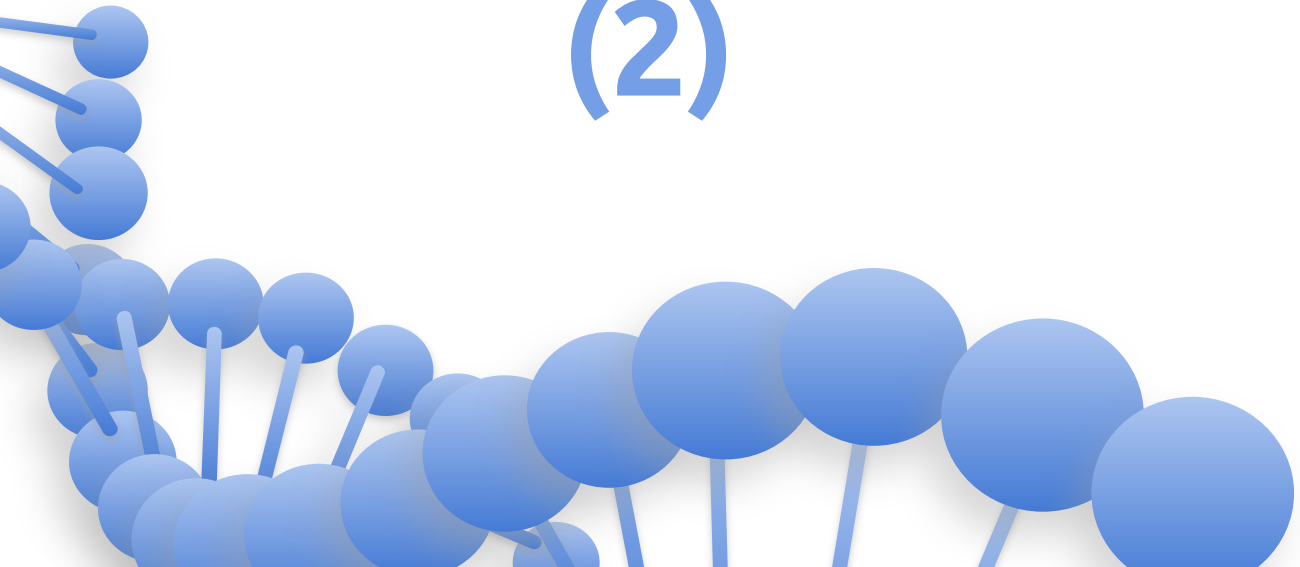
Molecular Biology (2)



Lecture 5

Color Index

- Girls' slides
- Boys' slides
- Doctors' notes
- Important
- Extra info

Editing File

Biochemistry 442

MED 442

Objectives

- Understand DNA replication.
- Know the transcription of genetic material into mRNA.
- Get an idea about the translation of mRNA into functional protein.

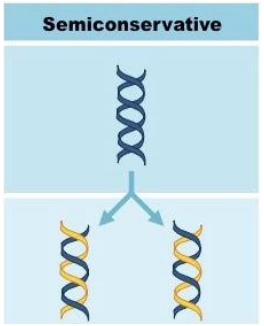
DNA is the genetic material, therefore it must:

- Replicate faithfully (من غير أخطاء)
- Have the coding ability to produce proteins for all cellular functions.

Features of Eukaryotic DNA Replication

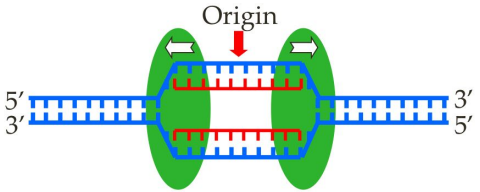
1 **Semiconservative** with respect to parental strand:

Daughter DNA molecules contain one parental strand (old) and one newly-replicated strand.



2 **Bidirectional** with **multiple origins of replication.**

Bidirectional: it goes both ways.
Origins of replication: specific sequence where the parent DNA strands separate.

