Molecular Biology 2

Editing File

Color Index:
- Main Text (black)
- Female Slides (Pink)
- Male Slides (Blue)
- Important (Red)
- Dr's Notes (Green)
- Extra Info (Grey)

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 functional protein

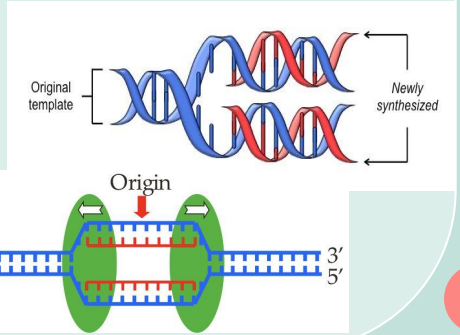
DNA is a genetic material, therefore it must:

- Replicate faithfully "بطريقة صحيحة وبلا أخطاء"
- Have the coding ability to produce proteins for all cellular functions

Features of eukaryotic DNA replication:

- Semiconservative with respect to parental strand:
  Daughter DNA molecules contain one parental strand and one newly-replicated strand.
  Semi=half
  Conservative=old
- Bidirectional with multiple origins of replication.

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



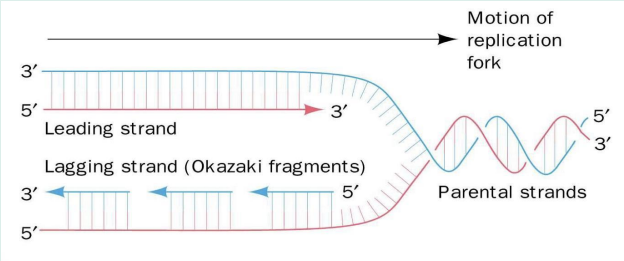
Features of eukaryotic DNA replication:

Helpful video

- Primed by short stretches of RNA
- Semi-discontinuous:

In DNA replication, both daughter strands are synthesized in their 5' → 3' directions

Note: DNA replication is 'semi-discontinuous' because one of the strands is synthesized continuously, while the other strand, is discontinuously by the formation of Okazaki fragments.



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

Leading strand: a new strand that is synthesized **continuously in 5' to 3' direction**
Lagging strand: a new strand that is synthesized in **fragments** (discontinuously) **in 5' to 3' direction**
(Okazaki-fragments)

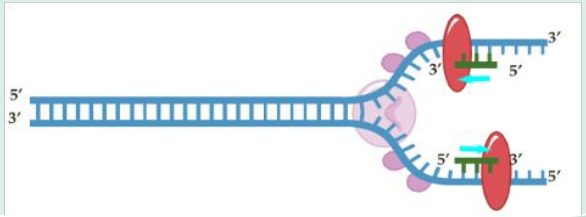
Proteins Involved in DNA Replication		
Protein name	**Function**	**باختصار**
DNA Helicase	Binds to DNA sequences called “origins” and unwinds DNA strands (by breaking the hydrogen bond between the bases)	يفصل الـ DNA
Single-Stranded DNA Binding Proteins	Prevent single DNA strands **from rewinding** (prevent hydrogen bond formation)	يمنع الالتفاف (من انهم يرجعون يلتفون على بعض)
(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) 1. Add DNA nucleotides to RNA primer. 2. Proofreads bases added and replaces incorrect nucleotides. 3. 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 الـ 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

Steps in DNA replication

1. **Helicase protein** (pink) binds to DNA sequences called origins and unwinds DNA strands.

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

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



Steps in DNA replication

4 **DNA polymerase:**

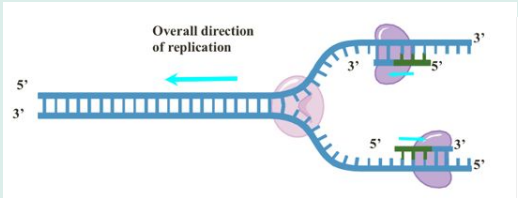
- Adds DNA nucleotides to the RNA primer.

