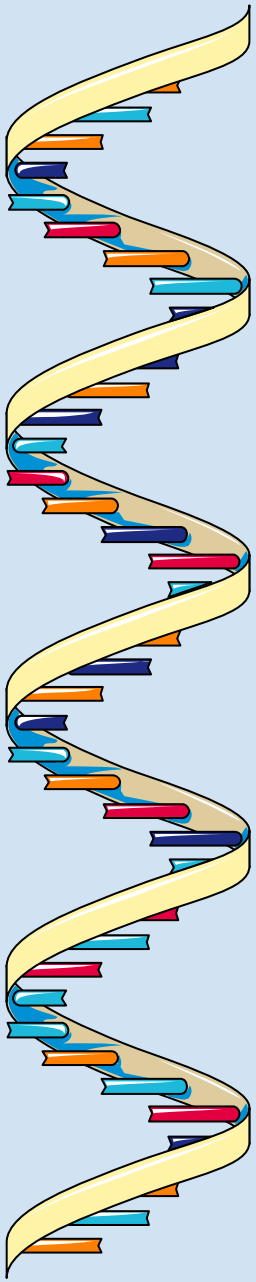
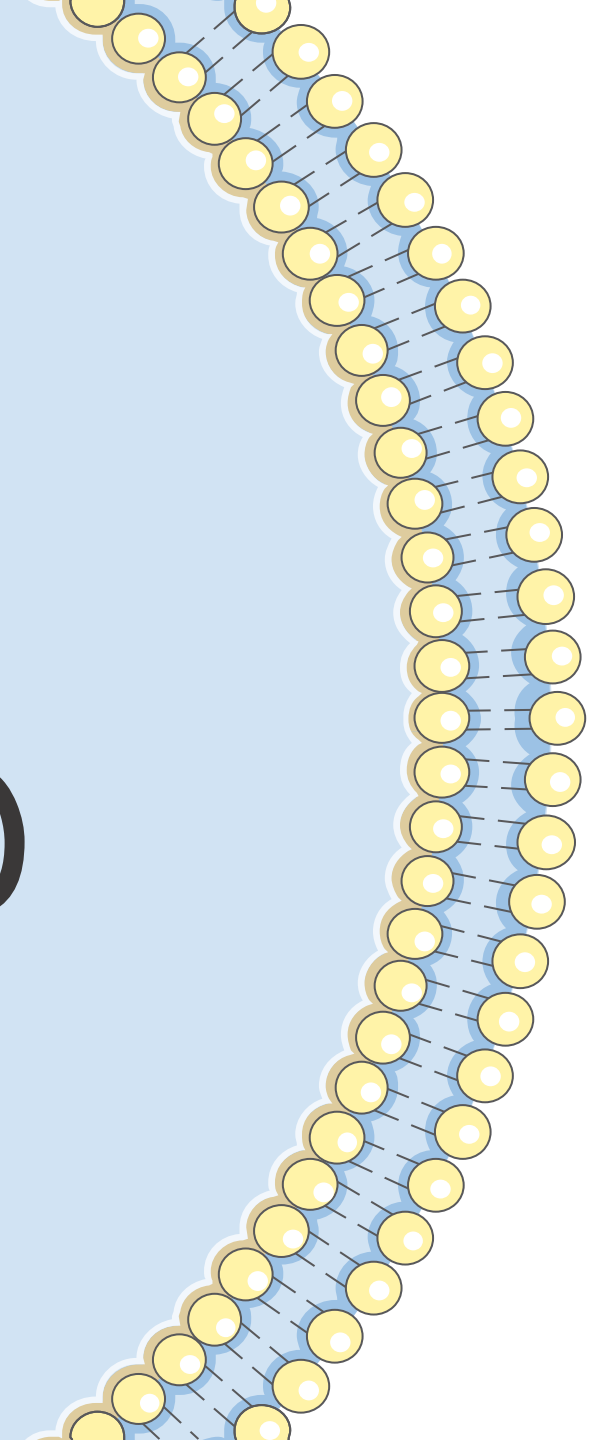


Molecular Biology (2)



MED441
KING SAUD UNIVERSITY

Revised & Reviewed by: Abdulaziz & Bahammam
Faye Wael Sendi

4
V1

Foundation Block - KSU

Color Index:

- Main text
- Important
- Notes
- Boys slides'
- Girls slides'
- Extra

Editing File

Objectives

- ✓ To understand DNA replication
- ✓ To know the transcription of genetic material into messenger RNA
- ✓ To get an idea about the translation of mRNA into a functional protein.

