بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 5

DNA Replication pt.1

Written by : DST

Reviewed by : NST

Molecular Biology (4)

DNA replication

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School of Medicine

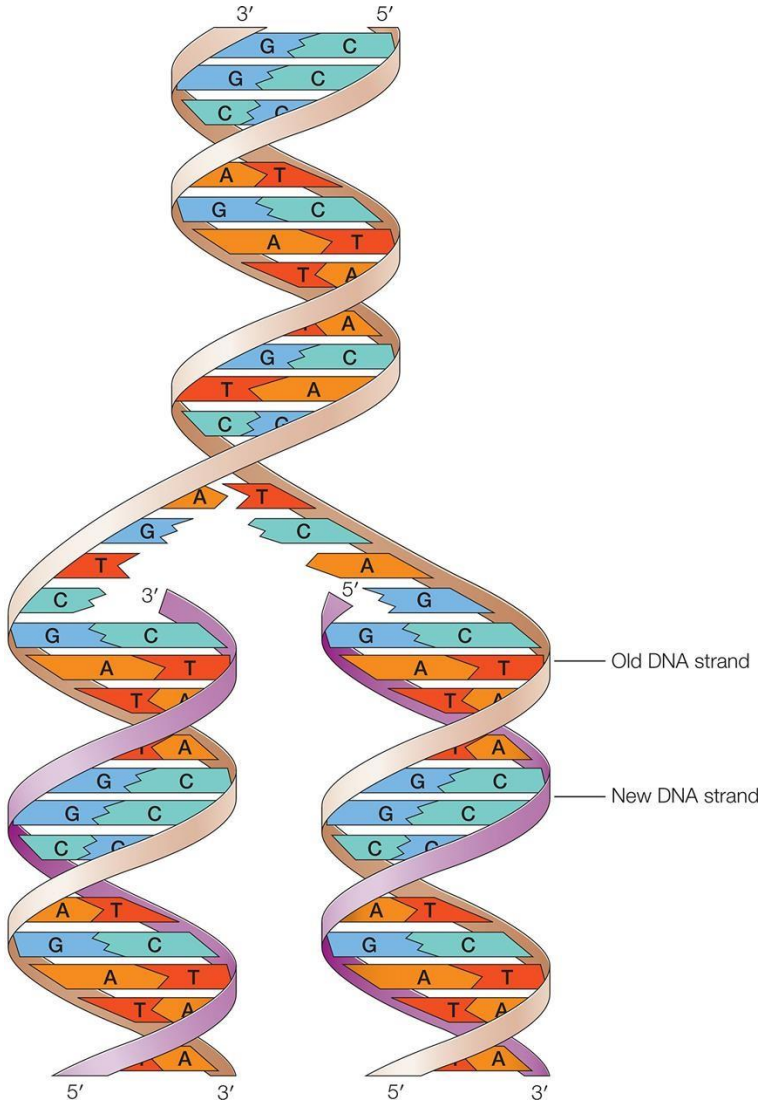
Second year, Second semester, 2024-2025

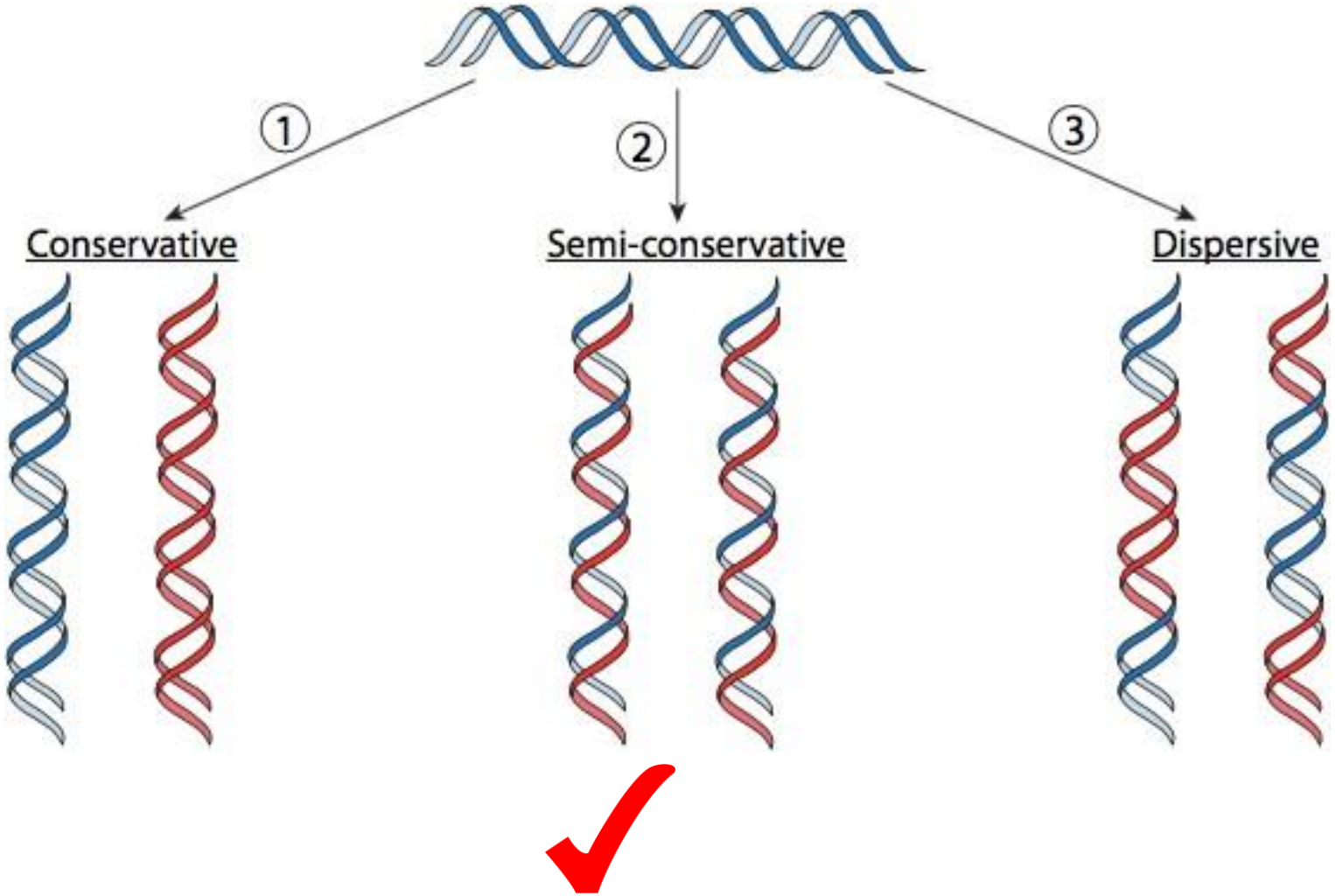
(اللّٰهُمَّ عَلّمني ما يَنفَعُني، و انفَعني بما علَّمتَني ، و زِدني عِلمَا)

❑ Some basic information

- The entire DNA content of the cell (or an organism) is known as a "genome".
- DNA is organized into chromosomes.
  - Bacterial genome: usually one, circular chromosome.
  - Eukaryotic genome: multiple, linear chromosomes complexed with proteins known as histones, and the complex is known as chromatin.
- DNA must be accurately copied (replicated).
- DNA synthesis is carried out by DNA polymerases.
  - In bacteria (E. coli: DNA polymerases I, II, and III)
  - In Humans (DNA polymerases α, δ, and ε) (alpha/delta/epsilon, there are more but we'll mainly focus on these)
- The substrates are deoxyribonucleotides. (Specifically: deoxyribonucleoside triphosphate)

❑ The hypotheses and fact



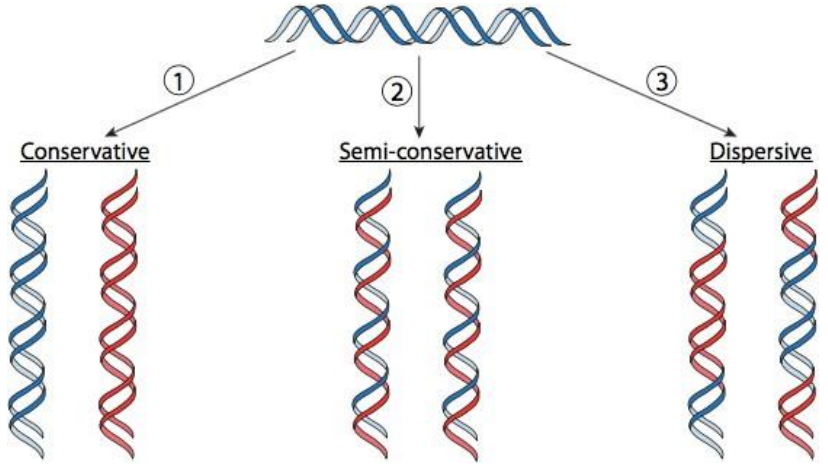


Go to the next slide for explanation

❑ Explanation for the previous slide :

