Molecular Biology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

Done By

Ayat Nabil

Contributed in the Scientific Correction

Rahaf Abu Zayed, Rahaf Mohammad

Contributed in the Grammatical Correction

Rahaf Abu Zayed, Rahaf Mohammad

Doctor

Walhan Alshaer

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ) [البقرة: 32]
(وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا) [الإسراء: 85]

Some Basic Information:

😊- اقرأوا الملاحظات صفحة 10 في الشيت.

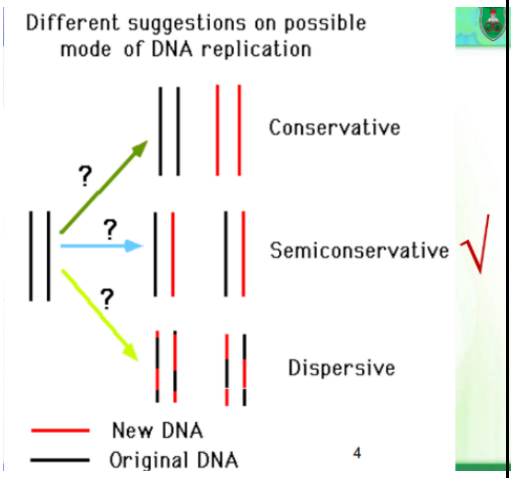
- DNA is the storehouse of the genetic information and the entire DNA content of the cell is known as genome.
- DNA is organized into chromosomes. Also, we have some non chromosomal DNA like mitochondrial DNA, plasmid DNA and chloroplast of plant cells.
- Bacterial genome: usually one and circular chromosome.
  While in:
- Eukaryotic genome: multiple, linear chromosomes complexed with proteins known as histones. {Histones are positively charged proteins}

➤ The genetic information is found in the DNA, and this genetic information should be copied and transmitted to daughter cells after division.

DNA Replication: the mechanism of producing copies of DNA.

How does the DNA replicate?

We have three suggested models for the DNA replication:



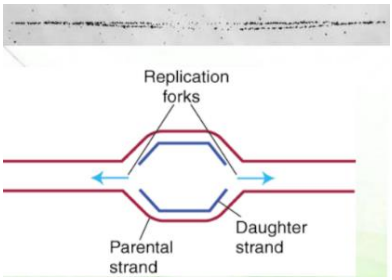
1. The first one is the Conservative one, the resulting two double strands contain one original and one new, and they are separated as in the figure.(The original and new strands are separated; one of the resulting two double strands contains the original strands while the other contains the new strands, so the parental DNA is "conserved")
2. In the Dispersive model, the resulting two double strands contain a mix of new and original DNA as in the figure.
3. However, scientists revealed that DNA replication should be Semiconservative, in which each of the resulting double strands contains an original strand and a new strand. This model was considered the correct model, while the other two models were considered wrong.

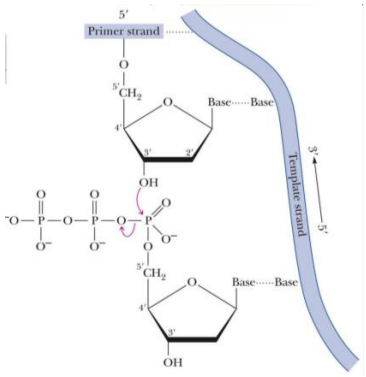
- Replication moves progressively along the parental DNA double helix bidirectionally.

  The DNA is double stranded and antiparallel, and those two strands should be separated to allow the synthesis of new strands. Each parental strand serves as a template for the synthesis of new daughter strand and the direction of synthesis is from 5' end to the 3' end. Therefore, the replication of DNA is considered bidirectional.

- Because of its Y-shaped structure, this active region is called a replication fork.

