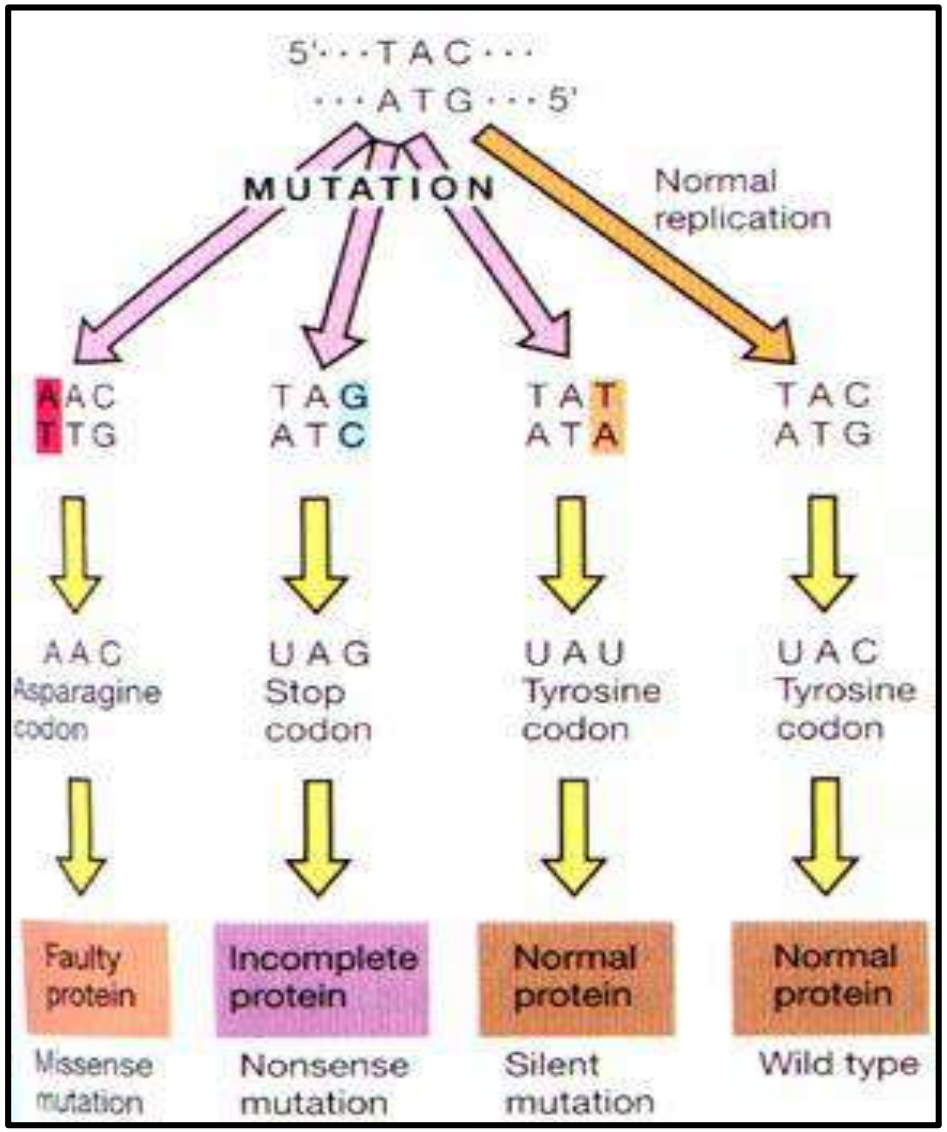
Types of mutation

1. Gene mutations

- **Based on transcription, point mutation are of three types**

c. Non sense mutation

- **It is the mutation in which mutated codon is a stop codon.**
- **Non sense mutation causes incomplete synthesis. Such incomplete protein is always non-functional.**

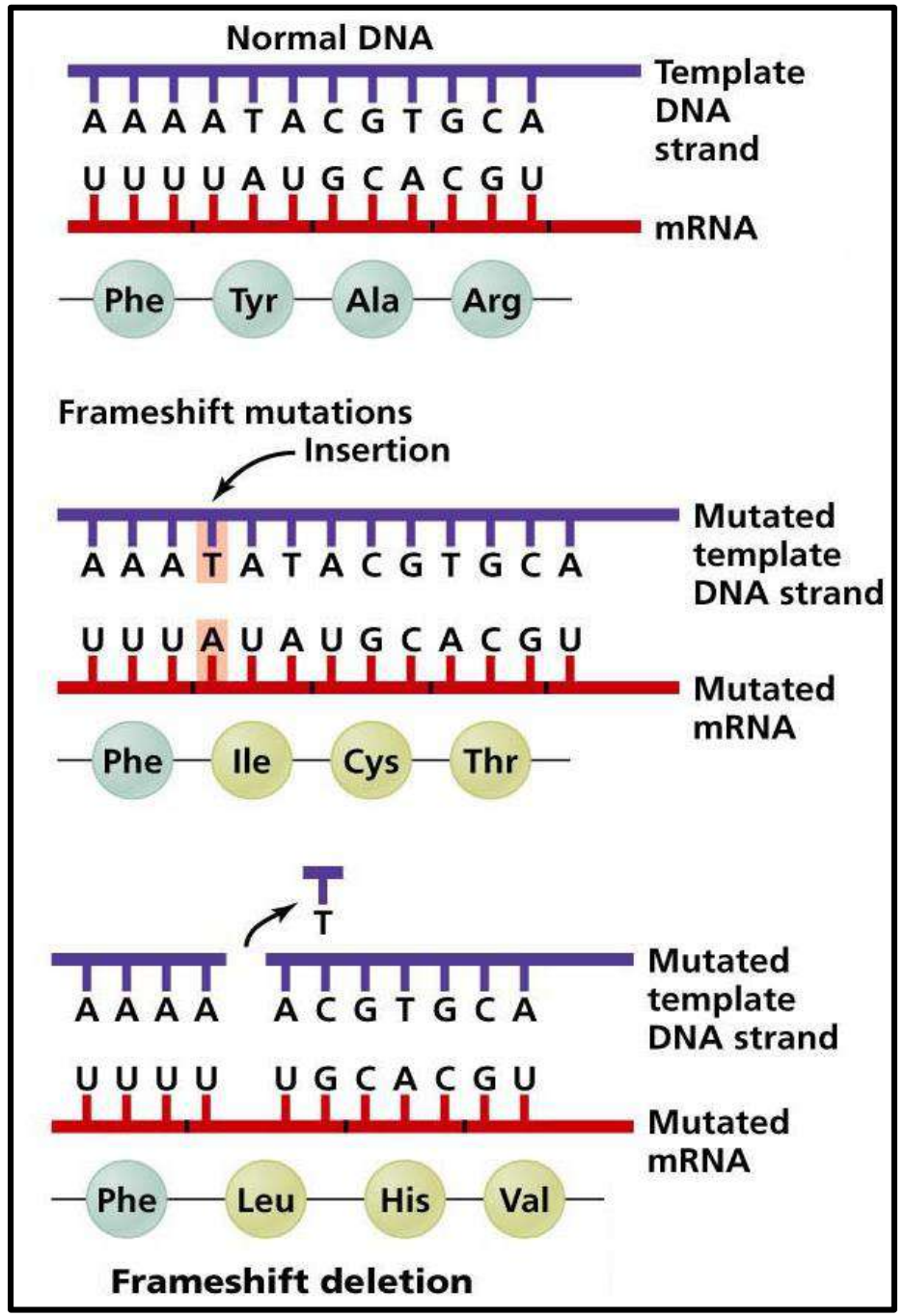


DNA Replication

Types of mutation

1. Gene mutations

- **Frameshift mutation occurs when one or more nucleotides are inserted into or deleted from the DNA.**
- **This mutation results in a shift in the reading of mRNA codons, so different protein is produced.**



DNA Replication

Types of mutation

2. Chromosomal mutations

- **Chromosomal mutation is any change that occurs within the chromosome.**
- **Unlike gene mutations that involve the alteration of a gene or a segment of DNA in the chromosome, chromosomal mutations occur and change the entirety of the chromosome itself.**
- **Such mutations can be attributed to any mistake or problem that occurs during cell processes like mitosis and meiosis.**
- **There are four types of chromosomal mutations: deletion, duplication, inversion and translocation.**

DNA Replication

Types of mutation

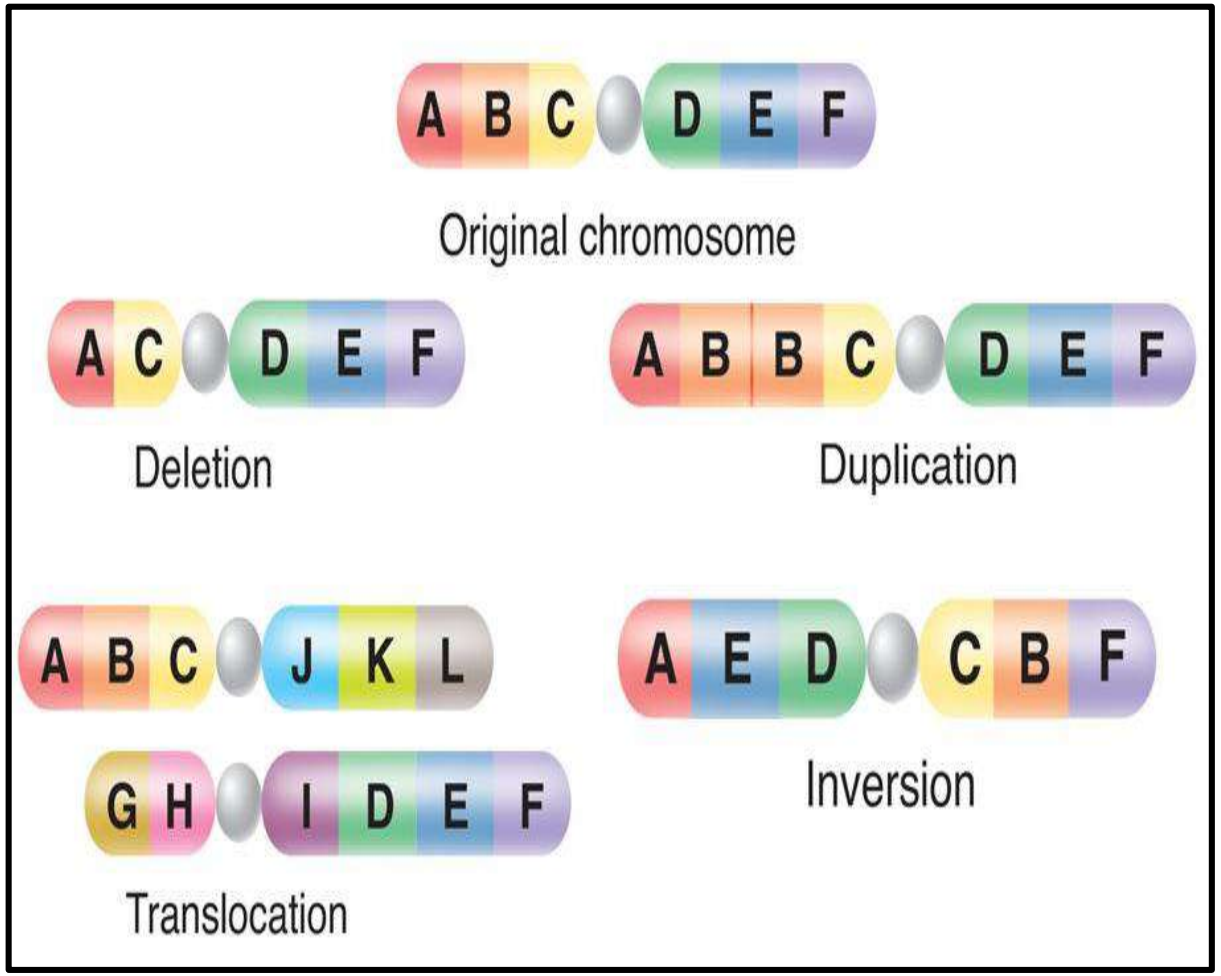
2. Chromosomal mutations

a. Deletion

- **This type of mutation occurs when a part of the DNA is not duplicated or is lost during DNA replication.**

b. Duplication

- **This type of mutation occurs when an extra copy of a region (or regions) in the DNA is produced.**
- **This duplicated region can either be located in its normal location in the chromosome or sometimes be located in other parts of the chromosomes.**



