جامعة الملك سعود
King Saud University

Biochemistry team 436

Molecular Biology 2

- Color Index:
  - Important.
  - Extra Information.
  - **Doctors slides.**

436 Biochemistry team

Revised by

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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.

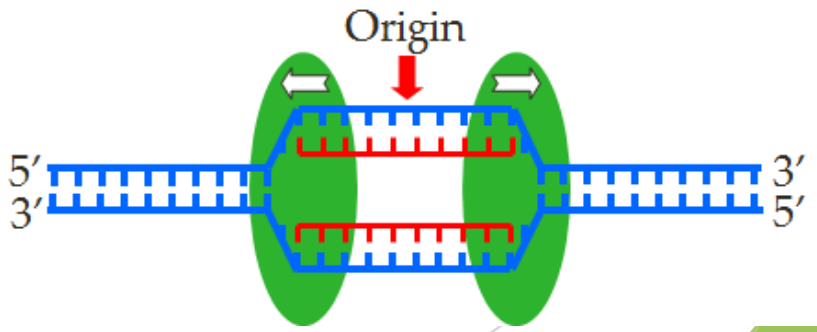
DNA is the genetic material, therefore :

- 1- it replicates faithfully.
- 2-has the coding ability (ability to transfer information) to produce proteins for all cellular functions.

Features of Eukaryotic DNA Replication

1- Semiconservative → **Daughter DNA molecule contains one parental strand and one newly-replicated strand**

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



Origin of replication: is a particular sequence in a genome at which replication is initiated.

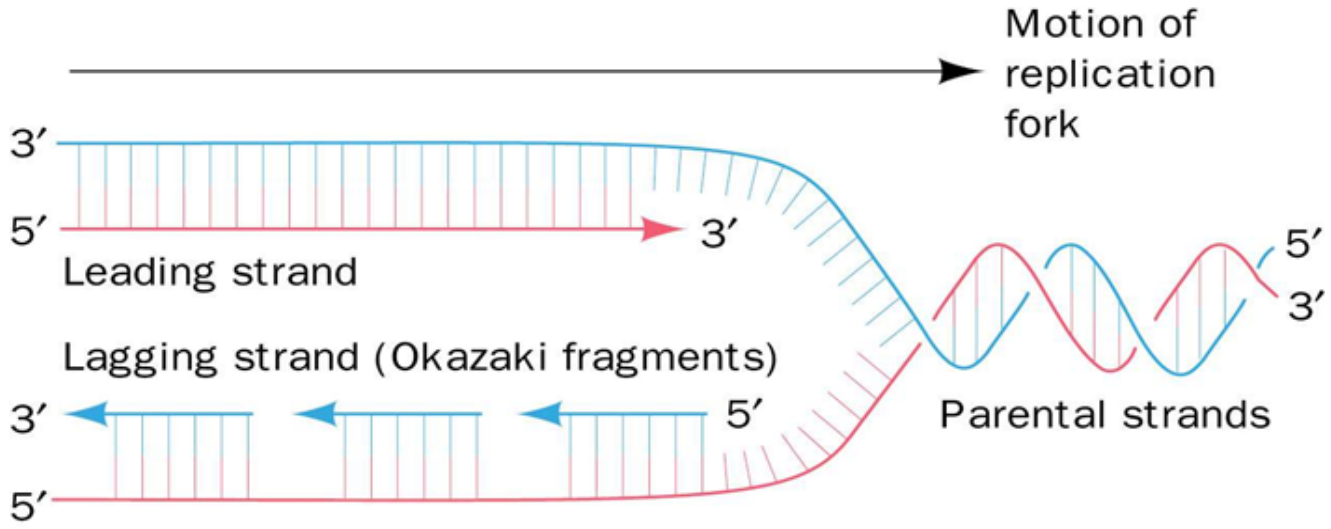
3- Primed by short stretches of RNA.

There must be a basic structure to build upon "a foundation". The enzymes for replication can't start from scratch; therefore Primers (RNA nucleotides) are used (and they are later removed)

4- Semi-discontinuous

The leading strand is synthesized continuously and the lagging strand is discontinuous (in fragments)

The directions will always be 5' -> 3' (either away or into the fork).



للتذكر: هل لك سبب تلوم بُه ؟ لا، توبه هو بريء

Proteins involved in DNA replication

Name	Types	Functions
DNA Helicase	_____	-An enzyme that binds to DNA sequences called Origins and unwinds DNA strands.
Single-stranded DNA binding proteins	_____	Prevents single strands from winding.
DNA Primase	_____	An enzyme that makes a short segment of RNA primer which is complementary to the DNA
DNA polymerases	α (Alpha) , β (Beta) , γ (Gamma) , δ (Delta) , ε (Epsilon).	An enzyme that adds DNA nucleotides to the RNA primer. -Proofreads bases added and replaces incorrect nucleotides.
DNA ligase	_____	An enzyme that forms bonds between the sugar-phosphate backbone.
Topoisomerases	Topoisomerases I	(cut one of the DNA strands)
	Topoisomerases II	(cut both of the DNA strands)
Telomerases	_____	An enzyme that adds nucleotides to telomeres (a reign at the end of a chromosome)

Eukaryote enzymes are different than prokaryote enzymes.