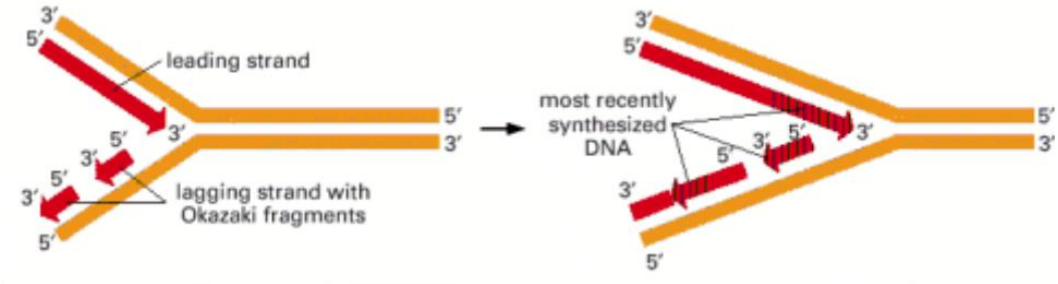
  As you can see in this figure, the bidirectional synthesis of the new DNA strands, and because this active region looks like a Y-shaped structure, it's called the replication fork.





Here in this figure you can see the formation of a phosphodiester bond between two nucleotides from 5' to 3' end.

➤ A long strand and shorter pieces (Okazaki fragments) of DNA are present at the growing replication fork.



The synthesis of the two new strands differs, the first strand starts growing in the replication fork in a continuous manner (the upper red arrow) without interruption or gaps, while the second strand is synthesized as short pieces of DNA called Okazaki fragments, and

each fragment's size ranges between 150-200 bases, in fact there will be gaps between the okazaki fragments and these gaps will be later on filled and ligated to form a continuous complete strand called the **lagging strand**.

Components of DNA replication

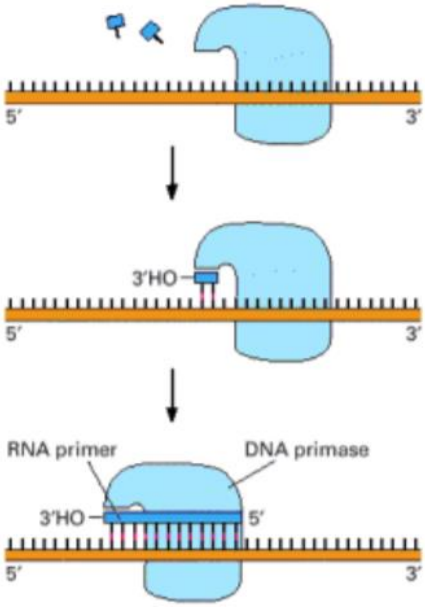
(and some differences between prokaryotes and eukaryotes)

RNA primer

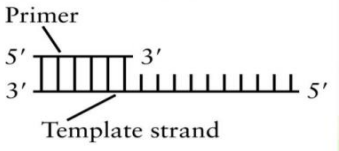
- DNA polymerases cannot initiate replication de novo. So, they require a RNA primer that is complementary to the DNA template to be added first.

  Actually, we need the RNA primer. Why?

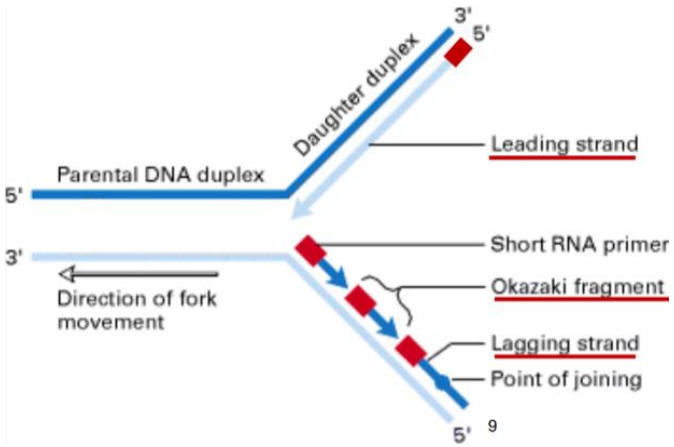
  DNA polymerases mediate the synthesis of DNA and they cannot initiate the DNA replication by itself. DNA polymerases need a 3'-OH end to start synthesis and such 3'-OH end can be provided by a short RNA sequence called RNA primer.