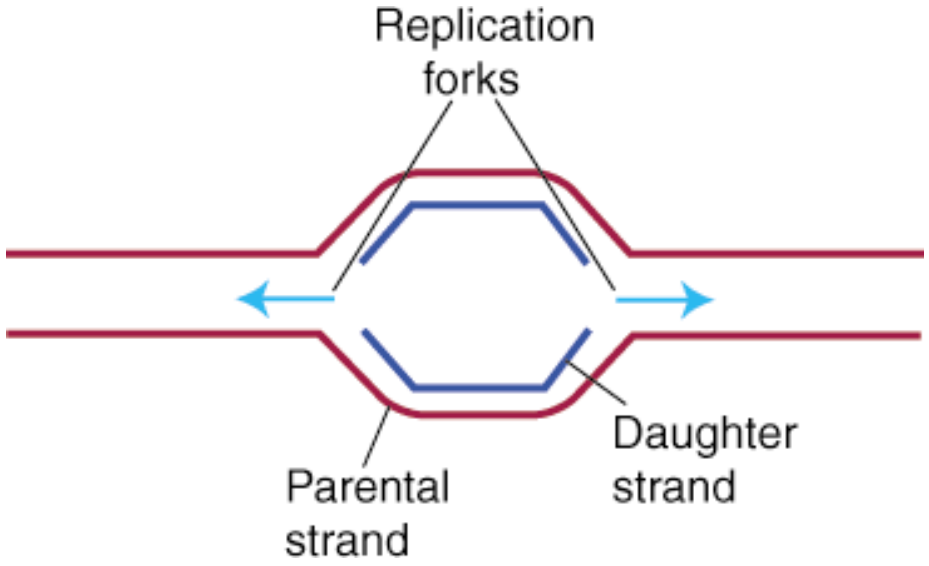
- In the past scientists didn't know how DNA is replicated so they came up with this 3 hypothesis:

1. Conservative: when DNA is replicated the old DNA is conserved (that's why we call it conservative) and the new one contains two new strands
2. Dispersive: the new DNA is composed from random fragments from the new and the old DNA strands
3. Semi conservative (the true one): each daughter cell will have the DNA composed from one strand from the new DNA and the other strand from the old one as during DNA replication the parental strands are separated, and the new ones are copied from each one of those strands



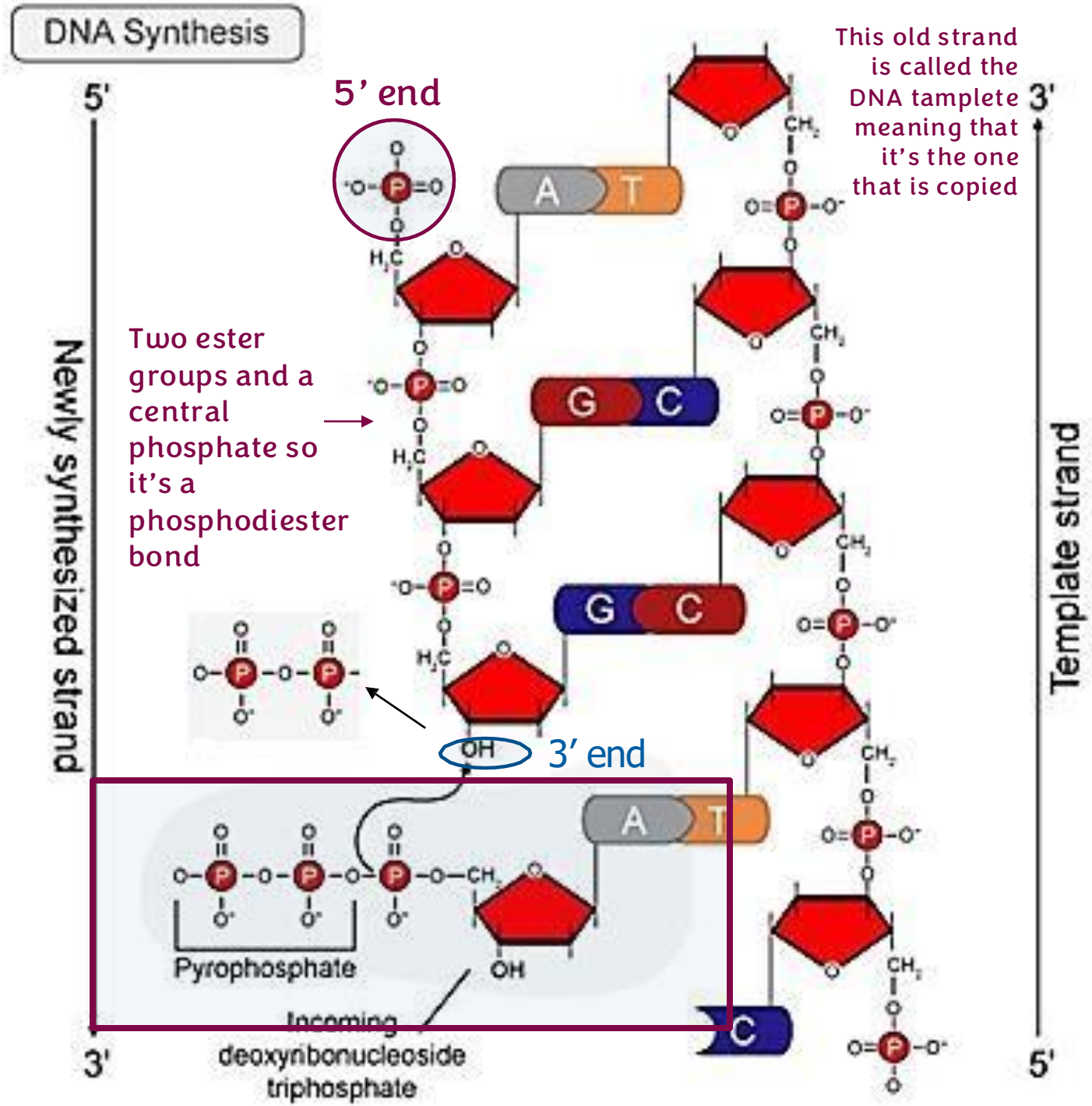
❑ Bidirectional

- Replication is bidirectional. (It moves in two opposite directions)
- This replicative region is called a replication fork.
- If we divided this replication bubble, we will have one fork to the left and the other is to the right
- This is an EM image that shows the replication bubble that consists of two forks