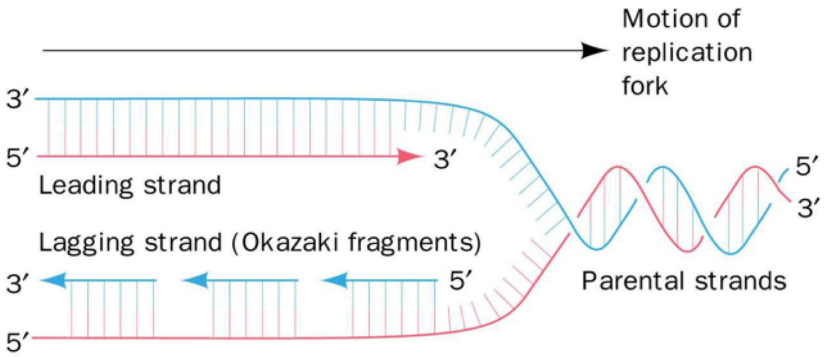


Features of Eukaryotic DNA Replication

3 Primed by short stretches of **RNA**.

It's a primer (initial fragment) that helps DNA polymerase know where to add the DNA nucleotide. The primer≈10 nucleotides.

4 **Semi-discontinuous**



Leading strand:
يكون مستمر

Lagging strand:
يكون متقطع

In DNA replication, both daughter strands
(leading strand red, lagging strand blue) are synthesized in their **5' → 3' directions**
Leading strand: a new strand that is synthesized **continuously**
Lagging strand: a new strand that is synthesized **in fragments (Okazaki-fragments)**

Proteins Involved in DNA Replication		
Protein name	**Function**	**باختصار**
DNA Helicase	Unwinds (melts) the DNA at the site of origin **by breaking the hydrogen bond between the bases**	يفصل ال DNA
Single-Stranded DNA Binding Proteins	Prevent rewinding of the single DNA strands **(prevent hydrogen bond formation)**	يمنع انه يرجع دبل ستراندز
(DNA) Primase	Make short segments of RNA primer complementary to DNA	يسوي RNA البادئة
DNA Polymerases (5 types: α; β; γ; δ; ε)	(each with different job but we are not going that deep) Add nucleotides to RNA primer. Proofreads bases added and replaces incorrect nucleotides. (Removes RNA primers/fill the gaps/spell check)	يبني ويتأكد من بنائه
DNA Ligase	Join the segments that DNA polymerase made instead of RNA nucleotides by form bonds between sugar-phosphate backbone	انزيم يربط بين ال Lagging strands
Topoisomerases: Topoisomerase I Topoisomerase II	**prevent supercoiling of the chromosome**	يمنع الالتفاف الفائق
Telomerases	**prevent the shorting of the chromosome (maintain the chromosome length).**	يمنع تقصير الكروموسومات

NOTE:
it's important to memorize the protein name and function specially the first 5

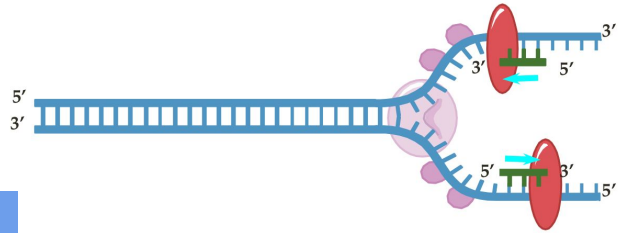
Helpful Video :)

Steps in DNA Replication

1. **Helicase protein (pink)** binds to DNA sequences called origins and **unwinds DNA strands.**
   - opens the DNA strands and forms replication fork

2. **Single-stranded DNA binding proteins (purple balls)** prevent single strands from rewinding.

3. **DNA Primase protein (red)** makes a short segment of RNA primer complementary to the DNA
   - always the synthesis direction 5′⟶3′
   - made by DNA polymerase.