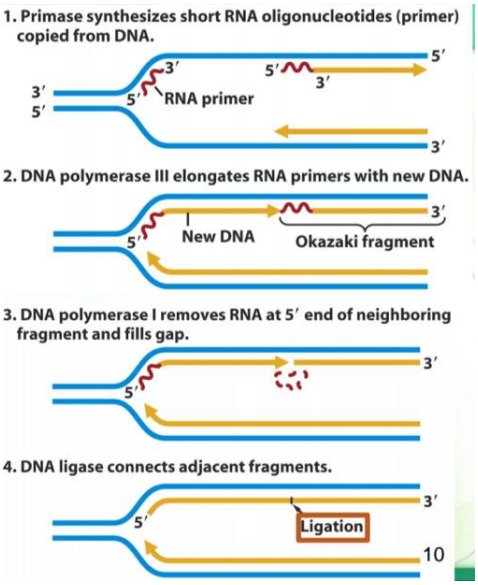
- RNA primer is synthesized by a primase.



This RNA primer can be synthesized by an enzyme called DNA primase (which is RNA polymerase and it's responsible for synthesis of short RNA sequences that range in size between 9-12 bases). So, the DNA primase adds the primer sequence, then DNA polymerase recognizes the primer and the 3'-OH end to start synthesis of the new DNA strand.



In this figure notice the position of RNA primers for the leading strand and lagging strand (in red). For the leading strand we need a single primer to start the synthesis of the whole sequence (elongate) while for the lagging strand we need multiple primers (one primer for each Okazaki fragment).



This figure summarizes the whole process of adding primers, elongation of DNA, removing primers and the ligation of adjacent fragments at the end (synthesis of new DNA strands - leading strand and lagging strand -)

NOTES about the steps:

2 ⟶ the DNA polymerase III recognizes the 3'-OH end provided by the RNA primer and start synthesis of new DNA.

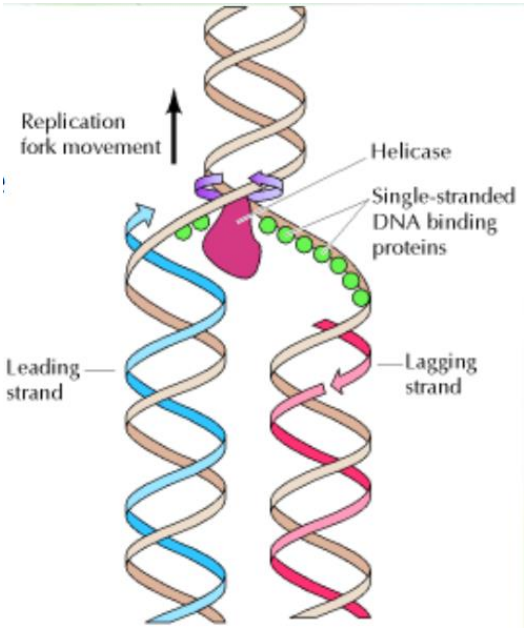
4 ⟶ fragments are ligated and joined together (connected) to form complete and continuous new DNA strands.

DNA helicases and SSB proteins

(important players in DNA replication)

- For DNA synthesis to proceed, the DNA double helix must be opened up ahead of the replication fork.

- Opening up the DNA double helix is done by two types of proteins contributing to this process:
  a) DNA helicases
  b) single-strand DNA-binding proteins called replication protein A (RPA).



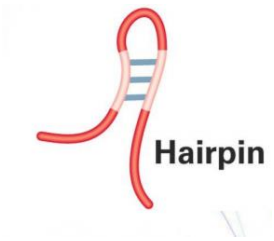
DNA helicases

- DNA helicase bind to the double stranded DNA and start separation and unwinding the double strand into single strands by breaking the **hydrogen bonds** between the annealed nucleotide bases.
- DNA helicases use ATP (as a source of energy) to open up the double helical DNA as they move along the strands.
- In bacteria, helicases form a complex with the primase called **primosome**.