***** The Pol α complex (pol α-DNA primase complex)

EUKARYOTIC DNA REPLICATION (continued)

- Figure 29.23

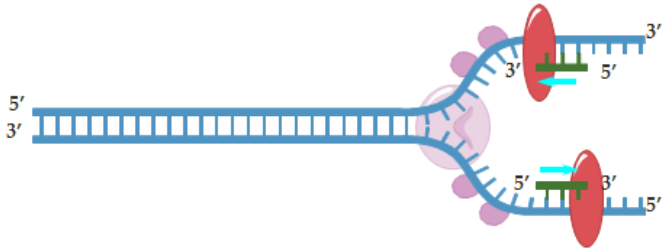
Activities of eukaryotic *DNA polymerase* (*pol*) *3′→5′ *exonuclease* activity.

POLY-MERASE	FUNCTION	PROOF-READING*
***Pol* α** (alpha)	• Contains primase • Initiates DNA synthesis	–
***Pol* β** (beta)	• Repair	–
***Pol* γ** (gamma)	• Replicates mitochondrial DNA	+
***Pol* δ** (delta)	• Elongates Okazaki fragments of the lagging strand	+
***Pol* ε** (epsilon)	• Elongates the leading strand	+

Steps in DNA replication

Untwists DNA strands, making a fork-like structure → called replication fork

- Helicase.
- If there are multiple origins then there are multiple helicase.



Prevents single strands from twisting again.

- Single-stranded binding proteins.
- يمسك كل شريطة على طرف بحيث يبعدهم عن بعض عشان ما يرجعوا يتصلوا

Makes RNA primer.

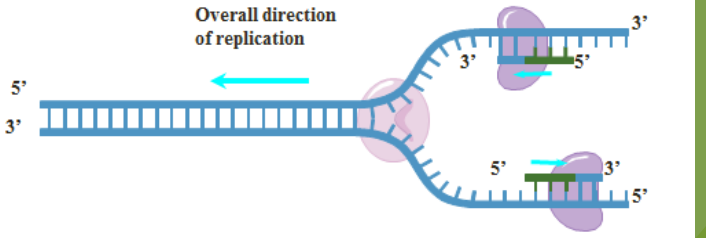
- Primase
- The primer can only sit on the 3’.

Adds nucleotides to the primer and checks if the bases added are correct or not, if not → removes incorrect nucleotides.

- DNA polymerase.
- يضيف الوحدات وبعد كذا يتأكد من شغله و اذا سوا شيء خطا يمسحه.

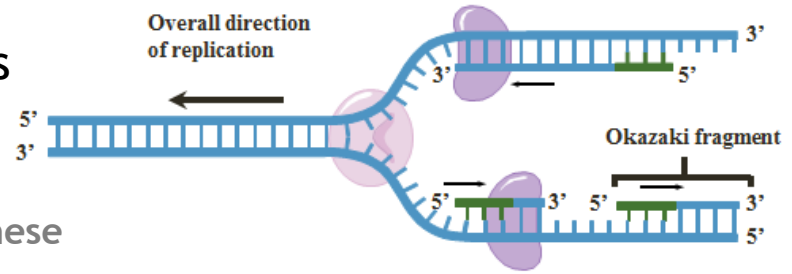
Why do we need Single-stranded binding proteins?

The DNA has a lot of hydrogen bonds between its base pairs, and because hydrogen bonds are very strong they may form back on their own, so we need those proteins to keep the two strands apart to prevent hydrogen bonds from forming and reconnecting the strands while replication is still happening.



- Leading strand synthesis is continuous in a 5’ to 3’ direction.
- Lagging strand discontinuous synthesis produces 5’ to 3’ DNA segments (okazaki fragments).

The two strands are antiparallel. However, DNA polymerase can ONLY work in a 5’ → 3’ direction so we are left with gaps where the DNA polymerase cant continue working. These fragments are called okazaki fragments



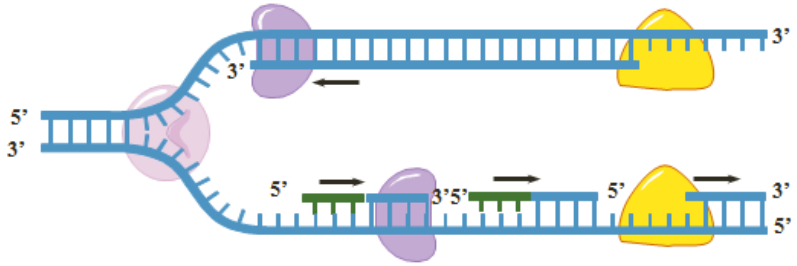
Continue:

Removes the RNA primers by exonuclease activity.

- DNA polymerase.
  - In the lagging strand
  - Exonucleases are enzymes that remove nucleotides.

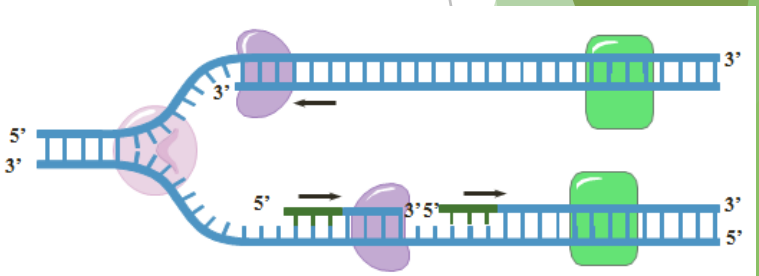
Fills the gaps that we got after removing the RNA primers.

- DNA polymerase.
  - In the lagging strand.