DNA Replication

Types of mutation

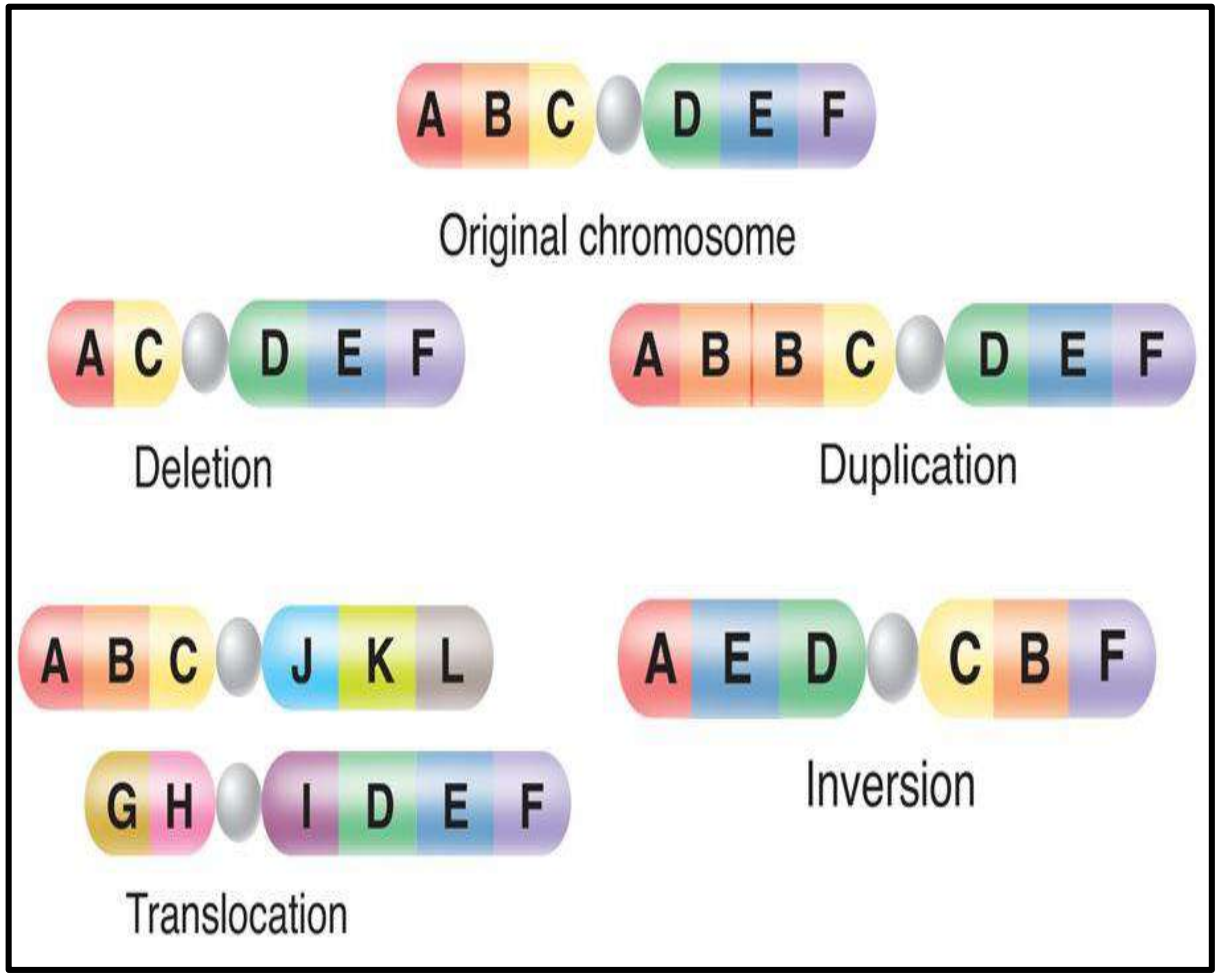
2. Chromosomal mutations

c. Inversion

- **During inversion, a portion in the chromosome is reversed and gets inserted back into the chromosome.**

d. Translocation

- **Translocation happens when a fragmented chromosome tends to join with a nonhomologous chromosome.**
- **This newly-formed segment then detaches from the chromosome and moves to a new position on another chromosome.**



DNA Replication

Types of mutation

2. Chromosomal mutations

- **Aneuploidy is a type of mutation in the chromosome number wherein the ploidy (chromosome number) of the new individual is different from its wild type.**

- **This is typically a result of the nondisjunction of chromosomes during mitosis or meiosis, hence producing offspring with either extra or lost chromosomes.**

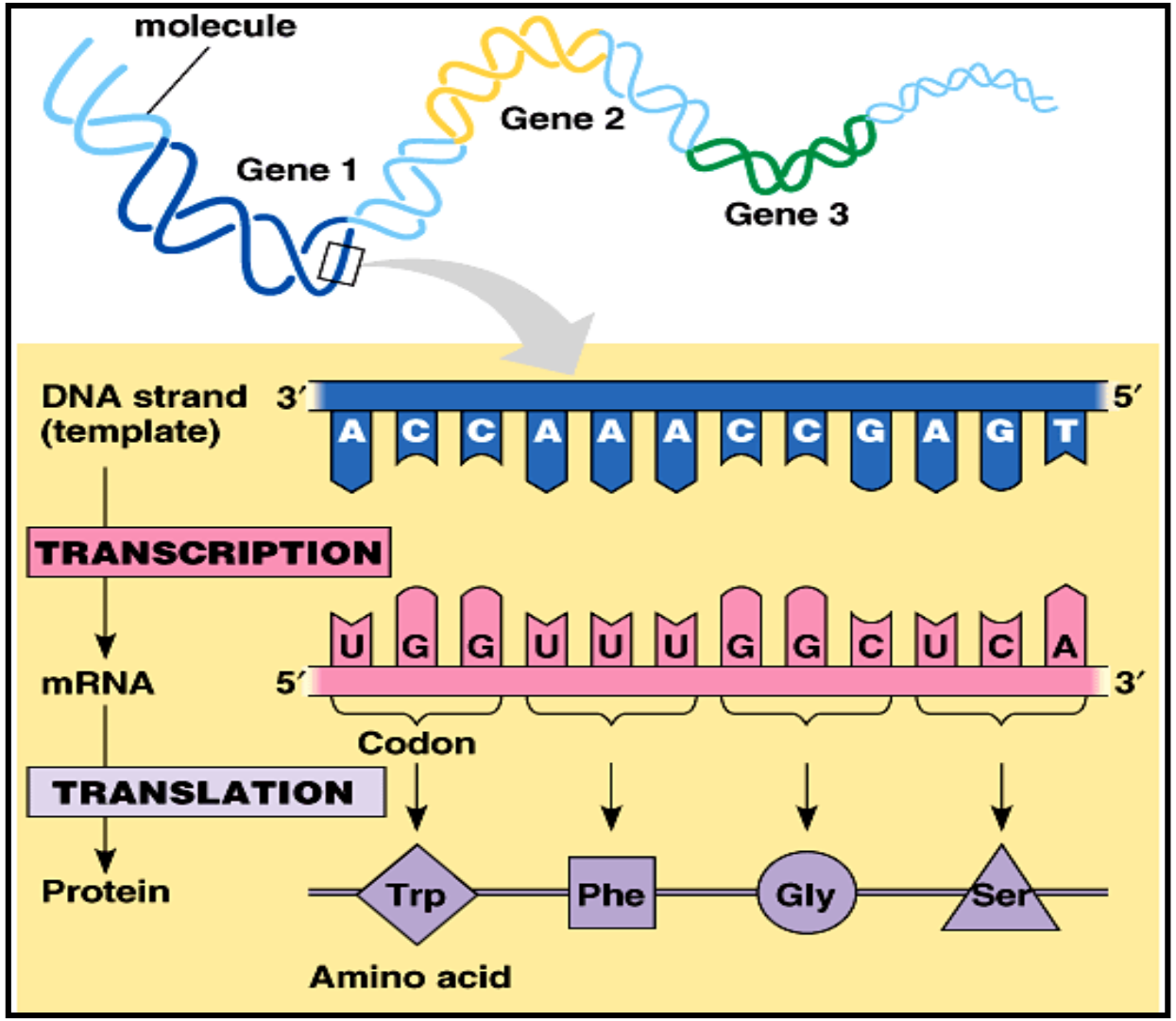
Protein Synthesis: From gene to protein

- Protein synthesis is the process by which the cells make new proteins as a result of signals from specific factors (as growth factors) to specific genes.

- Genes contain the information required for synthesis of proteins, but genes cannot produce proteins directly, so messenger RNA (mRNA) is required.

Gene → mRNA → Protein

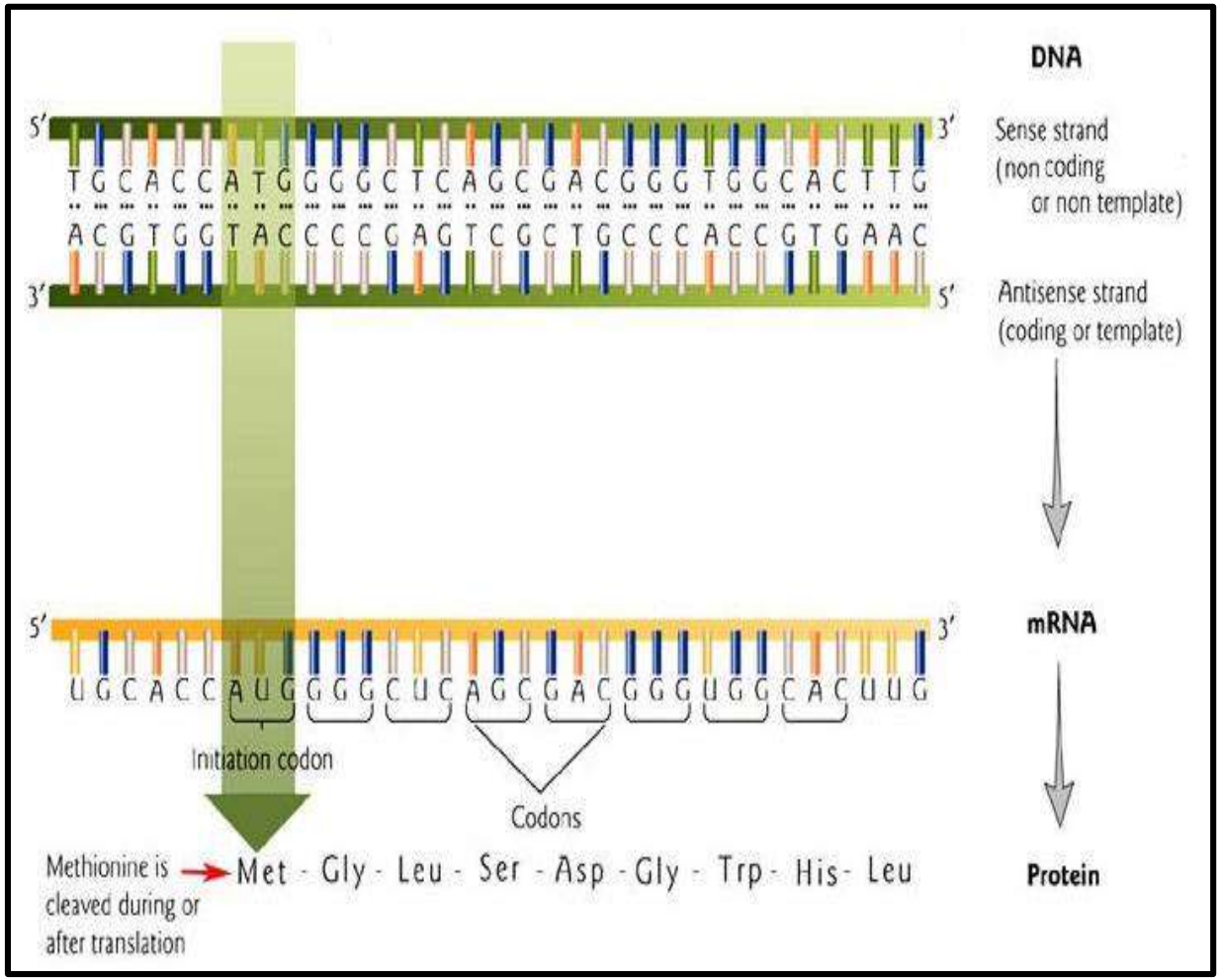
- To get a protein from a gene, two processes are required: Transcription and Translation.