❑ Bidirectional

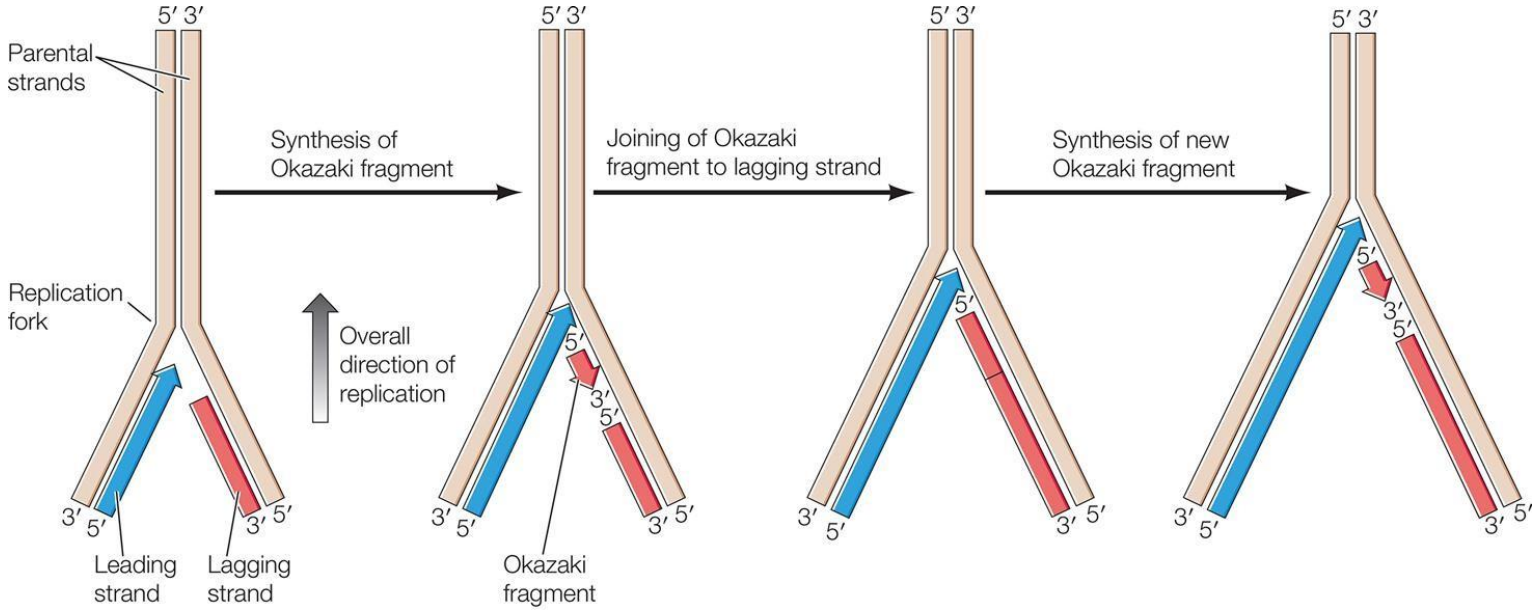
- When we add the nucleotides we need energy, so the energy here is coming from the nucleotide itself
- the substrate or the incoming nucleotide is added to the 3' end of the newly synthesized strand by the enzyme DNA polymerase (notice the box)
- Two phosphate groups are released generating energy when the new nucleotide is added so we end up with one phosphate group for each nucleotide and it's what connects them together by phosphodiester bonds
- Notice that we always add nucleotides from the 5' end to the 3' end and the new added nucleotide is the host for the next one
- The DNA strand on the right is anti parallel running from the 3' end to the 5' end and it's complementary to the strand on the left

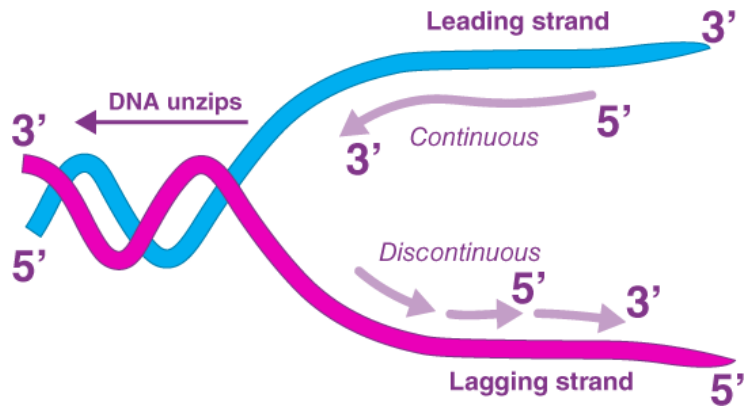


❑ Continuity of DNA synthesis

- The parental DNA is called a template.
- The new DNA is synthesized ONLY from the 5’-end to the 3’-end.
- One strand of DNA is continuously synthesized and called the leading strand.
- The other strand is synthesized discontinuously as shorter pieces known as Okazaki fragments and is called the lagging strand.

We call it this way as it’s lagging, waiting for the leading strand to allow it to be synthesized



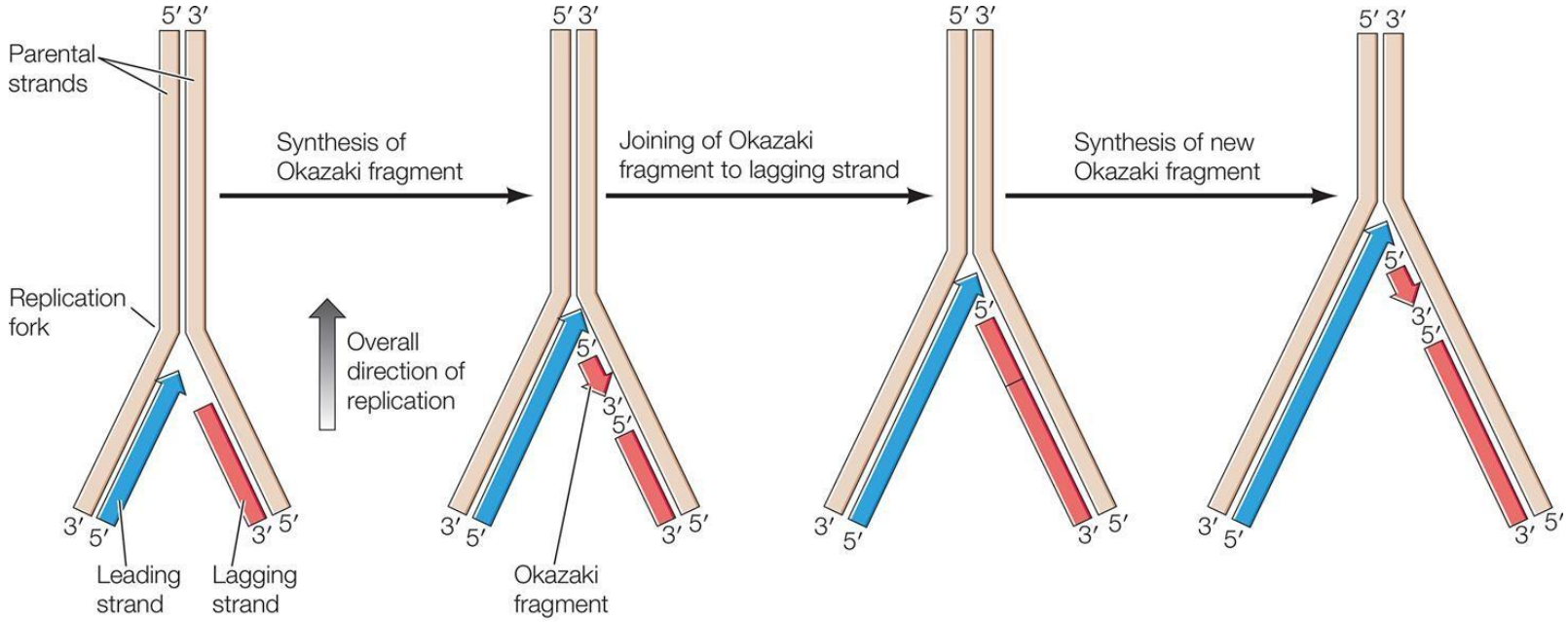


Go to the next slide for explanation

❑ Continuity of DNA synthesis

- So, what happens is that the leading strand is synthesized continuously and it opens the fork further allowing for the lagging strand to be synthesized
- The lagging strand is synthesized as fragments that are called Okazaki fragments once the leading strand opens up the fork for them and then those fragments are connected to each other by an enzyme called ligase to make up the lagging strand

- Extra note: the lagging strand is the strand that is running from the 3' end to 5' end as we said the double strands are anti parallel so one is 5' to 3' and the other is 3' to 5' and we said that the replication only happens from 5' to 3' that's what we form Okazaki fragments