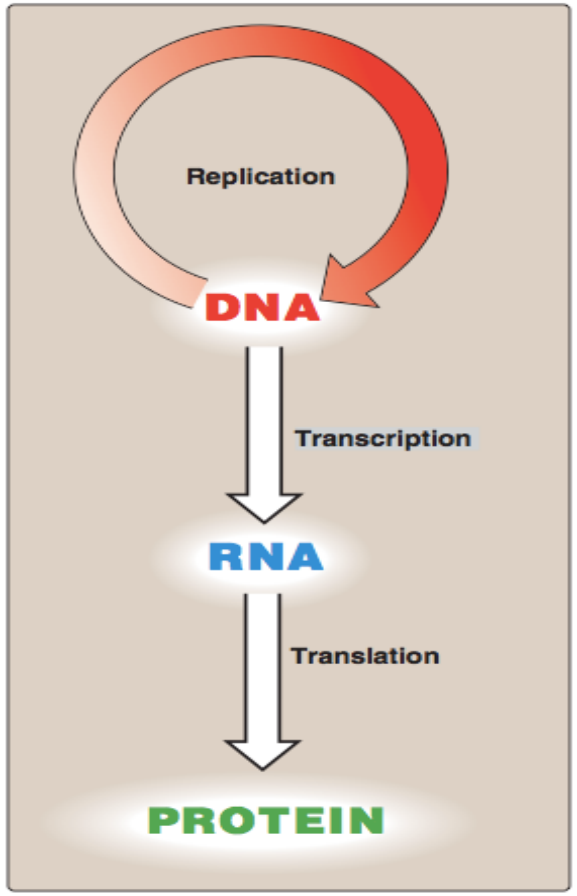
Connects the okazaki fragments by forming bonds between the sugar-phosphate backbone.

- Ligase
  - In the lagging strand



DNA Replication video

The central dogma of Molecular Biology



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

***RNA** is translated into **proteins**.*

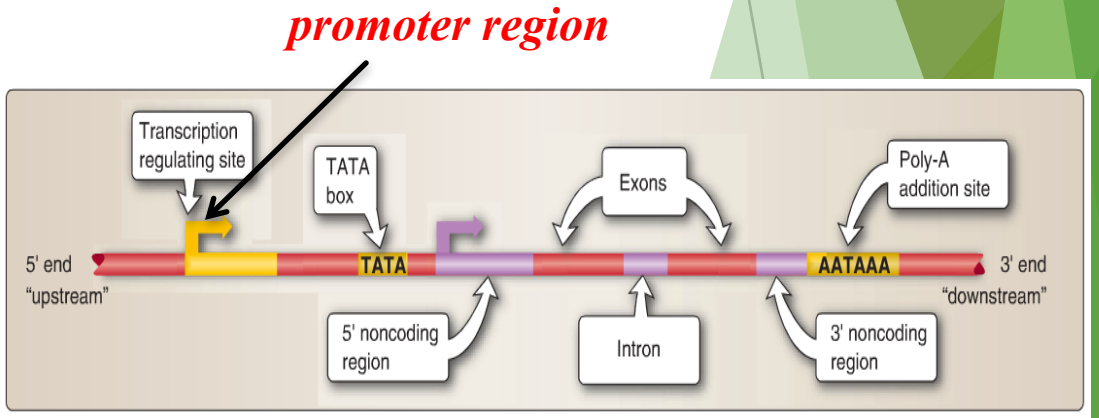
Transcription (mRNA synthesis)

- 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'**.

Steps of mRNA synthesis

Chain initiation:

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

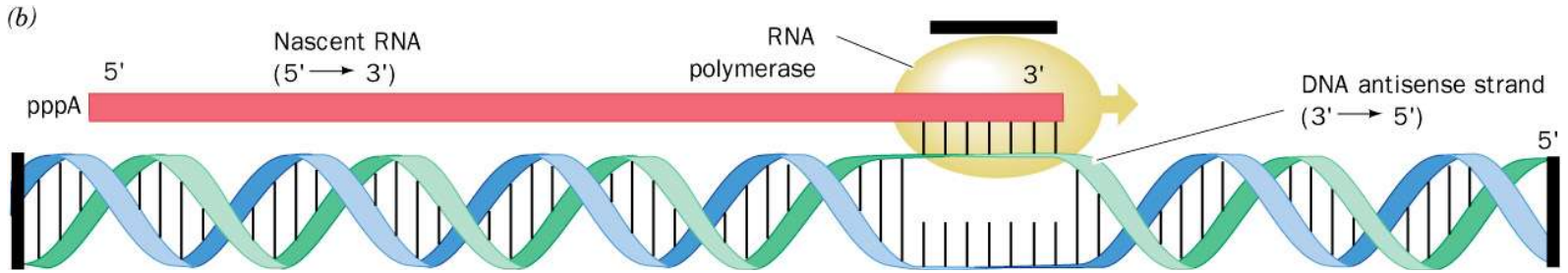


Notes :

Ta-Ta box is in the promotor region

Steps of mRNA synthesis

- **Chain elongation:**
  - A portion of DNA template unwinds (opens) 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 a sequence of **4-10** AT base pairs).

Note: in RNA we call it transcription bubble instead of replication fork

Post-transcriptional modification

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.**

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

Functions:

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

Notes:

Tail: poly (A) tail helps with recognition

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

*** Note:**

Introns will be removed before the transcription , but Exons present.