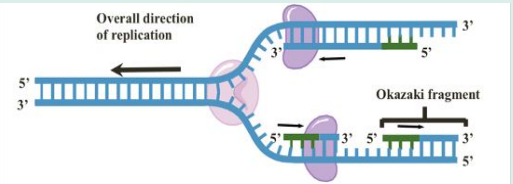
Leading strand is (5’-3’) toward the fork, while the **lagging strand** is (5’-3’) in the opposite direction of the fork

- Proofreads bases added and replaces incorrect nucleotides

If a wrong nucleotide is added that is not complementary to the parent strand, DNA polymerase will **recognise it and replace it**

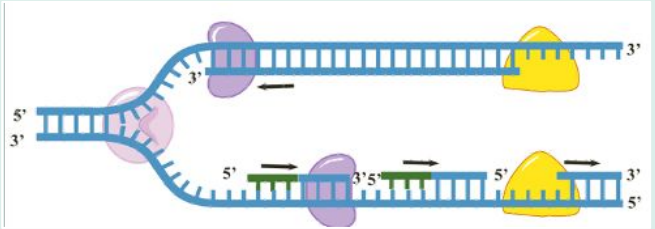
- Leading strand synthesis continues in a 5’ to 3’ direction.
- Discontinuous synthesis produces 5’ to 3’ DNA segments (Okazaki fragments).





Steps in DNA replication

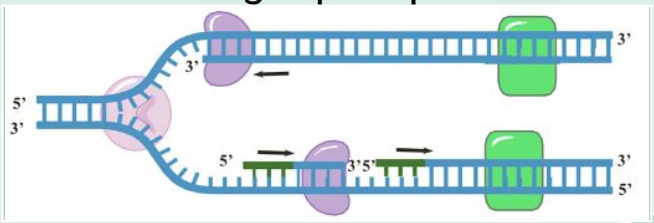
5 Exonuclease activity of **DNA polymerase** (yellow) removes RNA primers



6 **DNA polymerase** fills the gaps.

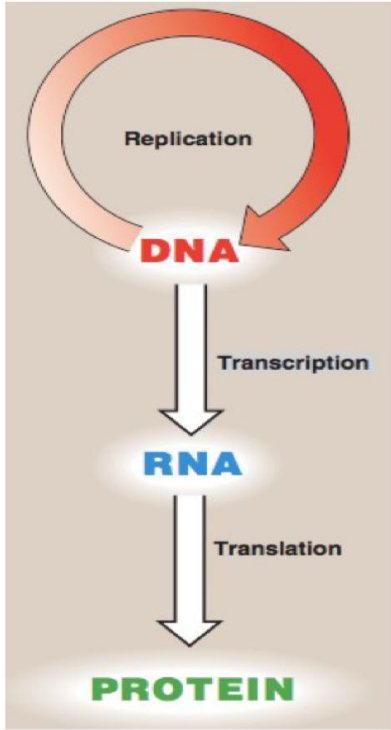
7 **Ligase** (green) forms (phosphodiester) bonds between sugar-phosphate backbone

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.
  (which means ~95% is “junk” DNA)
- DNA comes from DNA (in replication).



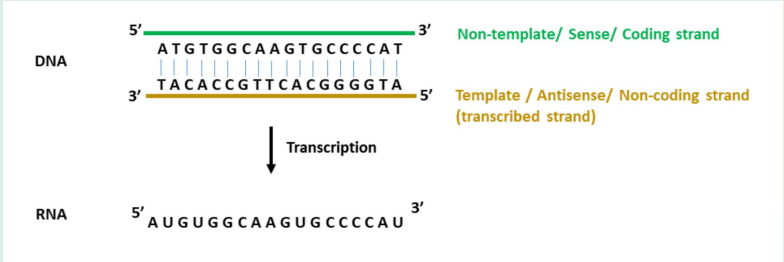
Helpful video

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′.



- Final mRNA = Sense Strand

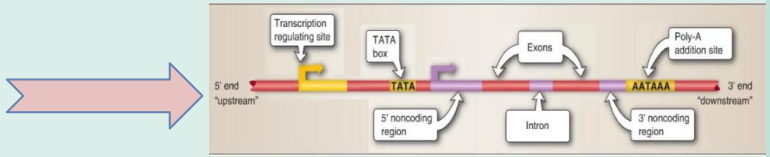
-Strand used for transcription is Antisense Strand (because it is "anti" opposite of the desired mRNA)

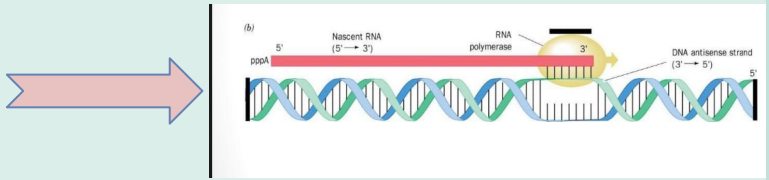
Steps of mRna synthesis

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).