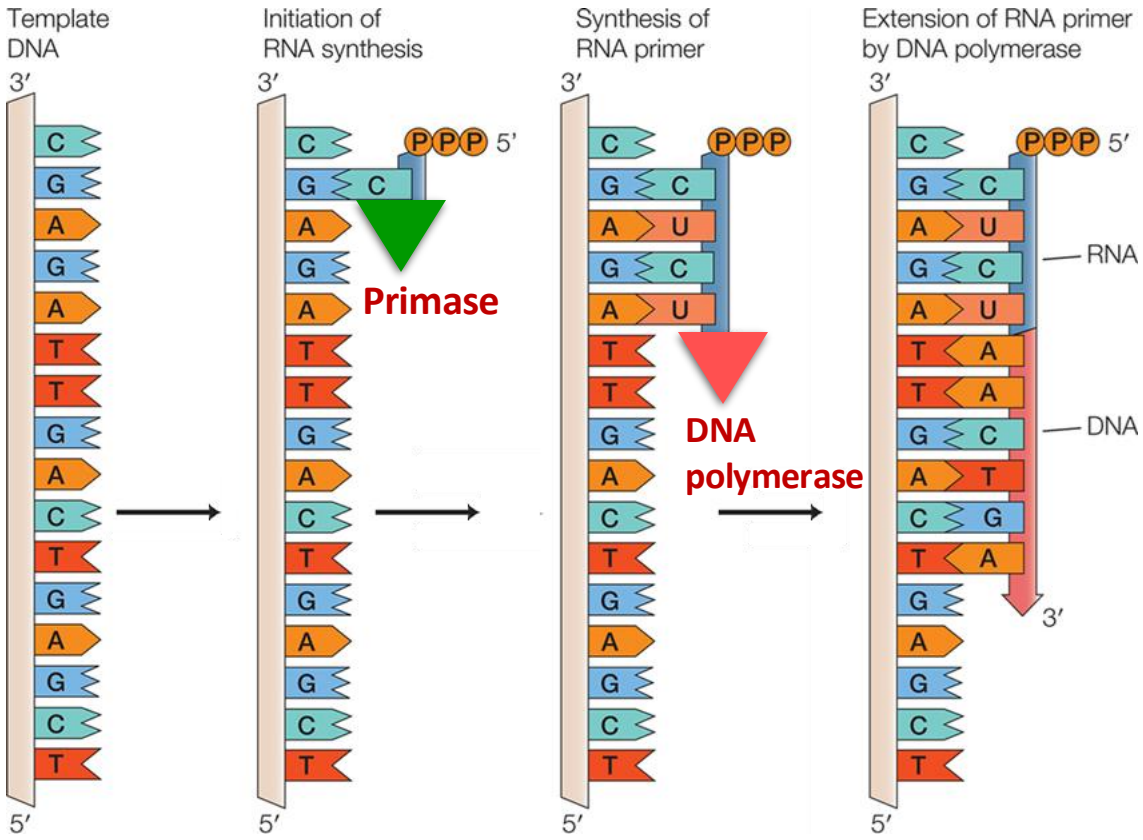


Components of DNA replication

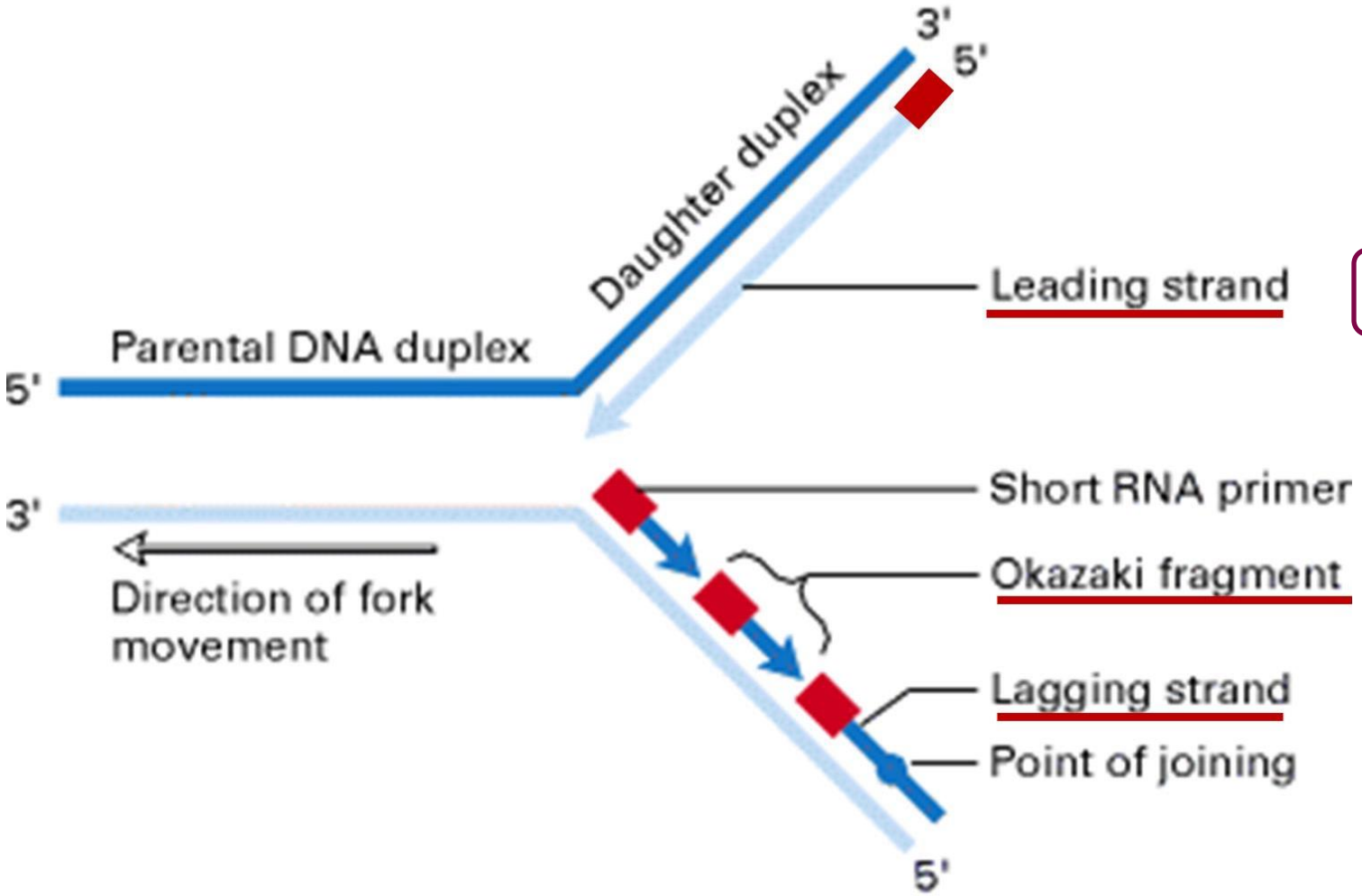
❑ Primase and RNA primer

- DNA polymerases cannot initiate replication *de novo* (from scratch).
- They require an RNA primer (3-10 nucleotides long) that is complementary to the DNA template to be added first.
- It is synthesized by a primase.

✓ To initiate DNA synthesis an RNA primer is first synthesized this primer is complementary to the DNA template but contains Uracil instead of Thiamine and uses ribonucleosides instead of the deoxyribonucleosides as substrates. Once the RNA primer is in place DNA polymerase binds to it and begins synthesizing the DNA strand