Steps of mRNA synthesis

Chain initiation:

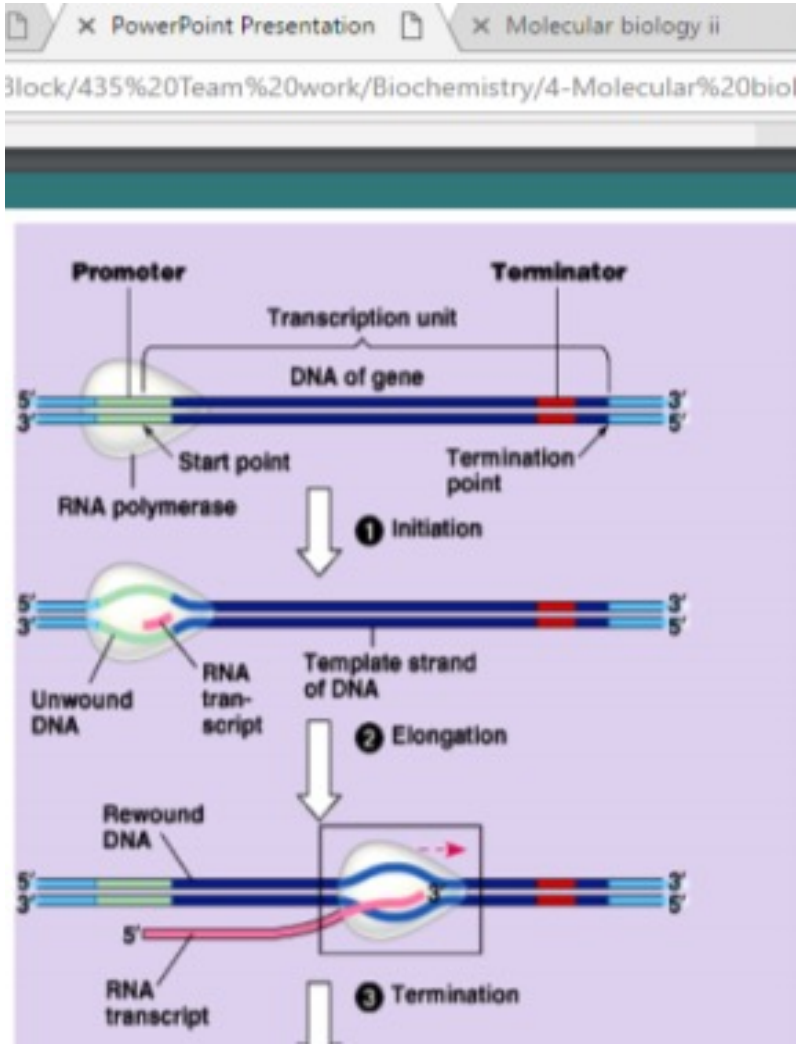
- RNA polymerase II binds to promoter region of DNA to start transcription.

Chain elongation:

- a portion of DNA unwinds (opens) at the point of RNA synthesis.
- RNA polymerase II moves along the template strand of the DNA Synthesizing the complementary single stranded mRNA molecules *the direction of transcription is from 5’ ☹3’*
- as the RNA polymerase II moves the double helix rewinds.
- 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).



Post-transcriptional modification

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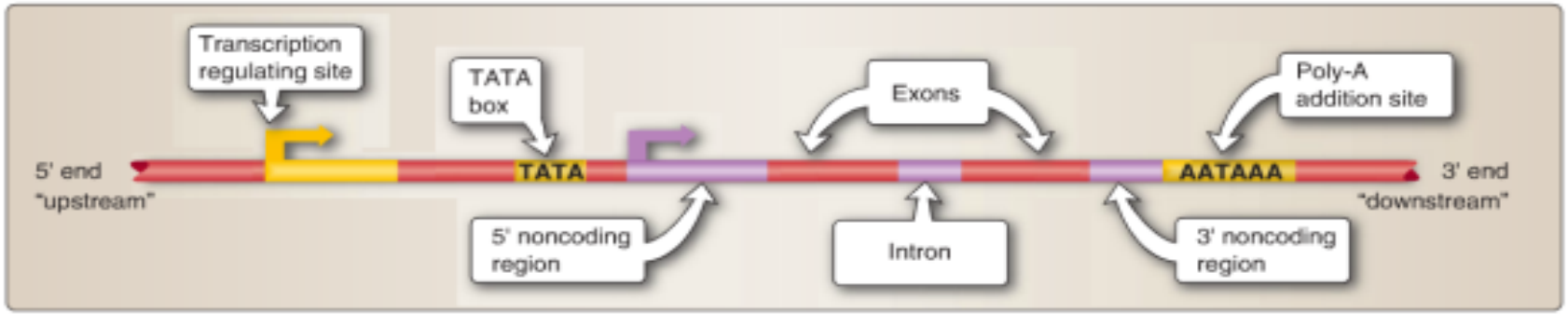


Regulation site: site where regulators binds and effect the productively of the enzyme
طريقة للتذكر:
Exons: from expressed آي تظهر في المنتج النهائي
Intron: from (In) اي شيء داخلي ما يخرج من الخلية
-added at 5 end is a cap , at 3 end is tail

Translation (Protein synthesis)