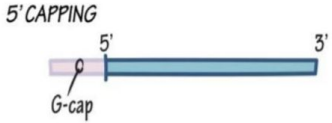
Thank you 442!

Post-transcriptional modification

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

Functions:

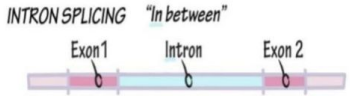
- To **prevent** mRNA degradation by **exonucleases**
- It helps the transcript **bind to the ribosome** during protein synthesis



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.**

Also called "splicing"

- **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

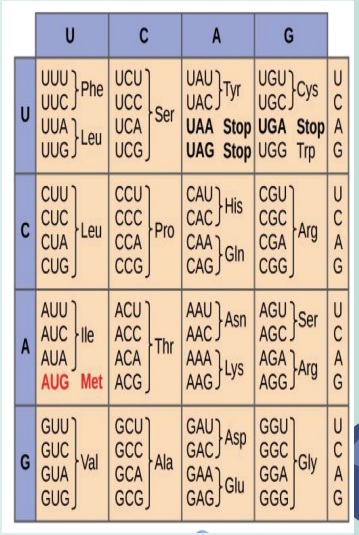
- mRNA has genetic codes for amino acids present in proteins.

- 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"team 441".

- 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 Helpful video

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: A**cceptor site that binds to tRNA, which holds the new amino acid to be added to the polypeptide chain

★**P site: P**eptidyl site that holds the tRNA carrying the growing polypeptide chain

★**E site: E**xit site that discharges the tRNA to leave the ribosome


E site
A site
P site
5'
3'

Steps of protein translation

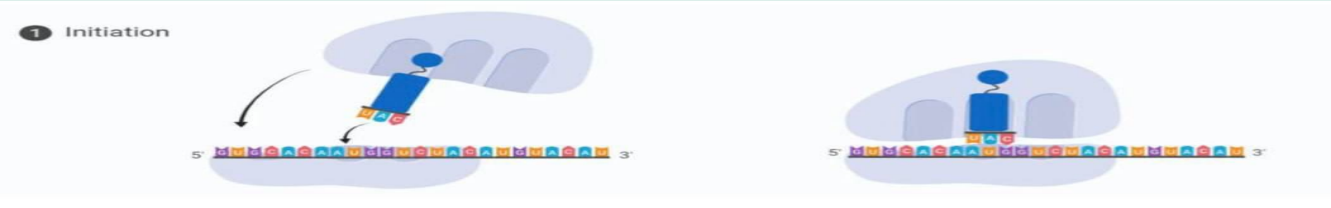
Initiation:

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



Steps of protein synthesis...