Helpful Video :)

Steps in DNA Replication

4

DNA polymerases (purple):

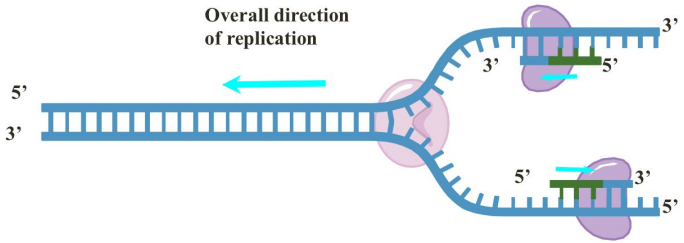
- **Adds** (complimentary) **DNA nucleotides** to the RNA primer.
  Leading strand is **(5'→3')** toward the fork, while the **lagging strand** is **(5'→3')** in the opposite direction of the fork.

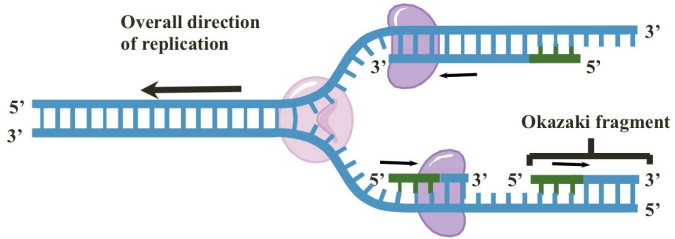
- **Proofreads** bases added and replaces incorrect nucleotides.
  If a wrong nucleotide is added that is not complementary to the parent strand, DNA polymerase will **recognize it** and **replace it.**

Reading is always in the **3'→5' direction,** while **synthesis** is always in the **5'→3' direction.**

- Leading strand synthesis **continues** in a 5' to 3' direction.

- **Discontinuous** synthesis (of the lagging strand) produces 5' to 3' DNA segments (Okazaki fragments).

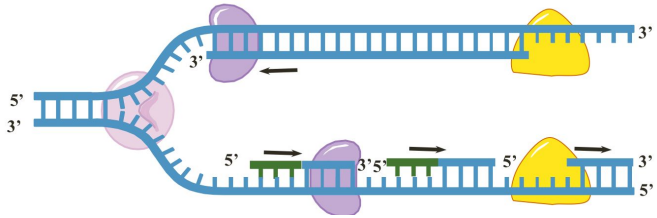




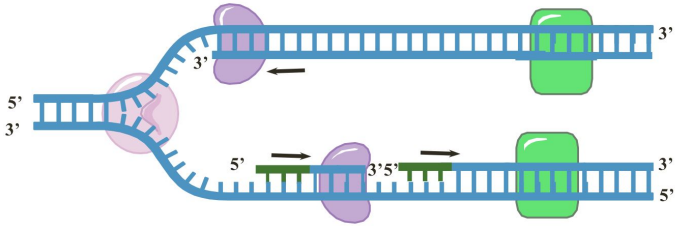
Steps in DNA Replication

5 Exonuclease activity of **DNA polymerase (Yellow) removes RNA primers.** **Exonucleases** are enzymes that remove nucleotides.

6 **DNA polymerases** fill the gaps.

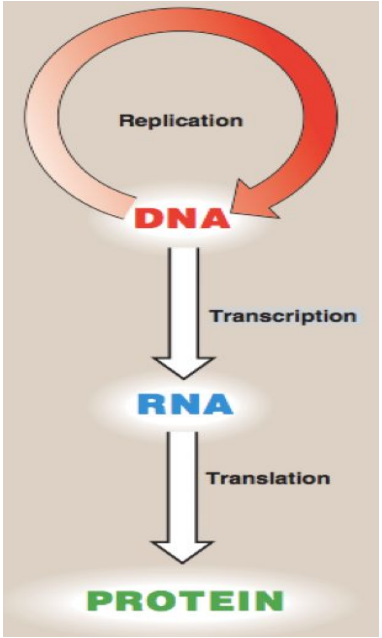


7 **Ligase (Green)** forms bonds between the sugar-phosphate backbone (phosphodiester bond). The DNA consists of sugar-phosphate chains on the sides and nitrogenous bases in the center.