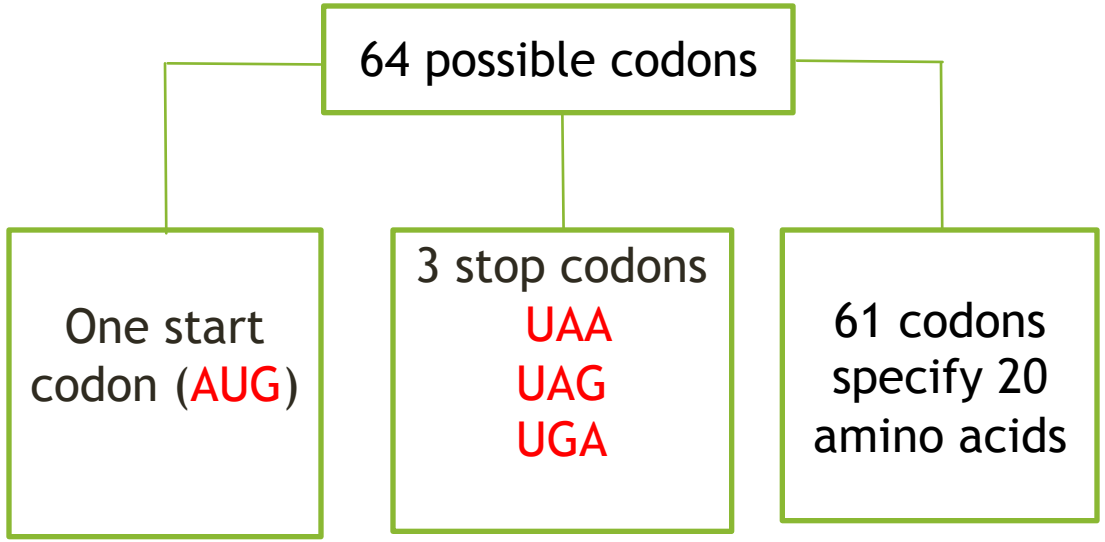
It is a process of protein synthesis from mRNA

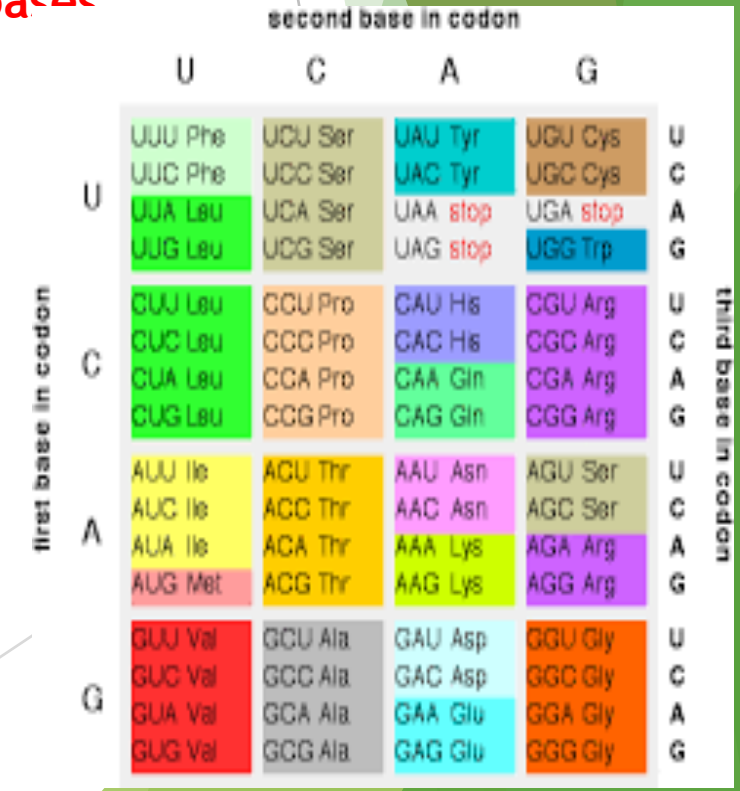
- mRNA has genetic codes for amino acids present in proteins.
- **The genetic code** is a dictionary that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids.
- **Each individual word in the code is composed of three nucleotide bases (codons).**

"الكودون هو ثلاثة نيوكليتايد على تكون شفرة لحمض أميني معين "

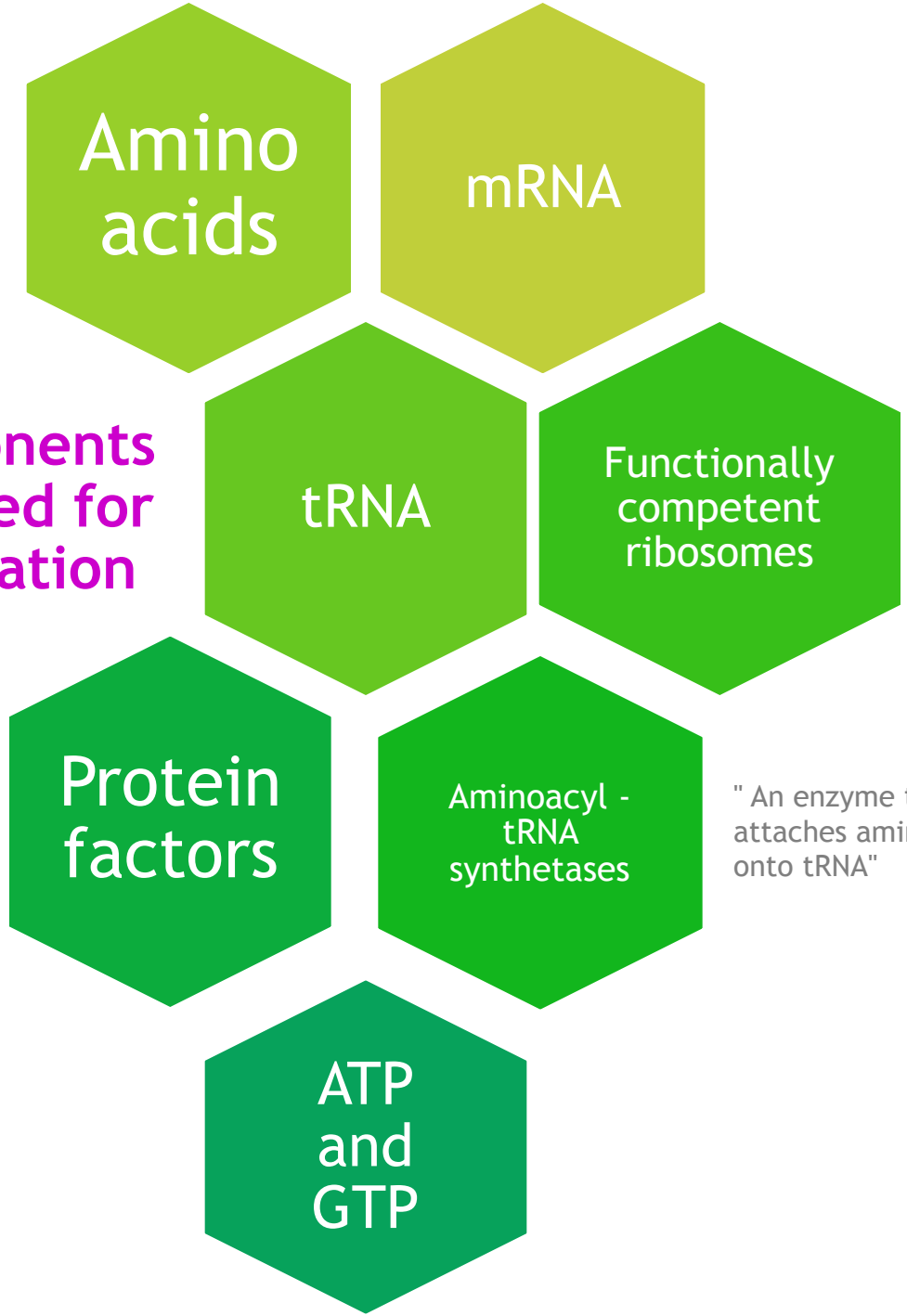
جملة لتسهيل حفظ كودونات التوقف:

"يُقال من يواعد يُواجه"
UGAl mn UAAed UAGeh

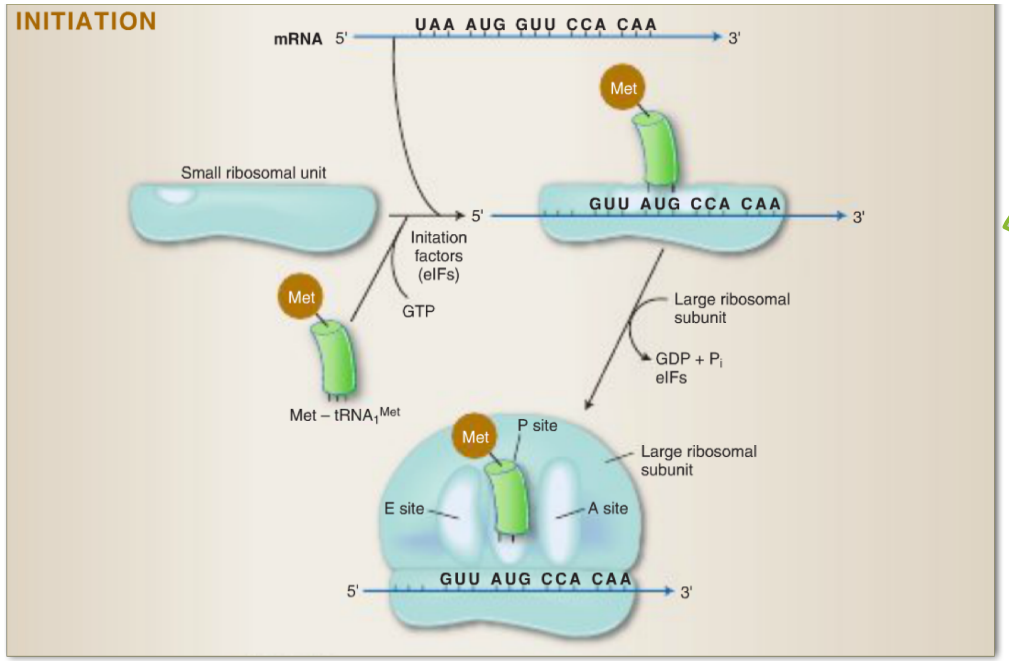




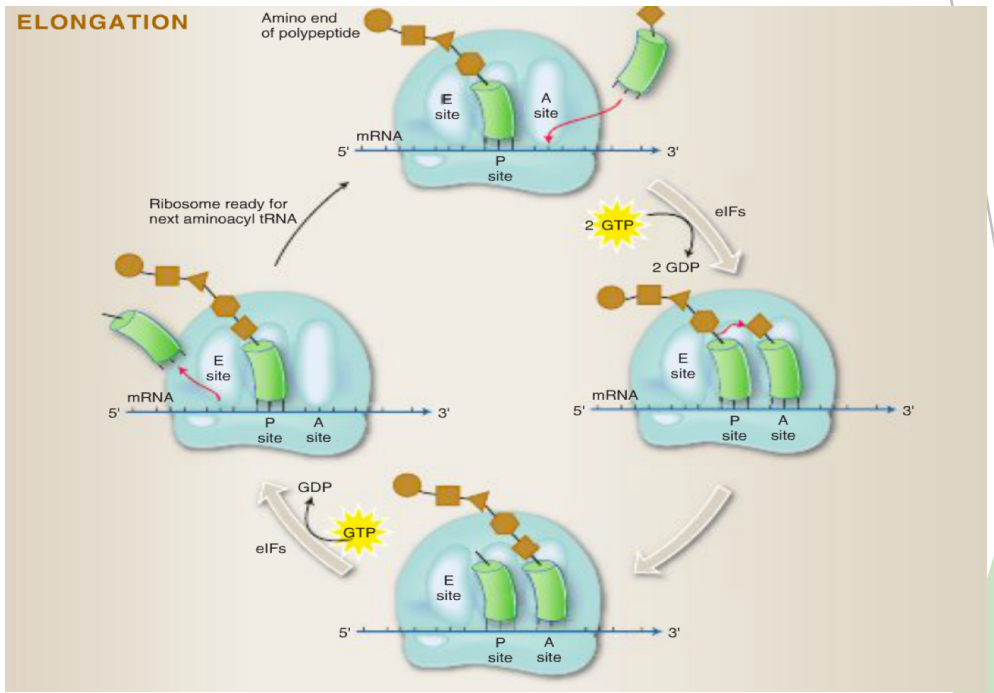
Components required for Translation

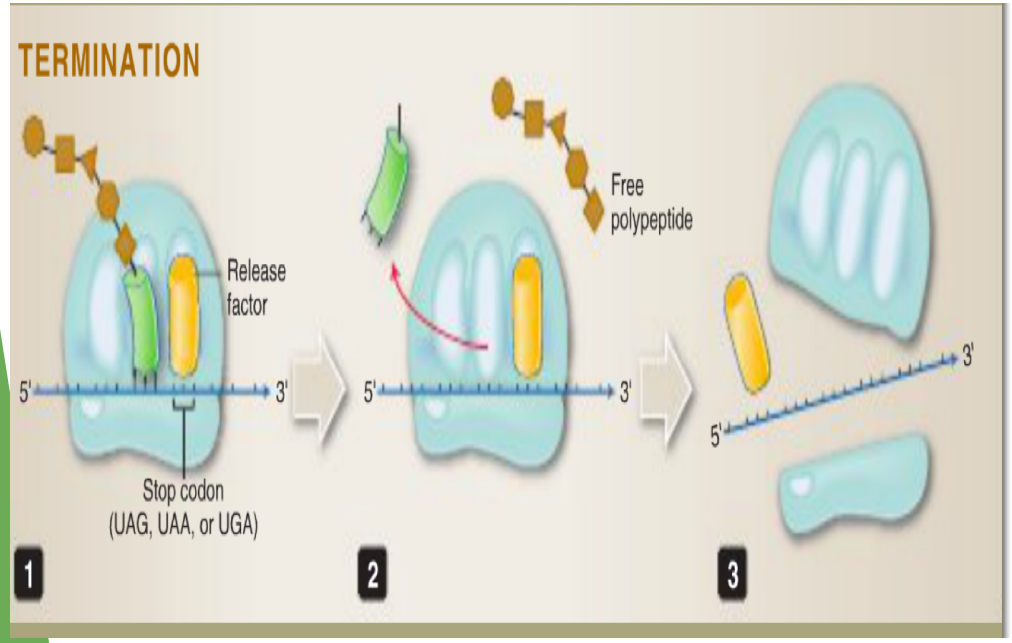


" An enzyme that attaches amino acid onto tRNA"



1. Initiation: in general it means {making complements}
Puts (ribosoms +tRNA+ mRNA} together and make it ready to start