❑ The need of primers

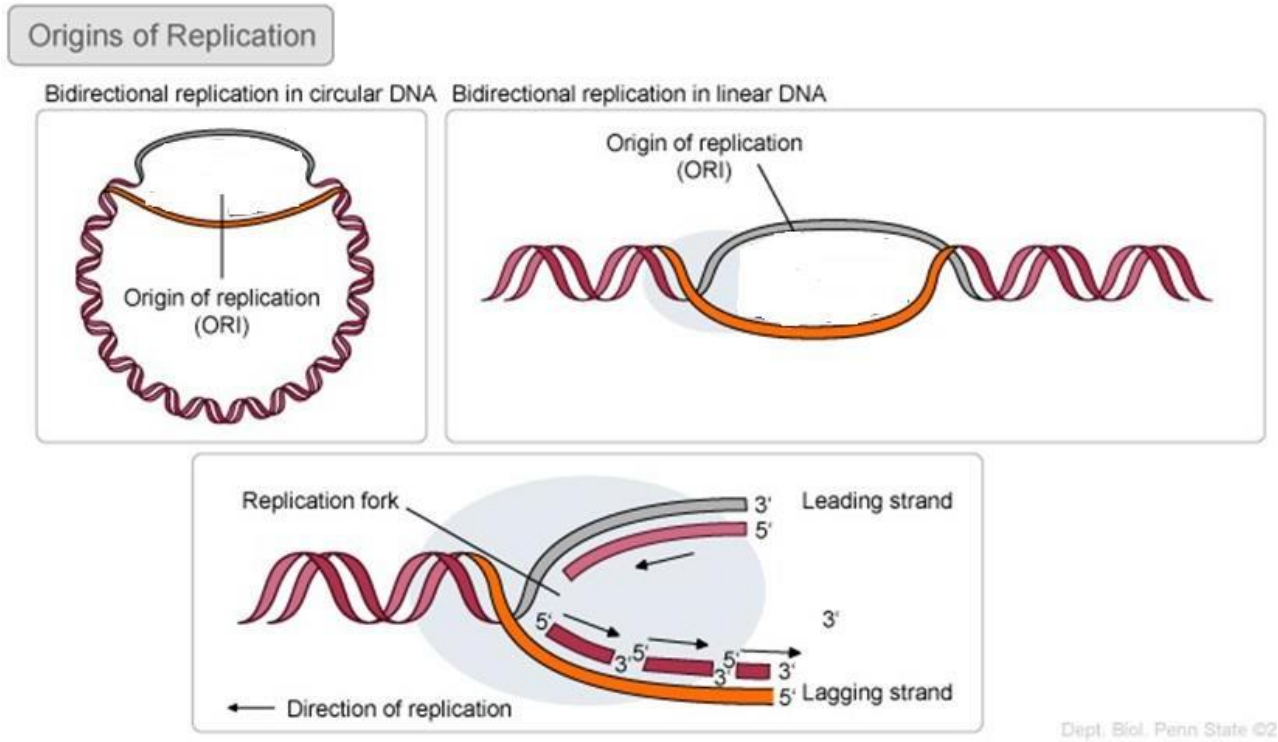


It requires one primer

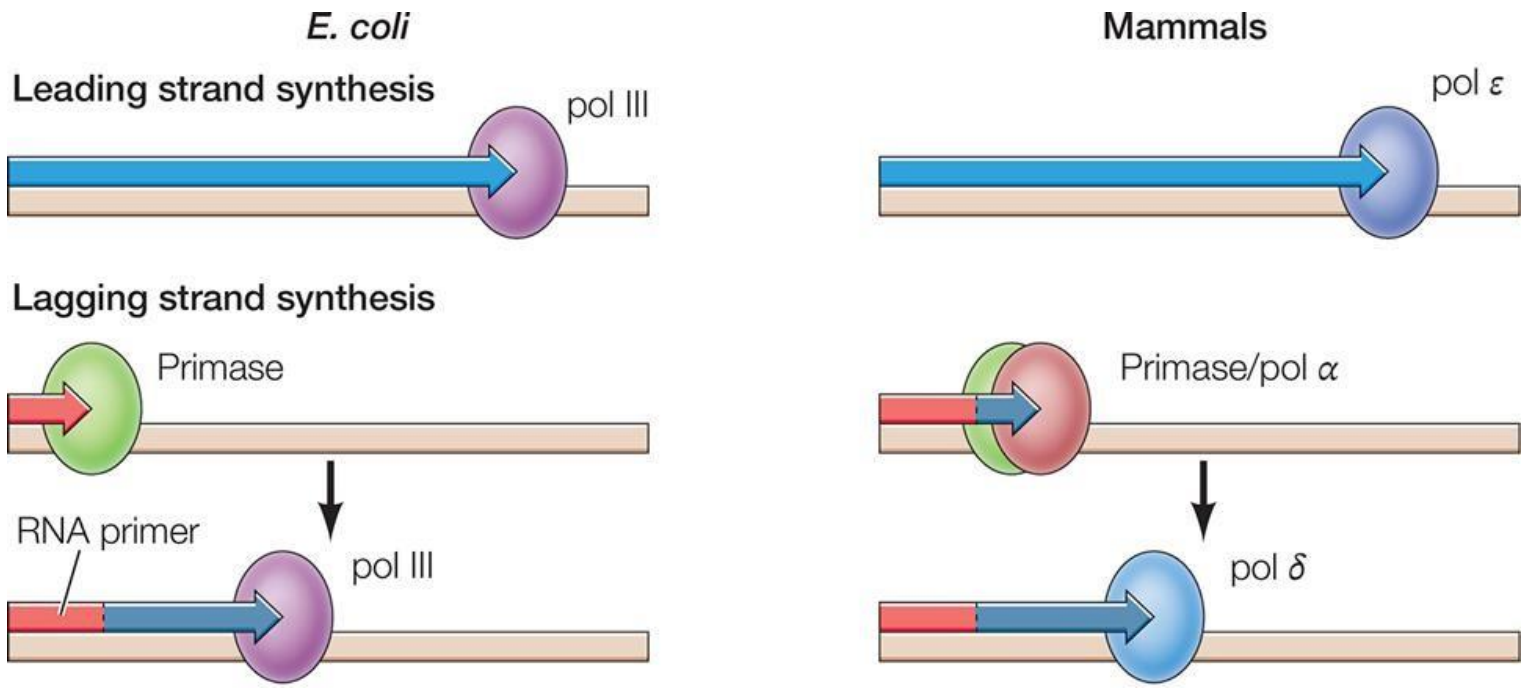
Each Okazaki fragment needs a primer, and when we connect the fragments together, **we must remove the primers**

Exercise

- I have shown you how DNA synthesis proceeds in the replication fork. Draw how DNA replication proceeds in the whole bubble.



The replicative process



- In bacteria, DNA polymerase III is the major replicative enzyme

- In human cells:
- DNA polymerase α is complexed with primase initiating the synthesis of DNA, and then
- DNA polymerase ε (epsilon) synthesizes the leading strand.
- DNA polymerase δ (delta) synthesizes the lagging strand.

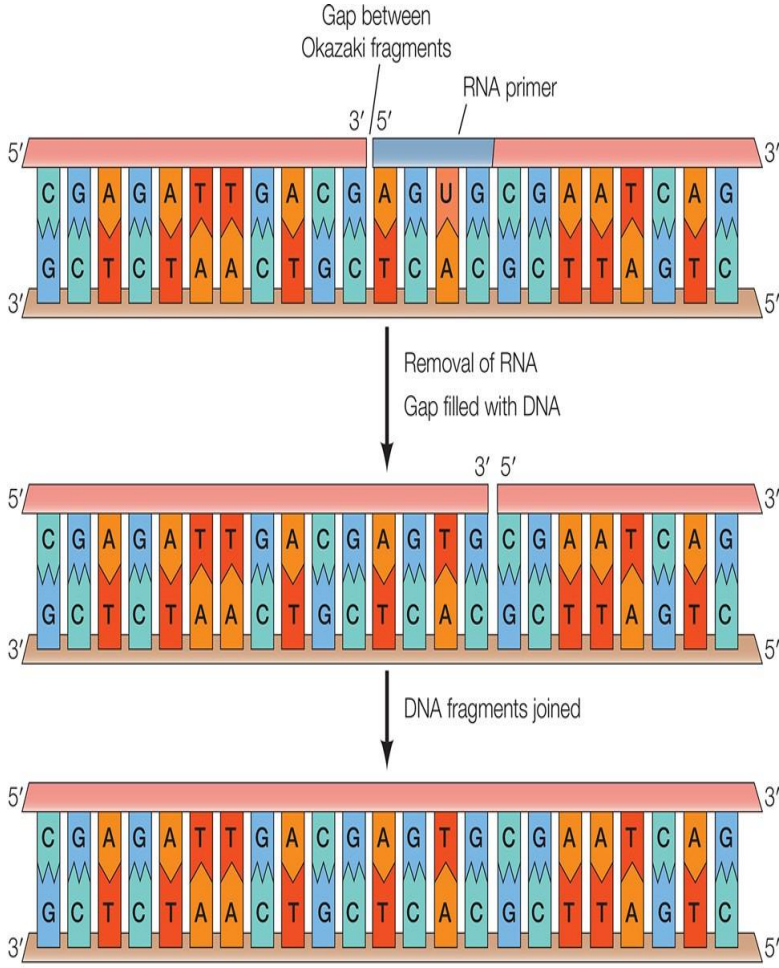
➢ the primase binds to the DNA polymerase alpha and they set on the DNA then the primase synthesize the primer so the DNA polymerase alpha can synthesize the first portion of DNA then they dissociate so the **DNA polymerase delta** comes and **form the Okazaki fragment** in the **lagging strand** and in the leading strand the **DNA polymerase epsilon** takes over.

Removal of primers

- In bacteria, RNA primers are removed by **DNA polymerase I**, which has two activities:

  - ✓ a 5′ to 3′ exonuclease activity hydrolyzing the primer in the 5′ to 3′ direction
  - Exo : from the end /nuclease:breaks down nucleic acid.
  - Meaning that when DNA polymerase comes in it moves along the strand **removing the primers from 5' to 3'** and replacing deoxyribonucleoside instead of ribonucleoside.