Protein Synthesis: From gene to protein

1. Transcription

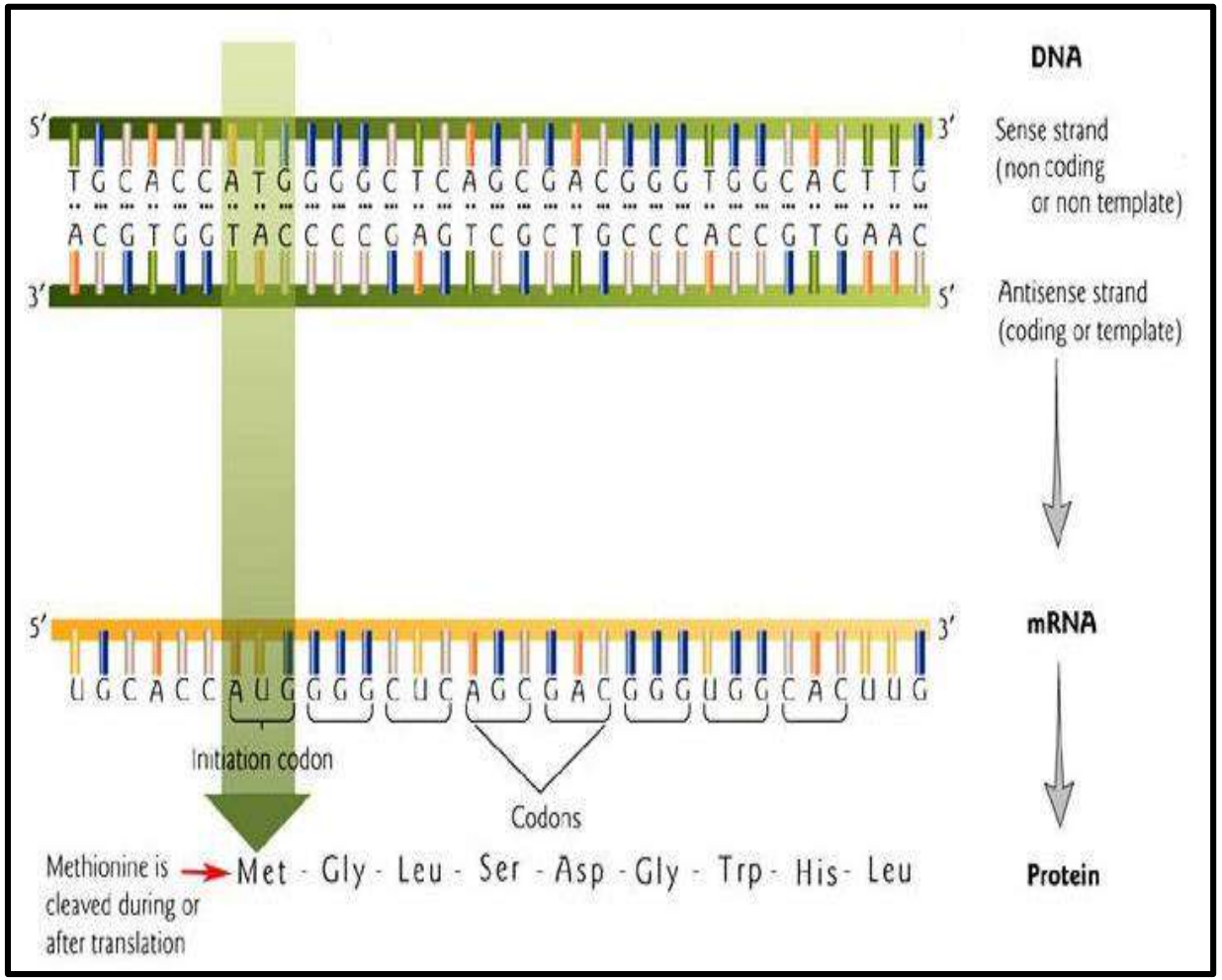
- **Transcription is a process by which genetic information from a specific gene is transcribed into RNA.**
- **It occurs in the cell nucleus, where only one strand of the DNA double helix is transcribed called the template (antisense strand).**
- **Antisense strand is the strand from which the mRNA is transcribed (mRNA is its complementary)**
- **The second strand is called coding (sense) strand.**
- **Coding strand is the strand which has the same sequence as the mRNA transcript and it contains the codons that are translated into a protein.**



Protein Synthesis: From gene to protein

1. Transcription

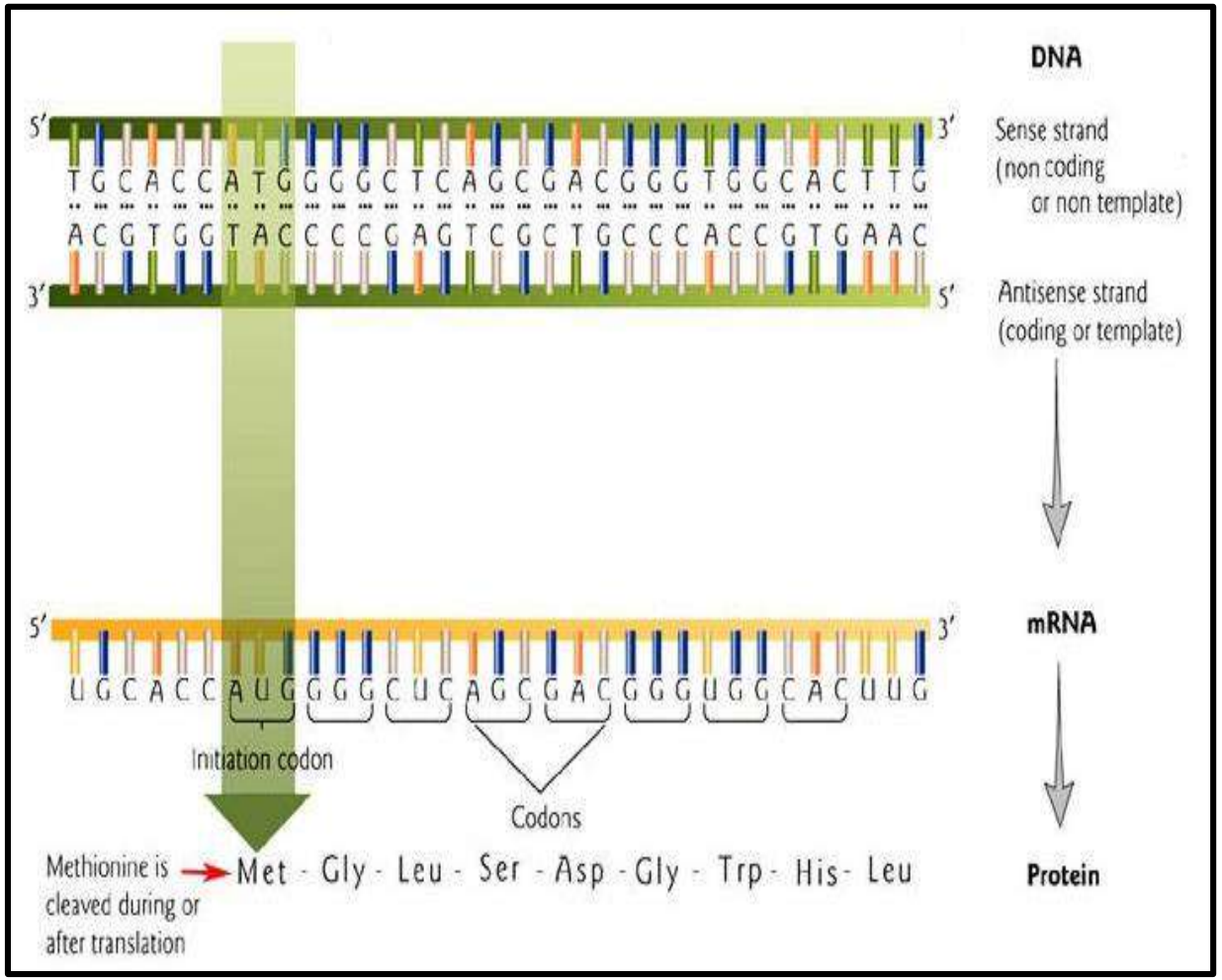
- **RNA polymerases (I, II, III) are the main enzymes in transcription process.**
- **Types of RNA polymerases**
  - **RNA polymerase I which is used for rRNA synthesis.**
  - **RNA polymerase II which is used for mRNA and microRNA synthesis.**
  - **RNA polymerase III which is used for tRNA synthesis.**



Protein Synthesis: From gene to protein

1. Transcription

- **Types of RNA**
  - ***Messenger RNA (mRNA):*** **It carries the information from a gene to make a protein.**
  - ***Transfer RNA (tRNA):*** **It transfers amino acids to the ribosome to make a protein.**
  - ***Ribosomal RNA (rRNA):*** **It is a part of the structure of ribosomes.**
  - ***MicroRNA (miRNA):*** **It is a small sequence of nucleotides which regulate gene expression.**



Differences between DNA replication and transcription

Item	DNA Replication	DNA Transcription
Template	Double strands	Single strand (antisense)
Primer	Yes	No
Enzyme	DNA polymerase III	RNA polymerases
Product	Leading and lagging strands	Single strand RNA
Base pair	A-T, C-G, T-A, G-C	A-U, T-A, G-C, C-G
Target	Whole strand	Specific region in the template

Protein Synthesis: From gene to protein

1. Transcription

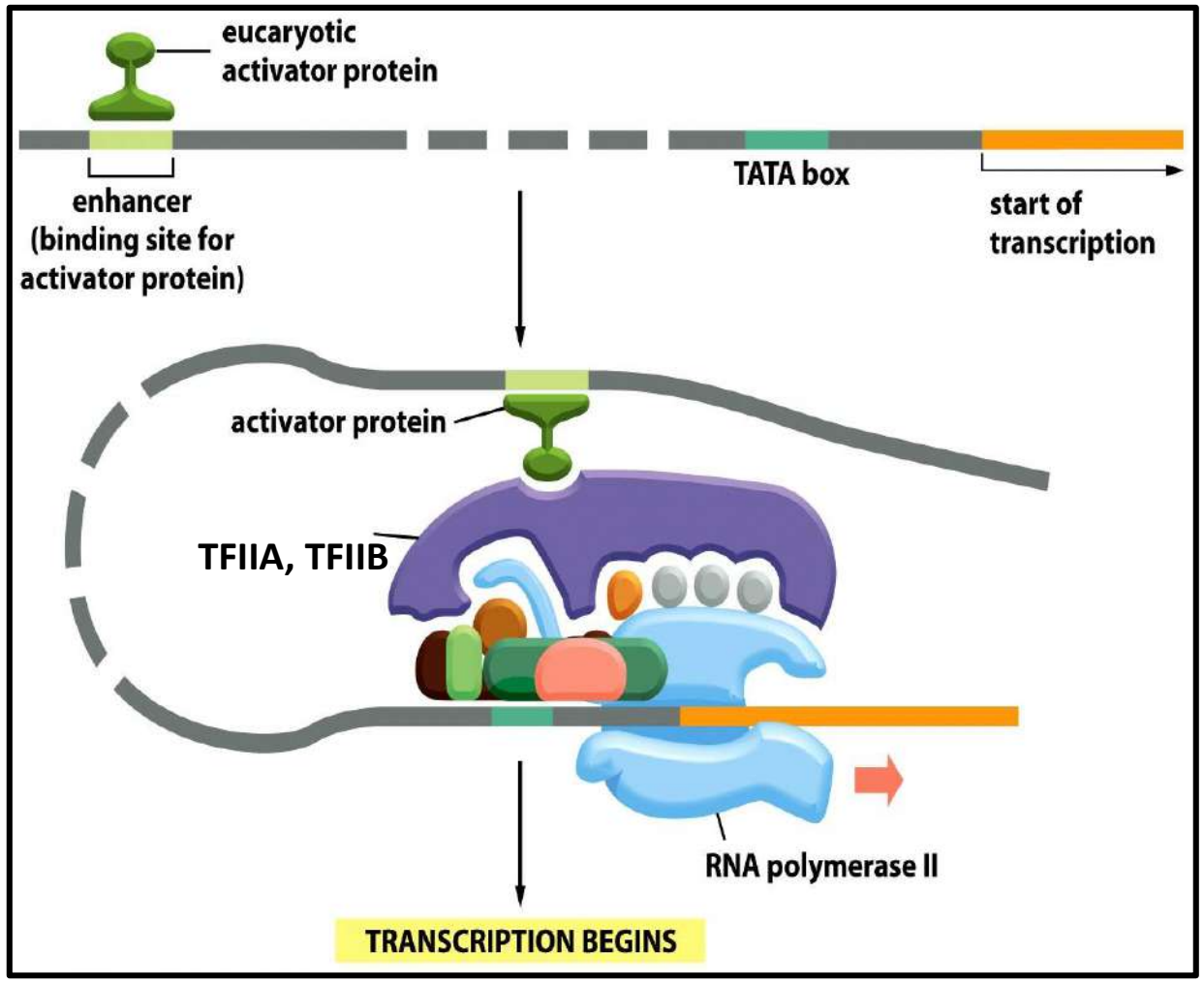
Stages of transcription process

1- Initiation Stage

- **It is the first step in transcription process which requires the presence of a promoter sequence in the template strand.**

- **Promoter is a specific nucleotides sequence in the template strand to which RNA polymerase II can bind and initiate the transcription process.**

- **The most characterized promoter in eukaryotes is a short DNA sequence known as a TATA box, which is a small sequence near the beginning of the selected gene.**