DNA is the genetic material

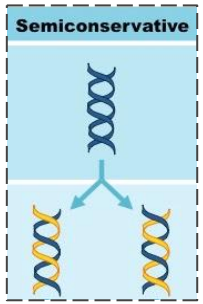
Therefore it must:

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

Features of Eukaryotic DNA Replication

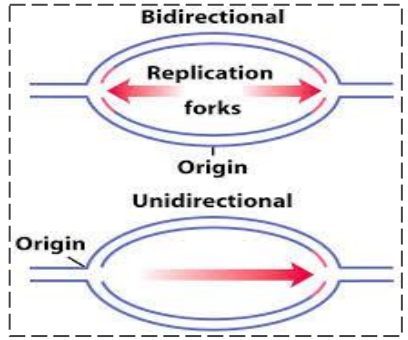
01

Semiconservative with respect to parental strand:
Daughter DNA molecules contain one parental strand and one newly-replicated strand.



02

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



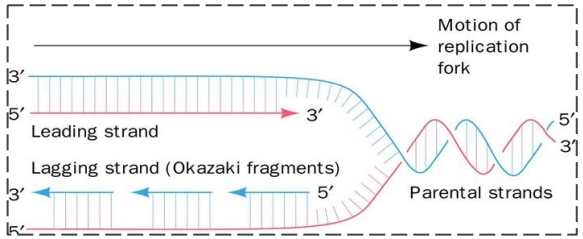
03

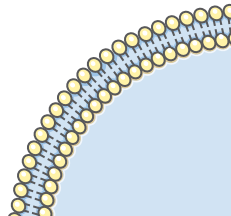
Primed by short stretches of RNA.
It's a primer (initial fragment) that help DNA polymerase to know where to add the DNA nucleotide. The primer≈10 nucleotides.

** 'semi' means half, so *semiconservative* : a half is from parent DNA
semi-discontinous : a half has intervals

04

Semi-discontinous.
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 synthesized continuously
Lagging strand: a new strand that synthesized in fragment (Okazaki-fragments)





Proteins involved in DNA Replication

helpful video

helpful video

Protein name	Function
DNA Helicase.	Unwinds the DNA at the site of origin by breaking the hydrogen bond between the bases
Single-stranded DNA binding proteins.	Prevent rebinding of the DNA helix (prevent hydrogen bond formation)
(DNA) Primase.	Make short segments of **RNA prime** complementary to the DNA.
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. (Remover RNA primers / fill the gaps / spell check)
DNA ligase.	Join the segments that DNA polymerase made instead of RNA nucleotides, to form bonds between sugar-phosphate beckbone.
Topoisomerases: Topoisomerase I + Topoisomerase II.	prevent supercoiling of the chromosome.
Telomerases	prevent the shorting of the chromosome (Maintain the chromosome length).

Steps in DNA Replication

helpful video

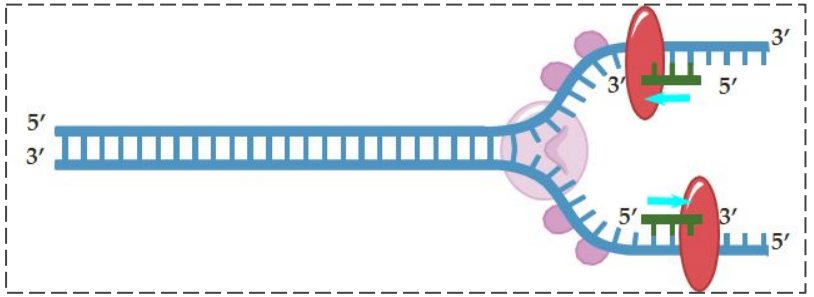
helpful video

01

Helicase protein. (pink)

binds to DNA sequences called origins and **unwinds DNA strands.**

-open the DNA strands and form **replication fork**



02

Single-stranded DNA binding proteins. (purple)

prevent single strands from rewinding.