  - ✓ A DNA polymerase activity where it fills in the gap. (DNA synthesis from 5' to 3')
  - Then a ligase enzyme links /connects them together

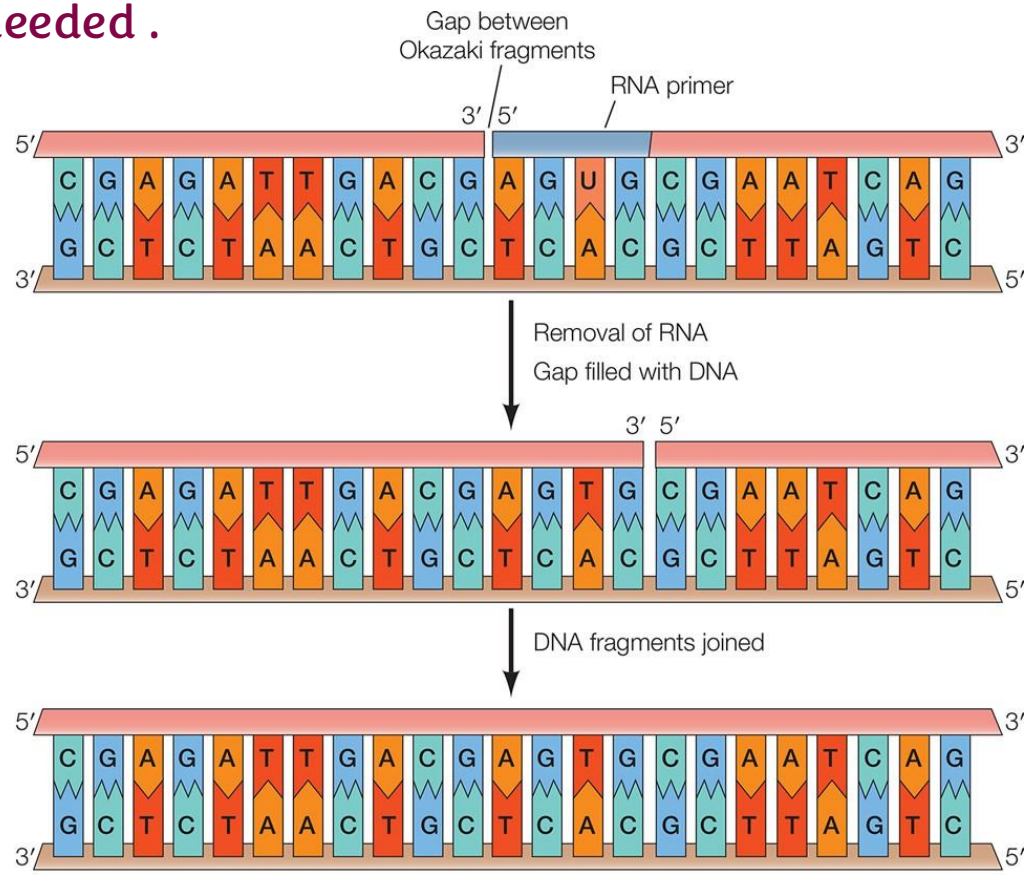


Removal of primers

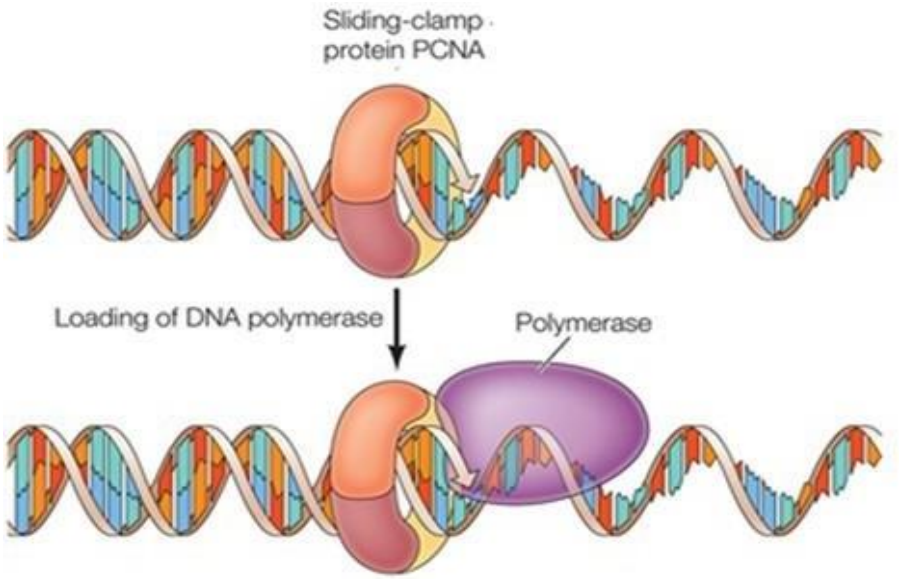
Same mechanism as bacteria, but different enzymes are needed .

- In human cells, 3 enzymes are involved:

  - ✓ RNase H, a 5′ to 3′ exonuclease that removes the primers.
  - ✓ polymerase δ that fills in the gaps
  - ✓ DNA ligase that joins the fragments.
  - **Notice that the ligase is important in both humans and bacteria.**

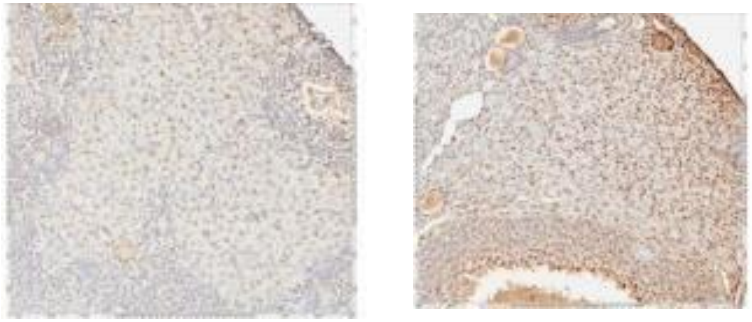


Clamping and sliding



- **The sliding-clamp protein** , which is called **proliferating cell nuclear antigen [PCNA] in human cells** is associated with the major replicative polymerases loading them onto the primer and stabilizing their association with the DNA template.
- What it first do that it guides the DNA polymerase to where the primer is and it binds to the DNA polymerase as it's synthesizing the DNA so it strengthen the interaction between it and the DNA
- Dividing cells must have a high level of PCNA
- Note: PCNA is a diagnostic marker of proliferating cancer cells. And tells if the cancer cells are aggressive or not

Normal cells Cancer cells

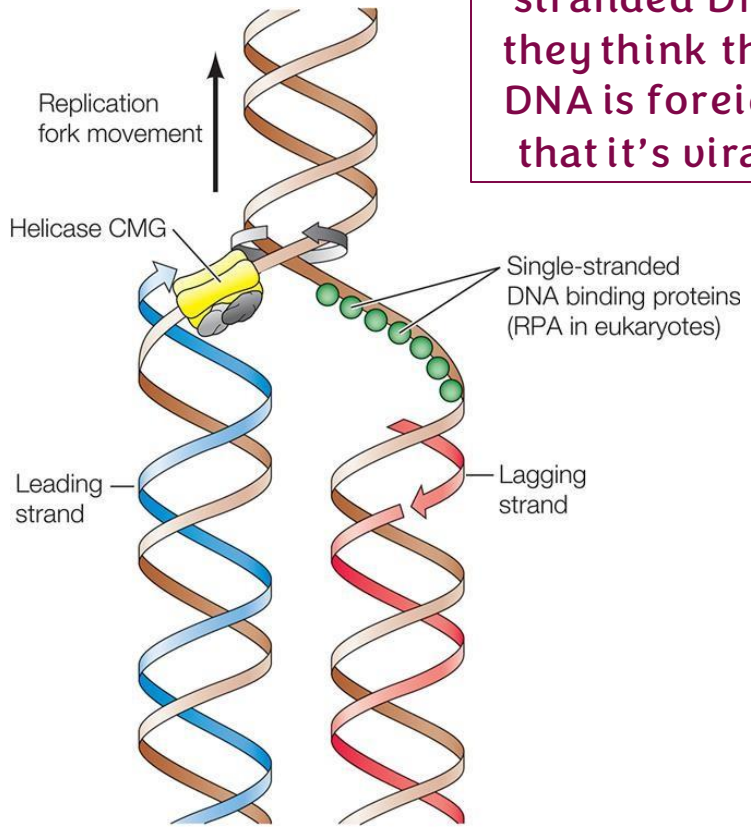


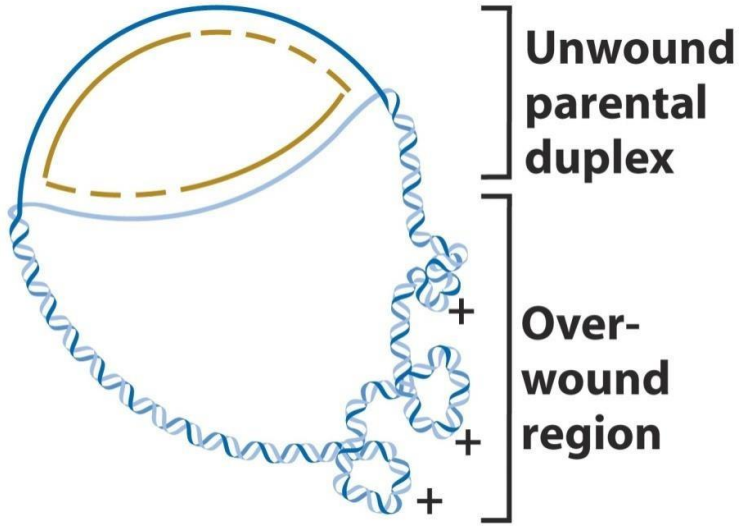
The brown color indicates the expression of a protein and as you can see in cancer cells there is more brown color so more protein is abundant mainly PCNA