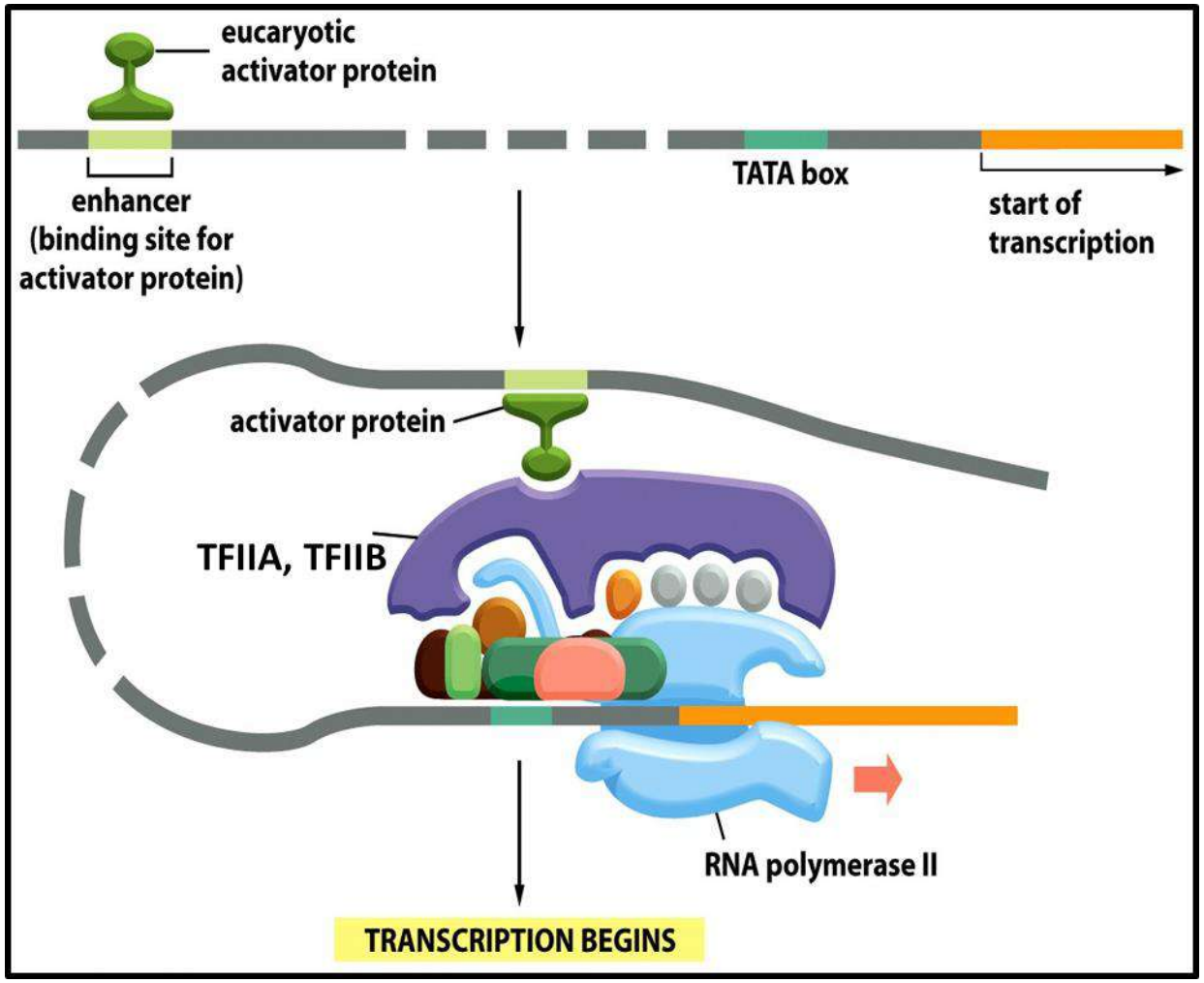
Protein Synthesis: From gene to protein

1. Transcription

Function of promoter

1. **Determination of the template strand for RNA polymerase II.**
2. **Determination of the starting point of transcription on the gene.**

- **Firstly, an activator protein binds with a specific sequence called an enhancer located near the promotor.**
- **This enhances binding of helper proteins called general transcription factors as well as RNA polymerase II to the promoter.**



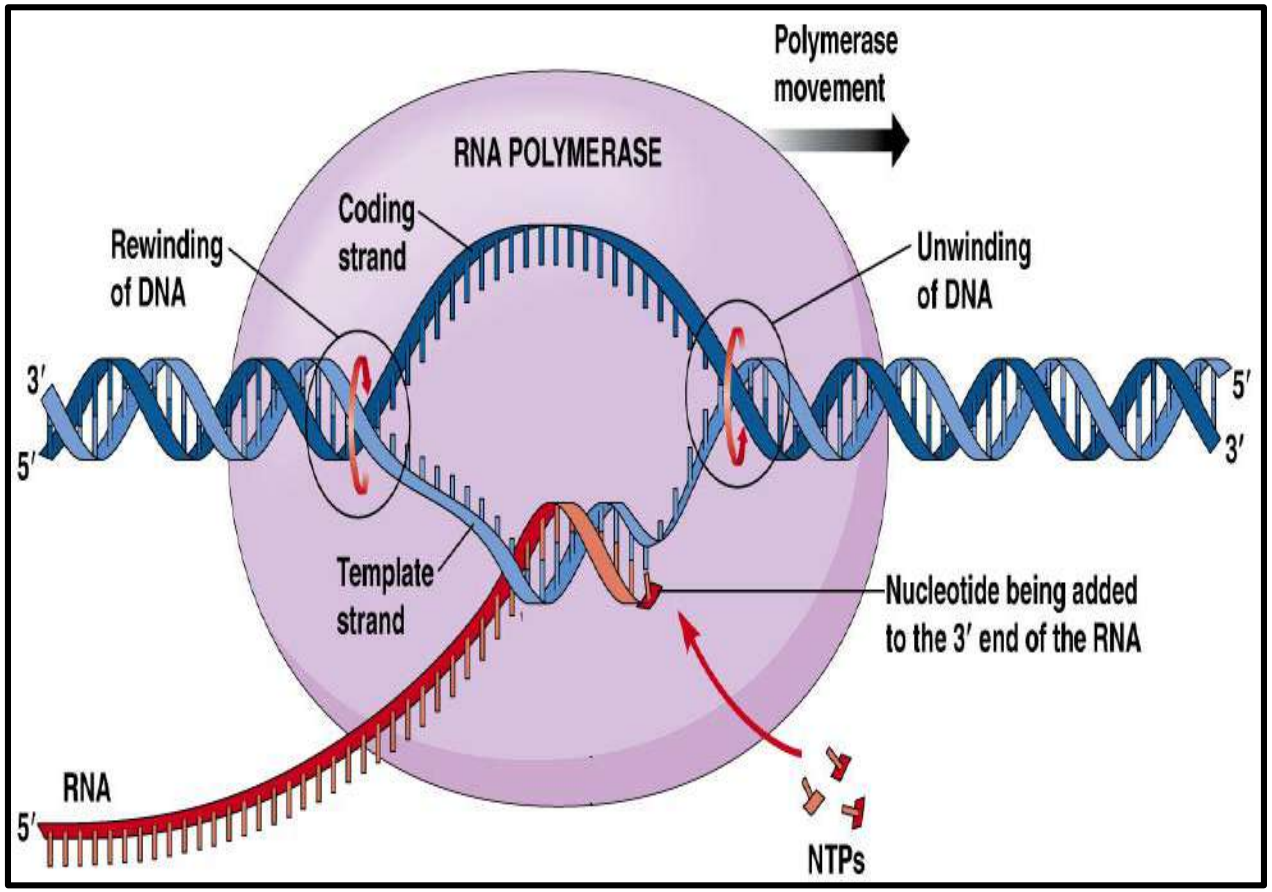
Protein Synthesis: From gene to protein

1. Transcription

Stages of transcription process

2- Elongation Stage

- **Once the RNA polymerase II has bound to the promoter, it begins to separate the DNA double strands about 20 bases at a time and reads the template strand from (3') end to (5') end.**
- **During this step, RNA polymerase II adds a complementary RNA nucleotide to the 3' end of the RNA strand, meaning that mRNA synthesizes in the 5' → 3' direction.**
- **This produces mRNA molecule which is an exact copy of the coding strand except that thymine is replaced with uracil, and the nucleotides are composed of ribose sugar.**



Protein Synthesis: From gene to protein

1. Transcription

Stages of transcription process

3- Termination Stage

- **It is the last stage of transcription process in which the RNA polymerase II cannot add any nucleotide to mRNA.**
- **RNA polymerase II gets signals to stop once it transcribes a sequence of DNA known as a terminator which is usually is TTATTT sequence.**
- **After that, mRNA molecule is separated from the template strand by ribonuclease.**

Protein Synthesis: From gene to protein

1. Transcription

Post-transcriptional modification

- All the primary transcripts of RNA are immature and unstable, so they must undergo processing steps to produce functional RNA (mature RNA) before leaving the nucleus.

- In the case of mRNA, the primary transcript of RNA is called precursor messenger RNA (pre-mRNA) which must be converted into mature mRNA prior to protein translation in a process called mRNA processing.

- mRNA processing consists of three major steps:-

  a. 5’ Capping (5' cap)

  b. Addition of 3' poly-adenylation tail (Polyadenylation)

  c. Splicing

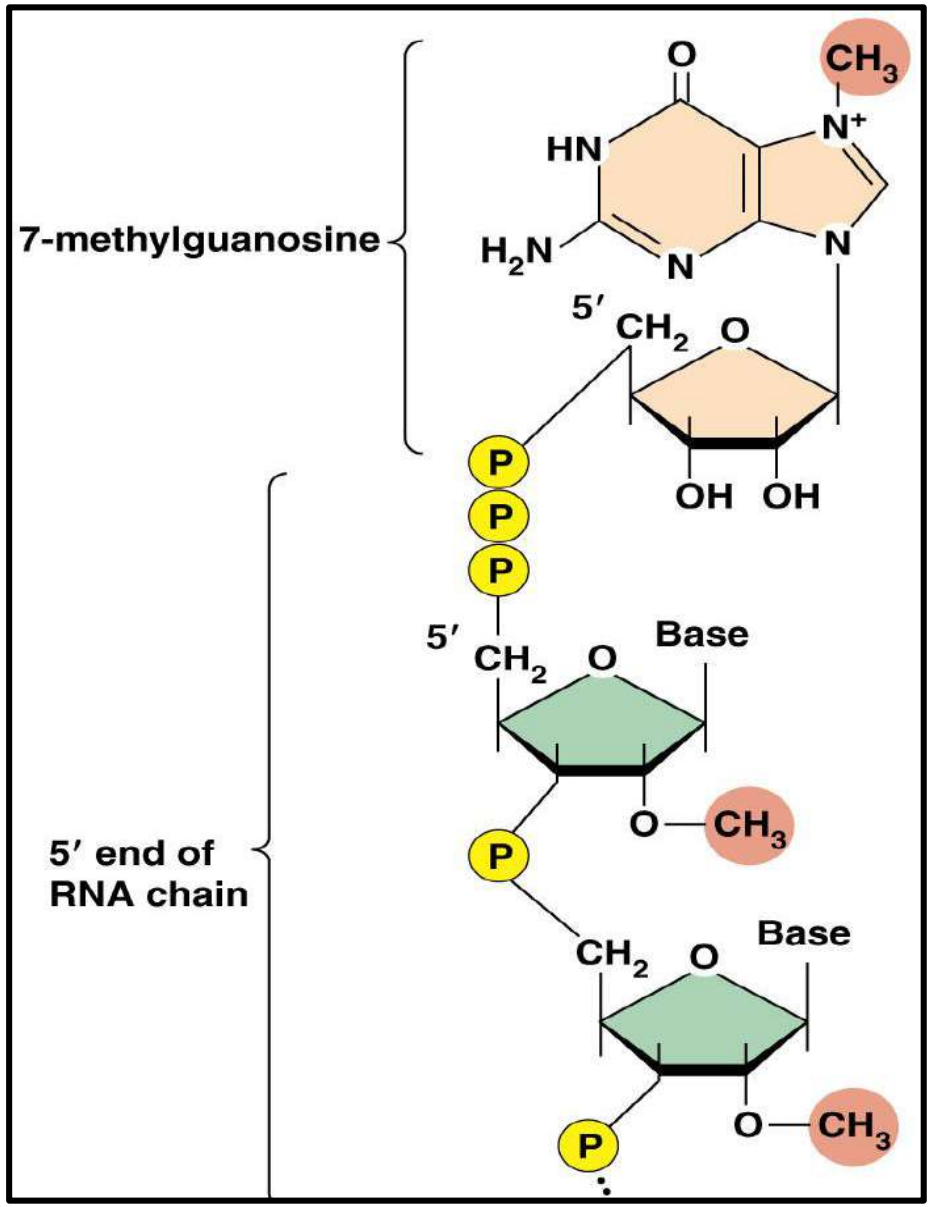
Protein Synthesis: From gene to protein

1. Transcription

Post-transcriptional modification

a. 5’ Capping (5' cap)

- It is addition of a modified guanine nucleotide to the 5' end of pre-mRNA shortly after the start of transcription.
- The 5' cap consists of 7-methylguanosine residue that is linked through 5'-5'-triphosphate bridge to the first transcribed nucleotide.
- Its presence protects mRNA degradation by RNA nuclease and facilitates the binding of mature mRNA to ribosome to initiate translation process, where translation initiation factors can identify 5’ cap.