03

DNA Primase protein. (red)

makes a short segment of RNA primer complementary to the DNA

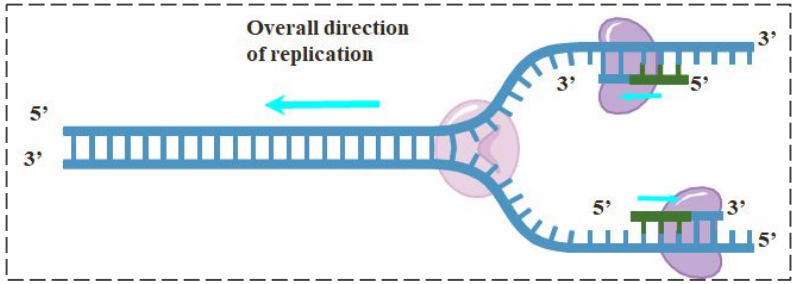
-always the synthesis direction 5′⟶3′

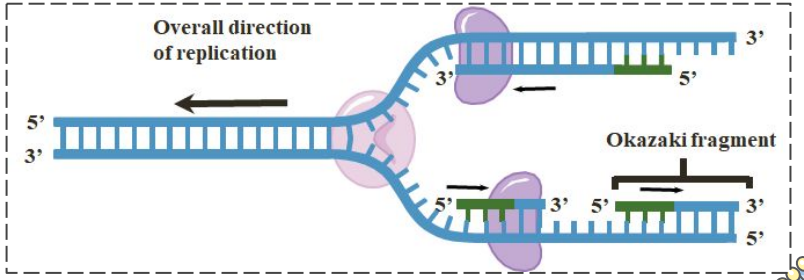
-made by DNA polymerase.

04

DNA polymerases

- Adds DNA nucleotides to the RNA primer.
  -start adding complementary nucleotide. **leading strand** is (5′→3′) toward the fork. but 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
- Leading strand synthesis **continues** in a 5′ to 3′ direction.
- **Discontinuous** synthesis produces 5′ to 3′ DNA segments (Okazaki fragments).





CONTINUE ...

05

DNA polymerases. (yellow)

Exonuclease activity of DNA polymerase **removes RNA primers**

Exonucleases are enzymes that remove nucleotides.

06

DNA polymerases

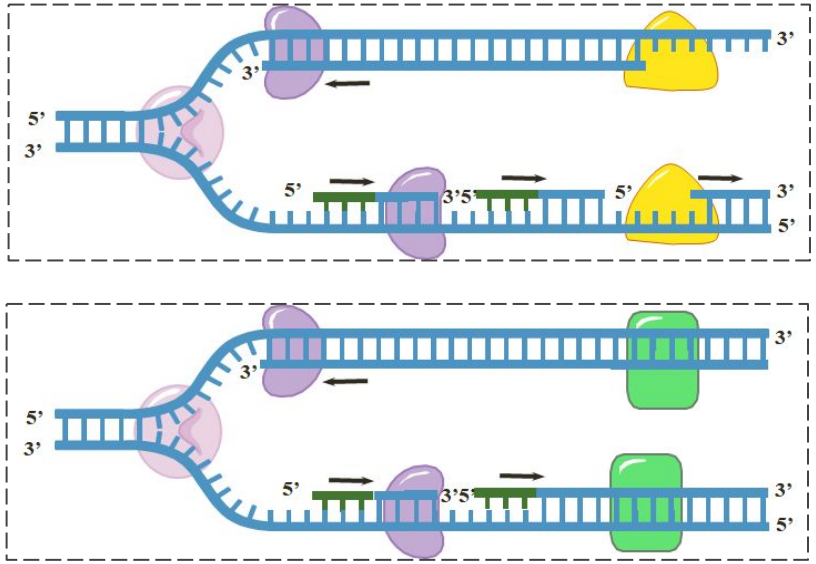
DNA polymerase fills the gaps.