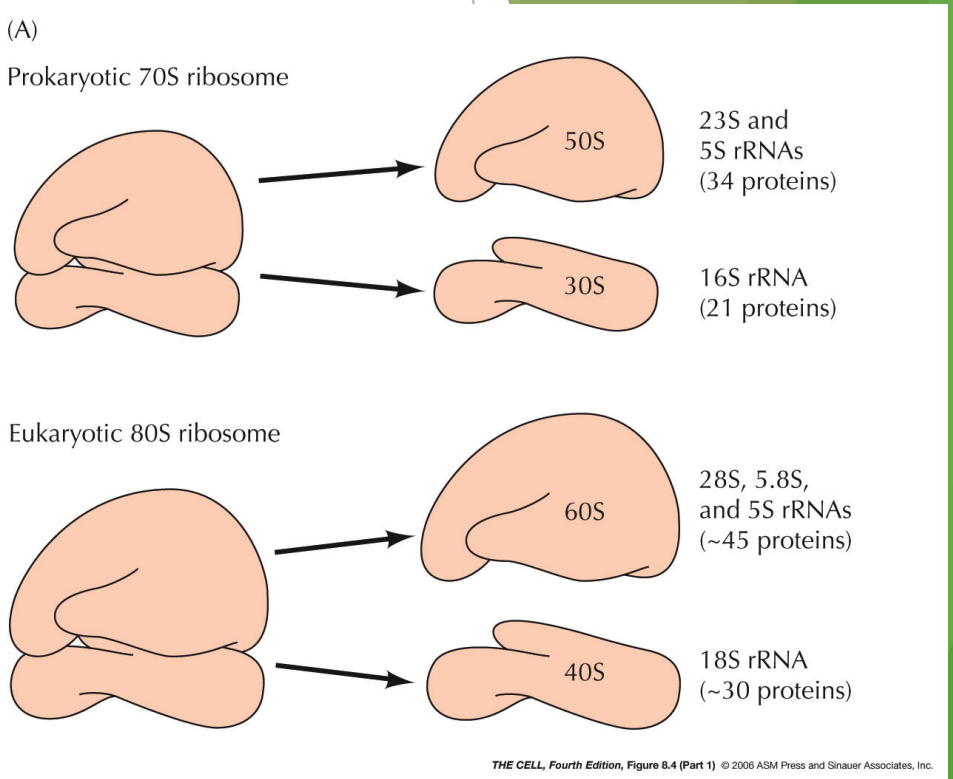


Elongation: it happens until the termination (stop codon)
* P =peptide side, A=incoming amino acids, E= Exit site

Mentioned in males lecture:

- All prokaryotes have 70S ribosomes. 70S ribosome is made up of a large subunit (50S) and small subunit (30S)
- Eukaryotes have larger ribosomes (80S) made up of 60S and 40S subunits.