Protein Synthesis: From gene to protein

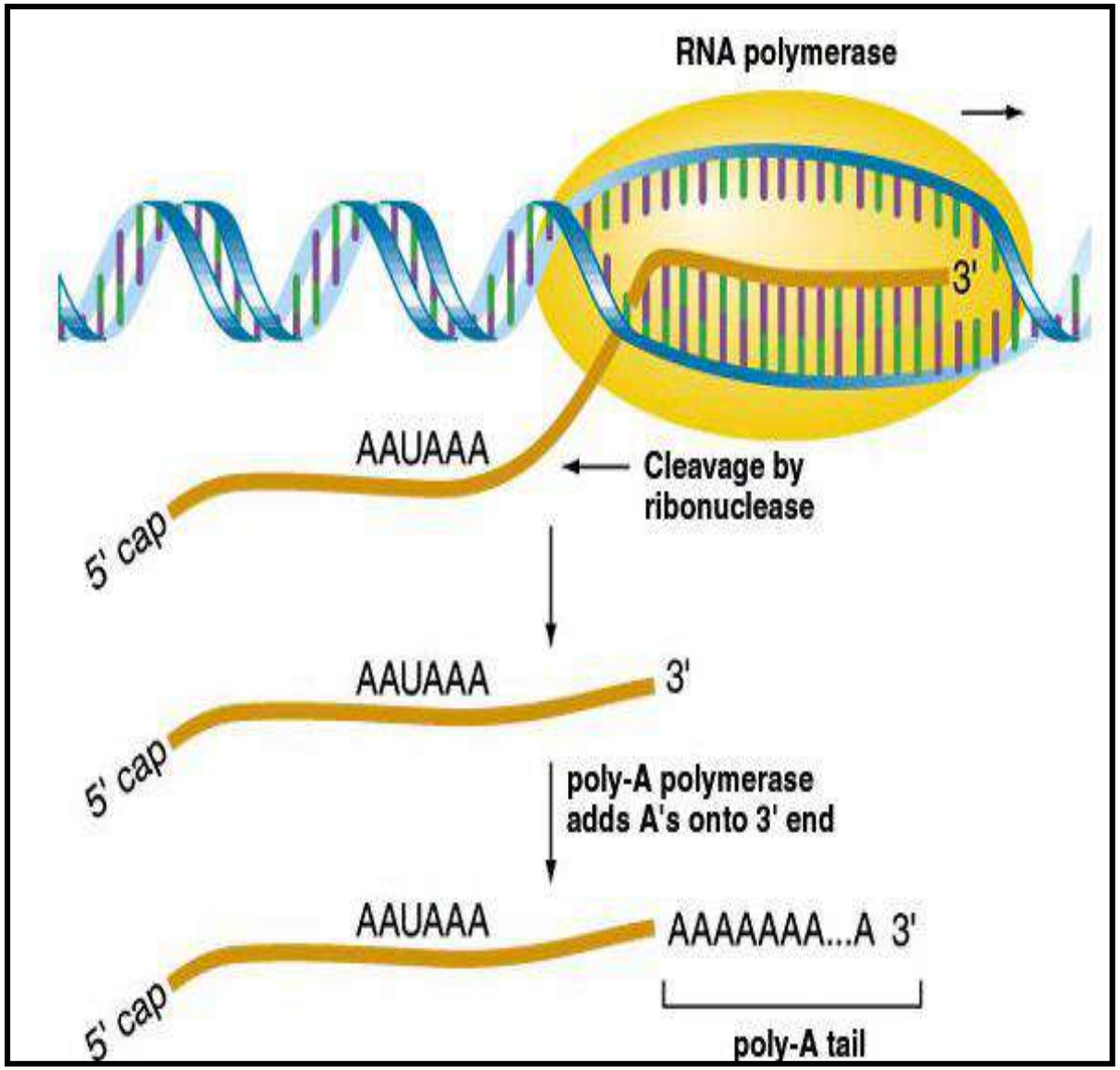
1. Transcription

Post-transcriptional modification

b. Addition of 3' poly-adenylation tail (Polyadenylation)

- **Polyadenylation is addition of polyadenine nucleotides to pre-mRNA at the 3' end forming poly(A) tail.**

- **It occurs immediately after transcription termination, when pre-mRNA chain is cleaved through the action of ribonuclease at AAUAAA sequence.**



Protein Synthesis: From gene to protein

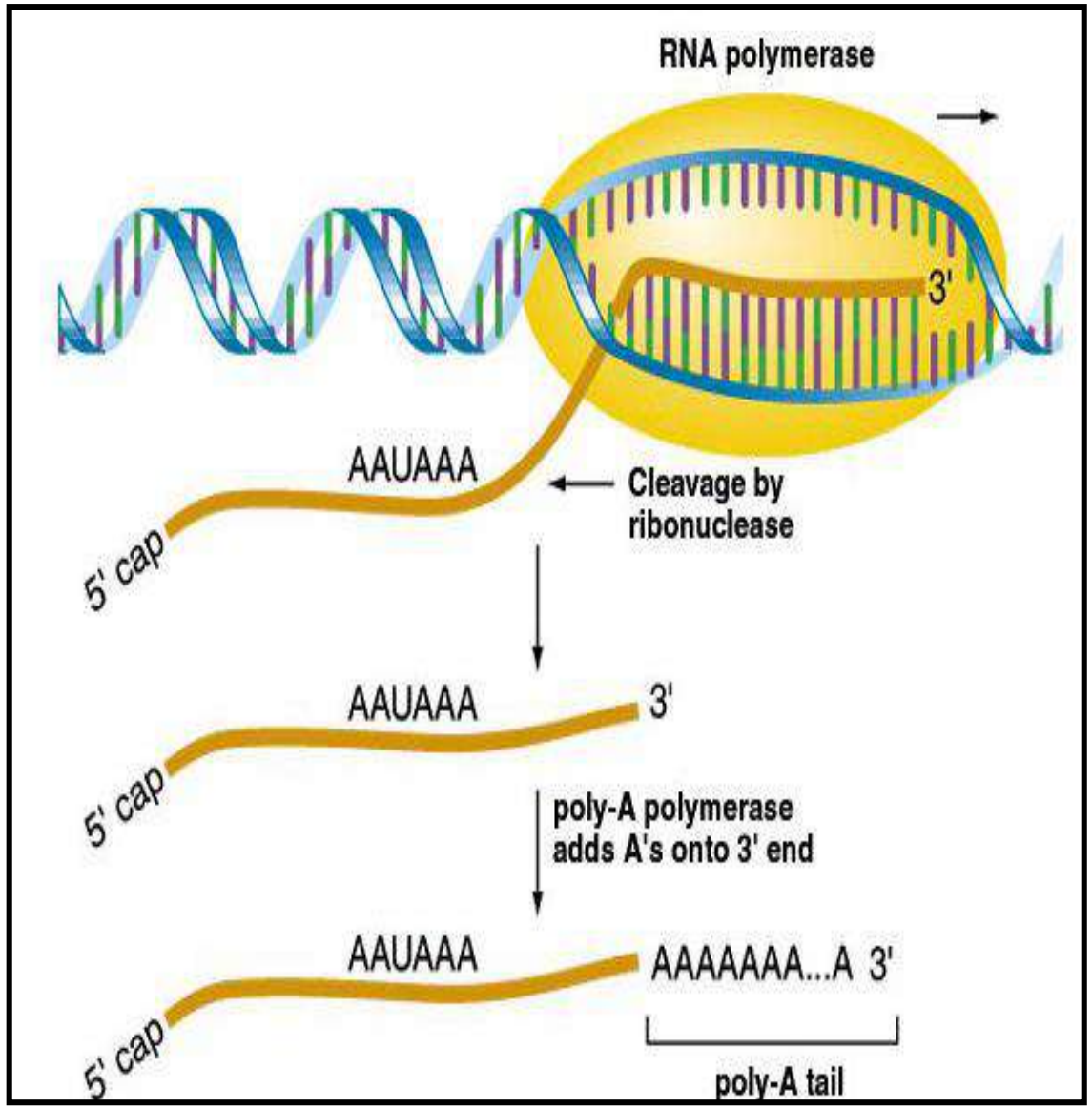
1. Transcription

Post-transcriptional modification

b. Addition of 3' poly-adenylation tail (Polyadenylation)

- **After the mRNA has been cleaved, around 100-300 adenine residues are added to the free 3' end at the cleavage site by the action of polyadenylate polymerase (PAP).**

- **This process enhances the stability of mRNA and regulates its transport to the cytoplasm, and enhances binding proteins involved in initiating translation.**

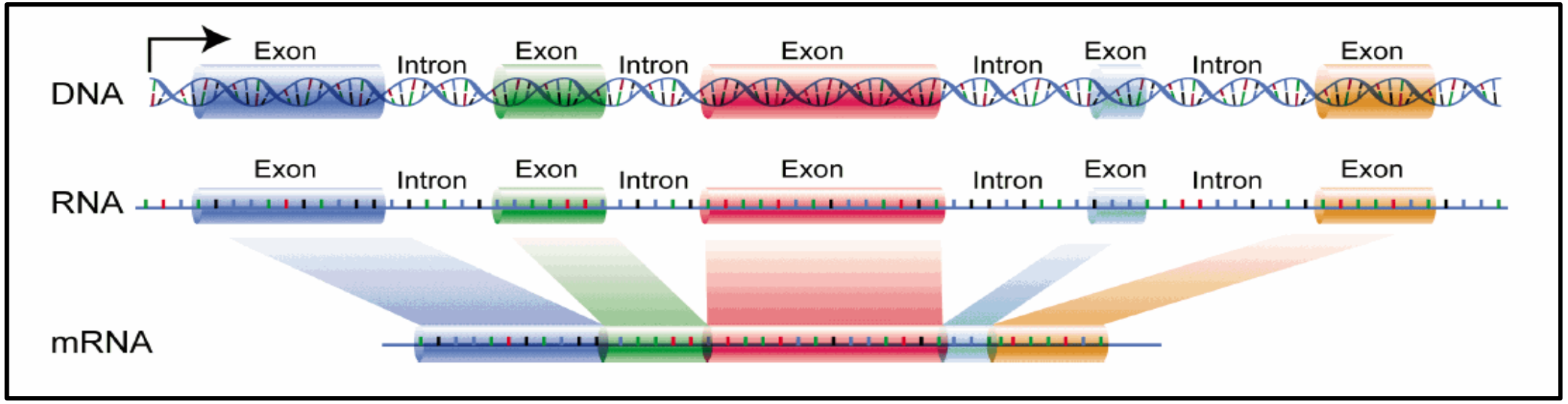


Protein Synthesis: From gene to protein

Post-transcriptional modification

c. Splicing

- **In general, eukaryotic genes are composed of exons, which correspond to protein-coding sequences, and intervening sequences called introns.**
- **Introns are non-coding sequences involved in gene regulation.**
- **All introns in a pre-mRNA must be completely removed before protein synthesis because their translation results in non-functional proteins.**