07

Ligase. (green)

Ligase forms bonds between sugar-phosphate backbone (phosphodiester bond)

The DNA is consist of *sugar-phosphate* chains on the sides and *nitrogenous bases* in the center

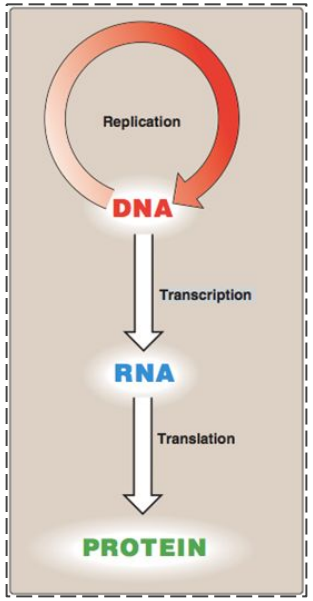


The central dogma of Molecular Biology

A portion of DNA, called a **gene**, is transcribed into RNA.

RNA is translated into proteins.

only 5% of DNA wil transcribed into mRNA



Transcription (mRNA synthesis)

1. A portion of DNA (a gene) is transcribed into messenger RNA (mRNA).
2. Only one of the DNA strands is transcribed (antisense strand).
   - Antisense strand: It is the strand that contains the opposite codon of the required mRNA to be transcribed.
3. The RNA polymerase II is responsible for this process.
4. The direction of transcription is 5'--->3'.

helpful video

helpful video

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 (opens) at the point of RNA synthesis.

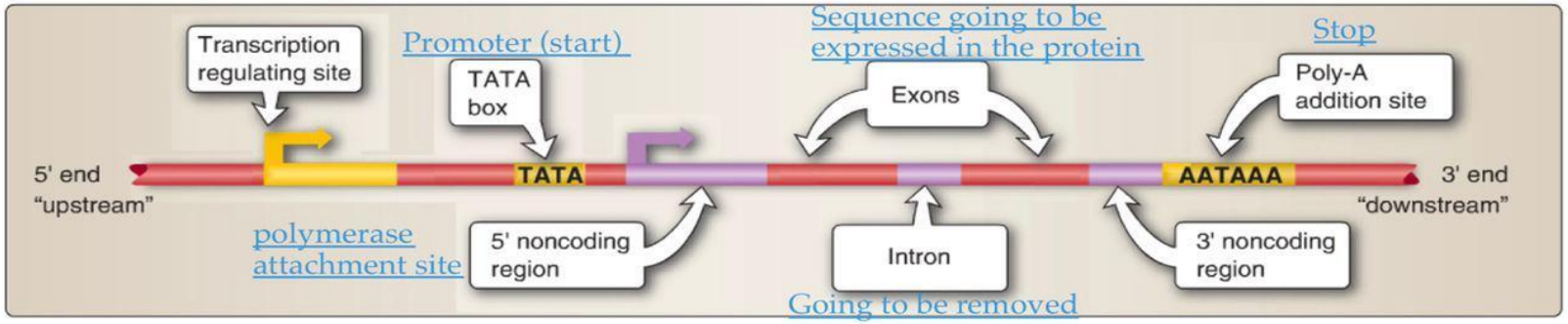
Chain elongation (continued): This forms a short length of RNA-DNA hybrid.

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

Steps of mRNA synthesis:

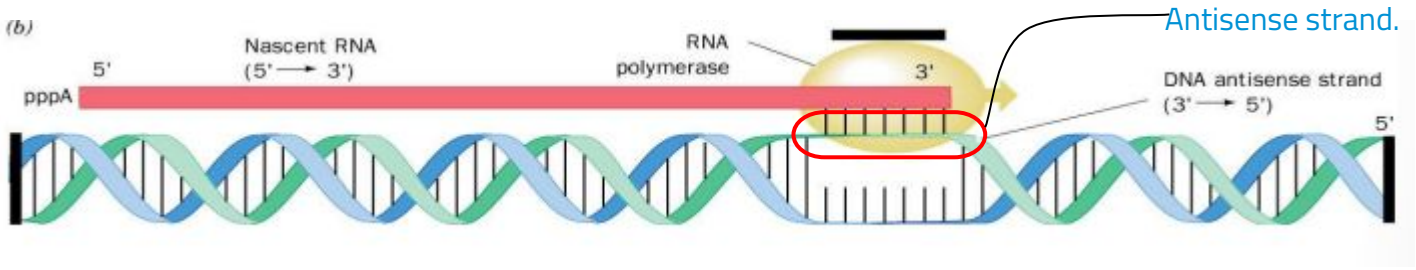
shows:

- Initiation (first step), where RNA polymerase II binds to promoter region of DNA to start the transcription process. **(always at 5′ side)**
- Termination (last step), where RNA polymerase II stops transcription. **(always at the 3′ side)**



shows:

- Elongation (second step), where a portion of DNA unwinds (opens) at the point of RNA synthesis, which forms a short length of RNA-DNA hybrid.



Post-transcriptional modification

helpful video

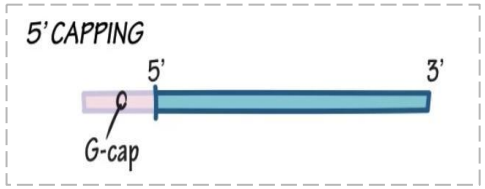
*The pre-mRNA has to go through some modifications to become a mature mRNA, the modifications occur in the cell nucleus before the RNA is translated.

Post-transcriptional modification (3 steps)

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

Function:

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



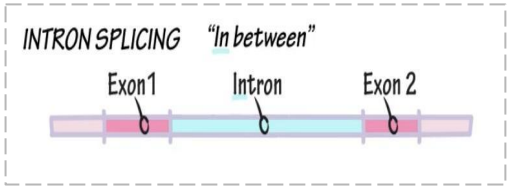
*__Exonucleases__ are enzymes that remove nucleotides.

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

Function:

- To **protect** the mRNA from **degradation**
- For ribosomal RNA **recognition**

3- Intron removal for releasing **mature mRNA from nucleus.**



Translation (Protein synthesis)

- Translation is 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 connect the nucleotide bases in mRNA and the amino acids.
- Each individual word in the code is composed of **three nucleotide bases** (**codons**).
  *each codon specifies a particular amino acid
- **64 possible codons:**
  1. **61** codons specify 20 amino acids. there are different codes give the same amino acid.
  2. **One start codon (AUG)**. AUG code is for **Methionine,** every translation start with AUG.
  3. Although methionine (Met) is the first amino acid incorporated into any new protein, it is not always the first amino acid in mature proteins, it may be removed after translation.
  4. **3 stop codons: UAA, UAG and UGA**

First position (5′ end)	Second position				Third position (3′ end)
	U	C	A	G	
U	UUU Phe	UCU	UAU Tyr	UGU Cys	U
	UUC	UCC Ser	UAC	UGC	C
	UUA Leu	UCA	UAA Stop	UGA Stop	A
	UUG	UCG	UAG Stop	UGG Trp	G
C	CUU	CCU	CAU His	CGU	U
	CUC	CCC Pro	CAC	CGC	C
	CUA Leu	CCA	CAA Gln	CGA Arg	A
	CUG	CCG	CAG	CGG	G
A	AUU	ACU	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC	AGC	C
	AUA	ACA	AAA Lys	AGA Arg	A
	AUG Met[b]	ACG	AAG	AGG	G
G	GUU	GCU	GAU Asp	GGU	U
	GUC Val	GCC Ala	GAC	GGC Gly	C
	GUA	GCA	GAA Glu	GGA	A
	GUG	GCG	GAG	GGG	G

Just memorize the **start** + **stop** codons

Components required for Translation

01 **Amino acids.**

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

03 **Aminoacyl-tRNA synthetases.**
Aminoacyl-tRNA: (tRNA + amino acid)
synthetases: enzyme that make Aminoacyl-tRNA

04 **mRNA**

05 **Functionally competent ribosomes.**
Functional Ribosomes consists 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 is needed.