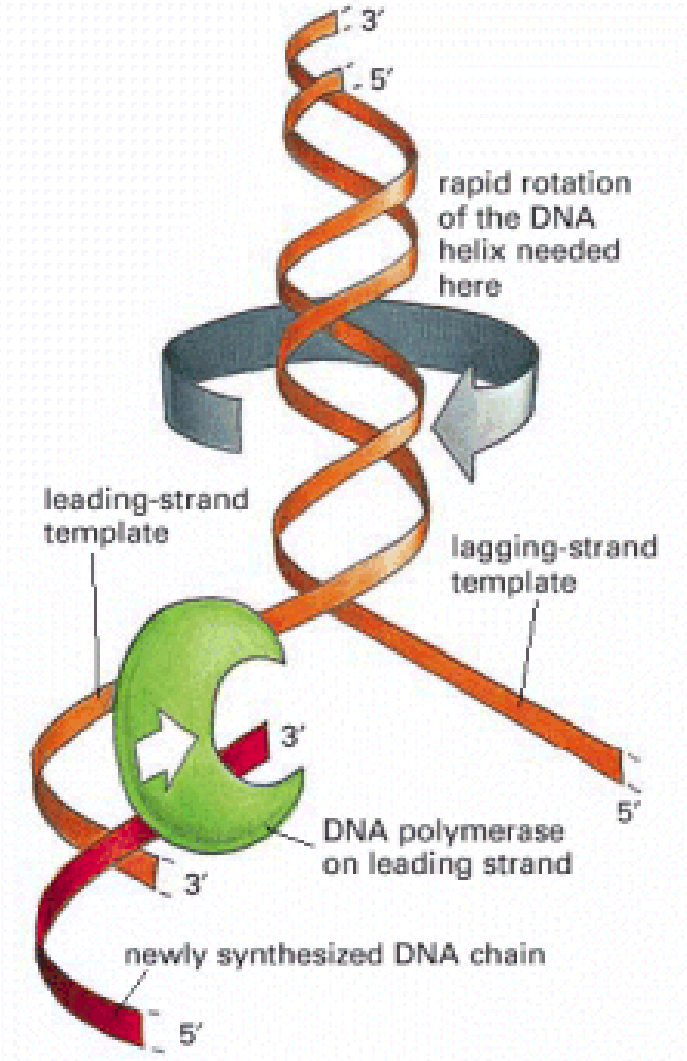
DNA helicases and SSB proteins

Cells doesn't like to see a single stranded DNA cuz they think that this DNA is foreign and that it's viral DNA

- The double-stranded DNA is opened up by DNA helicases (they separate the two strands from each other, allowing the DNA polymerase to read each strand and start replication)
- single-stranded DNA-binding proteins called replication protein A (RPA) do these:
  - ✓ Prevent the formation of short hairpin structures,
  - DNA &RNA are dynamic molecules so if a part of the DNA was complementary with another one in the same strand it will form a hairpin shape which will block the DNA polymerase from moving forward as it thinks that this is a dsDNA and it can't synthesize DNA in this case
  - ✓ Protect single-stranded DNA from being degraded, and
  - ✓ Prevent the renaturation of DNA.
  - Remember that the two stranded are complementary to each other so they can come back and renature.





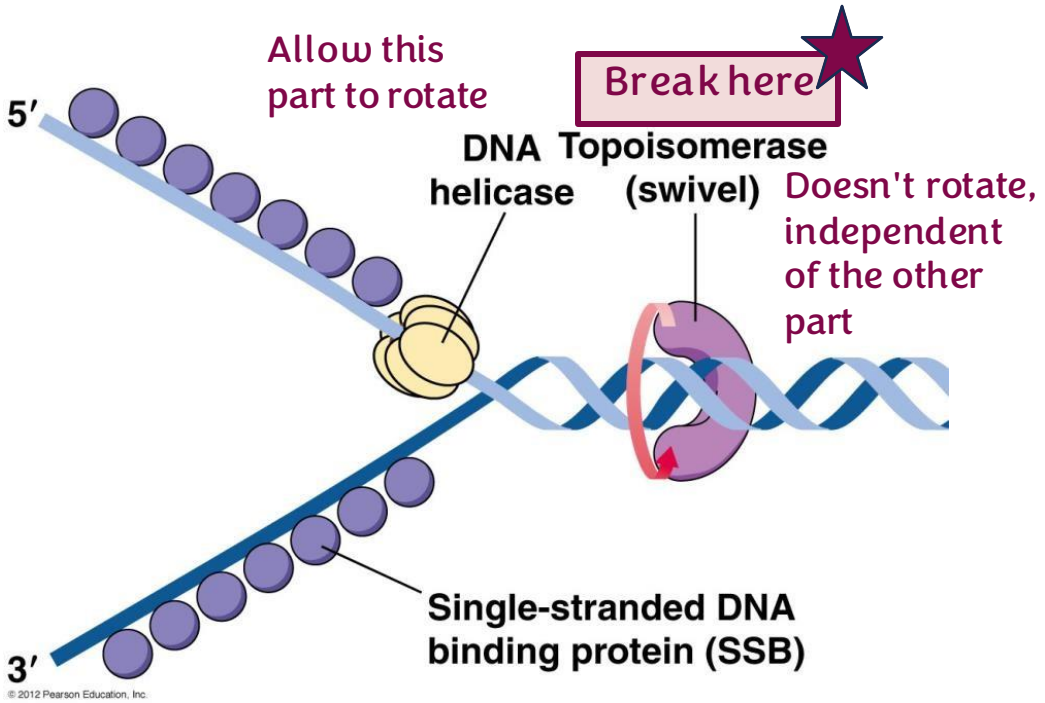


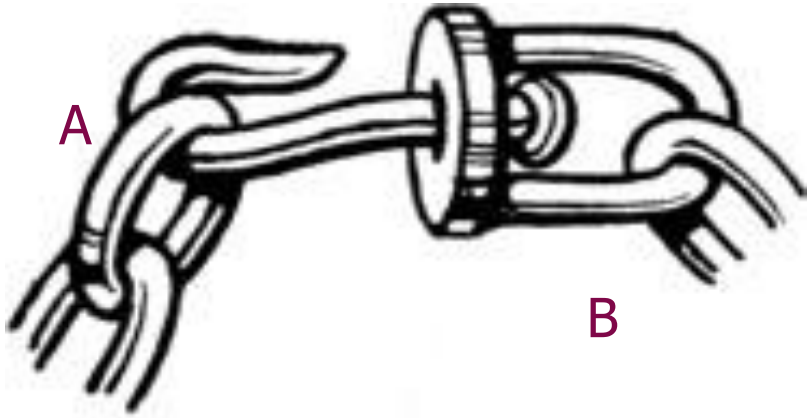
- We have another challenge which is when helicase separate the dsDNA it will cause a rotation in the regions that it didn't separate yet causing the formation of over wound regions whether it's a linear or circular DNA preventing the movement of DNA polymerase

DNA topoisomerases

- It's an enzyme that changes the structure of a molecule.