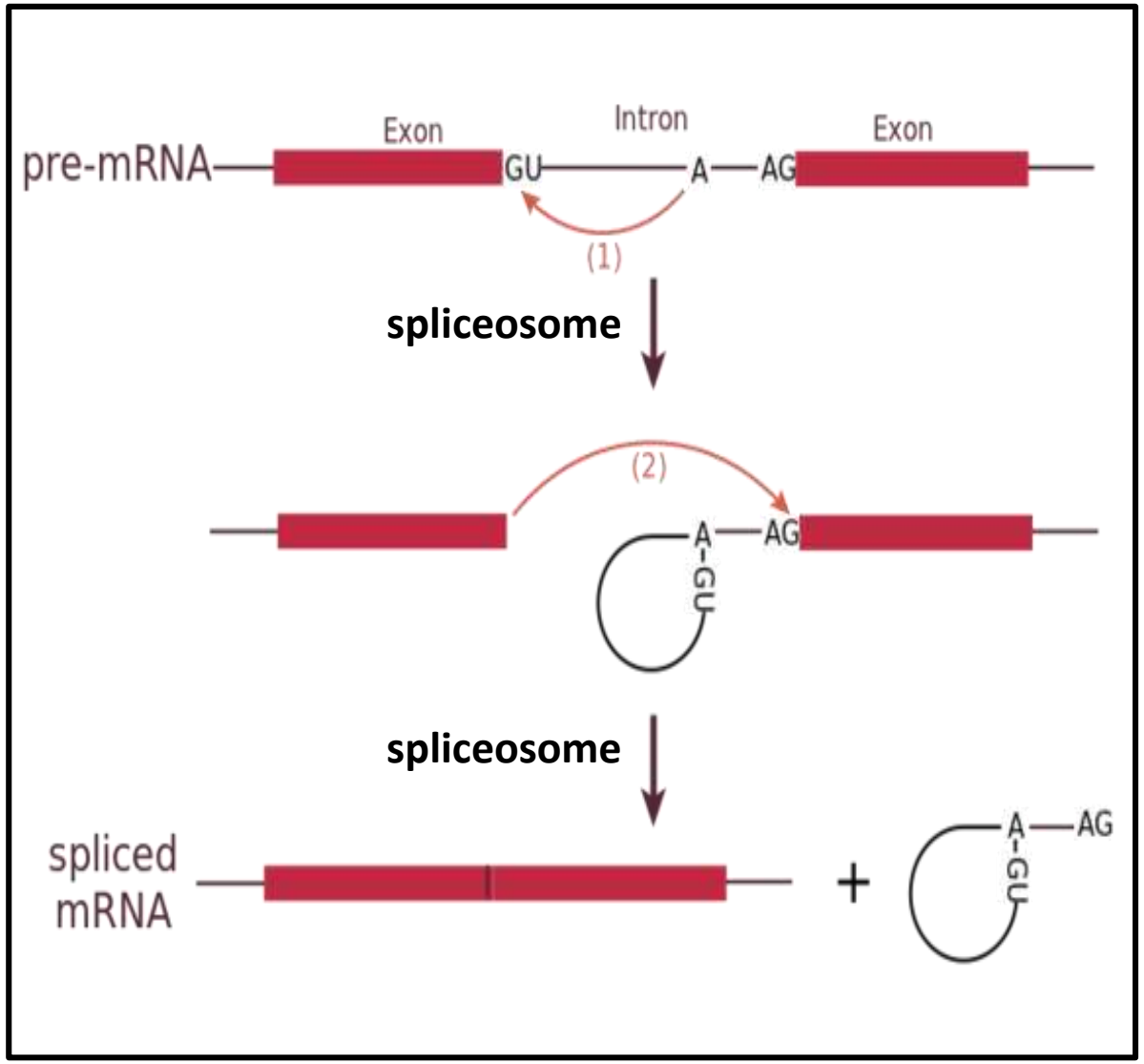


Protein Synthesis: From gene to protein

Post-transcriptional modification

c. Splicing

- **Introns are removed and degraded while the pre-mRNA is still in the nucleus in a process called splicing.**
- **The splicing of pre-mRNAs is conducted by complexes of proteins and RNA molecules called spliceosomes.**
- **Each spliceosome is composed of five subunits called snRNPs (small nuclear ribonucleoparticles).**
- **Spliceosomes recognize sequences at the 5′ end of the intron because introns always start with the nucleotides GU and they recognize sequences at the 3′ end of the intron because they always end with the nucleotides AG.**

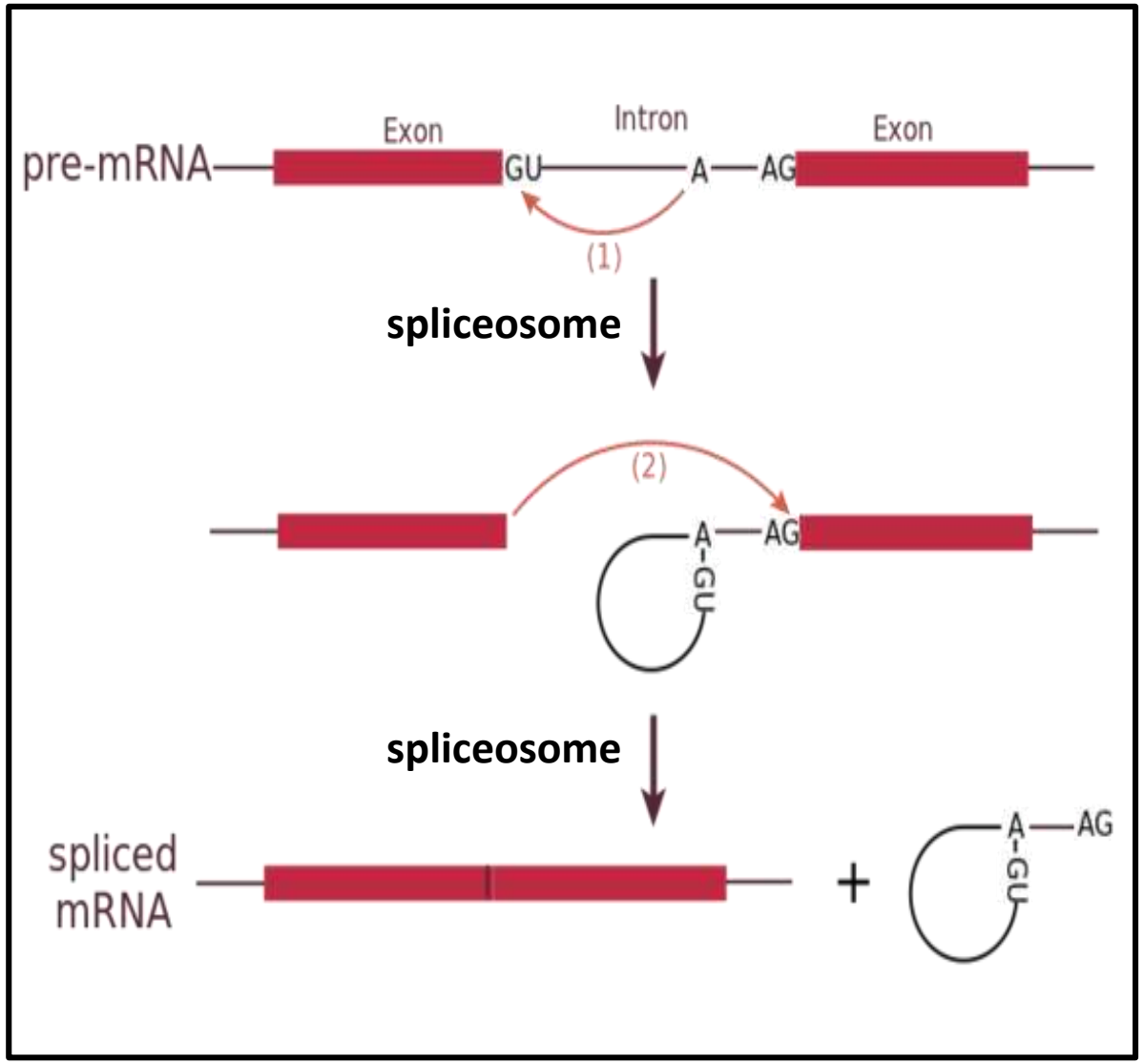


Protein Synthesis: From gene to protein

Post-transcriptional modification

c. Splicing

- **The spliceosome cleaves the pre-mRNA's sugar phosphate backbone at the G that starts the intron and then covalently attaches that G to an internal A nucleotide within the intron.**
- **Then the spliceosme connects the 3′ end of the first exon to the 5′ end of the following exon, cleaving the 3′ end of the intron in the process.**
- **This results in the splicing together of the two exons and the release of the intron.**



Protein Synthesis: From gene to protein

2. Translation

- **Translation is the second process of protein biosynthesis which occurs in the cytoplasm, where the ribosomes are located.**

- **It is the process by which mRNA produced by transcription process is translated by the ribosome to produce a protein.**

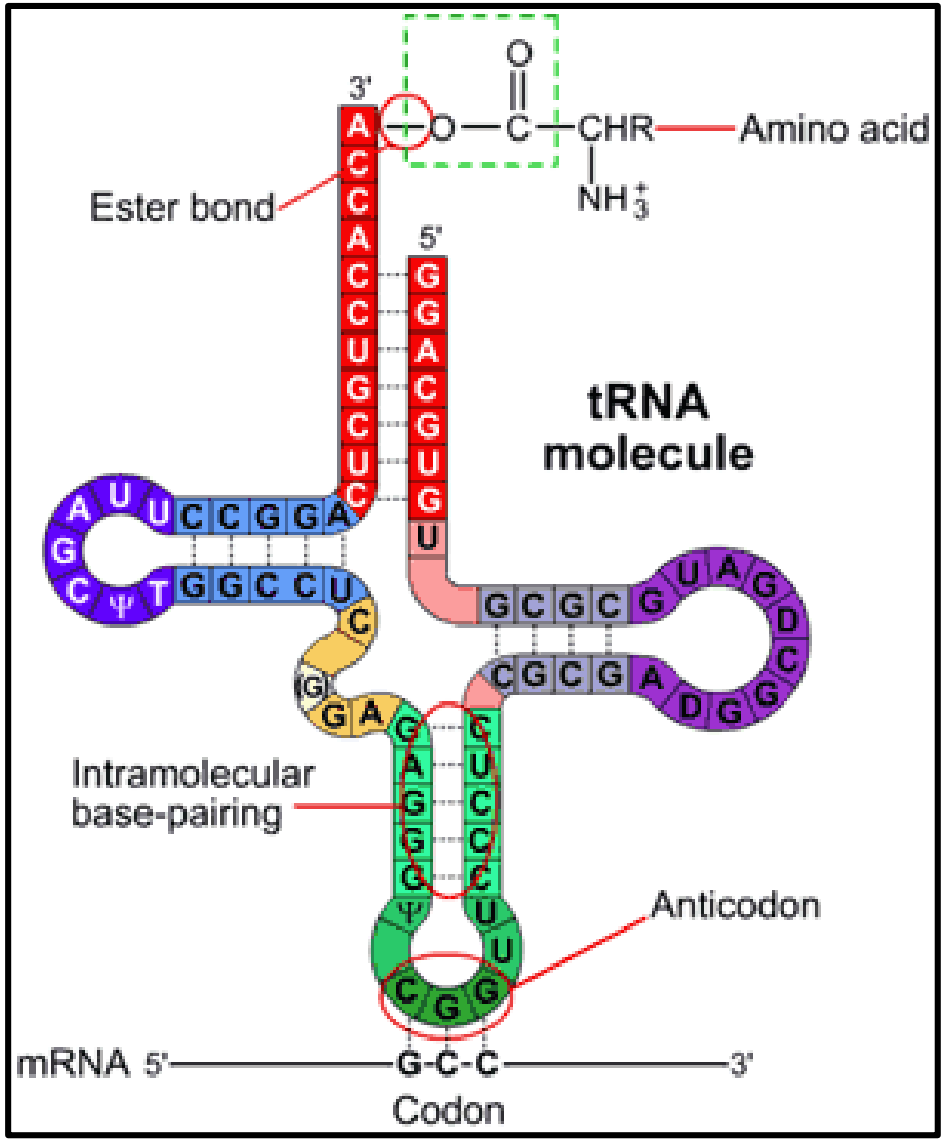
- **Transfer RNA (tRNA) is considered as a critical component in this process.**

Protein Synthesis: From gene to protein

2. Translation

Transfer RNA (tRNA)

- Transfer RNA (tRNA) converts the information in mRNA codon into specific amino acid and binds with it, then carrying the selected amino acid to the ribosome to form a protein.
- At one site of the tRNA, it has a specific segment called the anticodon which is a complementary segment to a codon in mRNA.
- By the anticodon segment, tRNA binds to the (A) site at the ribosome.
- On the other site of the tRNA, the amino acid that corresponds to the anticodon is covalently attached.

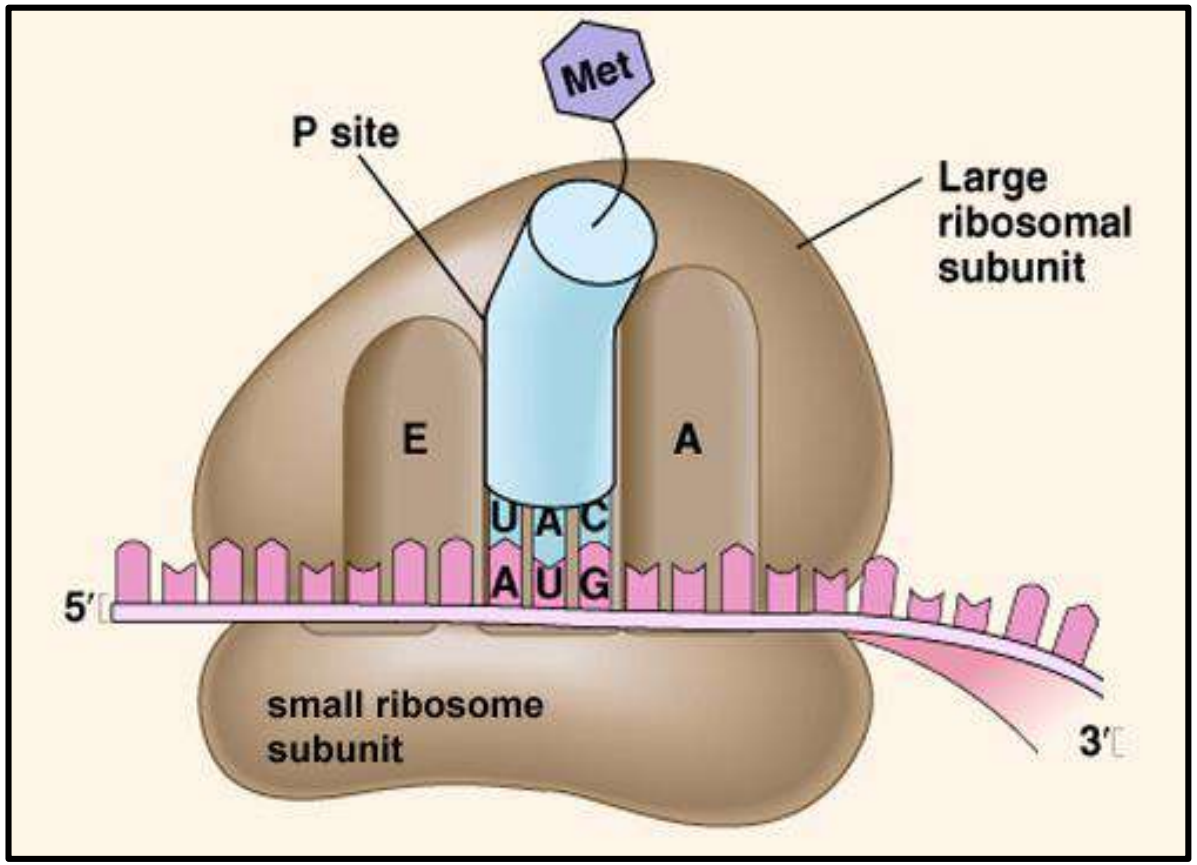


Protein Synthesis: From gene to protein

2. Translation

Ribosomes

- Ribosomes are small structures composed of proteins and ribosomal RNA (rRNA), which act as the primary site of biological protein synthesis.
- Each ribosome is divided into two subunits: the small subunit and the large subunit.
- Before translation process and after finishing mRNA translation, the ribosomes exit as a separated subunits.
- Small subunit: binds with mRNA at 5’-end.