Single-strand DNA-binding (SSB) proteins

- Single-strand DNA-binding (SSB) proteins bind tightly to exposed single-stranded DNA strands without covering (or hiding) the bases, which remain available for templating.
- These proteins can do several functions (have important roles):

a) Prevent the formation of the short hairpin structures.



Short hairpin structures are a kind of complex structures as a result of **complementarity** between the bases among the single strand (of the same sequence), and these hairpin structures may interfere with the replication machinery.

So, single-stranded DNA-binding protein help solve and inhibit the formation of these complex structures and allow the replication machinery to proceed smoothly.

b) Protect (inhibit) single-stranded DNA from being degraded.

c) Aid (help) helicases by stabilizing the unwound, single stranded conformation.

As we mentioned before, DNA synthesis is mediated by DNA polymerases in prokaryotes and eukaryotes.

DNA polymerases in prokaryotes

In prokaryotes we have five different DNA polymerases with different functions:

- DNA polymerase III: DNA polymerization at the growing fork in E. coli.
  - The complex of primosome and DNA polymerase III is known as **replisome.**

REMEMBER:

Helicase + Primase ⟶ Primosome

Primosome + DNA polymerase III ⟶ Replisome

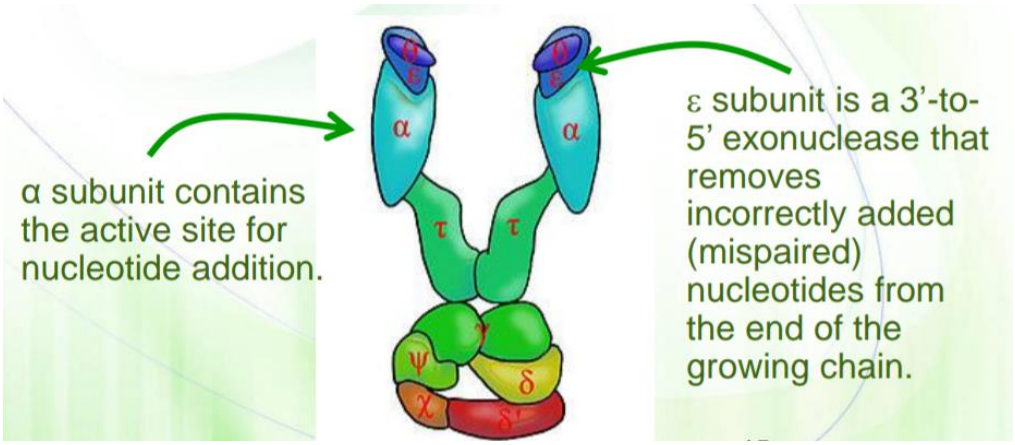
- DNA polymerase I has several functions:
  - **5'-to-3'** exonuclease activity (removal of RNA primer) of each Okazaki fragment.
  - Fills in the gaps between the lagging-strand fragments.
  - Playing a role in DNA repair.
- DNA polymerase II, IV, and V: DNA repair

DNA polymerase III

- The DNA polymerase III is a very large protein composed of 10 different polypeptides with different functions.

Three important subunits in the DNA polymerase III:

1) α subunit: is responsible for the polymerization or polymerase activity.
2) ε subunit (pronounced Epsilon): responsible for **3' to 5'** exonuclease activity (the proofreading or correction of incorrectly added bases (mispaired))
3) θ subunit: stimulates the ε subunit proofreading. So, it helps the ε subunit in the proofreading or the **3' to 5'** exonuclease activity.



DNA polymerase III

REMEMBER:

Exonucleases are enzymes that cleave DNA or nucleic acids from either end, so they cleave the first nucleotide from either end.

How accurate is DNA replication?