Steps in Protein Translation

helpful video: start at 3:40

*Important to know that:

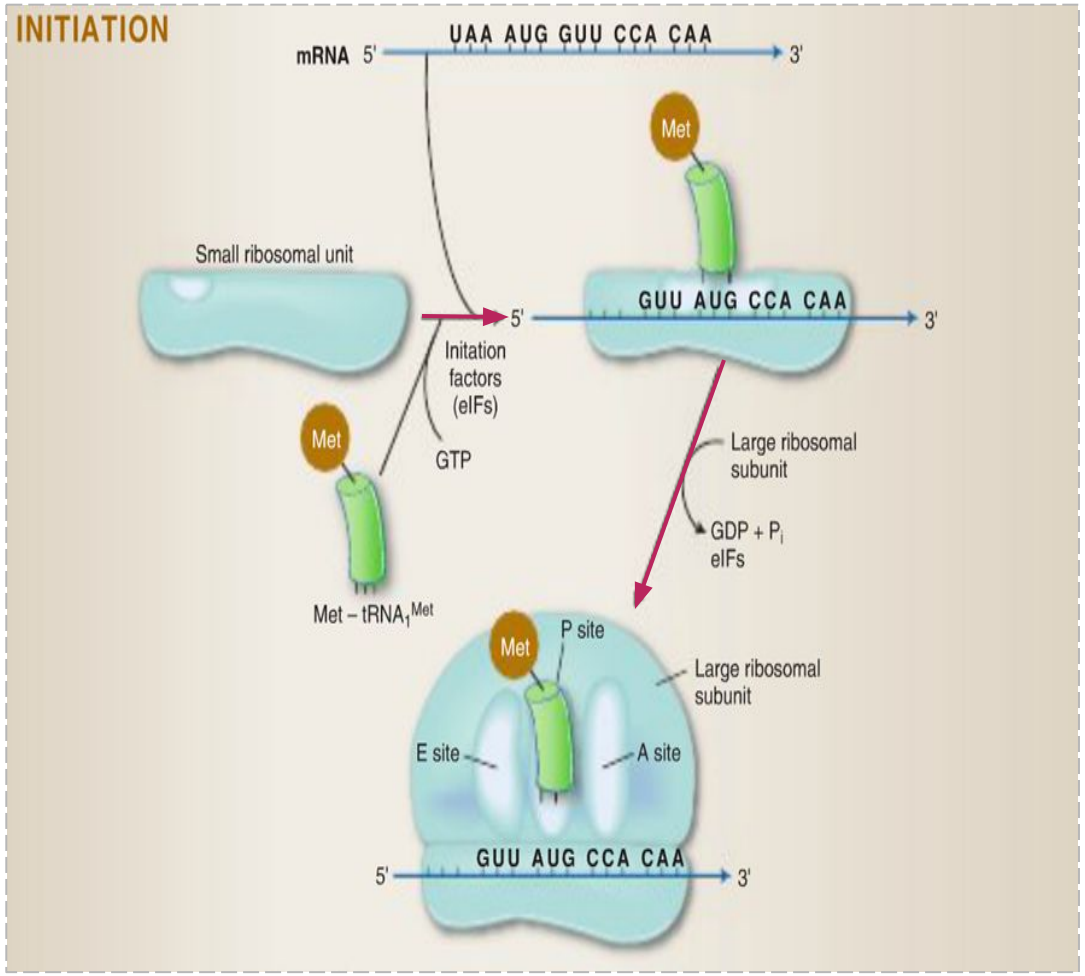
- **tRNA has two important areas:**
  - 1- the **anticodon** which forms base pairs with its complementary sequence on mRNA.
  - 2- a region for **attaching a specific amino acid.**
- **Ribosome has 2 subunits that join to form a functional ribosome:**
  - 1- **small subunit:** where the mRNA come and site
  - 2- **large subunit:** has three 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, hold tRNA carrying the **growing** polypeptide chain.
    - **E site: Exit** site, that **discharge** tRNA to leave the ribosome

1- Initiation:

It requires ribosomal subunits, mRNA, aminoacyl-tRNA for methionine, initiation factors and energy. These all join to 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.