The Central Dogma of Molecular Biology

- A portion of DNA, called a **gene**, is transcribed into RNA.
- RNA is translated into proteins.
- Only 5% of DNA wil transcribed into mRNA.
- DNA comes from DNA



Transcription “mRNA Synthesis”

- A portion of **DNA** (a **gene**) is transcribed into messenger RNA **(mRNA)**.
- Only one of the DNA strands is transcribed **(antisense strand)**.
- The **RNA polymerase II** is responsible for this process.
- The direction of transcription is **5’--->3’**.

The **antisense strand** contains the **opposite codon** of the required **mRNA** to be transcribed.

mRNA is the **sense strand**

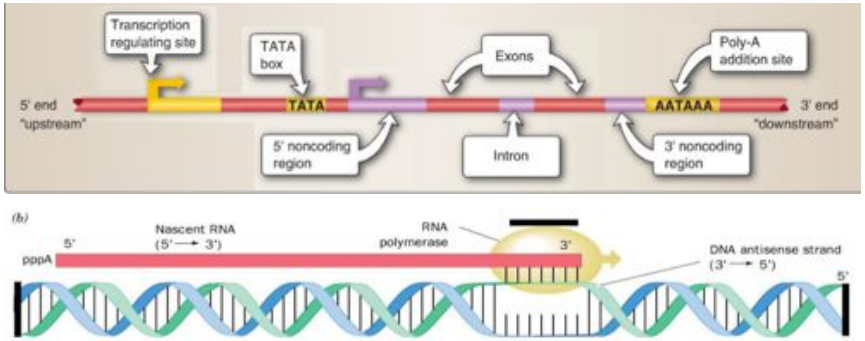
Transcription “mRNA Synthesis”

Steps of mRNA synthesis

Chain initiation: **RNA polymerase II** binds to **promoter region** of DNA to start transcription.

Chain elongation: a portion of DNA template unwinds (DNA melting) at the point of RNA synthesis.
This forms a short length of RNA-DNA hybrid.

Chain termination: DNA contains specific sites which stop transcription (at the sequence of 4-10 AT base pairs).

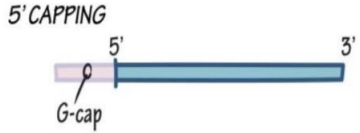


Post-Transcriptional Modification

Capping: addition of a methylated guanine nucleotide **at 5' end of mRNA**

Functions:

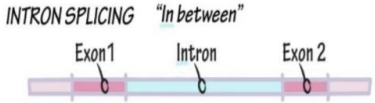
- To **prevent** mRNA degradation by **exonucleases**
- It helps the transcript **bind to the ribosome** during protein synthesis



Polyadenylation: Addition of a poly(A) tail (a highly conserved AAUAA sequence) **at 3' end of mRNA**.

Functions:

- For rRNA **recognition**
- To **protect** the mRNA from **degradation**



Intron removal: for releasing **mature mRNA from nucleus.**

- **Introns** are **non-coding areas**
- **Exons** are **coding areas**

The pre-mRNA has to go through some modifications to become a mature mRNA, the modifications occur in the cell nucleus before the RNA is translated.