- S= Svedberg units to measure the size of the subunits



- In humans the start codon is **Methionine.**
- In bacteria the start codon might code with different codons.

*summary for all the lecture

DNA replication

1- the rest of proteins involved in DNA replication A- topoisomerases I & II B- telomerase
2- leading strands synthesis continues in a 5 to 3 direction.
And the lagging strand discontinuous synthesis 5 to 3 (okazaki fragments)

DNA Helicase
- Protein unwinds DNA strands

Single stranded
- Protein prevent single strands from rewinding

Primase
- Protein makes a short segment of RNA primer

DNA polymera
- Protein adds nucleotides to RNA primer
- Removes RNA primers
- Fills the gabs

Ligase
- Protein form bonds between sugar-phosphate backbone

mRNA synthesis (transcription)

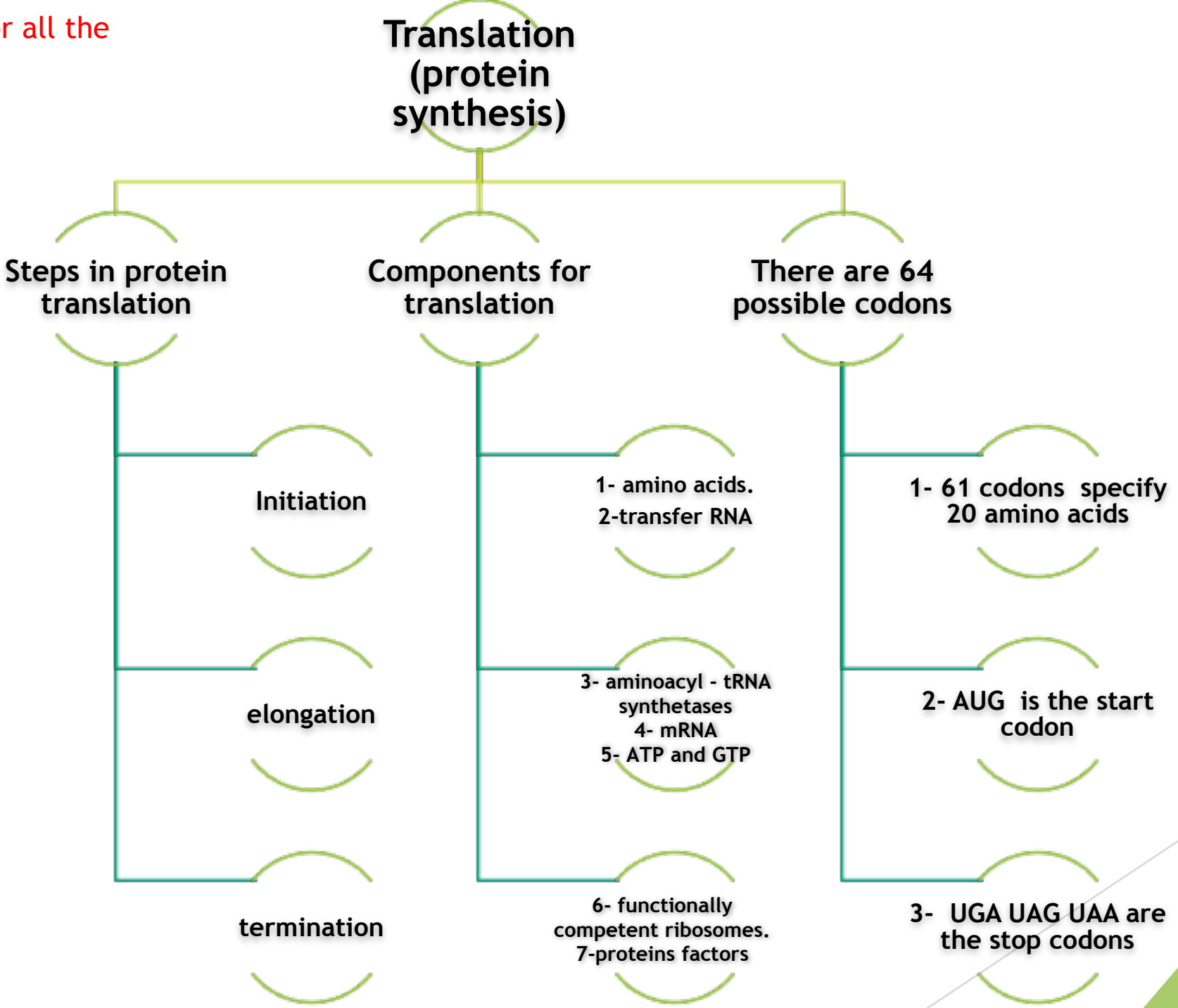
Chain initiation
- RNA polymerase II binds to promoter region of DNA to start transcription.

Chain elongation
- A portion of DNA template unwinds at the point of synthesis and this will form short length of RNA-DNA hybrid.

Chain termination
- DNA contains specific sites which stop transcription.

Post-transcriptional modification
1- capping : at the 5 end of mRNA
2- polyadenylation : at 3 end of mRNA

*summary for all the lecture



Videos

From DNA to protein

DNA Replication

Protein synthesis

DNA

QUIZ

Girls team members:

1- أسيل السليماني.
2- نوره الشبيب.
3- ريم السرجاني.
4- غادة المزروع.
5- سارة الشمراني.
6- نجود العنزي.
7- شهد السويدان.
8- هيفاء الوعيل.
9- منيرة الضفيّان.
10- لمى الفوزان.
11- بثينة الماجد.

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