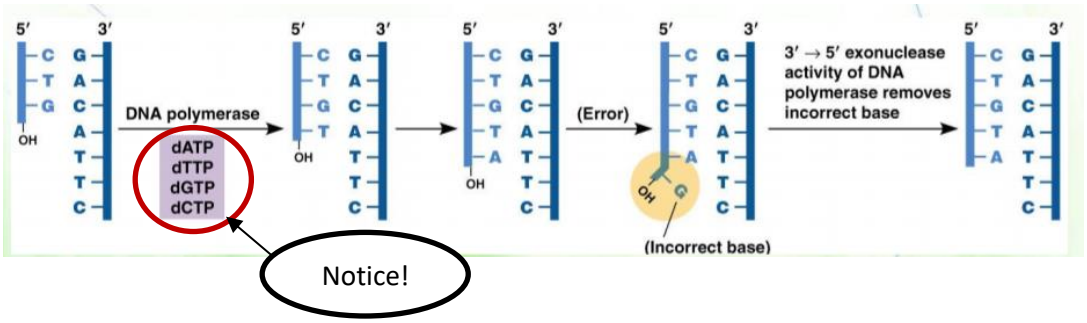
- In fact, there are different mechanisms to ensure high fidelity of DNA.
- The frequency of errors during replication is only one incorrect base per 10^8 nucleotides incorporated.
- DNA polymerases synthesize the DNA with high fidelity.
- **How is fidelity high?**
  - The DNA polymerase can catalyze the formation of phosphodiester bonds when the right hydrogen bonding takes place between the bases.

    REMEMBER: base pairing → G with C, A with T

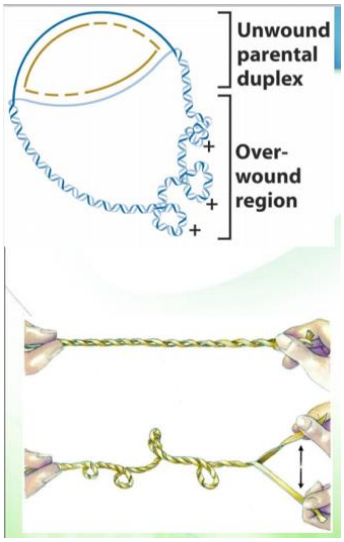
    BUT this process produces one error base per 1000 correct bases synthesized. (accuracy=1/1000)

  - Proofreading mechanism (a **3'→5'** exonuclease activity)- **Remember** ε subunit of DNA pol III.

➔ This exonuclease activity increases the fidelity rate into 1 error per 10^8 correct nucleotides added.

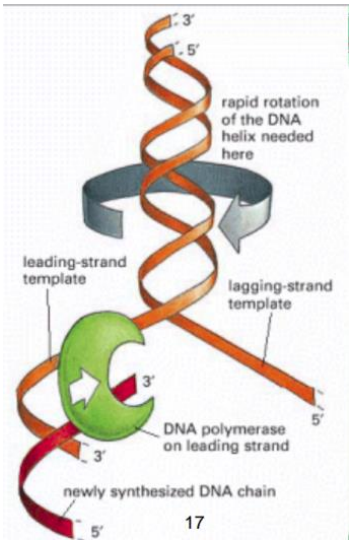


Solving the problem of supercoils



As the two strands of double helix are separated a problem occurs, mainly the appearance of **positive** supercoils as you can see in the figure below.

This issue must be solved before the replication fork can proceed. These supercoils (or sometimes called super twists) can interfere with the replication machinery. Solving this problem is mediated by a group of enzymes called topoisomerases.



DNA topoisomerases

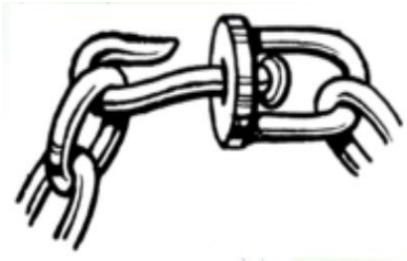
- DNA topoisomerases are responsible for removing supercoils in the helix.
- A swivel is formed in the DNA helix by proteins known as DNA topoisomerases.