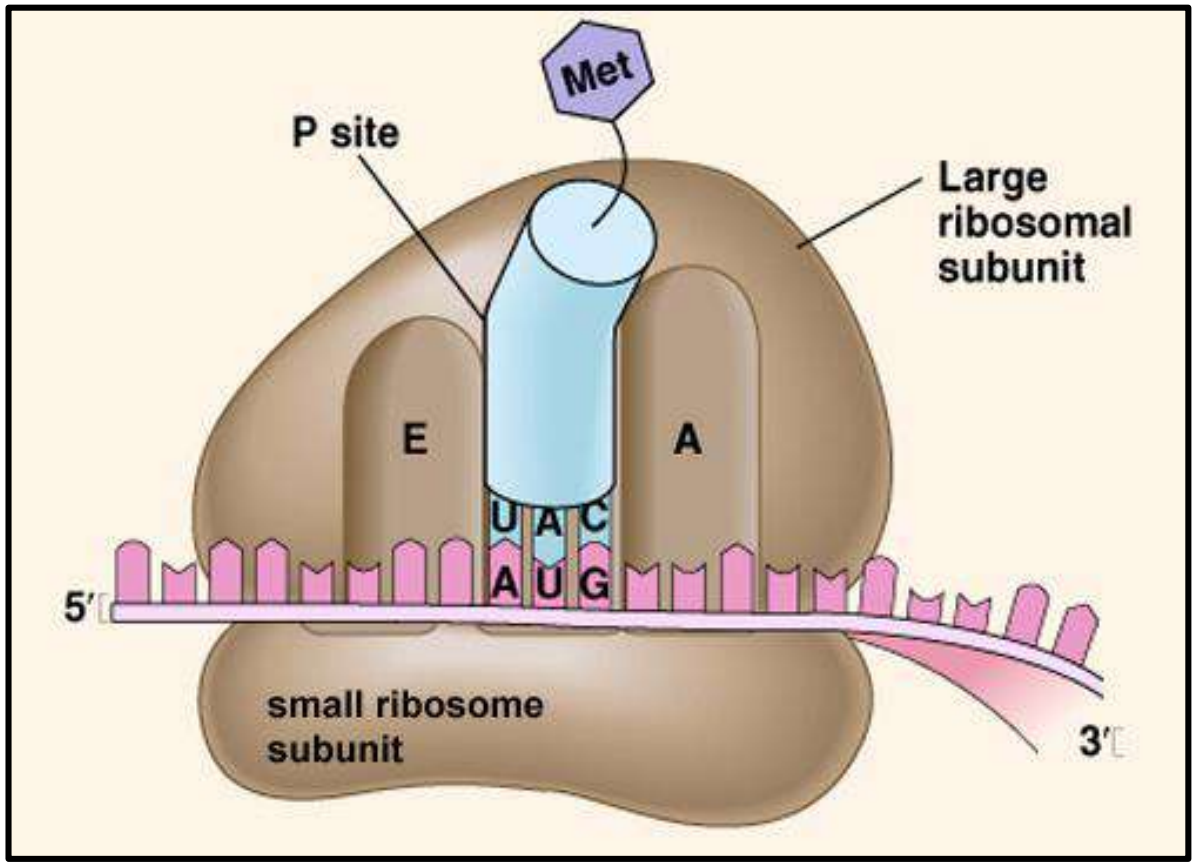


Protein Synthesis: From gene to protein

2. Translation

Ribosomes

- **Large subunit has three binding sites for tRNA:-**
  - **The A (amino acid) site: is the site that binds with the tRNA which has the selected amino acid.**
  - **The P (polypeptide) site: is the site that binds with the tRNA carrying the growing polypeptide chain.**
  - **The E (exit) site: is the site where the empty tRNA leaves the ribosome to the cytoplasm to pick up another amino acid and begins the process again.**



Protein Synthesis: From gene to protein

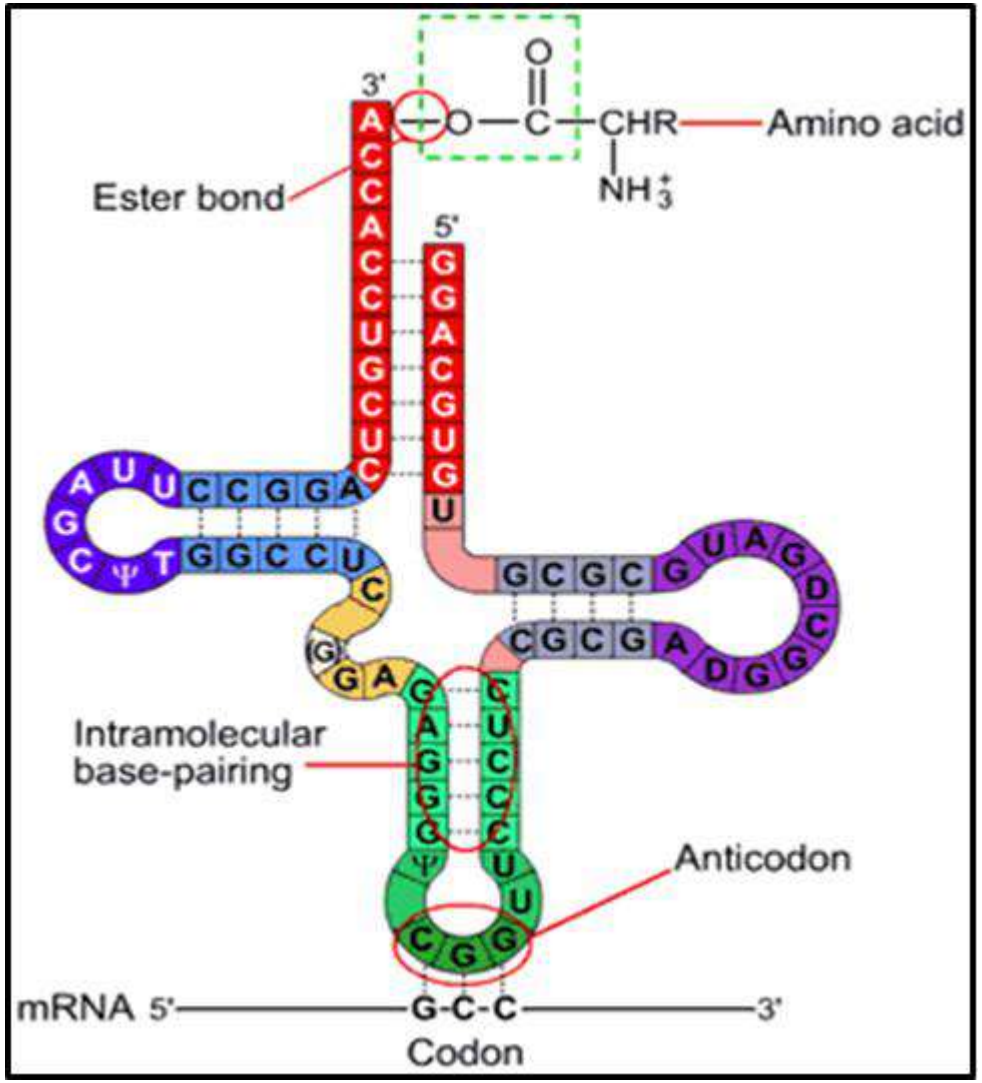
2. Translation

Stages of translation process

1- Activation stage

- **It is binding of the first amino acid (methionine) covalently with the correct tRNA forming aminoacyl-tRNA molecule or charged tRNA.**

- **The amino acid is joined at its carboxyl group to the 3' OH of the tRNA by aminoacyl tRNA synthetase.**



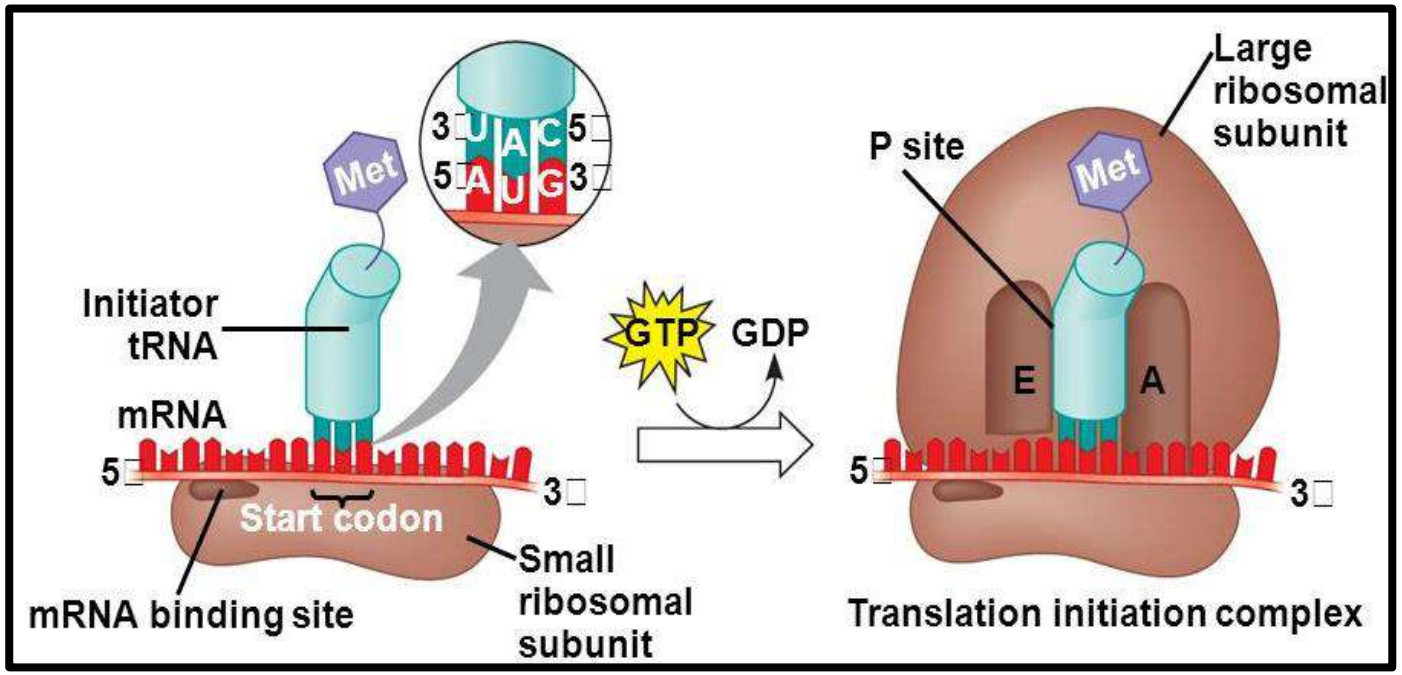
aminoacyl-tRNA

Protein Synthesis: From gene to protein

Stages of translation process

2- Initiation stage

- To start translation process, initiation complex must be formed which consists of the charged tRNA, mRNA, small and large subunits of the ribosome.
- Firstly, the small subunit of the ribosome binds to 5' end of mRNA, then the charged tRNA that carries the first amino acid (methionine) attaches to mRNA by its anticodon to the start codon (initiator codon) which is AUG.
- After that, the large subunit joins to the system forming the initiation complex.
- In this stage, the methionine charged tRNA is found in the (P) site.