Translation “Protein Synthesis”

A process of **protein synthesis from mRNA**

- mRNA has genetic codes for **amino acids** present in **proteins.**
- The **genetic code** is a dictionary that **identifies the correspondence** between a sequence of **nucleotide bases** and a sequence of **amino acids.** It is a code that connects the nucleotide bases in mRNA and the amino acids**”team441”.**
- Each individual word in the code is composed of **three** nucleotide bases (**codons**). Each codon specifies a particular amino acid **“team441”**.
- **64 possible codons:**
  - **61** codons specify **20** amino acids (one amino acid can have many codons that represent it)
  - **1** start codon (**AUG**) (Also code for methionine)
  - **3** stop codons **UAA, UAG and UGA**

	U	C	A	G	
U	UUU Phe UUC UUA Leu UUG	UCU UCC UCA Ser UCG	UAU Tyr UAC **UAA Stop** **UAG Stop**	UGU Cys UGC **UGA Stop** UGG Trp	U C A G
C	CUU CUC CUA Leu CUG	CCU CCC CCA Pro CCG	CAU His CAC CAA Gln CAG	CGU CGC CGA Arg CGG	U C A G
A	AUU AUC Ile AUA AUG Met	ACU ACC ACA Thr ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC GUA Val GUG	GCU GCC GCA Ala GCG	GAU Asp GAC GAA Glu GAG	GGU GGC GGA Gly GGG	U C A G

Components Required for Translation

01. **Amino acids.**

02. **Transfer RNA (tRNA).**
reads the code and bring the amino acids

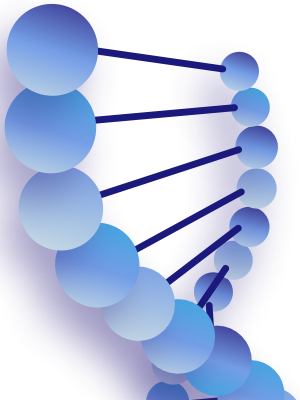
03. **Aminoacyl-tRNA synthetases.**
enzyme that makes Aminoacyl-tRNA (connect tRNA to the specific amino acid)
Aminoacyl-tRNA: (tRNA + amino acid)

04. **mRNA.**

05. **Functionally competent ribosomes.**
Functional Ribosomes consist of 2 subunits:
- Small ribosomal subunit (has mRNA binding site)
- Large ribosomal subunit

06. **Protein factors.**
Catalytic or stabilizer for the synthetic machinery.

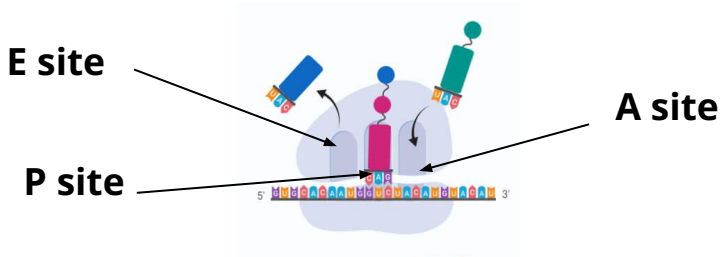
07. **ATP and GTP.** **(Energy)**



Protein Translation

Important to know that (441):

- **tRNA has 2 important sites:**
  1. The **anticodon** that forms base pairs with its complementary sequence on mRNA
  2. A region for **attaching a specific amino acid**
- **Ribosomes have 2 subunits that join to form functional ribosome:**
  1. **Small subunit:** where the mRNA comes and binds
  2. **Large subunit:** has 3 sites for tRNA:
     - ★ **A site: Acceptor** site that binds to tRNA, which holds the new amino acid to be added to the polypeptide chain
     - ★**P site: Peptidyl** site that holds the tRNA carrying the growing polypeptide chain
     - ★**E site: Exit** site that discharges the tRNA to leave the ribosome