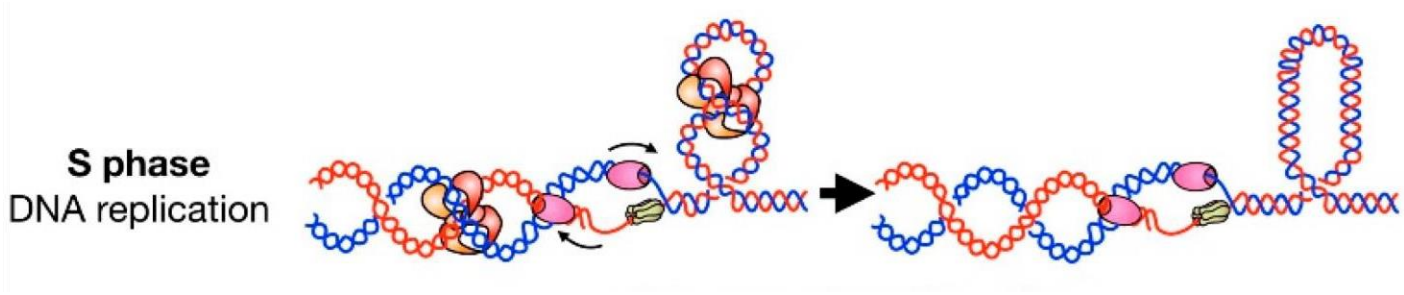
- A swivel is formed in the DNA helix by DNA topoisomerases.
- A DNA topoisomerase breaks then re-forms phosphodiester bonds in a DNA strand.
- Type I topoisomerases break just one strand of DNA
- Type II topoisomerases introduce two breaks: one break on each strand.





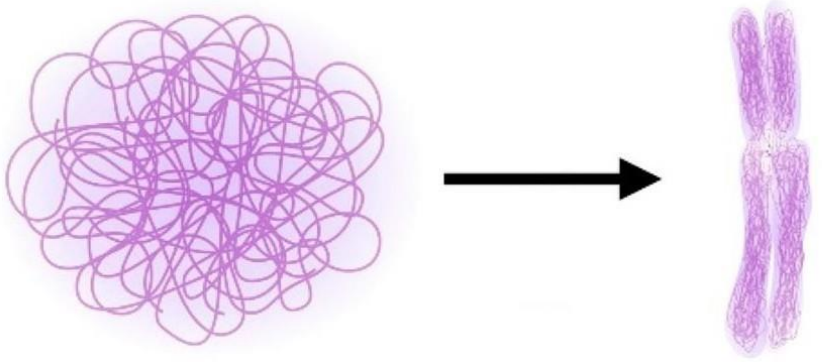
This is a swivel, where one half "A" could be rotated without affecting the other half "B".

Topoisomerase functions similarly by rotating only a small portion of the DNA to remove overwound regions while maintaining the replication fork and the DNA ahead intact.

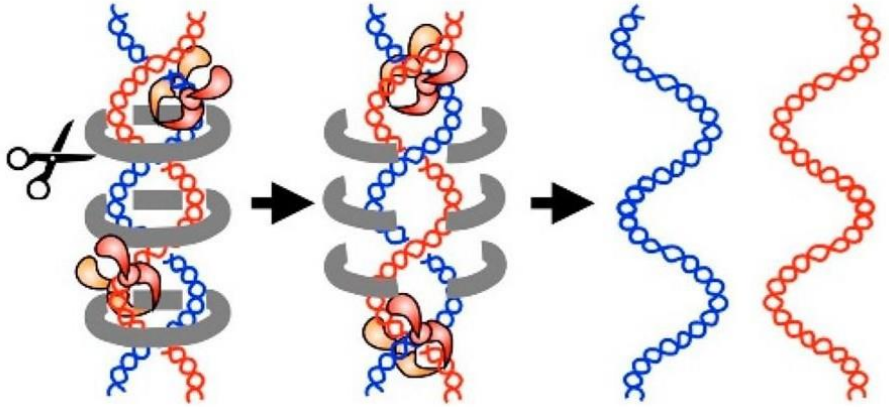


Other functions of topoisomerase II

- Note: topoisomerase II is also required for
  - mitotic chromosome condensation
  - Right before cells divide, their DNA must be replicated, and the chromosomes must be condensed, this process is facilitated by topoisomerase II
  - the separation of daughter **(sister)** chromatids at mitosis.



- **Since this enzyme has a role in DNA replication and in cell division, it's targeted by inhibitors to treat cancer by preventing cancer cells from dividing:**
- Antineoplastic (Anti-tumor) anti-topoisomerase II inhibitors include:
  - Anthracyclines
  - Doxorubicin
  - Mitoxantrone



They prevent cancer cells from dividing cuz they inhibit topoisomarase II.

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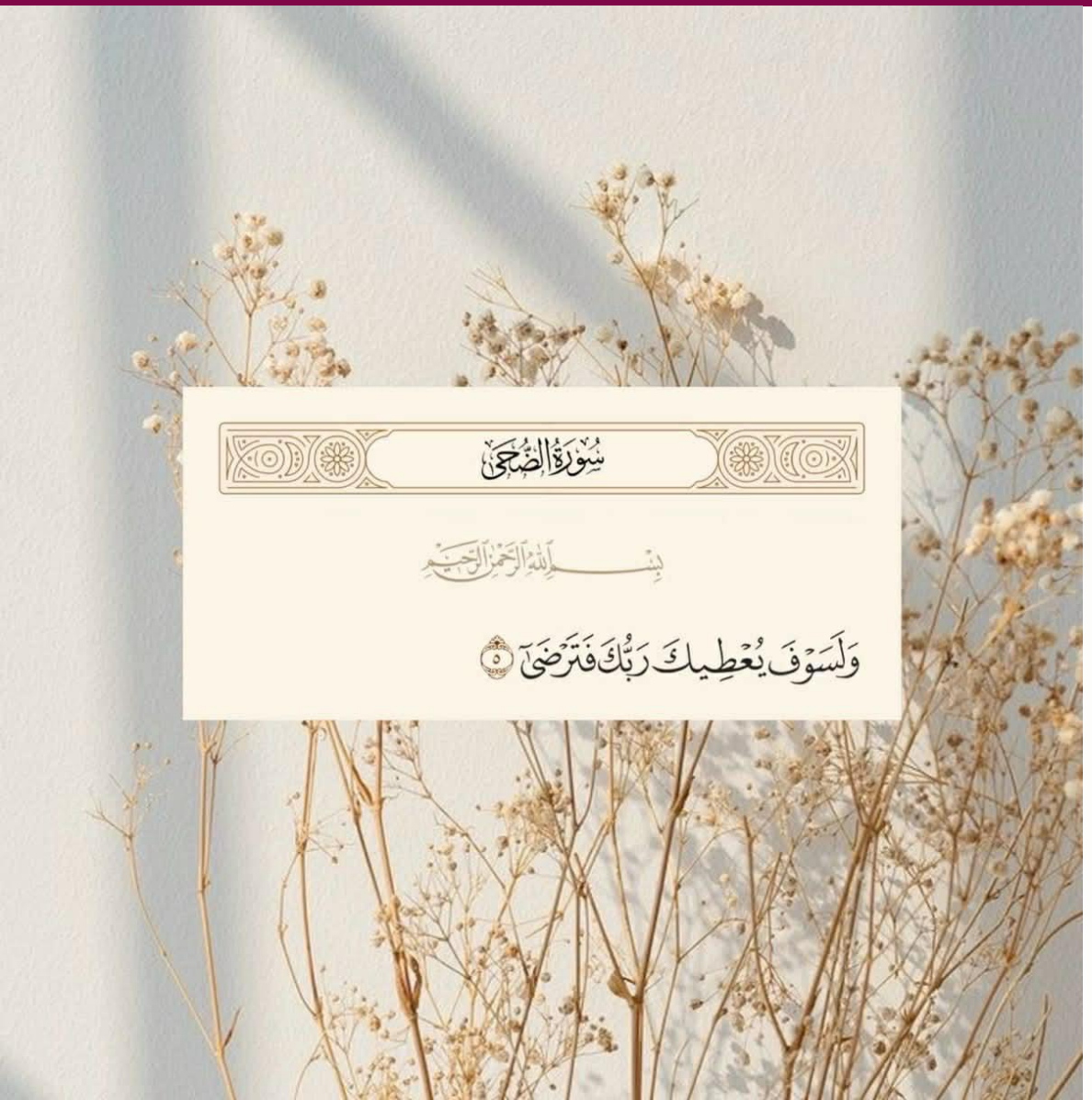

(وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَى)

أنت لا تعرف كيف سيغيّر الله المشهد لأجلك، وكيف سيعيد ترتيب الأقدار لأجل ندائك ورجائك، **(فَاسْتَجَبْنَا لَهُ)** هذه لوحدها كفيلة أن تجعل من المستحيل ممكنًا، ومن الصعب سهلاً، ومن البعيد قريبًا فأبشر، يا من لجأتَ لمن إذا أراد شيئًا قال له: كن، فيكون...

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			