Steps in Protein Translation

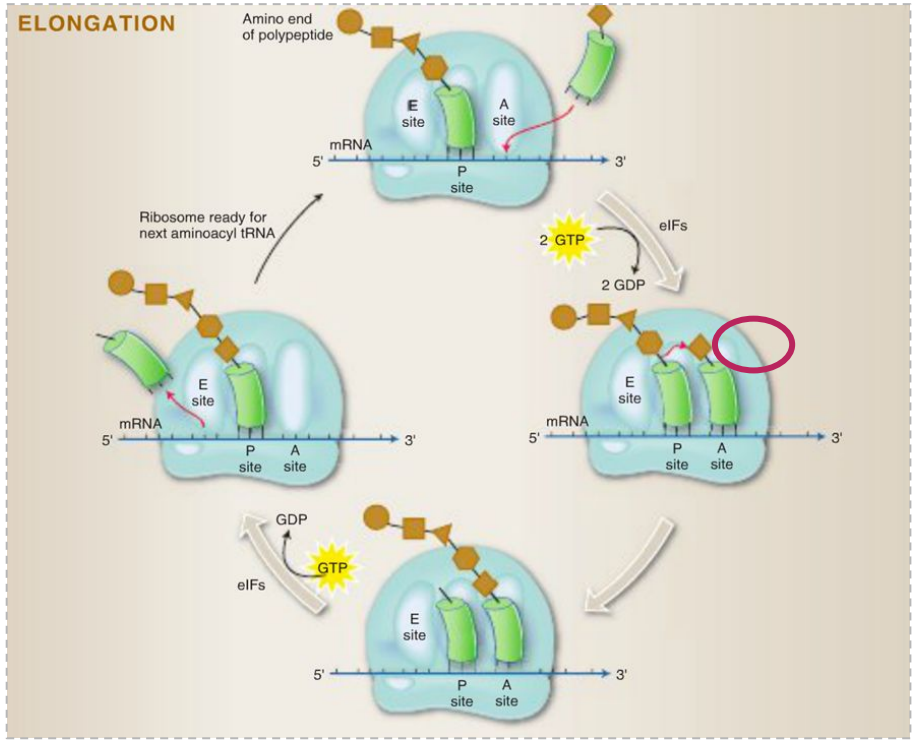
2- Elongation:

1- An aminoacyl-tRNA will attach to **A site**. the attachment will be facilitated by **elongation factors.**

2- peptidyl transferase : it is an enzyme that **separates** the growing chain bond with tRNA in the **P site**, and **transfers** the growing polypeptide chain to tRNA at **A site**

3-then the ribosome will move 3 nucleotides. So 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.

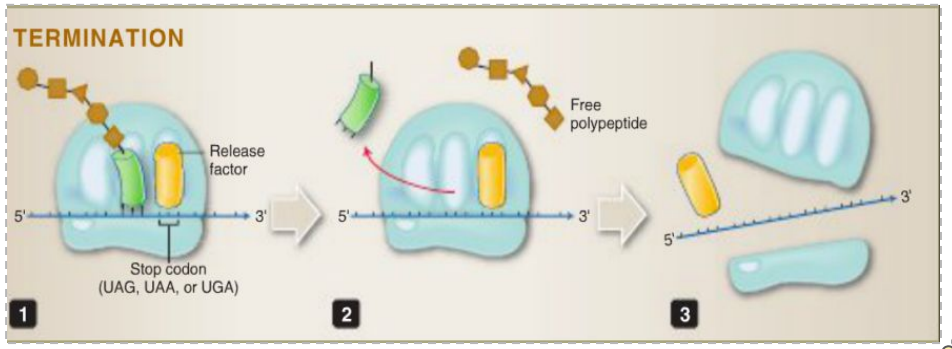


3- Termination:

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 - EXTRA SLIDE -

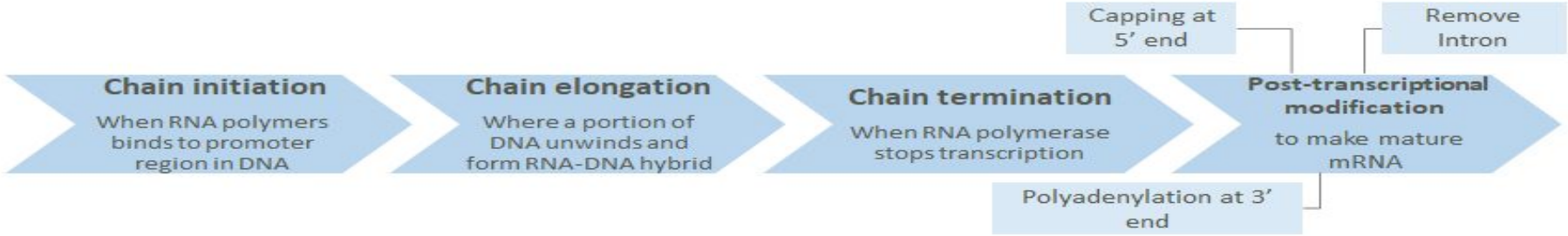
helpful video

Features of Eukaryotic DNA Replication

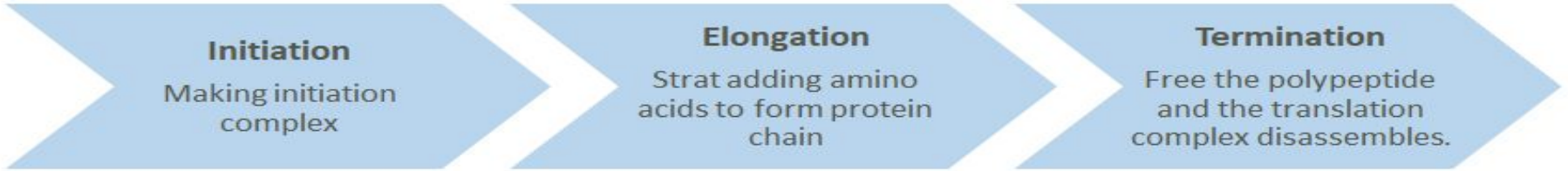
1- Semiconservative 2-Bidirectional with multiple origins of replication 3-Primed RNA. 4-Semi-discontinous

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 eukaryotic cells with some differences.

Quiz

Q1: DNA polymerases is responsible for?							
A	Proofreads bases	B	Leading strand synthesis	C	Adds DNA nucleotides	D	All

Q2:In post-transcriptional modification, where does capping have place?							
A	at the 5` end	B	at the 3` end	C	at introns	D	at the promoter region

Q3:How many proteins are involved in DNA replication?							
A	4	B	11	C	5	D	7

Q4: What is DNA polymerases function							
A	Unwinds DNA strands	B	Forms phosphodiester bonds	C	Fills the gaps	D	Prevent single strands from rewinding

Q5:What is the function of A site in ribosomes?							
A	empty tRNA leaves the ribosome through it.	B	binds incoming aminoacyl-tRNA.	C	holds tRNA carrying the polypeptide chain.	D	dissociation of ribosome.

Answer Key: 1)D 2)A 3)D 4)C 5)B

Q6:Mention all stop codons?

Q7:What is the codon of methionine?

Q8:What is the function of DNA primase?

Q9:What is the function of Polyadenylation

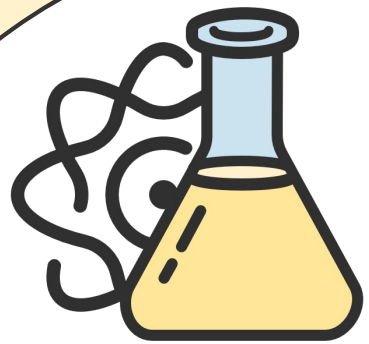
Answer:

6-Three stop codons: UAA, UAG and UGA

7-AUG.

8-makes a short segment of RNA primer complementary to the DNA.

9-To protect the mRNA from degradation + for ribosomal RNA recognition



Biochemistry 441

Girls

Boys

Leader: Ghadah Alarify

Members:

Yara Almufleh
Reema Alrashedi
Wareef Almousa
Joud Alangari
Fay Alluhaidan
Sarah Alhamlan
Arwa Almobeirek
Jumana AL-qahtani
Latifa Alkhdiri
Alanoud Alhaider
Futoon Almotairi
Manal Aldhirgham
Raaoum Jabor
Norah alawlah
Shahad Helmi
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Abdulrahman Alnoshan
Ahmed Alqahtani
Hamad Alshaalan
Anas Alharbi
Mohammed Alwahibi
Saad Alghadir
Firas Alqahtani

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