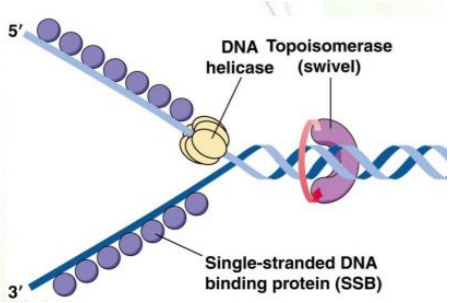
How do they work?

- A DNA topoisomerase breaks then re-forms phosphodiester bonds in a DNA strand.
- There are two different topoisomerases, named topoisomerase **I** and **II**.
    - Topoisomerase I produces a transient single-strand break (or nick) (a reversible cut in the single strand of the double helix).
    - Topoisomerase II binds to the double helix, cuts and rejoins **both** strands.

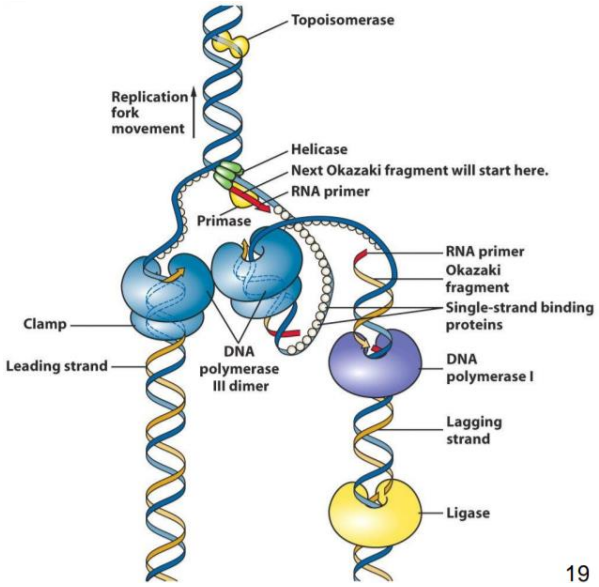
➔ Topoisomerase I works without the need of ATP, so it is ATP-independent.

➔ Actually, they use the energy from phosphodiester bond they cleave, and using this energy they reseal the strand again.





DNA replication machinery is coordinated



Based on what we described before, you can see that the DNA replication machinery is coordinated. We have multiplayers and every player does its job in high precision and accuracy. This figure summarizes the basic concepts of DNA replication.

Dr. Walhan recommended you read it to catch and have some key points for the DNA replication.

This is the end of this lecture for Part 1 of DNA Replication

Good Luck!

(اللَّهُ الَّذِي خَلَقَ سَبْعَ سَمَاوَاتٍ وَمِنَ الْأَرْضِ مِثْلَهُنَّ يَتَنَزَّلُ الْأَمْرُ بَيْنَهُنَّ لِتَعْلَمُوا أَنَّ اللَّهَ عَلَىٰ كُلِّ شَيْءٍ قَدِيرٌ وَأَنَّ اللَّهَ قَدْ أَحَاطَ بِكُلِّ شَيْءٍ عِلْمًا) [الطلاق: 12]

- المختلف شوي بالشيت هذا إنه ملوّن اللون الأسود هو الكلام اللي بالسلايدز واللون الخمري هو كلام الدكتور وشرحه واللون الأزرق وهو بس ملاحظات لتجميع الأفكار فقط
- التلوين بس مشان إذا في ناس بتحب تدرس السلايدز تدرسهم عراحتها وبترجع لكلام الدكتور هان بتشوفه
- الشيت أغلبه بنعرفه من قبل وسهل وحلو رح يكون إن شاء الله استمتعوا بدراستكم
- هلأ ارجعوا لبداية الشيت وبلشوا موفقين بإذن الله