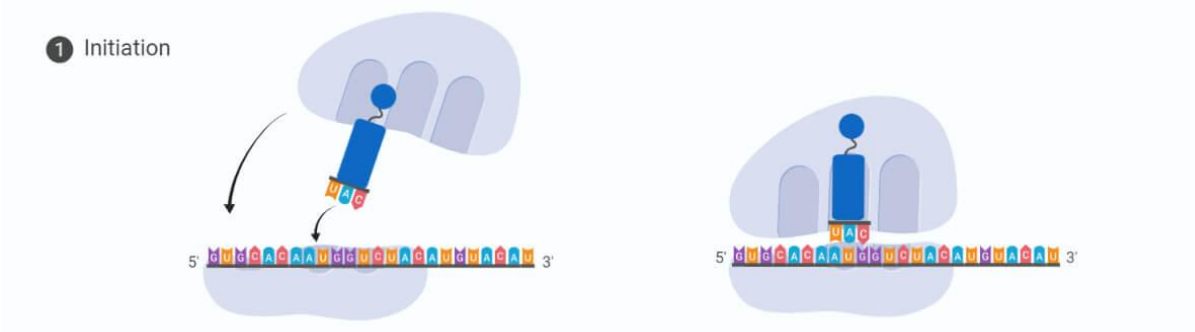
Steps of Protein Translation

Initiation:

It requires **ribosomal subunits**, **mRNA**, **aminoacyl-tRNA** for methionine, **initiation factors** and **energy**. These all form the **initiation complex**.

1. Small ribosomal subunit bind to the aminoacyl-tRNA and mRNA which carries the **start codon**.
2. The large ribosomal subunit binds to the small ribosomal subunit to complete the **initiation complex.**

The first stage is about binding the start codon to form the initiation complex

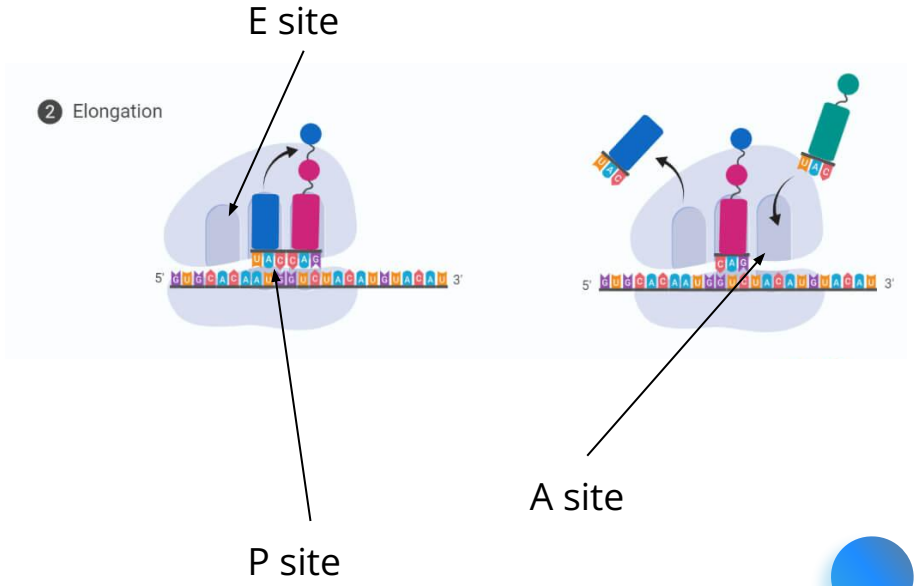


Helpful Video :)

Steps of Protein Synthesis

Elongation:

1. An aminoacyl-tRNA will attach to **A site**. the attachment will be facilitated by elongation factors.
2. **Peptidyl transferase** is an enzyme that **separates** the **growing chain** from tRNA in the **P site**, and **transfers** the growing polypeptide chain to tRNA at **A site**
3. The empty **tRNA at P site** moves to **E site** and **discharge**, and the tRNA with the **growing chain** moves to the **P site**.
4. The **A site** will be **ready to receive** another aminoacyl-tRNA and repeat the steps.