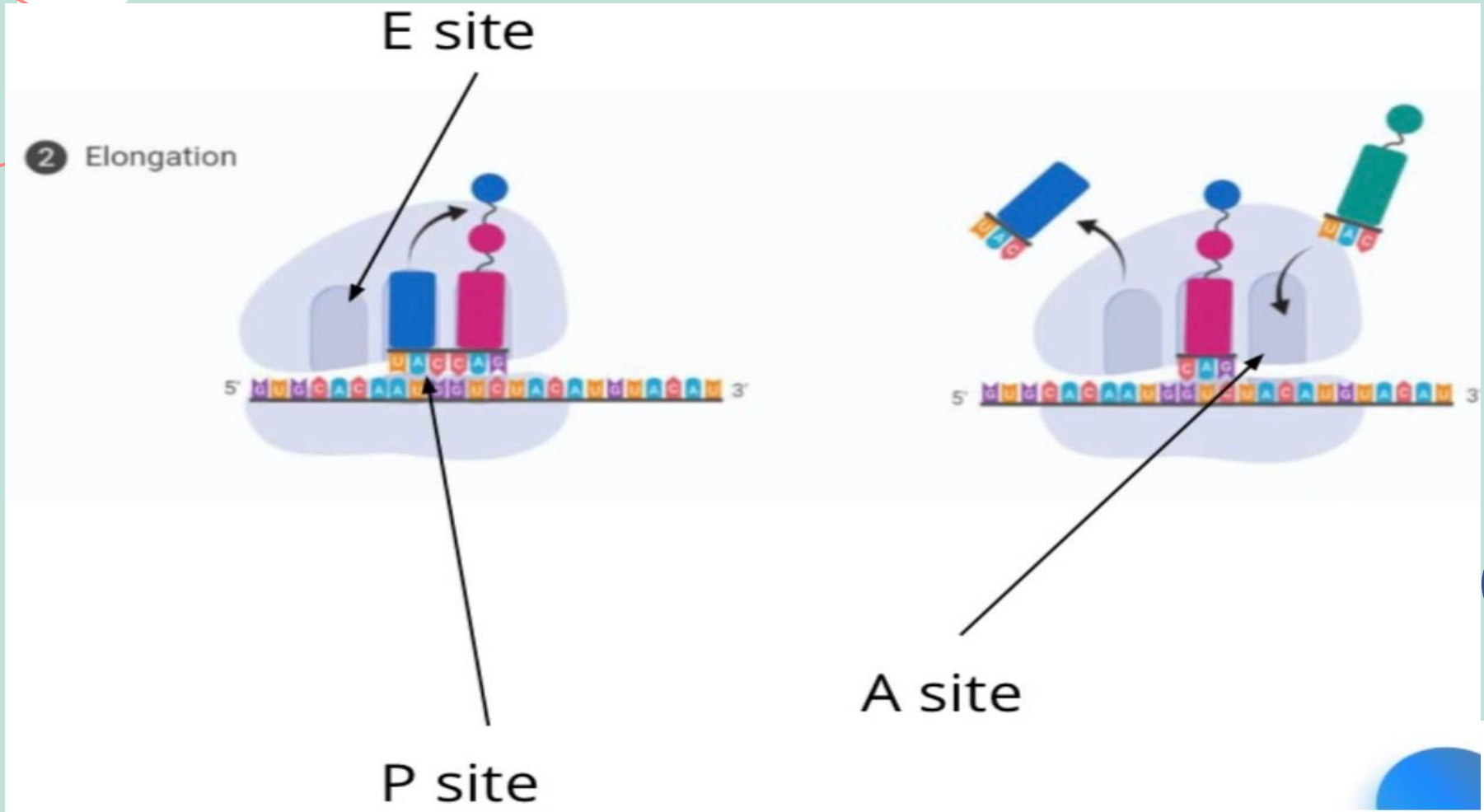
Elongation:

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.


E site
2 Elongation
P site
A site

Steps of protein synthesis...

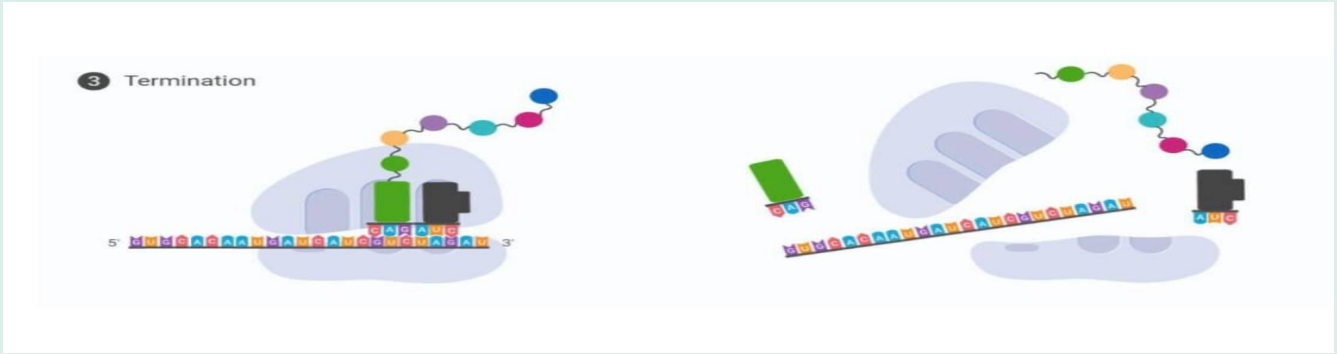
Termination :

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.

Termination



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)

Thank you 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

B/4
C/3
C/2
A/1

Q1: the primer is composed of?			
A) RNA	B)codons	C)Gene	D)nitrogen base
Q2: The 3 bases that are complementary to one of the mRNA codons are called,and are present on?			
A)Semi codons,ribosomes	B) Antagonist codons,tRNA	C)anticodons,tRNA	D)reverse codons,rRNA
Q3: Replication, Transcription, Translation respectively take place in?			
A) All in nucleus of cell	B) Nucleus,cytoplasm,cytoplasm	C) Nucleus,nucleus,cytoplasm	D) All in cytoplasm
Q4: In the final step of translation,what binds to the A site?			
A)A stop codon	B)A release factor	C)Methionine	D)Nothing

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