Protein Synthesis: From gene to protein

2. Translation

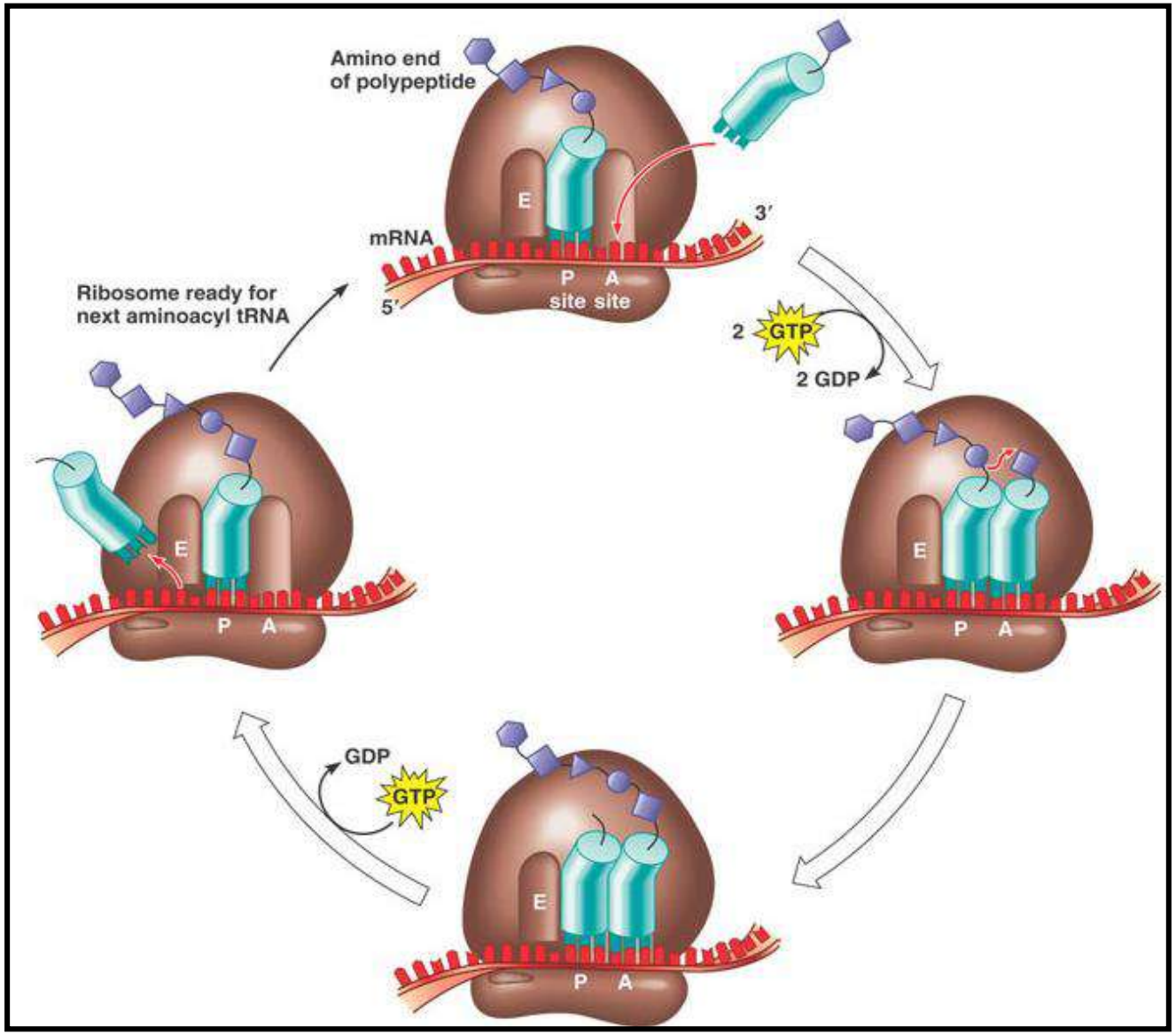
Stages of translation process

3- Elongation stage

- **Elongation is the addition of amino acids to the growing protein chain according the order of codons in mRNA as follows:**

a- After formation the initiation complex, the aminoacyl-tRNA whose anticodon is complementary to the second codon in mRNA is picked up to the (A) site.

b- When the correct aminoacyl-tRNA enters the (A) site, the ribosome breaks the bond between the tRNA in the (P) site and its amino acid (or polypeptide chain) and links it with the new amino acid in the (A) site by a peptide bond. This reaction results in an empty tRNA in the (P) site.



Protein Synthesis: From gene to protein

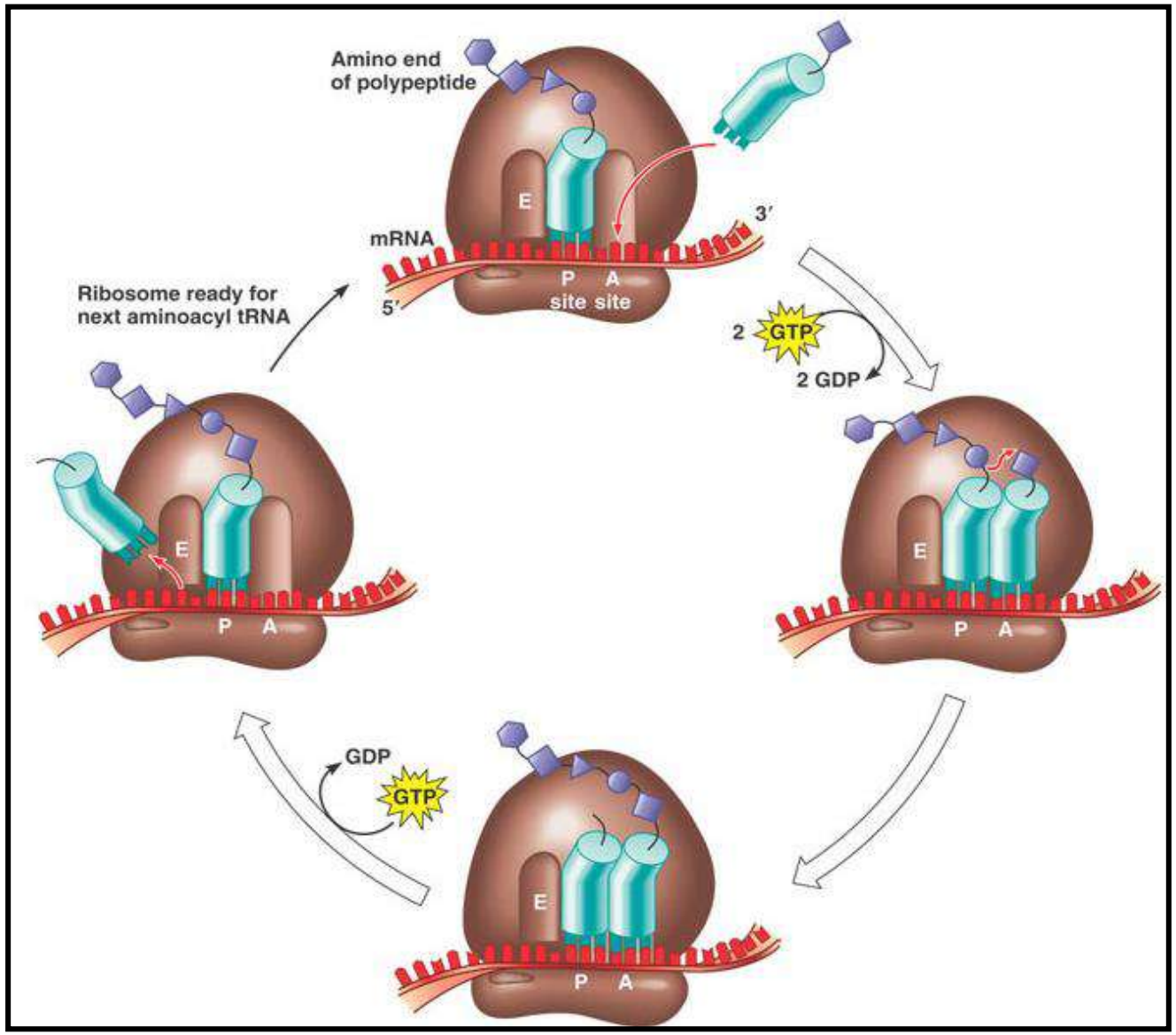
2. Translation

Stages of translation process

3- Elongation stage

c- The empty tRNA is displaced from (P) site to the (E) site to return to the cytoplasm to pick up another amino acid.

d- The peptidyl tRNA is translocated from the (A) site to the (P) site to allow binding a new charged tRNA to the (A) site to complete the process.



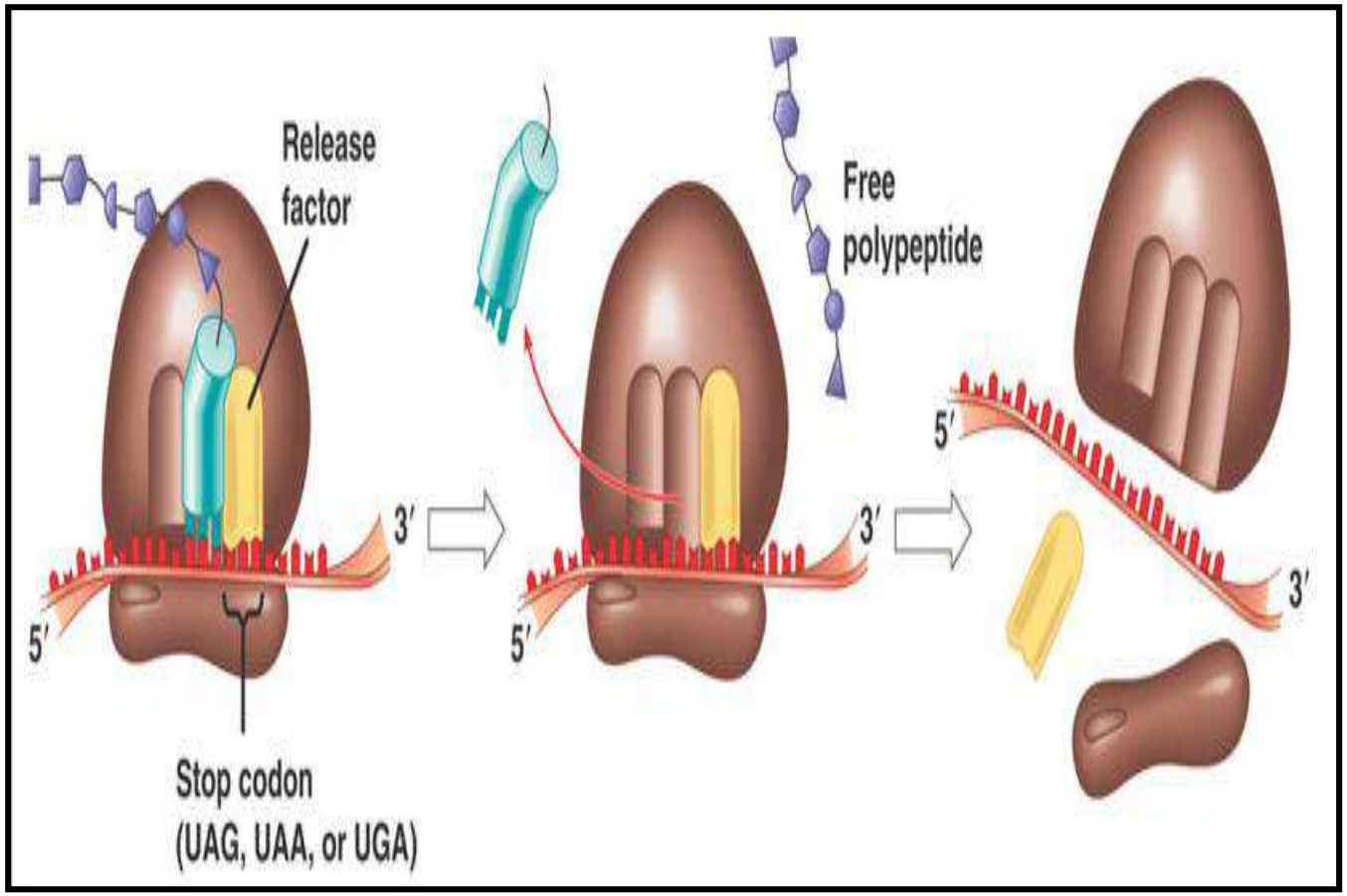
Protein Synthesis: From gene to protein

2. Translation

Stages of translation process

4- Termination stage

- Termination of the polypeptide happens when the (A) site of the ribosome faces the stop codon which is usually be one of the following: UAA, UAG or UGA.
- This leading to separation of the polypeptide chain from tRNA by a factor called releasing factor.
- The C-terminal of that protein is the ? amino acid, while the N-terminal is the ?



Protein Synthesis: From gene to protein

2. Translation

Posttranslational Modifications

- **Posttranslational modifications are the chemical modifications of a protein after its translation as the followings:-**

1. **Formation the secondary and tertiary structures for the formed protein.**
2. **Attachment of the formed protein with functional groups such as acetate, phosphate, lipids and carbohydrates.**
3. **Removing methionine from the formed protein.**
4. **Separation of the formed protein into two or more chains as insulin.**