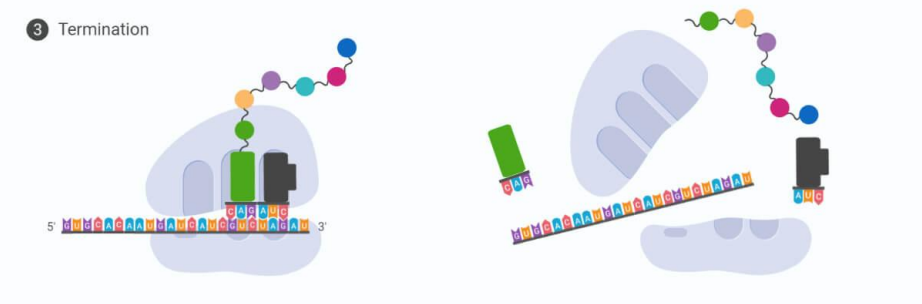


Steps of Protein Synthesis

Termination :

Occurs when one of the three **stop codons** (**UAA,UAG,UGA**) reaches the **A site**.

1. **Release factor** binds to the **stop codon** and cut the bond between the polypeptide and its tRNA in the **P site.**
2. This frees the polypeptide and the **translation complex disassembles**.



Summary:

1. **Initiation:** making initiation complex and attaching the start codon
2. **Elongation:** adding amino acids and forming protein chain.
3. **Termination:** free the polypeptide and disassemble translation complex.

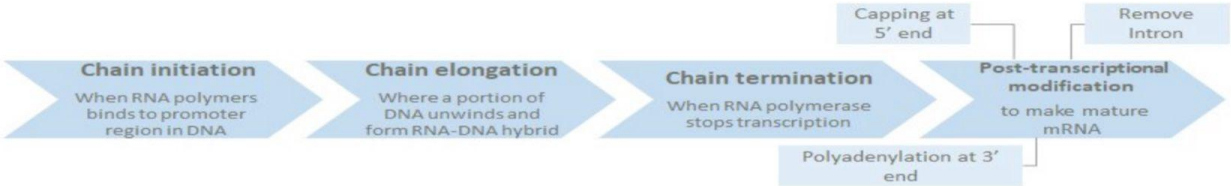
Summary
(441)

Features of Eukaryotic DNA Replication

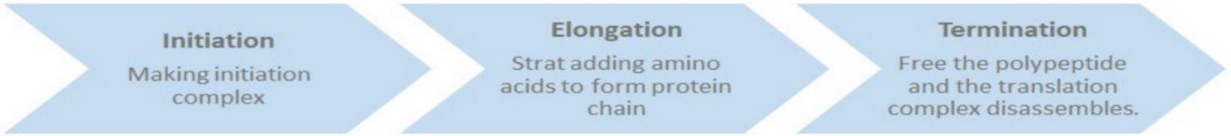
1- Semiconservative 2-Bidirectional with multiple origins of replication 3-Primed RNA. 4-Semi-discontinous

DNA Replication

transcription : mRNA synthesis



Protein Translation



Take Home Messages

- DNA is the genetic material, so it must replicate faithfully and have the coding ability to produce proteins for all cellular functions.
- Only one strand of DNA (antisense strand) is transcribed into mRNA.
- The synthesized mRNA is protected from the destruction and prepared for translation through post-transcriptional modification.
- mRNA transcription and protein synthesis processes are the same in both prokaryotic and eukaryotic cells with some differences.

Quiz

1- Which of the following is components required for translation			
a) amino acid	b) tRNA	c) DNA ligase	d) A&B
2- Which one of the following is responsible for transcription			
a) RNA polymerase I	b) RNA polymerase II	c) DNA polymerase II	d) DNA polymerase I
3- A portion of _____ called _____ , is _____ into RNA			
a) gene, DNA , translations	b) DNA, gene , transcribed	c) DNA , gene , translations	d) gene , DNA , transcribed
4- In which step of mRNA synthesis there's a chain that forms a short length of RNA-DNA hybrid ?			
a) elongation	b) initiation	c) termination	d) capping
5- Which one of the following is start codon			
a) AUG	b) UAG	c) UGA	d) UAA

1. D
2. B
3. B
4. A
5. A

Our Team

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